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on
Food Safety, Food Security, and Sustainable Development

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Asian Food Safety and Security Association (AFSA)

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Asian Food Safety and Security Association

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A brief note on AFSA

The Asian Food Safety and Security Association (AFSA) was founded to provide opportunities for researchers, teachers, students, entrepreneurs, non-governmental organizations (NGOs), and government organizations to undertake collaborative research among member countries to improve life quality. To address new and complex challenges across various disciplines and conduct advanced research necessitate a platform of specialists from multiple disciplines to exchange ideas, address challenges, and discuss strategies to enhance the ongoing global efforts to deliver safe foods for the better health and environment of developing-country people. As a result, AFSA organizes regional and international conferences in Asian nations every other year to discuss key food safety, food security, health, and climate change issues and publishes high-quality articles in the conference proceedings. AFSA is a collaborative platform for academics, researchers, students, institutions, funding organizations, corporations, and society to conduct, organize, and assess multidisciplinary research programs, training, and projects.

PREFACE

This proceedings is a compilation of the keynote addresses and oral and poster presentations given at the International Conferences on Food and Agriculture Advanced Technology for Sustainable Development (FAATSD 2022) held at the Industrial University of Ho Chi Minh City (IUH) on November 24-27, 2022, and co-organized by the Asian Food Safety and Security Association (AFSA), the Industrial University of Ho Chi Minh City (IUH), the Asia-Pacific Institute of Food Professionals (APIFP), and EcoTech Village belongs to Techfest Vietnam, Ministry of Science and Technology, Vietnam. This proceedings is divided into five sections: Agriculture and Advanced Technologies; Food Processing and Food Economy; Food Biotechnology and Food Waste; Food Safety, Food Security, and Climate Change; and Miscellaneous. Section A featured CRISPR-CAS technology for wheat blast detection, a new climate-intelligent farming system, a biofilm shield for sustainable agriculture, and eggplant genetic diversity to improve food safety and security. Section B discusses opportunities and problems in the coffee industry, cuttlefish sashimi meat, packaging conditions, biological activities, and e-commerce, consumer trust, and other issues influencing the food sector. Section C examined genetically engineered fish and sea-fish and their benefits and drawbacks, as well as enhancing food values through the use of extracts, enzymes, and meal replacements. Section D discussed sustainable resource management, rational use of antibiotics as agricultural chemicals, comparative food safety systems, vegetable consumption and use of non-chlorine sanitizer in vegetables, toxins and heavy metals bio-decontamination, and fecal contamination in water systems. Section E examined consumer buy intention, factors influencing purchase intention, customer behavior marketing-mix factors, molecular docking model, de novo MicroRNAs methodologies, and some other synthesis. As our world grows more linked and our food supply chains span great distances, assuring the safety and security of what we eat has become an increasingly serious concern. This proceedings highlights innovative approaches, technologies, and strategies used to improve food safety and security, ranging from block chain in supply chains to precision agriculture, and serves as a comprehensive resource for policymakers, food industry professionals, academics, and students.

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About Asian Food Safety and Security Association (AFSA) (Past, Present, and Future)

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Background

The passion of some Asian scientists who believe that food and agriculture science, bio-science, and biotechnology contribute to improvement in the quality of life and welfare and understand the value of laboratory work and direct exchange of ideas and information between experts, entrepreneurs, and government officials would result in positive changes in ensuring food quality and safety. In 2005, the Bangladesh-Japan Association for Science and Technology (BJAST) was founded in Osaka, Japan, and decided to hold workshops every year in Japan with alternative years in Bangladesh. The 5th annual general meeting of BJAST was held at Dhaka on December 26 - 28, 2010, where 14 countries of Asia participated and unanimously decided on the formation of the Asian Food Safety and Security Association (AFSSA). However, after discovering another French Agency for Food Safety (AFSSA) with a similar abbreviation, we modified the acronym to AFSA rather than AFSSA. The Asian Food Safety and Security Association (AFSA) is a non-profit charity organization founded in 2010 in Dhaka, Bangladesh, with the following basic goals: 1) To foster practical collaboration among member countries in areas such as climate change, food safety, and food security; 2) To communicate research findings and future research in food safety and security; and 3) To promote Food Safety and Security knowledge for disease prevention and control through training, conferences, workshops, and information distribution via various channels. 4) To assist educational institutions in developing food safety and security expertise by supplying equipment, publications, materials, concepts, and so on. 5) To establish, document, maintain, and communicate standardized Food Safety and Security protocols and procedures; 6) To assist national and regional research activities, as well as to preserve food safety and security, by building the Asian Food Safety and Security Network. 7) To foster strong relationships among Asian countries and to establish links with essential partners and other organizations; 8) To assist in securing funds for students to attend seminars, conferences, and training programs. 9) To launch a journal on food safety and security in Asia, emphasizing current trends and requirements. To begin, a 20-member board of advisors comprised of notable professionals worldwide and an executive committee comprised of one president, two vice presidents, one general secretary, two joint secretaries, one treasurer, and five members were constituted for a five-year tenure. Following that, a new EC committee was formed every five years following AFSA norms.

AFSA programs:

On September 15-17, 2012, the first AFSA conference on food safety and security was held at the Rinku Campus of Osaka Prefecture University in Osaka, Japan. This conference was held at Osaka Prefecture University in Japan. The two-day scientific and poster presentation sessions included four plenary speeches and four keynote talks on food safety and security, five microbial control oral presentations, and five food nutrition oral presentations, as well as 57 posters on food microbiology and its detection that were displayed and discussed, providing multiple opportunities for experts and participants to discuss key food safety and food security issues. Experts from government organizations, international organization, businesses, and universities from many nations might share their ideas, solve difficulties, and debate methods and collaborative projects to improve the world's ongoing efforts to provide safe foods for better health and the environment of the people of developing countries.

On August 15-18, 2014, the 2nd AFSA conference was conducted at Dong Nai University of Technology in Bien Hoa City, Vietnam, and this 2nd AFSA conference tackled crucial food safety and food security concerns. The plenary and concurrent sessions addressed the challenges that Asia faces in ensuring food safety in the development of new food products and processing technologies, globalization of food trade, protection of foods derived from biotech, microbiological risks, the emergence of new and antibiotic-resistant pathogens, particularly emerging pathogens, directing research to high-risk areas, focusing intervention, and establishing target risk levels. The two-day scientific and poster presentation sessions gave experts and participants numerous opportunities to debate crucial food safety and security problems. Experts from governmental organizations, international organizations, entrepreneurs, and universities from various countries exchange

ideas, address challenges, and discuss strategies and collaborative programs to strengthen ongoing global efforts to deliver safe foods for better health and the environment of developing-country people. The review committee chose 32 articles for publication in the 2nd AFSA proceeding, several of which were published in the Journal of Food and Nutrition Science (JFNS).

On September 15-17, 2016, the third AFSA conference was conducted at KIIT University in Bhubaneswar, India. This bi-annual conference is sponsored by the Asia Pacific Institute of Food Professionals (APIFP), the International Committee on Food Microbiology and Hygiene (ICFMH), the University of Dhaka, and KIIT University in Bhubaneswar, India. At this conference, 170 higher-level research papers from 13 countries were presented, lively debates regarding food safety were held, and 14 articles were chosen for publishing in the 3rd AFSA proceedings.

On August 10-12, 2018, the 4th AFSA conference was held in the Angkor Paradise Hotel in Siem Reap, Cambodia. This symposium was co-organized by Cambodia's Royal University of Agriculture and Thailand's Mekong Institute. Asia Pacific Institute of Food Professionals (APIFP), International Committee on Food Microbiology and Hygiene (ICFMH), USAID, CIAT Asia Regional Office, Hanoi, Vietnam, Global Knowledge Center on Crop Biotechnology, ISAAA SEAsia Center, Los Banos, Philippines Horticulture Innovation Lab and Postharvest Technology Center, UC Davis, USA, and CESAIN, RUA, Cambodia provided additional support. Many renowned guests and researchers from 18 countries attended the 4th AFSA conference, where 250 higher-level research papers were presented, heated discussions took place, and only 26 high-quality publications were published in the conference proceedings.

The 5th AFSA conference on food safety and security was scheduled for September 16-18, 2020, at Chulalongkorn University in Bangkok, Thailand. However, because of the worldwide COVID-19 pandemic and the travel embargo, organizing conferences in 2020 and 2021 was impossible. Following that, one of the AFSA EC members sought a joint international conference in Ho Chi Minh City, Vietnam, on November 24-27, 2022. Almost 90% of AFSA members decided to plan the International meetings in Vietnam collaboratively. As a result, we agreed to conduct the International Conferences on Food and Agriculture Advanced Technology for Sustainable Development (FAATSD 2022) at the Industrial University of Ho Chi Minh (IUH) from November 24-27, 2022. The Asian Food Safety and Security Association (AFSA), the Industrial University of Ho Chi Minh City (IUH), the Asia-Pacific Institute of Food Professionals (APIFP), and EcoTech Village are organizing this conference in collaboration with the Ministry of Science and Technology of Vietnam. There are 62 high-quality presentations and interactive discussions were given during this conference, and Tylor and Francis Press have agreed to publish 25 full papers and seven invited pieces in a book collection. The 6th AFSA conference on food safety and security was supposed to be held on 25-27 September 2024 at Chulalongkorn University, Bangkok, Thailand. Department of Food Science and Technology of Chulalongkorn University will host this conference.

Future perspectives

Our never-ending quest for improved quality of life (QOL) and the welfare of humans through Agriculture, food-bioscience, and biotechnology calls for a constant flow of new, creative knowledge. We began our adventure in Dhaka, Bangladesh, in 2010 and continually held the conference every two years across Asian countries. This series of discussions was started by the passions of some scientists in Asia who realize the value of collaborative work and open communication of ideas and information and feel that food and agriculture science, bioscience, and biotechnology increase the quality of life and welfare of people around the world and by holding such conferences, there is an opportunity for interaction between academia and regulatory authorities, students and scientists, non-profit organizations, international organizations, local entrepreneurs, and industries. In addition, the following food safety and security trends and challenges should be resolved.

1. **Technological Advancements**: Technological advancements, including block chain, artificial intelligence, and data analytics, will likely play a significant role in enhancing food safety and security across Asia. These technologies can help track and trace food products, detect contaminants, and monitor supply chains more effectively.
2. **International Collaboration**: Given the global nature of food supply chains, collaboration between Asian countries and international organizations will be essential. Sharing best practices, harmonization of regulations, and cooperation in food safety and security initiatives will become increasingly important.
3. **Rapid Urbanization**: Asia is experiencing rapid urbanization, changing dietary patterns and food distribution systems. Managing food safety in densely populated urban areas will require innovative solutions and regulatory adjustments.
4. **Climate Change**: Climate change poses challenges to food production and security. Extreme weather events, altered growing seasons, and changing pest patterns can impact food safety. Adaptation and mitigation strategies will be necessary.

5. **Consumer Awareness**: As consumers become more health-conscious and concerned about the origin of their food, there will be a growing demand for transparent labeling and safer food products. This will pressure governments and food producers to ensure food safety and security.
6. **Regulatory Frameworks**: Governments in Asia will need to continually update and strengthen their regulatory frameworks to keep pace with evolving food safety risks. This includes setting and enforcing standards, conducting inspections, and imposing penalties for non-compliance.
7. **Education and Training**: Building capacity in the food industry through education and training programs will be crucial. This includes training farmers, food handlers, and inspectors on best practices in food safety.
8. **Food Fraud Prevention**: Preventing food fraud, including adulteration and counterfeiting of food products, will be a priority. Enhanced testing methods and supply chain transparency will be needed to combat such issues.
9. **Biotechnology and GMOs**: The use of biotechnology and genetically modified organisms (GMOs) in agriculture will continue to be debated. Regulatory frameworks and public perception of GMOs will influence their role in food security.

The future perspectives on food safety and security in Asia could be changed, and the specific priorities and challenges could vary by country and region within Asia. Thus, AFSA encourages collaboration with government institutions, international organizations, entrepreneurs, and universities from various countries to share ideas, address challenges, and discuss strategies and collaborative programs to strengthen ongoing global efforts to deliver safe foods for better health and the environment of developing-country people.

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Section A: Agriculture and Advanced Technologies

Development of a Point-of-Care Method for the Detection of Destructive Wheat Blast Fungus Using CRISPR-Cas Technology

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Abstract

Wheat blast is a destructive disease caused by the filamentous fungus *Magnaporthe oryzae Triticum* (MoT), which poses a serious threat to food security in some South American countries, Bangladesh and Zambia. In 2016, the first epidemic outbreak of wheat blast devastated 15,000 hectares of wheat in Bangladesh with yield losses of up to 100%. In a rapid response, next-generation sequencing, open data sharing, and open science approaches were used for the precise determination of the genetic identity and origin of wheat blasts in Bangladesh as a lineage of South American *M. oryzae*. To achieve the goal of developing a point-of-care diagnostic method for the wheat blast, genomes of *M. oryzae* isolates from rice and wheat were sequenced and two unique DNA fragments, MoT-6098 and MoT-6099 were discovered in the genome of MoT and a Polymerase Chain Reaction (PCR) method and a loop-mediated isothermal amplification technique for the detection of MoT was developed. A new cost-effective, rapid, and upgraded method was developed using CRISPR-Cas 12a technology for MoT detection in infected wheat seeds and plants. This method was further improved as a field-deployable lateral flow assay, Bio-SCAN. Deployment of this rapid and point-of-care method in plant quarantine offices offers an opportunity for restriction of the further spread of MoT fungus to new wheat-growing areas/countries. This method can also be used by farmers for precise detection of MoT in infected plants, seed lots, and alternate hosts as well as monitoring and surveillance of wheat blast disease in the practical field.

Keywords: Blast, Bio-SCAN, CRISPR-Cas**,** *Magnaporthe oryzae Triticum* (MoT), SARS-CoV-2

Introduction

The cereal killer fungus, Magnaporthe oryzae Triticum (MoT), earned its epithet after triggering an epidemic outbreak of wheat blast disease in Bangladesh in February 2016(Kamoun et al., 2019). This notorious fungal pathogen invaded and caused devastation in around 16% of wheat-growing regions of Bangladesh. *Magnaporthe oryzae* (Mo, anamorph *Pyricularia oryzae*) is a filamentous ascomycete that causes blast diseases, infecting more than 50 kinds of grasses which include some of the most commercially significant food crops like rice, wheat, maize, pearl millet, and finger millet (Islam et al., 2016; S. Igarashi, 1986). Different lineages of M. oryzae exhibit a significant degree of host-specificity (Gladieux et al., 2018; Urashima et al., 1993). Blast disease in rice, for example, is caused by *Magnaporthe oryzae oryzae* (MoO), while blast in wheat is caused by the Triticum pathotype of M. oryzae (MoT). Wheat blast is a relatively new disease that is considered a danger to wheat production in around three million hectares of wheat-growing areas in South America and roughly one million hectares in Bangladesh (Islam et al., 2020).

The wheat blast infection was initially observed in South America, specifically in the Paraná State of Brazil in 1985, and subsequently spread to the adjacent wheat-growing areas (Ceresini et al., 2018; Kohli et al., 2011; S. Igarashi, 1986). Beyond South America, MoT was first identified in 2016 in Bangladesh that spread to an estimated 15,000 hectares in eight districts (Pabna, Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, and Bhola) of the country's wheat fields and reported to cause a crop loss of up to 100% (Figure 1). The pathogen expanded to 12 additional districts (Jamalpur, Tangail, Cumilla, Rajbari, Magura, Mymensingh, Madaripur, Faridpur, Natore, Narail, Naogaon, and Rajshahi) in Bangladesh between 2016 and 2019 as well as to two districts (Nadia and Murshidabad) in West Bengal, India (Islam et al., 2020). Tembo et al (2020) reported the prevalence of wheat blast in Zambia, Africa in 2020 (Tembo et al., 2020).

In the globe, next to corn and rice, wheat is the third most widely grown cereal species (Food and Agricultural Organization, http://faostat.fao.org). It is the major source of plant proteins in the human diet and an important source of protein in animal feed. A frequent danger to the security of the world's food supply is the advent of fungal diseases, which have an adverse effect on plant yield (Fisher et al., 2012). The main wheat-cultivating regions in the nearby South Asian countries are still free from the wheat blast but its possible spread has sparked worry on a global scale (Islam et al., 2020). A key danger factor for its spread to the nearby major wheat-growing nations in Asia is its recent arrival in Bangladesh as well as the fungus

being a seed- and air-borne pathogen. The spread of this dangerous infection will be devastating in India and China, which are adjacent and are the second and first major producers of wheat in the world, respectively (Islam et al., 2020). According to a climate analogue model, around 7 million hectares of wheat are at risk from wheat blasts, and 5% blast-related wheat loss might result in losses of up to 132 million USD (Mottaleb et al., 2018)

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Figure 1. Bleached head symptoms in a blast-affected wheat field in Meherpur of Bangladesh in 2016.

The first notable symptom of the wheat blast is the water-soaked, diamond-shaped lesions on the leaves. With disease development, these lesions become eye-shaped and gray-colored that expand and merge with adjacent lesions. The main indications of the outbreak are fully or partially bleached (dead) spikes. Complete or partial bleaching of the head above the spot of infection is frequently seen, along with either no grain or grain that has shrunk and has low test weight and inadequate nutrition (Islam et al., 2016). Infection during the early stages of blooming causes sterility and empty grains. Multiple spots of infection in the rachis can be seen, and infection usually spreads upward from the node. In contrast, infection during the grain-filling stage results in tiny, shriveled, and discolored grains that are unsuitable for human consumption (Islam et al., 2016; Surovy et al., 2020). Gray specks appear on bleached heads, indicating fungal sporulation at the site of infection (Islam et al., 2016). When environmental factors are favorable (18-30 °C and > 80% relative humidity) during the ear emergence or grain-filling stage, this disease can quickly spread and damage the wheat crop (Islam et al., 2019). One of the main issues with managing wheat blast is that the MoT often doesn't manifest visible signs in wheat until the commencement of the panicle, by which time the infection would have spread and given farmers little or no time to take any action. As a result, disease control based exclusively on the identification of visible signs is mostly futile. Again, diagnosing wheat blast disease in the field at the heading stage is challenging because it shows symptoms similar to those associated with Fusarium head blight (Pieck et al., 2017). Furthermore, there is a scarcity of highly efficient and reliable anti-MoT fungicides (Castroagudín et al., 2015; Fernández-Ortuño et al., 2006). Hence, the proper and early diagnosis of MoT is crucial since management measures would be ineffective otherwise. Even though various diagnostic techniques were devised for the wheat blast, they are not very feasible or completely dependable (Gupta et al., 2019; Pieck et al., 2017). As the greatest method for managing disease before it spreads widely is the accurate early pathogen detection in the field sample, a convenient, specific, rapid, and cost-effective diagnostic tool is a burning need to assess seed health and allow the prompt implementation of risk mitigation. With this critical concern in mind, scientists have devised two prompt and precise wheat blast diagnosis methods that make use of cutting-edge technology called CRISPR. Kang and co-workers developed a method for identifying these sequences by integrating Cas12a protein, RPA (recombinase polymerase amplification), and NALFIA technologies (Kang et al., 2021). Again 2022, Sanchez and the group, led by Magdy Mahfouz, developed another method using Cas9 protein, RT-RPA, and NALFIA (Sánchez et al., 2022). Simple, rapid, specific, and sensitive point-of-care detection methods are needed to contain the spread of MoT. CRISPR/Cas9-based lateral flow assays are emerging as a powerful alternative for wheat blast diagnostics. Bio-SCAN (biotin-coupled specific CRISPR-based assay for nucleic acid detection) is an accurate MoT detection platform that requires no sophisticated equipment or technical expertise. Compared to other CRISPR-Cas-based pathogen detection assays, Bio-SCAN requires no additional reporters, probes, enhancers, reagents, or sophisticated devices to interpret the results. Furthermore, Bio-SCAN reagents can be assembled locally for any emerging variant in limited-resource areas with an estimated cost of less than $10 per test (Ali et al., 2021). Importantly, the Bio-SCAN detection platform has already facilitated rapid, sensitive, and specific mass screening of SARS-CoV-

2 (Ali et al., 2022) and potentially other infectious pathogens like MoT (Sánchez et al., 2022)in no laboratory and low-resource settings in less than 1 h from sample collection to result and complies with most criteria for POC use. In this chapter, we present the discovery of genome-specific primers for plant disease diagnosis, currently available methods for the detection of the wheat blast, CRISPR-based lateral flow assay, and point-of-care blast pathogen, MoT. Detection of the wheat blast is effectively achieved by combining isothermal amplification, CRISPR, and NALFIA technologies. The devices are benchmarked against commercial diagnostic devices in terms of ease of use, time of testing, and cost. This chapter discusses molecular methods and updates the rapid and convenient methods developed for the detection of wheat blast disease with special reference to a putative point-of-care CRISPR-Cas technology.

Currently Available Method for Detection of Wheat Blast Fungus

Molecular diagnostics has demonstrated significant application potential for many plant diseases concerning the control and prevention of these diseases due to its high sensitivity and outstanding specificity. Currently, several methods, including polymerase chain reaction, loop-mediated isothermal amplification, and CRISPR are being employed to identify MoT.

1. Polymerase Chain Reaction (PCR)

PCR is now considered the current gold standard for molecular diagnostic testing. Using the MoT3 transposon and the PoT2 transposon, Pieck *et al.* (2017) developed a primer, C17, and using an end-point and real-time PCR assay, they identified *M. oryzae* at the pathotype level disturbing it from other fungi of other genera(Pieck et al., 2017). A novel polymorphism in the Triticum lineage was used by Thierry *et al.* (2020) to develop a real-time PCR-based MoT detection technique. However, there was no field sample or seed testing done to validate this procedure (Thierry et al., 2020). MoT-6098 and MoT-6099 DNA sequences are currently being used as primers to identify the presence of MoT strains by PCR (Kang et al., 2021) diagnostics for wheat

2. Loop-mediated Isothermal Amplification (LAMP)

The loop-mediated isothermal amplification (LAMP) technique is a unique nucleic acid amplification method that amplifies a small amount of template DNA in a reaction into billions of copies in an hour in a single tube. This process necessitates six primers, two of which are loop primers. LAMP amplifies DNA while generating pyrophosphates that produce turbidity, which makes visualization easier than with PCR. In comparison to PCR and real-time PCR, LAMP is more stable. Thus, it serves as a screening tool for clinical applications and a variety of nucleic acid studies because of its high sensitivity and robustness (Dhama et al., 2014; Notomi et al., 2000) Yasuhara-Bell *et al* (2018) developed loop-mediated isothermal amplification assays based on Pot2 and MoT3 for precisely identifying MoT in lab and field samples (Yasuhara-Bell et al., 2018). All *M. oryzae* pathotypes could be detected using the PoT2 assay as a general screening test. The MoT3 test then was used to verify the presence of *M. oryzae Triticum* following the discovery of a positive result (Yasuhara-Bell et al., 2018). However, because MoT3 is not very accurate, MoT 6098 and MoT 6099 are presently utilized to detect MoT isolates from infected samples (Gupta et al., 2019). These primers allowed Kang and colleagues to differentiate MoT from MoO with accuracy. One of the advantages LAMP offers is that the result can be determined by visual inspection of the LAMP product without any apparatus or data analysis. MoT-infected samples, for example, colored green, whereas MoO-infected samples turned brown (Kang et al., 2021).

3. CRISPR: CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR is the hallmark of a bacterial defense system that has been used to detect RNA viruses, diagnose diseases, and identify various bacterial pathogens. The CRISPR-based platform is considered a good diagnostic platform for infectious diseases. The emerging CRISPR diagnostic tool will have great potential for detecting viral and tumor-derived nucleotides, DNA methylation, and single nucleotide polymorphisms (Jia et al., 2020). In the field of genome engineering, the term "CRISPR" or "CRISPR-Cas9" is often used loosely to refer to the various CRISPR-Cas9 and -CPF1, (and other) systems that can be programmed to target specific stretches of genetic code and to edit DNA at precise locations, as well as for other purposes, such as for new diagnostic tools (Jia et al., 2020) Integration of CRISPR has offered molecular nucleic acid detection high specificity and sensitivity. Researchers used Cas endonucleases to create a variety of molecular point-of-care devices by taking advantage of their extraordinary selectivity and distinctive, programmable function. Recently, a ground-breaking detection method was devised that combines the nucleic acid rapid lateral flow immunoassay (NALFIA) technology with the programmable nucleotide target carried by Cas proteins like Cas12a and Cas13a. This technique offered a simple-to-use approach for plant disease diagnosis that is accurate, quick, specific, and affordable. Recently, two methods were developed to detect wheat blast pathogens using Cas12a and Cas9 endonuclease. Kang and team devised a precise, sensitive, and affordable nucleic acid detection technique that effectively recognized the MoT-specific DNA sequences in infected wheat plants by combining indiscriminate Cas12a ssDNase

activation with RPA and NALFIA (Kang et al., 2021). For the quick identification of wheat blast and other significant plant diseases in the field, this unique approach is easily adaptable and field deployable. The complete procedure to identify the MoT fungus in plant samples takes around 30 minutes at 25–40 °C. Also, it does not need users to have advanced technical abilities or complicated laboratory equipment. However, this strategy has not yet undergone extensive field validation (Kang et al., 2021). Sanchez et al devised another method by combining reverse transcription-recombinase polymerase amplification (RT-RPA), recombinant biotin-labeled nuclease-dead version of Cas9 (bio-dCas9), and field-deployable Lateral Flow Immunoassay (LFA)(Sánchez et al., 2022). These two studies successfully demonstrated the potential of CRISPR-based nucleic acid detection platforms combined with nanotechnology in the diagnostics of wheat blast disease.

Discovery of Genome-Specific Primers for Plant Diseases

Early, rapid, and specific detection and identification of plant pathogen(s) is the first essential step toward effective plant disease management and prevention of pathogen(s) spread (Mirmajlessi et al., 2015). Plant disease management necessitates the need to reduce the spread of the pathogen. The extent of the optimum implementation of control strategies depends not only on the presence of a pathogen but also on the pathogen inoculum load. Thus, the capability of quantifying the pathogen load represents an important aspect of plant disease management (Mirmajlessi et al., 2015). A variety of molecular methods have been developed for specific detection and identification of phytopathogenic fungi and bacteria. Generally, the sequences and the genomic targets of conserved universal genes with enough sequence variation between species are the best choice for designing PCR-based diagnostic assays. DNA-based diagnostic methods such as PCR have been widely used as an effective approach to detect pathogens from diseased plants, which offer advantages over traditional antibody-based detections which are expensive and time-consuming processes (Cho et al., 2011). Depending on the genomic region chosen to design PCR primer sets, highly specific diagnostic tests can be obtained, allowing the detection of the specific pathogen species and strains from related species or within the same species, respectively (Kang et al., 2021; Sánchez et al., 2022). Primer design requires knowledge of the target DNA sequences, and multiple strategies are therefore being developed to design primers for specific detection and disease diagnosis (Lees et al., 2002; Williams et al., 1990). Currently, there has been growing efforts to search for new marker gene or sequences to be developed as detection markers for bacterial, fungal, and viral pathogens of plants, humans, and animals with high reliability. A perpetual increase in the availability of whole genome sequences of either bacterial or fungal or viral pathogens has shifted the attention to the use of comparative genome analysis, which is the most effective way to select candidate marker genes of the respective pathogen(s) (Kang et al., 2021; Papaiakovou et al., 2017; Rahman et al., 2021). The rDNA operon has frequently been used to design primers that allow highly sensitive detection, but due to its universal nature, the level of discrimination lies at the species levels (Schaad et al., 2003). The ITS region within prokaryotic and eukaryotic rDNA operons has been described as a stable genetic marker and was used to design primers (Bilodeau et al., 2012; Schena et al., 2004). Thus, the ITS (internal transcribed spacer) region is the most widely sequenced for plant pathogens. Another genomic portion of the rDNA cistron is the spacer between IGS (intergenic spacer) or the non-transcribed spacer (NTS) that was used to design primers (Bilodeau et al., 2012; Suarez et al., 2005). IGS sequences are more difficult to amplify and sequence, but they can be more variable than the ITS sequences. Thus, they are exploited to design diagnostic assays when there are not enough differences available across the ITS (Schena et al., 2004).

Discovery of Genome-Specific Primers for Wheat Blast

Efficient pathogen diagnostics and genotyping methods enable effective disease management and breeding, improve crop productivity and ensure food security. To develop an accurate and sensitive method, Pieck et al.(2017) identified DNA markers that appeared to be unique to MoT strains(Pieck et al., 2017). One of the markers, MoT3, showed specificity when tested on DNA from 284 *M. oryzae* strains from 11 host species, collected from several countries. A 360-base pair (bp) fragment of the MoT3 marker was amplified in the MoT strains but not in the *M. oryzae* Oryzae*(*MoO) strains. However, the fragment is located within a single-copy gene encoding a retinol dehydrogenase, which is present in MoT, MoO, and *M. oryzae* Lolium (MoL) strains. A recent study analyzed the polymorphism among 81 previously assembled Magnaporthe genomes and found that the MoT3 sequence is not present in all the MoT strains (Pieck et al., 2017). A new DNA marker, C17, was identified that is specific to the Triticum lineage with high sensitivity (Pieck et al., 2017). In quantitation real-time (qRT)-polymerase chain reaction (PCR) reactions, it successfully amplified DNA from 100% of the 30 wheat-borne strains used in the study. However, it is not clear whether both MoT3 and C17 are useful for rapid MoT detection in infected wheat plants or not in the field. Furthermore, MoT3 is not very reliable (Gupta et al., 2019). In search for a more accurate and reliable species-specific primer for MoT, Kang et al (2021) performed de novo sequencing of the genome of two wheat blast strains followed by a

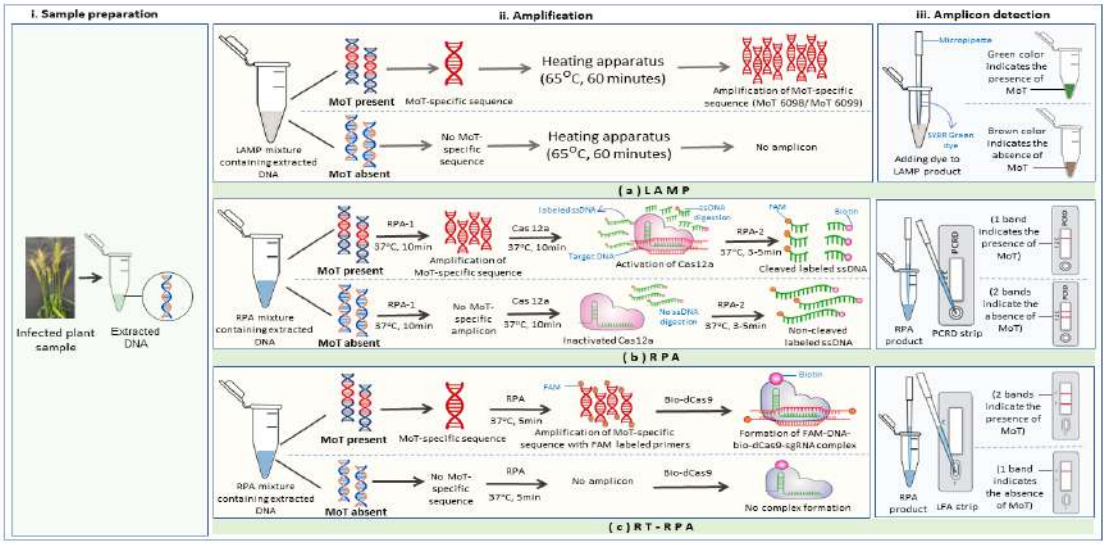
pair-wise gene sequence alignment analysis between the MoO and MoT genomes, which led them to the identification of two genome-specific DNA fragments: MoT-6098 and MoT-6099 in MoT. Finally, they designed a few sets of primers based on the MoT-6098 and MoT-6099 sequences and verified the sensitivity of the primers via PCR and LAMP (Kang et al., 2021).

CRISPR-based Lateral Flow Assay for Wheat Blast

In recent years, several new technologies have been developed to rapidly detect plant diseases in both laboratory and field conditions. For example, loop-mediated isothermal amplification (LAMP) (Dhama et al., 2014; Notomi et al., 2000) and recombinase polymerase amplification (RPA) (Piepenburg et al., 2006) are being widely used for plant disease detection (Figure 1). It is worth mentioning that a revolutionary detection method for human diseases, which integrates the programmable nucleotide target carried by Cas proteins, such as Cas12a and Cas13a, with the NALFIA method, was recently developed (Gootenberg et al., 2018). This method is an accurate, rapid, specific, and cost-effective alternative that can be easily adapted for plant disease detection. Recent advances in CRISPR technology have achieved remarkable success in the field of molecular diagnosis of plant pathogens (Kang et al., 2021; Sánchez et al., 2022), and become a hot topic in COVID-19 diagnosis (Broughton et al., 2020; Nouri et al., 2022). Several recent studies have developed CRISPR-based biosensing technologies such as DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter), a system that couples RT-LAMP and Cas12 with the use of a reporter to obtain an LFAreadout (Broughton et al., 2020); SHERLOCKv2, a system coupling Cas13a, and RPA to detect both DNA and RNA offering both multiplexing and quantification of the target system (Gootenberg et al., 2018), CIA-based LFB (CRISPR/Cas12 based lateral flow biosensor) coupling Cas12 and LAMP that can detect upto single copy of pathogen in a given sample (Mukama et al., 2020); miSHERLOCK (Minimally instrumented SHERLOCK), a platform using Cas12 and RPA, that provides visual and mobile phone–enabled output interpretation(de Puig et al., 2021).

Kang et al., for the first time, developed a novel method of plant disease detection using RPA, Cas12a, and NALFIA for the rapid and accurate field-level detection of a highly destructive wheat blast pathogen MoT(Kang et al., 2021). First, they collected wheat plant samples that were blast infected and extracted the DNA from those samples. Then they added the extracted DNA in the RPA mixture containing reporter (Biotin and FAM-labeled ssDNA) and sgRNA which was designed to recognize MoT-specific sequences (Figure 2).

Figure. 2: A proposed point-of-care detection method for the wheat blast



(i). DNA extraction:

Leaf or seed samples are collected and crushed in a lysis buffer for DNA extraction.

(ii). Amplification of the target DNA:

MoT-specific sequences (MoT-6098/MoT-6099) are amplified by LAMP, RPA, or RT-RPA. a) LAMP mixture containing MoT-specific primers amplifies the target DNA sequences. b) The RPA mixture containing MoT-specific primers amplifies the target sequences. Once the sgRNA recognizes and binds to the amplicons, Cas12a is activated which then cleaves off the FAM and biotin-labeled ssDNA (reporter) c) In RT-RPA, the RPA mixture contains FAM-labeled

primers with which the target DNA sequence is amplified(Sánchez et al., 2022). The mixture also contains a programmable single guide (sg) RNA designed for target pathogens. The sgRNA recognizes and binds to the FAM-labeled target DNA sequence, forming a FAM–DNA–recombinant biotin-labeled nuclease-dead Cas9 (bio-dCas9)–sgRNA complex.

(iii). Amplicon detection:

In the case of LAMP, a dye is added to the LAMP product where green color indicates the presence of MoT and brown color denotes the absence of MoT (a); RPA (b) and RT-RPA (c) products are then transferred to an LFA strip using a micropipette. The LFA strip shows the result within 5 min with C referring to the control line and T to the test line.

When the sgRNA recognizes and binds to the target sequences, the Cas12a activates and initiates the trans-cleavage activity. The activated Cas12a then cleaves off the reporter. The RPA product is then mixed with a buffer and added to the LFA strip for visual naked-eye result detection. After around 5 minutes, bands can be seen. One band at the control line denotes the presence of MoT, two band shows that MoT is absent in that sample (Figure 2). The entire process requires only 30 min at 25–40°C to detect MoT in infected wheat plants in the field (Kang et al., 2021). Moreover, the process requires no complex laboratory equipment, technical skills, or instrument for result analysis. However, large-scale field validation of this method remains to be performed.

Recently, Ali et al. devised a biotin-coupled specific CRISPR-based test for nucleic acid detection (Bio-SCAN) (Ali et al., 2022). Devised by combining reverse transcription-RPA (RT-RPA), recombinant biotin-labeled nuclease-dead version of Cas9 (bio-dCas9), and LFA, Bio-SCAN successfully detected the human pathogen severe acute respiratory syndrome-coronavirus 2 (SARS-CoV2) with 100% specificity (Ali et al., 2022). In a recent study, Sánchez et al. optimized this POCT (point-of-care testing) lateral flow assay, Bio-SCANtechnology as a platform in agriculture for the accurate and prompt detection of phytopathogens, transgenes, and herbicide-resistant alleles(Sánchez et al., 2022). This field-deployable assay can precisely identify elite germplasm and detect mutations, transgenes, and phytopathogens in <1 h, starting from sample isolation to resulting output using lateral flow strips.

The highly sensitive, cost-effective, and easy-to-use Bio-SCAN platform requires only a single guide RNA (sgRNA) and recombinant biotin-labeled nuclease-dead version of Cas9 (bio-dCas9) to detect a one-step FAM-labelled amplicon produced by RT-RPA for a target sequence with commercially available streptavidin-biotin-based LFA strips(Ali et al., 2022). A control line and a test line on the strips immediately validate the presence or absence of the target nucleic acid (Figure 2).

To optimize Bio-SCAN for agricultural purposes, genotyped various wheat germplasms for the Lr34 and Lr67 alleles conferring broad-spectrum resistance to stripe rust, confirmed the presence of synthetically produced herbicide-resistant alleles in the rice genome (Sánchez et al., 2022). They also confirmed the presence of transgenic elements in the genomes of transgenic rice and tobacco plants with 100% specificity. Furthermore, Bio-SCAN successfully detected various phytopathogens, including viruses, bacteria, and fungi, in wheat and *Nicotiana benthamiana* (Sánchez et al., 2022). For instance, Bio-SCAN assay demonstrated its sensitivity to precisely detect the resistant alleles of Lr34 andLr67 in wheat cultivars; the synthetic mutants SGR3, SGR5, and OsmALS in rice; the transgenic promoters Ubiquitin and cauliflower mosaic virus (CaMV) 35S; the plant pathogenic viruses tomato yellow leaf curl virus (TYLCV), tobacco mosaic virus (TMV)and potato virus Y (PVY) in *Nicotiana benthamiana*; the fungi *Pucciniastriiformis* f. sp. *tritici* and MoT in wheat and the bacterial pathogens *Pseudomonas* and *Agrobacterium tumefaciens* in *N. benthamiana* (Sánchez et al., 2022). Therefore, Bio-SCAN is undoubtedly a robust, reliable, and user-friendly method that can greatly facilitate crop protection, crop breeding, and genetic engineering. However, field validation of this new technology is required (Islam and Kasfy, 2022).

One of the best features of CRISPR-based disease detection technologies is that they can identify any pathogen by simply taking the gRNA targeting specific primers/unique nucleic acid fragment(s) for that particular pathogen (Ceresini et al., 2018). The whole CRISPR-based detection process takes~1h and requires few reagents; hence, it is considerably less expensive than other laboratory-based methods. Since the techniques use RPA rather than LAMP, target sequence amplification can be conducted at room temperature without any sophisticated heating apparatus. In addition, this method reduces the analysis time and cost of pathogen identification. However, the PCRD strip used in CRISPR-based-lateral flow assays for rapid detection of phytopathogens is expensive for large-scale deployment of this method in the field, especially in low-income countries.

Putative Point-of-Care Diagnostics for Wheat Blast

The boom in POC testing today is mostly driven by wearable microsensors and lab-on-a-chip technology. However, designing a POC assay for plant diseases like wheat blast is a challenging task, even with CRISPR-based systems. The ultimate goal of CRISPR molecular diagnostics is to combine sample processing, detection, and readout in a single tube. One attempt to combine these steps in a single tube has already been recently reported in human diseases like COVID-19(Li et al., 2021). Multiplexing and data sharing represent additional desirable features that can

be incorporated into home-use diagnostic kits. Li et al. (2021) developed a colorimetric RT-LAMP assay to detect SARS-CoV-2 in nasopharyngeal swabs using a single-step sample-to-answer approach (Li et al., 2021). Fozouni and coworkers (2021) developed an amplification-free CRISPR-Cas13a assay for the direct detection of SARS-CoV-2 from nasal swab RNA that can be read with a mobile phone (Fozouni et al., 2021).

The assay achieved ~100 copies/mL sensitivity in under 30 min of measurement time and accurately detected pre-extracted RNA from a set of positive clinical samples in under 5 min. The fluorescence can be measured with a mobile phone camera in a compact device that includes low-cost laser illumination and collection optics. This approach has the potential to enable a fast, accurate, portable, and low-cost option for point-of-care SARS-CoV-2 screening (Fozouni et al., 2021). To further reduce the number of steps involved in sample processing, several one-step quick extraction protocols have been developed. Quick Extract (Lucigen) was developed to lyse viral particles via a one-step heat treatment in lysis buffer at 95 °C for 5 min. The inactivated sample is compatible with One-step RT-LAMP/Cas12 detection (Joung et al., 2020). A simpler, 5 min, room temperature lysis protocol was developed in SHINEv2 (Arizti-Sanz et al., 2021; Ghouneimy and Mahfouz, 2022).

Encapsulating reagents in a compact device and processing these reagents by automation could provide another approach to creating a user-friendly CRISPR-based diagnostic kit for the wheat blast. For instance, a simple test, miSHERLOCK (minimally instrumented SHERLOCK), has been designed to minimize the liquid handing steps in SARS-CoV-2 detection (de Puig et al., 2021). Moving one step toward semi-automated systems, DISCoVER is an emerging sample-to-answer platform that combines lysis, amplification, and detection in microfluidic modules (Ghouneimy and Mahfouz, 2022).

CRISPR-based diagnostics offer the opportunity for multiplexing at the POC. Multiplexing (multiple targets detected per sample) and high throughput (allowing many samples to be processed) are important features that allow massive testing at the population level, especially during pandemics. Digital microfluidics (DMF) is a disruptive technology capable of controlling discrete, minute volumes of reagents. DMF enables extensive multiplexing and parallelization by controlling droplets, each acting as a biological or chemical reactor. The technology features all the advantages of traditional microfluidic platforms such as volume miniaturization, improved sensitivity and reaction time, decreased cross-contamination, portability, and full automation. Moreover, DMF features accurate control over discrete droplets, a great multiplexing potential, easy integration with signal detection modules, and elimination of propulsion devices for reagent movement (Millington et al., 2010). DMF could potentially be combined with multiplexing for POC sample-to-answer comprehensive syndromic testing of the MoT. The impact of these technological advancements will be mostly seen in low-income countries where healthcare infrastructure is weak and there is a clear lack of lab-based testing available.

Conclusion and Future Perspectives

Wheat blast is a devastating fungal disease caused by the *M. oryzae Triticum* pathotype. This report summarizes the chronological development of molecular methods for the detection of wheat blast. Recently, two genome-specific primers, MoT-6098 and MoT-6099 were discovered and they were used for the development of a rapid, convenient, and specific method for the detection of wheat blast using CRISPR technology. Large-scale field validation and improvement of the CRISPR-based lateral flow assay for wheat blast detection are needed for cost-effectiveness and leading this method as a point-of-care and user-friendly.

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Genetic Diversity of Eggplant to Mitigate the Food Security and Sustainable Development in Bangladesh

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Abstract

The study was conducted at the experimental field of Olericulture Division, Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur during 2021-22 to assess the extent of genetic diversity among 23 eggplant germplasm. The collected 23 germplasm originating from Plant Genetic Resources Center (PGRC), BARI was subjected to cluster analysis. The germplasm was constellated into five distinct groups, with a range of 2 germplasm in cluster II to 7 germplasm in cluster V. In all cases, the inter-cluster distance was larger than the intra- cluster distance. The intra cluster distance was maximum in cluster V (1.574) and minimum in cluster II (0.673). Regarding inter cluster distance, cluster III showed maximum genetic distance with cluster V (18.525) followed by the genetic distance from cluster III and IV (13.184), cluster II and cluster III (12.289) and cluster I and cluster III (10.267). Considering the group distance and inter-genotypic crosses between the members of cluster III and V, cluster III and IV and cluster II and III would exhibit high heterosis and was also likely to produce new recombinants with desired traits. But in case of the cluster means values and yield contributing performance cluster I, cluster II, cluster III, cluster V performed well. Therefore, inbreds belong to cluster I, cluster II, cluster III, cluster V will be given higher priority for crossing in future eggplant hybridization program and will ensure good quality eggplant hybrids. Thus this study will contribute to mitigate the food security and sustainable development in Bangladesh.

Keywords: Genetic diversity, food security, sustainable development, eggplant, (*Solanum melongena* L.), Bangladesh.

Introduction

Eggplant (*Solanum melongena* L.) is the fourth most important crop of the Solanaceae, which is widespread due to its high nutrition value and the taste of the fruits. Eggplant is widely cultivated as one of the most important vegetables in both subtropical and tropical areas worldwide. It can play a vital role in achieving nutritional security (Sarker et al., 2006). Eggplant is the most popular vegetable crop in terms of total acreage (51165 ha) and production (516007 t) in Bangladesh, with an average yield of 10.09 tons per hectare (BBS, 2020), which is very low as compared to that in other tropical countries. This low yield may be due to a lack of high yielding varieties with pest resistance. High yielding varieties are an important factor for maximizing the yield of eggplant. For a long time, eggplant has been growing year-round in our country. But due to a lack of good winter varieties, the yield of eggplant is not optimistic, which is only 10.83 tons per hectare during the winter season.

Farmers are consequently eager to find a winter variety that is resistant to pests and diseases, particularly bacterial wilt and fruit and shoot borer. Using hybrid exploitation in eggplant, there is a good probability of increasing production. Eggplant fruits are high in minerals such as calcium, magnesium, potassium, iron, zinc, and copper. It is also a fair source of fatty acids and it is used for medicinal purposes, incurring diabetes, asthma, cholera, bronchitis, and diarrhea. It is reported to stimulate the metabolism and reduce blood cholesterol. Fresh or dried leaf and fruit produce a significant reduction in blood cholesterol levels (Agoreyo et al., 2012, Nyadanu and Lowor, 2015).

Exploitation of hybrid vigour has become a potential tool for improvement in eggplant. Nagai and Kada (1926) were the first to observe hybrid vigour in eggplant. Hybrids and improved agronomic techniques have resulted in a steady linear increase in the performance of vegetable crops (Ellstrand et al., 1999; Lippman and Zamir, 2007; Quamruzzaman et al., 2006, Sing et. al., 2013a).

Exploitation of hybrid vigour has become a potential tool for improvement in eggplant. The commercial exploitation of this phenomenon has been possible in the eggplant, especially with the increasing popularity of F_1 hybrids in eggplant. It is imperative to obtain such hybrids having excellent quality coupled with high yields. Genetic diversity is the extent of genetic variation that exists among selected cultivars, breeding lines, or species. Knowledge of genetic diversity among existing cultivars of any crop is essential for the long-term success of a breeding program and maximizes the exploitation of the genetic resources. If the structure of the genetic diversity is known within a large collection of germplasm, it may be of great help to make decisions on management procedures and breeding strategies to use in a breeding

program. With the development of advanced biometrical techniques such as multivariate analysis, quantification of the degree of divergence among the biological population and assessing the relative contribution of different components to the total divergence at intra- and inter-cluster levels have now become possible. Such a study also permits the selection of the genetically diverse parents to obtain the desirable recombinant in the segregating populations upon crossing. Hybridization is the common practice of combining the desirable characteristics of two or more lines or varieties into a single variety. In several cases, the progenies become far superior to the parents in vigor, i.e. hybrid vigor or heterosis. Inclusion of more diverse parents (within a limit) is believed to increase the chances of obtaining stronger heterosis and give a broad spectrum of variability in segregating generations. Available research also reported that crossing between moderately diverse parents showed maximum heterosis. The current study was done to examine the level of genetic variety in 28 eggplant germplasm, which will aid in the selection of prospective parents to generate transgressive segregates.

Materials and Methods

Experimental site

The experiment was conducted at the Olericulture Division of Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI) during 2021-2022.

Plant materials, experimental design and crop management

The seeds of 23 germplasm of eggplant were collected from PGRC, BARI and sown on the seedbed on 15 September, 2021. Thirty days old seedlings were transplanted in the main field on 14 October, 2021. The experiment was laid out in a RCB design without replication. The unit plot size was 7.0 ´0.7 m and 10 plants were accommodated in a plot with a plant spacing of 70 cm apart in single row maintaining a row-to-row distance of 70 cm with 30 cm drain. The land was fertilized with cowdung –N-P-K-S-Zn-B @ 10,000-170-50-125-18-4.3-1.70 kg/ha, respectively. One third of the cow dung and half of P and full of S, Zn and B were applied during final land preparation. Rest of cow- dung and P and 1/3 of K were applied as basal in pit. One fifth of urea and K were applied after 20 days of transplanting. After that, rest of urea and K were applied in equal four installments at 20 days interval. The intercultural operations (weeding, irrigation, insecticide spray etc.) were done as and when necessary.

Data recorded

Data on days to 1st harvest, number of marketable fruit, average fruit weight (g), fruit length (cm), fruit diameter (cm), fruit yield (kg/plant), plant height at last harvest (cm), plant height at 1st harvest (cm), FSB infested fruit (%), Bacterial wilt infection (%), fruit yield (t/ha) were recorded from five randomly selected plants per germplasm.

Statistical analysis

Plot means for 10 quantitative characters were used for the statistical analysis. Genetic diversity was studied following Mahalanobis's (1936) generalized distance (D2) extended by Rao (1952). Based on the D^2 values, the germplasm was grouped into clusters following the method suggested by Tocher (Rao, 1952). Genetic diversity was studied following Mahalanobis's (1936) generalized distance (D2) extended by Rao (1952). Statistical analyses were carried out using Genstat 5 software.

Results and Discussion

The analysis of variance showed significant differences between the germplasm for all the characters studied, which indicated the presence of sufficient variability in the germplasm. Based on the degree of divergence, 23 germplasm were grouped into five clusters (Table 1). The distribution pattern revealed that the maximum number of germplasm (7) was included in cluster V, followed by cluster IV (6) and cluster I, III (4), while cluster II included the minimum (2) germplasm.

Table 1. Distribution of 23 germplasm of eggplant in different clusters

Clusters	Germplasm no./ cluster	Germplasm
I	4	2645, 2653, 2675, 2789A
II	2	2682, 2695
III	4	2663, 2680, 2695A, 2789
IV	6	2664, 2665, 2721, 2721A, 2740, 2790
V	7	2658, 2672, 2715, 2757, 2762, 2764, 2776

The inter-cluster distances in all of the cases were larger than the intra-cluster distances, indicating wider diversity among the germplasm of the distant group (Table 2). The intra-cluster distance was maximum in cluster V (1.574) and minimum in cluster II (0.673), indicating the germplasm in cluster V was more heterogeneous and those in cluster II were closely related. The range of the intra cluster distance values indicated the homogeneous nature of the germplasm within the clusters. Regarding inter cluster distance, cluster III showed maximum genetic

distance with cluster V (18.525) followed by the genetic distance from cluster III and IV (13.184), cluster II and cluster III (12.289) and cluster I and cluster III (10.267) suggesting wide diversity between them and the minimum distance was found between the germplasm of cluster I and II (4.550). Moderate inter cluster distance was also found between cluster I and V (9.442), cluster II and V (9.981). The result was supported by a scatter diagram (Fig 1).

Table 2. Mean intra (bold) and inter cluster distances (D^2) for the 23 eggplant germplasm obtained on the basis of the 9 morphological characters

Clusters	I	II	III	IV	V
I	**0.741**	4.550	10.267	4.295	9.442
II		**0.673**	12.289	6.432	9.981
III			**0.834**	13.184	18.525
IV				**1.359**	6.29
V					**1.574**

Differences in cluster means existed for all the characters. Cluster I recorded the lowest mean value for Bacterial wilt infection (0.0%) and highest mean value for fruit length (15.8cm), plant height at last harvest (112 cm), while cluster II was exhibited the lowest mean value for days to 1st harvest (99days), bacterial wilt infection (0.0%). Cluster III was exhibited lowest mean value for days to 1st harvest (99days) and highest mean value for Number of marketable fruit (29.5), while cluster V was responsible for highest mean vales for average fruit weight (167g), fruit diameter (5.3cm), fruit yield (40.17t/ha) and lowest mean value for ESFB infestation (15.2%). Jagadev *et al*. (1991) reported that the characters contributing maximum to the divergence should be given greater emphasis for deciding the type of cluster for the purpose of further selection and the choice of parents for hybridization.

Table 3. Cluster means for nine characters in 23 eggplant germplasm

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to 1st harvest	101	99	99	102	101
Number of marketable fruit	22.7	22.5	29.5	17.5	18.4
Average fruit weight (g)	130	134	90	143	167
Fruit length (cm)	15.8	14.2	7.2	11.4	10.5
Fruit diameter (cm)	4.2	3.7	4.9	5.0	5.3
Fruit yield (t/ha)	38.61	39.39	34.42	32.71	40.17
Plant height at last harvest (cm)	112	97	107	108	103
ESFB infestation (%)	19.0	18.0	16.0	18.1	15.2
Bacterial wilt infection (%)	0.0	0.0	7.5	3.3	4.2

Based on principal component axes I and II, a two -dimensional scattered plotting diagram (Z1 and Z2) reflecting the position of germplasm are presented in Fig. 1. It was revealed that from the diagram there were mainly five clusters. Most distantly located germplasm was within cluster III [2663, 2680, 2695A, 2789] and cluster V [2658, 2672, 2715, 2757, 2762, 2764, 2776] followed by cluster III [2663, 2680, 2695A, 2789] and cluster IV [2664, 2665, 2721, 2721A, 2740, 2790] and cluster II [2682, 2695] and cluster III [2663, 2680, 2695A, 2789]. Distribution pattern of germplasm in the scattered diagram also revealed that considerable variability exists in the germplasm studied. Contributions of the characters towards divergence are presented in Table 4. The canonical analysis revealed that, values in both vectors (Vector I and II) for average fruit weight (0.2437 and 0.2433) were positive. Such results indicated that this character contributed maximum towards the divergence of the germplasm. It is interesting to note that the greater divergence among the materials in the present study due to average fruit weight will offer a good scope for improvement of yield through rational selection of parents for producing heterotic eggplant hybrids. The major contribution of this character to divergence was well confirmed by their cluster mean, where the ranges varied, indicating the major role of these characters as differentiated at the inter-cluster level. The cluster means for average fruit weight was 167g for Cluster V showing a significant difference.

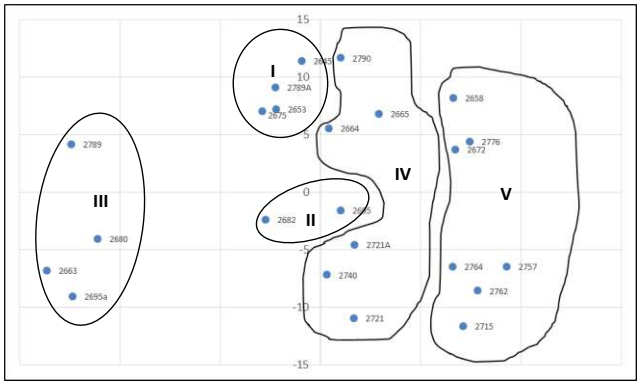

Fig. 1. Distribution of 23 eggplant germplasm in a two-dimensional scatter diagram based on PCA scores superimposed with clusters.

Table 4. Latent vectors for nine quantitative characters of 23 germplasm of eggplant

Characteristics	Vector (Z_1)	Vector (Z_2)
Days to 1st harvest	0.0617	-0.2505
Number of marketable fruit	0.0108	-1.2535
Average fruit weight (g)	0.2437	0.2433
Fruit length (cm)	-0.0054	0.1583
Fruit diameter (cm)	-0.3089	-0.1185
Fruit yield (t/ha)	-0.0212	0.8096
Plant height at last harvest (cm)	0.0115	-0.2192
ESFB infestation (%)	-0.0192	0.2151
Bacterial wilt infection (%)	0.0511	-0.0651

Conclusion

Considering the group distance and inter-genotypic crosses between the members of cluster III and V, cluster III and IV and cluster II and III would exhibit high heterosis and be likely to produce new recombinants with desired traits. But in case of the cluster means values and yield contributing performance, cluster I, cluster II, cluster III, cluster V performed well. Therefore, inbreeds that belong to cluster I, cluster II, cluster III, cluster V will be given higher priority for crossing in future eggplant hybridization program and will ensure good quality eggplant hybrids. Thus this study will contribute to mitigate the food security and sustainable development in Bangladesh.

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Section B: Food processing and Food Economy

Study to Develop Sashimi Meat from the Cuttlefish (*Sepia officinalis*) and Evaluate its Quality as Ready-to-Eat Products

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Abstract:

This study aimed to produce value-added Ready-to-Eat (RTE)/Ready-to-Cook (RTC) sashimi meat by developing a commercial-based method to get maximum revenue compared to unprocessed whole cuttlefish. Hazard Analysis and Critical Control Plan (HACCP) plan for this product was developed and approved by the Fish Inspection and Quality Control (FIQC), Department of Fisheries, Dhaka, Bangladesh. Receiving, defrosting, grading, washing, pre-processing, trimming, stirring, final trimming, export grading, final washing, weighing and panning, freezing, de-panning and packaging, metal detection, and storage are the major steps of the process flow. A total of 20.35 MT whole blocks of frozen (-20^{0}C) cuttlefish samples were purchased from the fish trawlers. The sizes ranged from 50g to 300g. After processing cuttlefish, preparing sashimi meat, weighing, panning, and 4 hr freezing in a blast freezer, packing and storing the final products in a cold store was carried out. Finally, 10.30 Mt of final products was produced, of which RTE sashimi meat was 5.71 MT and RTE head parts were 4.6 MT. The total yield was 50.62 percent, with the meat accounting for 55.40 % and the head accounting for 44.60 %. By-products/wastage was 49.38, of which, defrosted wastage was 1.5 %, other fish mixed with a block was 0.5%, trim meat wastage of 4%, egg wastage of 3%, drop wastage of 4%, 10 to 20 g under the size of 4.5%, and the rest of the wastage of 36%. The products graded on 8/12, 13/20, 21/30.31/40.41/60/61/80, and 81 up per kg. Grade 41/60 contains 37%, and grade 8/12 contains 0.36%. Five samples, S-1, S-2, S-3, S-4, and S-5, were tested for sensory and microbiological parameters. According to the Tory freshness scale, S-1 was 8.95, S-2 was 7.55, S-3 was 8.75, S-4 was 6.5, and S-5 was 7.15, where the average scoring was 7.78. Aerobic Plate Count (APC) S-1 6.9/10 cfu/g, S2-8.6x10, S3-7.4x10, S4-9.2x10, S5-8.0x10, the average score was 8.0, *Escherichia coli* was less than <0.30 MPN (most probable number)/g in every sample, *Salmonella* spp, *Vibrio*. According to the regulations of Japan and Bangladesh, the test results confirmed that the produced meat was RTE. After adding value, the revenue increased by 49%, which was statistically significant (P<0.5%).

Keywords: Cuttlefish, Sashimi, Ready-to-Eat, Processing, By-products Sensory, Microbiological quality

Introduction:

The cuttlefish is a marine Mollusca (Haszprunar, 2012), class cephalopods, order Sepiida under the family Sepiidae characterized by a thick internal calcified shell called the cuttlebone (Britannica, 2021, Barratt and Allock, 2012). *Sepia officinalis* is one of the common species (Mouritsen and Styrbaek., 2021) among 100 living cephalopods in the shallow marine water in the Bay of Bengal ((Britannica, 2021). It is a short-lived (1 to 2 yr) species with a single breeding season toward the end of its life cycle (Barrett, et al., 2022). This species has a wide geographic distribution and is not a threatened species, thus offering large-scale culture or capture fishery (Barratt and Allock, 2012). It is a commercially important species in many countries (Barratt and Allock, 2012). Cuttlefishes are primarily bottom dwellers in various habitats that inhabit sandy or muddy substrates and can tolerate brackish waters (Barratt and Allock, 2012). This species is caught in the Bay of Bengal as a by-catch by artisanal and industrial trawlers (Chavan, et al., 2019; DoF, 2022). Cephalopods (Cuttlefish) are caught around the world both by industrial trawlers and artisanal trawlers (Sykes, et al., 2014) on a large scale and small scale for human consumption (Vaz-Pries, et al., 2004). According to archaeological evidence, humans had been catching cephalopods for food at least 4,000 yr ago (Mouritsen and Styrbaek., 2018). Cephalopods, especially cuttlefish, octopus, and squid, had used in ancient Greece in large formal banquets. Generally, cuttlefish is consumed in a fresh and raw state and prepared for a wide range of cuisine by boiling, steaming, frying, grilling, marinating, smocking, and fermenting (Mouritsen and Styrbaek., 2018). The Japanese are the master in preparing a large variety of culinary with cuttlefish (Sujit, 2009). The Far East countries like China and Japan have the richest traditions for consuming Cuttlefish [Cephalopods provide micronutrients, proteins, minerals, and omega-3 fatty acids (Mouritsen and Styrbaek., 2018)] but not in fat ($p > 0.05$) (Sykes, et

al., 2009). Cephalopods contain super unsaturated (DHA) Docosahexaenoic acid and (EPA) Eicosapentaenoic acid (Ozogul, et al., 2008). Mollusks are used as a balanced protein resource which has a significant share of seafood (mussels, squids, clams, octopuses, snails), which is used as a balanced protein resource (Haszprunar and Wanninger., 2012). Chakraborty (2020) mentioned that, if anyone consumes mollusks in their daily diet, it helps to the enhancement of immunity and reduce the risk of several ailments. Ramasamy (2012) stated that the cuttlefish contains total cholesterol and High-density lipoprotein (HDL) cholesterol levels ranging from 131.25 ± 0.89 mg/100 g (mantle) to 359.36±1.94 mg/100 g (arm) and 43.83 ± 0.81 mg/100 g (head) to 204.53 ± 1.78 mg/100 g (tentacle) (Ramasamy, et al., 2012)

The Japanese value cephalopod food the most and consume more cephalopods like octopus and cuttlefish than anywhere else (Sujit, 2009). Cephalopods are sometimes consumed in raw form, known as 'Sashimi.' It is highly cherished on ceremonial occasions like weddings and festivals (Silas et al., 1985). Sashimi is mouth-watering worldwide, and the delicious cuisine continues to grow in popularity in the USA. What is Sashimi? Sashimi is merely defined as servings of raw fish. Sashimi is served with wasabi without rice (Sujit, 2009). The most popular Japanese sushi is prepared with Sashimi meat (Brighton and Hove, 2008., Kulawik and Dordevic, 2020). Japanese cuisine is dominated by seafood (fish/shellfish/crustaceans/cephalopods) eaten raw, called Sashimi and Sushi (Mouritsen, 2009). The top-class and most popular sushi is hand-pressed nigiri-sushi, which is often topped with a piece of raw Ika (cuttlefish) made of the mantle of cuttlefish or squid sushi (Mouritsen, 2009). Muscolino, et al., (2014) stated that sometimes sashimi becomes very hazardous to human life if it does not meet the microbiological, physical, and chemical quality. In 2022 the cuttlefish catch was 353,000 MT worldwide from captured fisheries among 3.8 million MT cephalopod (FAO., 2022) and provided 2% protein for human consumption. As one of the principal consumers, Japan imported about 60% of the cuttlefish from different countries.

Globally cephalopod catch increased from 303,000 mt in 2016 to 353,000 mt in 2020 (FAO., 2022; Chavan, et al., 2019;), while Cephalopod catch in Bangladesh increased from 792 MT in 2015-16 (BBS, 2017) to 960 MT in 2022 (EPB, 2022). Common cuttlefish contain 37% of global cuttlefish catch (Barrett, et al., 2022; Mouritsen and Styrbaek, 2021). The average price of the exported cuttlefish from Bangladesh is USD 2.58/kg (EPB, 2021, EPB 2021). Almost 98% of cuttlefish were exported to China and Thailand as a whole, and only 1.8% were exported to Japan as processed food (EPB, 2022). Cephalopod accounts for around 2.5% of seafood production (Ospina-Alvarez, et al., 2022). The global trade in cephalopod is a multimillion-dollar business, but Bangladesh exports very insignificant quantities due to a lack of planning and care. Three countries (China, Spain, and Japan) led most of the global market movement between 2000 to 2022 (Ospina-Alvarez, et al., 2022). The whole cuttlefish's price is significantly less than the value-added Sashimi or other products. Sashimi is the most expensive, and processing takes place with exceptional food safety care. It requires a higher standard of hygienic production. Before processing Sashimi, the raw frozen cuttlefish should meet the freezing temperature of -20^0C (Novascotia, 2018). Nor below, which was quick freeing by a blast freezer with a temperature of -35^0C (Novascotia, 2018). The study aimed to produce value-added raw-ready-to-eat sashimi meat by evaluating its quality as raw ready-to-eat food. Moreover, to evaluate the income or revenue compared to the unprocessed whole cuttlefish.

Methods and Materials

Sashimi grade on-board frozen raw cuttlefish blocks were collected from the industrial shrimp trawlers of Deep Sea Fishers Ltd where the core temperature was -20^0C (Mouritsen O., 2018), which comply the Food and Drug Administration (FDA, 2022) required temperature (Kulawik and Dordevic, 2020) and free from the parasite (Commission Regulation (EU)., 2011). Cuttlefish blocks were transferred to receiving room of the ARK Sea Food Processing plant through a refrigerated van. Each mixed block contains 10 gm to 300 gm size cuttlefish. An expert checked the core temperature and quality (Moon-bong Kang, 2013), weighed the raw cuttlefish, and transferred them to the chill room for thawing through air blasting overnight where the room temperature of the chill room was -2^0C to 0^0C (Hu and Aijun., 2022)

After thawing, the cuttlefish was graded to 20 gm to 50 gm size, 51 to 100 g size, 101 g to 200g size, and 201g to above size, and applied sufficient flake ice on it (Hu and Aijun., 2022), 10 to 20 g size were discarded as wastage. After while preparing 50 PPM chlorine solution with 20 liters of chilled (< 8^0C temperature) potable water and washing for 25 sec (Fguide 54, June 08). To remove the smell of chlorine, it was washed with chilled potable fresh water and sent the samples to the pre-processing room (Hu and Aijun., 2022, Joseph and Perigreen., 1988). An incision was made on the back of the cuttlefish with a sharpened knife (Blanc, 200), to remove the cuttlebone, behead the head, and remove viscera by hand to make a fillet (Joseph and Perigreen., 1988) and deep into 2% NaCl water solution for 40 sec (Hu and Aijun., 2022). At the same time, eyes, long legs, nails, and mouth were removed, and head parts were cleaned and deep into the

exact solutions. After pre-preprocessing, the meat was mixed with sufficient ice and transferred to the trimming table (Kulawik and Dordevic, 2020) and after trimming, the meat was stirred with 3% NaCl solution for 7 min. The water was drained and the second layer (Leucophore) of meat was gently peeled off by final trimming.

After trimming, did (Hu and Aijun., 2022) the final grade that was 8/12 (80g up), 12/20 (50g -79g), 21/30 (33g-49g), 31/40 (25g-32g), 41/60 (17g-24g), 61-80 (12g-16g), and 81 + (11g-7g). The final products were washed with 20 ppm hypochlorite solutions (water temperature $<8^0$C) for 30 sec and washed with chilled potable water for 60 sec. Then, arranged stainless steel pan weighing 1 kg/grade and poured potable chilled water. Freezing the products by contract freezer contact freezer till inside temperature reach -20°C where the operating temperature was -45^0C. Contact freezer till inside temperature reach -20°C. Did sensory (Torry Assement Scheme) evaluation and microbiological tests to know quality attributes (Mouritsen and Styrbaek., 2018). Five random samples were sent to FIQC for Microbiological tests and to issue Salubrity Certificate for export. FIQC test: *E.coli*, *Salmonella*, APC, and *Vibrio* for Quality and safety tests as per the Japanese Health regulations (JETRO, 2009) and FDA hazard guides (JAS, 2022; FDA, 2022).

Day-wise receiving and production data were collected manually into an Excel sheet and entered data into M.S. Excel by the researcher and production manager. Both the data were then checked manually. All the data were analyzed using the M.S. data tool pack, and one-way ANOVA was used to check the significance of revenue compared between unprocessed and value-added products.

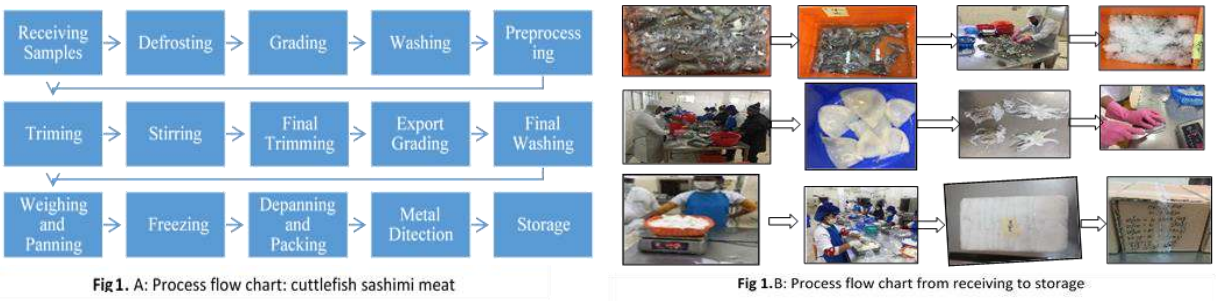

Fig 1. A: Process flow chart: cuttlefish sashimi meat

Fig 1. B: Process flow chart from receiving to storage

Fig 1. A Process flow chart of Cuttlefish Sashimi meat (Source: Hu and Aijun., 2022)

Results and Discussions

The Sashimi grade cuttlefish meat has processed thawing, air defrosting, removing bone, viscera, and skin, trimming, and quick freezing (Hu and Aijun., 2022, Moon-bong, 2013). Fig 1A and 1B shows the final process flow chart. A team of five members sits at different times to finalize the process flow chart as stated by Moon-bong Kang (2013), standardize the processing method (GAA, 2020), and Hazard Analysis Critical Contol Point (HACCP) plan per the recommendation of the FDA Hazards and Controls Guidance ((FDA, 2022). After that, the final process flow chart, a detailed HACCP plan, and a quality management system (GAA, 2020) were sent to the FIQC of the Department of Fisheries. The FIQC inspected the processing method, and quality management system, checked every document, approved the HACCP plan (Kulawik and Dordevic, 2020), and issued a HACCP Manual (GAA, 2020) for this product (CF/FC Version QC/10/0119). About 20,354 kg of onboard frozen (Hu and Aijun., 2022) cuttlefish whose temperature was -20^0C (Mouritsen and Styrbaek., 2018; Commission Regulation (EU), (2011) have been collected as samples. The weight size of the cuttlefish ranged from 21 to 300 gms. The Meat and head parts are considered products; the rest are by-products/wastage (Istiak, 2013). The result showed that the total yield is 10.30 mt, of which Sashimi meat is 5.71 mt and head parts are 4.60 mt (Fig 2).

The percentage of yield is given in Table 1 (Joseph and Perigreen., 1988). According to Joseph and Perigreen, 1988, the meat percentage is supposed to be

Table 1: Percentage of Yield of Various Parts of Cuttlefish

No	Different Items Mixed with Raw Cuttlefish Block	Percentage
1	Sashimi Meat	28.05
2	Head Parts	22.58
3	Frozen Block Defrost Wastage	1.5
4	Other Fish Mixed with Block	1.0
5	Trim Meat Wastage	4
6	Rotten Cuttlefish Mixed with Block	0.5
7	During the breeding season Contains Eggs	3
8	Tentacles are thrown into the garbage by unskilled labor	1
9	10 to 20 gm Size baby Cuttlefish	4.5
10	Skin + Bone + Ink + Water + Fin	33.87

35%, but as it is raw RTE (Lehel, et al., 2020) food, about 4% of meat is trimmed and removed (Table 1) from the extra membrane, and small-size cuttlefish

contains the rest of the percentage. Fig 3 shows that about 55 % is Ready to eat (RTE) raw (Lehel, et al., 2020) Sashimi meat, about 45 % Ready to cook (RTC) head parts, and the total yield percentage is 50.62.

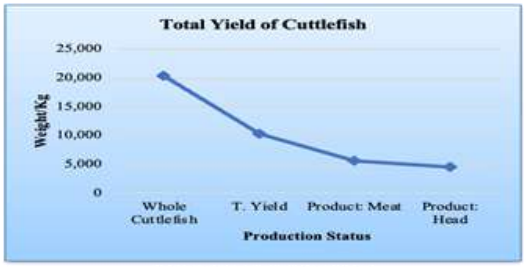

Fig 2: Total yield of cuttlefish

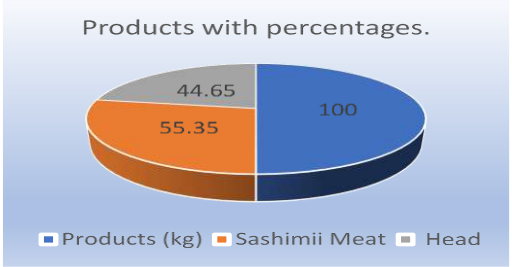

Figure 3: Products with Percentages

The yield percentage depends on various factors (Joseph and Perigreen, 1988). The size of the whole cuttlefish is essential to get maximum yield. The larger size provides maximum yield; 10-20 gm whole-size cuttlefish is unsuitable for getting maximum meat production. It is then treated as wastage. The result showed that 4.5 percent (Table 1 and Fig 5) were 10 to 20 gm size cuttlefish mixed with the whole block cuttlefish. Usually, fishing trawlers do mixed-grade blocks or two sizes of raw cuttlefish blocks. Mixed grade contains ten gms to 300 gms or more size cuttlefish block, 100 gm above size contains 100 gms to 300 gms or more sizes cuttlefish, and 100gm bellow block contains ten gms 100 gms size whole cuttlefish. 100gm above size is the most expensive raw materials processing method, freshness of the raw materials, water percentage. Table 1 and Fig 4 shows that the Cuttlefish blocks contained 1.5 percent ice, 1 percent other fish, and 0.5 percent rotten fish. Table 1 and Fig 4 shows that the skin, cuttlebone, Ink, water, and fins contain 33.87 percent of total production (Moon-bong Kang, 2013), which is higher wastage among the products. Season-wise yield is varied. The results showed that during the breeding season from March to May, about 3 percent of the yield was lost due to containing eggs (Barrett, et al., 2022)by some female cuttlefish.

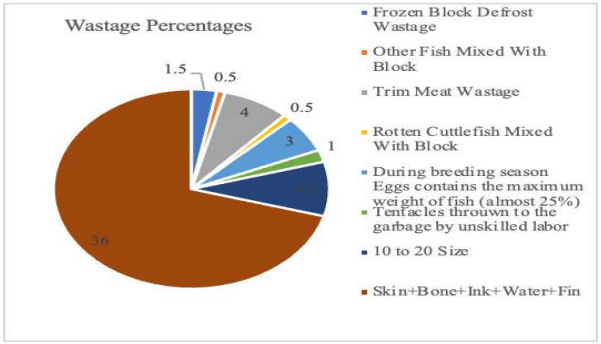

Fig 4: Wastage percentages

The production time of Sashimi meat, which consists of fillets (Brighton and Hove, 2008), is carefully controlled by all the quality parameters. Table 1 shows that about 4 percent of meat was trimmed from the fillet (Fig 3) to make it a good shape and size (Blanc, 200). The result showed that (Fig 8) after processing, freezing, and quality controlling, only 28 percent of meat is produced as sashimi grade raw RTE. RTC head part (Fig 4) is 22.58 percent (Fig 8). During the processing, the worker throws one percent of the meat into the basket due to their unskilled attitude.

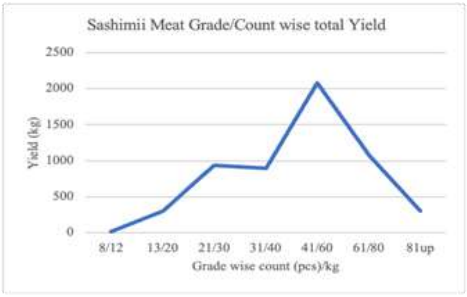

Fig 5: Sashimi meat grade/count wise total yield

According to the buyer's demand, the Sashimi meat (Fig 2) and head parts (Fig 3) have been graded into seven types. The result showed that Sashime meat grades or count (Fig 5) are 8/12 (80g up), 12/20 (50g - 79g), 21/30 (33g-49g), 31/40 (25g-32g), 41/60 (17g-24g), 61-80 (12g-16g), and 81 + (11g-7g) (Table 2 and Fig 4). Table 2 and Fig 4 show the grade-wise production percentage. Grade 41/60 is higher production which is 37%, and grade 8/12 is only 0.36%. It happened due to the different sizes of the whole cuttlefish. The yield of grade or count 41/60 has come from the 50 gms to 150 gms size whole cuttlefish. The unprocessed cuttlefish size from 50 to 150 gms is more among the samples.

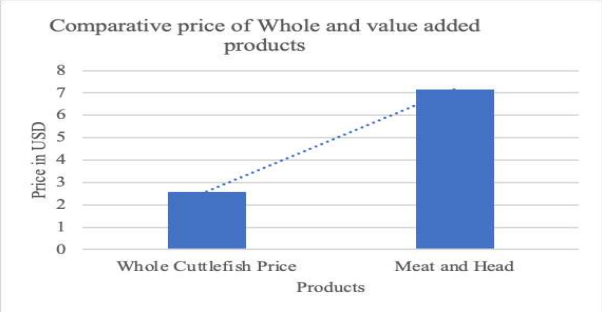

Fig 6: Comparative price of Whole and value-added products.

Fig 6.1: Sashimi meat ready to Eat

Fig 6.2: Cleaned head parts ready to cook

Fig 6.3: Weighing and grading Sashimi meat

The Sashimi processed by adopting the method has good quality, and the sensory, physical, and chemical properties and the safety sanitation quality (Lehel, et al., 2020) are in line with requirements (Hu and Aijun., 2022). Various quality assessment methods are popular (GAA, 2020), and this study has chosen two ways. One is Torry Sensory Scheme (SFIA, 2007), and another is the Microbiological test (Kulawik and Dordevic, 2020). Temperature control is crucial to get an effective result from the sensory and microbiological evaluation ((Lehel, et al., 2020; Istiak, 2015). Samples were collected in an insulated freezing box where the temperature was -18^{0}C. Before the production of Sashimi meat, sensory evaluation is a must. According to Sea Fish Industry Authority 2007 (SFIA 2007), Torry Freshness scoring is done by five panelists (1, 2, 3, 4, 5. Each panelist tested five samples individually. Then sample-wise, all results are calculated and made an average value. Fig -7.A and Table 3 show the results of sensory evaluation. Sensory attributes are A-odor, B-color, C-texture, D- stiffness, and E-Taste. Each sample has graded 1 to 10 scheme scales from unsuitable to excellent. According to the Torry Assessment Schemes result shows that the score of S-1 is 8.95, S-2 is 7.55, S-3 is 8.75, S-4 is 6.5, and S-5 is 7.15 respectively, where the average scoring is7.78, which is good quality (SFIA 2007).

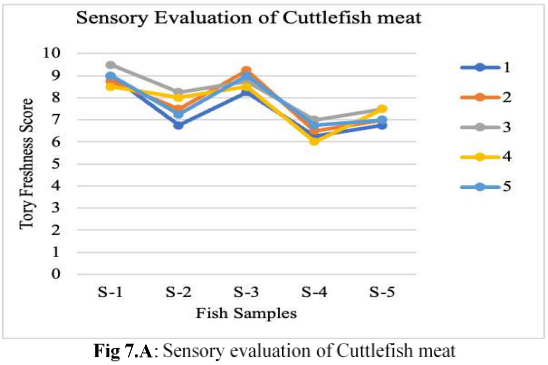

Fig 7.A: Sensory evaluation of Cuttlefish meat

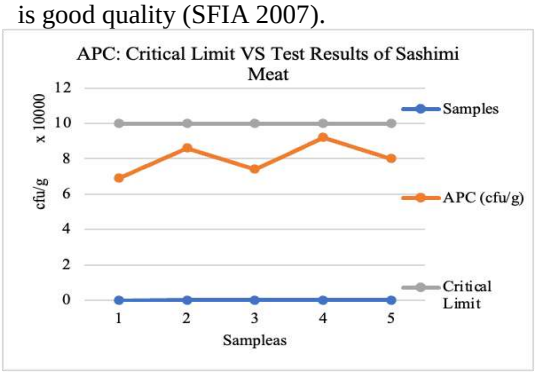

Fig 7.B: APC: Critical limit vs Test tesults of Sashimi meat

Table 2 shows the microbiological testing results of the samples which is very important to declare the cuttle fish fillet is sashimi grade for raw eaten (Muscolino, et al., 2014). Result shows that APC (cfu/g) S-1 6.9x10; S-2 8.6x10; S-3 7.4x10; S-4 9.2x10 and S-5 8.0 x10respectively.Fig 8 shows that the Aerobic Plate Count (APC) is av. 8.06x 10^4 cfu/g, which is less than the recommended critical limit of standard of the frozen food, (APC count <10^5 cfu/g) of Food Sanitation Law, (1947), Japan.

According to the standard of the frozen food under Food Sanitation Law, (1947) of Japan and

Bangladesh, test results have confirmed that the meat is Shashimi grade RTE (Lehel, 2020). FIQC inspector has collected five random samples. Microbiological tests have been done in the FIQC Lab; Chottogram: Accredited Scope: BDS ISO/IEC 17025: 2005. They are accredited by Bangladesh Accreditation Board (BAB).

Table 2: Microbiological test results of sashimi grade cuttlefish meat

Test Method	Test Parameter	Test Results				
		S-1	S-2	S-3	S-4	S-5
BDS ISO 4833:2009	Aerobic Plate Count (cfu/g)	6.9 x10	8.6 x10	7.4 x10	9.2 x10	8.0 x 10
ISO 16649-3: 2005	*Escherichia coli* (MPN/g)	<0.30	<0.30	<0.30	<0.30	<0.30
BDS ISO/TS21872-1: 2009	*Vibrio cholerae* in 25 g	Absent	Absent	Absent	Absent	Absent
BDS ISO 6579: 2009	*Salmonella* spp. in 25 g	Absent	Absent	Absent	Absent	Absent
BDS ISO/TS21872-1: 2009	*Vibrio parahaemolyticus* in 25g	Absent	Absent	Absent	Absent	Absent
BDS ISO 4831:2009	Total Coliforms (MPN/g)	Absent	Absent	Absent	Absent	Absent

The third row of the second column of Table 4 shows the results of *Escherichia coli* (MPN/g) that is S-1 <0.30, S-2 is <0.30; S-3 is <0.30, S-4 is <0.30 and S-5 is <0.30 respectively. The fourth row of the second column of Table 4 shows the results of *Vibrio cholerae* of 25 g samples absent in S-1, S-2, S-3, S-4, and S-5, respectively. Table 2 also shows the results of Salmonella spp. In 25 g samples, *Vibrio parahaemolyticus* in 25g, and Total Coliforms (MPN/g) are absent in every sample (JETRO, 2009).

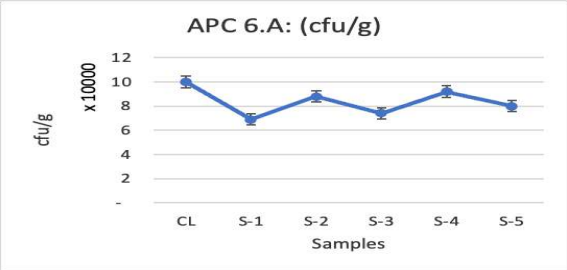

Fig 8: APC 6.A: (cfu/g)

According to Fig 6.B microbiological results of five samples where *Vibrio*spp, *Salmonella*, and Total coliforms are absent, and *E. coli* count is less than <0.30 (MPN/g) in the samples, which is less than the recommended critical limit of Sashimi Meat/unheating cuttlefish fillet ofthestandard of the frozen food under Food Sanitation Law, (1947) of Japan and FIQC, Bangladesh. Samples are tested in the FIQC Lab, Chottogram which is accredited in BDS ISO/IEC 17025 in 2005 by Bangladesh Accreditation Board (BAB). Usually, Bangladesh exports whole Cuttlefish to China, Thailand, and Korea (DoF, 2022). According to Fig 7, the result showed that the unprocessed Cuttlefish Price is USD 2.58/kg. So far, no value-added activities for cuttlefish have taken place in Bangladesh (Istiak S. M., 2018). Fig 8 shows that the processed cuttlefish Price is USD 7.16/kg. After analyzing the data, it proves that to produce 1 kg of cuttlefish RTE (Lehel, et al., 2020) and RTC products, about 2 kilograms of whole fish is required, which costs 5.16 USD. ANOVA single factor test proves that the profit gain is P<0.004 % significant. Therefore, the financial gain is 49 % higher than the same quantity of unprocessed cuttlefish price.

Conclusion and Recommendation

Nowadays, people are more concerned about seafood products' nutritious value, quality, safety, taste, and presentation. The increasing demand for Sashimi grade value added cuttlefish offers better prices than the traditional unprocessed fish. It has high commercial value worldwide, particularly in Japan and Spain, which consume the most. Culturally, Bangladeshi people are not used to having cuttlefish as a source of animal protein. This study proved that cuttlefish is a potential exportable seafood product to earn foreign currency by adding value. Therefore, the total yield of Sashimi meat and head part is about 50%, and the profit gain is 49% from the price of whole cuttlefish, which is statistically P<0.5% significant. Quality attributes comply the international standards. However, more studies need to be carried out to confirm this method.

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The Change of E-Commerce after the Covid-19 Pandemic Affects the Intention to Buy Food in Vietnam

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Abstract

E-commerce has become an area of great influence for both businesses and customers in the era of globalization. Especially in the period after the COVID-19 pandemic, e-commerce plays an important role in submitting products to the market, stimulating economic demand and helping consumer's access goods without having to worry about infection epidemic virus. Consumers' health consciousness is enhanced. At the same time, consumers have formed the habit of shopping for essential goods and spending at an appropriate level; Specifically, the food industry in Vietnam: vegetables, meat, and fish. Therefore, the authors have conducted a focused study based on the main factors: trust, customer behavior, strategy marketing, digital platforms, risk and E-commerce change applications determine which factors have the strongest impact on consumers' intention to buy food. The study was practiced in a combination of quantitative and qualitative research methods, with 310 online survey participants and the collected group results were entered into SmartPls software to check the reliability of Cronbach's Alpha scale, then perform Exploratory factor analysis (EFA), continue to analyze the SEM linear structural model. The results show that trust, customer behavior, digital platform, e-commerce change has a positive effect on consumer purchase intention to use in the food industry. In addition, factors such as risk and marketing strategy also affect consumers' purchase intention more or less. The Covid-19 pandemic has passed, both a difficulty and an opportunity to help businesses and individuals by business effectively on e-commerce platforms.

Keywords: intent to buy, food, Vietnam, change, e-commerce, Covid-19, consumers.

Introduction

Vietnam and the world have just experienced an economic "shock" due to the strong impact of the Covid-19 pandemic. According to statistics, there are a total of 11,383,248 infections, of which: 729,645 cases are being treated, 10,610,481 cases have recovered and unfortunately in Vietnam there have been 43,178 deaths as of December 15, 2022. After the e-commerce pandemic has changed, the economy worldwide has been developing in a positive direction. People are getting busier, there is not so much time left to go to (traditional) shops or markets to shop; instead, under the development of the Internet, consumers can completely buy directly on e-commerce channels such as Shopee, Lazada, Tiki… by phone, laptop, ipad anytime including when going to work and out. In the world, the form of buying online is no stranger, but it also accounts for a very high proportion. According to Mastercard's report, in 2020 people around the world spent more than 900 million USD, accounting for 20-30% of the total retail revenue of the e-commerce industry. U.S. retail sales will hit a record $4.26 trillion in 2021, up 8.1% from $3.94 trillion in 2020, according to consumer research firm Customer Growth Partners (CGP). Forecasts from eMarketer Market Research showed that total retail sales in China will reach 52.1% in 2021, up from 44.8% in 2020 from e-commerce transactions. According to eMarketer's forecast report, traditional sales in China are likely to decline by 9.8% in 2021, after falling 18.6% last year. Meanwhile, e-commerce sales will grow by 27.5% in 2020 and could grow by 21% in 2021 (Nam, 2021).

Returning to the Vietnamese market, the developed e-commerce market has been growing the most strongly in Southeast Asia. In the next few years, from 2020-2025, Vietnam's average growth rate is 299%. The scale of e-commerce will reach 52 billion USD to rank in the top 3 of ASEAN by 2025. Vietnam's e-commerce index (EBI) grew by 15% in 2021, reaching about $13.2 billion in 2020 (Ha, 2022). The digital economy has a strong impact on the e-commerce market in Vietnam, which has been and will develop further in the future.

The pandemic has brought many harms, but it has helped e-commerce thrive. Also since the pandemic, Vietnamese consumers have tended to shop actively in all sectors, including the food industry. In 2019, Vietnam's e-commerce officially surpassed the $ 10 billion mark, reaching $11.8 billion in 2020 and continuing to grow to $13.7 billion in 2021. In particular, the shopping value on Vietnam's e-commerce platform will continue to increase significantly and is expected to reach 260-285 USD/person in 2022 (Lin, 2022).In 2021, the share of B2C e-commerce revenue in the country's total retail sales of consumer goods and services will exceed 7%, increasing from 7.2% to 7.8%. Vietnam expects e-commerce revenue to reach $39 billion by 2025 (Anh, 2022).

Currently, the economic situation after the Covid-19 pandemic has gradually stabilized, people have gradually controlled the pandemic. At the same time, people have also experienced more difficult challenges than ever before: the human body weakens; therefore, health consciousness has driven the growth of the healthcare food industry. Keeping yourself mentally refreshed and healthy every day is a measure that helps improve the body's immune system and minimize the side effects of the COVID-19 pandemic. So what are the factors affecting shopping intentions for the food industry in Vietnam in the post-COVID-19 period? Where do consumers' biggest concerns come from? According to data collected by iPrice Group and Similar Web, in the first 6 months of 2021, e-commerce revenue reached 1.3 billion visits (Giang, 2021). In the top 4 e-commerce platforms in the world: Shopee, Tiki, Lazada, Sendo. Shopee is currently leading with the highest number of visitors of 73 million, an increase of 9.2 million compared to 2021 (VECOM, 2021).

Under the strong impact of the Covid-19 pandemic, Vietnam's economy has changed markedly. Consumers have transformed the form of buying food – goods from handicrafts to technology through e-commerce/websites. And businesses have caught up with the digital transformation of the economy: trading in functional foods, clean foods, health support tablets... help increase revenue and e-commerce is growing, along with many post-processing policies to help e-commerce develop: support shipping orders to home, multi-form payment, exchange/return.

In recent years, the e-commerce market has had a strong impact on our lives, bringing many benefits to businesses and customers. However, under that development, the fierce competition of the online market is also a pressure on businesses.

According to statistics from the internet world stats. Com website, according to the report "the rate has now increased due to Pingdom's Global Internet Conditions from 2000 to 2009." The number of Internet users in Vietnam increased by 10,882%. Vietnam's Internet usage rate accounts for about 26%, ranking 20th in the world. The number of website builders accounts for 38% and 14% of businesses participating in business on e-commerce platforms according to the Ministry of Industry and Trade (2011).

In 2021, our country was affected by the epidemic that led to companies not being able to work on-site and moving online through support applications. In that context, online business on e-commerce platforms has also developed strongly. Currently, our country has been developing many e-commerce platforms selling goods with a variety of items and high website visits such as Shopee.vn, Lazada.vn, Sendo.vn, and Tiki.vn. Or e-sales websites of businesses such as thegioididong.com, nguyenkim.com, cellphones.com. vn. A recent research report published on Dec. 3 found that consumers around the world have sharply increased spending on electronic products.

In 2021, Vietnam's e-commerce will have bright spots and continue to grow record. As a result, business-to-personal (B2C) e-commerce in Vietnam has grown by about 16-17% to reach 13.7 billion USD, accounting for nearly 6% of the total standards of consumer goods and services of the country. It is expected that e-commerce will continue to develop and create new consumer trends in 2022. By 2025, Vietnam's e-commerce scale will reach 52 billion USD, becoming the third largest e-commerce market economy in ASEAN.

In the context of e-commerce development in 2021, experts have outlined 3 development trends of e-commerce in 2022: The first is to personalize customer experience; the second is a wide range of forms of payment (in addition to cash); the third is eco-friendly consumption.

In the Vietnamese market, in 2021, the growth rate is over 20% and the scale is 16 billion USD. 2022 has well controlled the pandemic, so the growth rate will grow during the year and will be even higher in the future (Thu, 2022). However, to be able to meet this requirement requires businesses to have full knowledge of e-commerce and catch up with market trends to reach a large number of domestic and international customers.

From those reasons, the authors chose the topic: “The change of e-commerce after the Covid-19 pandemic affects the intention to buy food in Vietnam”.

This research topic aims to study the impact of e-commerce on consumer purchase intent on the food industry before and after the Covid-19 pandemic. From there, build a research model based on the premise and results of data analysis. At the same time, the best solutions and lessons learned help businesses doing business in the food industry on e-commerce platforms promptly adjust their policies to attract the most customers.

The Theory of Planning Behaviour – TPB

The TPB model has addressed factors that influence consumer intent and behavior, including attitudes, ethical norms, subjectivity, and cognitive behavioral control. This study is inherited from the TRA rational operation theory study. And in 1980, Martin Fishbein and Ajen did original research.

According to Ajzen, purpose is influenced by 3 factors: Cognitive control of behavior. This factor revolves around human perception. Specifically, the level of awareness can change easily or difficult according to different actions and situations. Second, subjective attitudes and norms. In this factor, attitudes towards behavior are related to the degree to which a subject judges. This assessment can be favorable or detrimental to an individual's behavior. Finally, there is the subjective norm, which is the perception of affected individuals as to whether or not the individual should perform the act (Ajzen, 1991).

Technology Acceptance Model – TAM

The TAM - Technology Acceptance Model was introduced by Davis (1989). The model refers to the prediction of the acceptability of an information

system in a more specific way and in turn proposes changes to the information system to achieve greater acceptance efficiency. Among them are influencing factors such as the perception of ease of use and the perception of usefulness.Model usefulness perception is defined as "the degree to which a person believes that using a particular system will enhance their job performance" (Venkatesh et al., (2003).

1. Trust (T)

Reliability of service or quality is the ability of a company to perfectly perform order-fulfilling activities according to customer perceptions (Mint, 2019). Trust is the expectation from interaction and belief involves the disclosure and acceptance of weaknesses (Kim and Park, 2012). Another study by Moorman, Zaltman and Deshpande defines trust as reliance on a rival or partner with the expectation that their words are trustworthy. The ability to solve problems online and develop e-commerce is a useful method to enhance customer confidence in suppliers (Belanger et al., 2002). Moreover, trust is emphasized as a major factor determining customer loyalty (Xiao et al., 2010).

The trust or trust of customers is an extremely important factor in business that speaks to the ability to maintain the trust of the company in the eyes and perceptions of customers. From the steps of care, using the company's products and services leaves an impression on customers, thereby creating the trust of customers and continuing to use the service or not. Beliefs influence online search intent (Yoon, (2002). Trust is the fundamental condition for users to participate in e-commerce (Corbitta et al., 2003). The ability to handle online communications and conduct e-commerce in a professional manner can enhance users' confidence in the capabilities of e-distributors (Belanger et al., 2002). Trust can raise awareness of usefulness (Gefen et al., 2003). Based on the studies mentioned, the authors hypothesize as follows:

Hypothesis 1_H1: *Trust has the same effect as e-commerce change.*

Hypothesis 2_H2: *Trust has a positive impact on customer behavior.*

2. Customer behavior (CB)

One of the factors that directly influences the change of e-commerce is customer behavior. Customer behavior (consumer behavior) has a lot of different ways of looking at it and research gives a different overall definition. Consumer behavior is defined as the behavior that consumers exhibit in seeking to buy or use goods or services (Echchakoui, 2016). Customer behavior is all direct and indirect actions and acts that consumers take to obtain goods and services at a specific place or time (Moon et al., 2015).

In addition, customer behavior is a set of mental and bodily activities related to the process of considering, selecting, and using goods and services (Singh and Singh, 2015). After the outbreak of the COVID-19 pandemic, the behavior of some consumers has changed including the needs and desires to make a purchase. Consumers are gradually becoming more concerned about their health and the changing economy because of the COVID-19 epidemic (Accenture, 2020). Since then, customer behavior has had an impact on e-commerce change. It requires e-commerce to change to accommodate the factors that influence consumer behavior. Based on the above studies, the authors have drawn the following hypothesis:

Hypothesis 3_H3: *Customer behavior positively affects e-commerce change.*

3. Marketing strategy (MS)

A successful marketing strategy will attract more customers to use the organization's goods, products or services. The buying behavior of buyers will be more or less affected by the marketing strategy of e-commerce channels. In addition, the marketing strategy will have a strong impact on the change of e-commerce. Currently, many people have chosen to buy online instead of going to in-person stores. Therefore, marketing strategy is a very important factor that businesses need to have.

According to Varadarajan (2010), marketing strategy consists of a set of important company decisions related to marketing activities in selected markets and segments in order to create and provide value to customers to achieve the company's goals (Varadarajan, 2010). Marketing strategy is the marketing reasoning by which a business hopes to achieve their marketing goals (Mai, 2011). Marketing strategy includes strategies related to the target market, marketing-mix and marketing budget. A marketing strategy is a system of long-term operational decisions that an organization needs to make in order to achieve its goals (Nga, 2005). A marketing strategy is feasible if it reflects the basic objectives, the directions of action to be implemented on the basis of proper analysis and assessment of environmental characteristics, ensuring the necessary conditions. In particular, it is necessary to combine the specific requirements of marketing activities with the requirements of other functional departments in the organization. If businesses want to survive and develop sustainably in the market, they need to have reasonable and effective marketing strategies (Tien, 2021).

The role of marketing is not new, but to implement it effectively is still a concern of many managers. Based on the above studies, the authors hypothesized the following:

Hypothesis 4_H4: *Marketing strategies positively affect e-commerce change.*

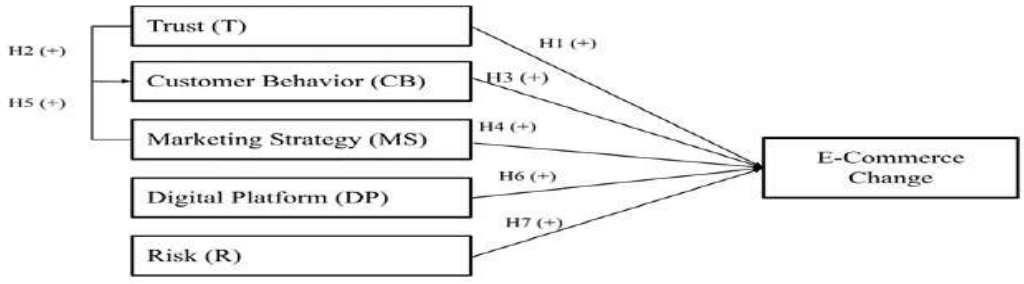
Hypothesis 5_H5: *Marketing strategies positively affect customer behavior.*

Digital Platform (DP)

The digital platform is an effect, a big challenge that requires entrepreneurs to change the form of buying and selling goods with e-commerce services (Andreas et al., 2020). It is a power to help managers

find a direction for development, find potential customers in domestic markets and network externalities (Rochet and Tirole, 2003). A digital platform is a work that describes the value of the market when influenced by other actors. A digital platform is a web-focused platform for delivering content (on platforms such as: Facebook, Twitter, blogs, websites, and sometimes SMS) (Schilling, 2002). This involves similar platforms: advertising, mailing, telemarketing, events. Digital platforms are a new generation of customer/employee/partner-centric internal and external platforms that require the flexibility to measure and compromise a wide range of input touchpoints. The study was conducted by (Nambisan et al., 2019; Mishra and Tripathi, 2020).
The platform provides accounts to help users manipulate the use of products / services on the system such as: SDK ... is the digital platform, according to (Constantinides et al. 2018). Digital platforms allow users and businesses to connect with each other on e-commerce channels with the participation of at least two parties; party A is the supplier and B is the business partner, aggregated information (Mishra and Tripathi, 2020). A digital platform is a platform that acts as a middle ground and helps connect groups of users together, also known as a multipurpose/ multifaceted platform (Boudreau and Hagiu, 2009). Based on the studies mentioned, the authors hypothesize as follows:
Hypothesis 6_H6: *Digital platforms positively affect e-commerce change.*

Risk (R)
According to Featherman and Pavlou, risk refers to consumers' perceptions of uncertainty and consequences of engaging in a particular activity. The risk structure consists of the following three components integrated into the conceptual framework: (1) financial risk; (2) operational risk, referring to probability. The functions of the application are processed and do not work as originally intended; (3) Security risk, referring to the possibility of losing control of personal information (Featherman and Pavloub, 2003). There is a lot of research on the relationship between risk and how useful perception is. Being aware of the level of risk significantly reduces the usefulness of paying bills directly on e-commerce platforms (Featherman and Wells, 2010). Similarly, research by Li and Huang (2009) shows that risk has a negative impact on how useful online shopping is. Specifically, Li & Huang (2009) clarified that shopping on the e-commerce floor includes many risks and is not as safe as traditional shopping (Li and Huang, 2009). The risk perceived by consumers has been shown to influence their online decisions. Furthermore, consumer perceived risk has a negative impact on consumers' purchasing intent. This means that, the higher the perceived risk, the less consumer intention to buy (Hwang and Kim, 2007). In addition, Chiu et al. found that consumer perceived risk diminishes the purchase intent of B2C e-commerce (Chiu et al., 2005). In other words, risk is one of the factors that directly affect consumers' intention to buy on e-commerce channels. It is a perceived scale that has a positive impact on consumers' online buying behavior, according to Khoa (2018). According to Ha cognitive risk helps bring satisfaction to customers, objectively affecting purchase intent (Ha, 2019). The risk of perceiving photos is a factor that directly affects the intention of gen Z to buy online (Thanh and On, 2021). Based on the studies mentioned, the authors hypothesize as follows:
Hypothesis 7_H7: Risks that directly affect e-commerce change.



Research method
The team performed the analysis based on two methods, qualitative research and quantitative research. In particular, qualitative research is carried out by the team through group discussions via zoom, google meet and face-to-face team meetings; thereby, the team created the experimental survey scale and questionnaire, the experimental survey results helped the group change the scale and establish the official questionnaire. To the next stage is quantitative research with a total survey sample of 310 using online surveys. The results collected by the group include:
In terms of age, from 15 to 25 years old, 146 customers accounted for the highest proportion with 47.1%. There were 106 customers aged 26-35 participating in the survey, accounting for 34.2%, aged 36-45 participating in the survey had 36 people and accounted for 11.6%. The ages of 46-55 surveyed 16 people, accounting for 5.2% and finally over the age of 55 had 6 participants, accounting for 1.9%.
Through this, it can be clearly seen that customers shopping on the e-commerce floor are diverse in age

from young to old; in which the ages of 15 - 25 accounted for the highest proportion. In terms of gender, 310 people participated in the survey; of which the percentage of men participating in the survey was 141, accounting for 45.5%, less than the proportion of women. Meanwhile, the percentage of women participating in the survey was 169, accounting for 54.5%. Thus, it can be clearly seen that women will often have more shopping needs than men.

In terms of living area, 44.5% of survey respondents live in South Vietnam, including 138 people; besides, 94 people accounted for 30.3% of participants living in Central Vietnam. Regarding North Vietnam, there were 67 participants, accounting for 21.6%; besides, 11 survey respondents live abroad and account for 3.5%. Thereby, it can be seen that the shopping level of southern consumers is higher than all the rest of the regions.

In terms of occupation, 147 respondents were students and accounted for 47.4%. 61 of the respondents were public employees, accounting for 19.7%. 11% of survey respondents were office workers (34 participants). The 14 respondents were non-working and accounted for 4.5%. Another 17.4% of participants (54) had other occupations.

Through these data, we can determine that students are the ones who need to shop the most. In terms of monthly income, the number of respondents without income accounted for 14.8% with 46 participants. The income level of less than 3 million VND accounts for a fairly high proportion with 25.5%. Next is the income from 3 million - 10 million accounting for a high proportion with 35.5% including 110 survey participants, the income level from 10 - 20 million accounting for 14.8% with 46 participants and finally the income level over 20 million accounting for the lowest 9.4% compared to other income levels.

Analysis and results

1. Analysis

To evaluate the information obtained after the survey process, the team conducted a two-step process for analysis. First, the authors used Cronbach's Alpha test to determine the reliability of the scale; next, use the Exploratory Factor Analysis (EFA) method to determine lateral consistency and use Confirmation Factor Analysis (CFA) to track whether the underlying variable data of the observed variable exhibits its characteristics. Second, use the Structural Equation Model (SEM); intended to consider whether the proposed model is consistent with original research objectives.

Cronbach's Alpha test

Cronbach Alpha is used to evaluate the reliability of the scales. This method helps to eliminate observed variables with small total correlation coefficients (< 0.5), if the Cronbach Alpha value of the latent variable is > 0.7, the scale is considered reliable (Hair et al., 2014).Table 1 shows the reliability of all six scales with Cronbach's Alpha index ranging from 0.8 to 0.9. Cronbach's Alpha value is highest in Reliability (0.9) and lowest in Risk (0.8). Therefore, the scales including: Marketing Strategy, Customer Behavior, Digital Platform, Risk, Reliability and E-commerce Change met the standards and were statistically significant.

Table 1. Results of Cronbach's Alpha for six coal measurements (Source: Processing results using SmartPLS 4.0)

No.	The scale	Number of observed variables	Coefficient Cronbach's Alpha
1	Marketing Strategy (hereafter MS)	3	0.876
2	Customer Behavior (hereafter CB)	3	0.838
3	Digital Platform (hereafter DP)	3	0.879
4	Risk (hereafter R)	3	0.800
5	Trust (hereafter T)	3	0.900
6	E-commerce change (hereafter ECC)	3	0.889

Explore factor analysis (EFA)

The team of authors conducted a study that looked at the values of the proposed ingredients. Thereby understanding and assessing how the change in e-commerce after the Covid-19 pandemic affects the food industry in Vietnam market during this period. During the implementation, the team used the SEM analysis method through the extraction of 6 elements of Promax rotation data: Marketing Strategy (MS), Customer Behavior (CB), Digital Platform (DP), Risk (R), Trust (T), E-Commerce Change (ECC).

In the table below, the team listed the extracted factors along with the observed variables and the corresponding load factor required (showing only the load factor > 0.3). All observation variables have a load factor > 0.5, so the observed variables perfectly measure the concepts we need.

The table was compiled by the authors from Rotated Component Matrix showing the convergence of observed variables to groups of factors. And, the team found that marketing strategy (MS1, MS2, MS3), customer behavior (CB, CB2, CB3), digital platforms (DP1, DP2, DP3), risk (R1, R2, R3), trust (T1, T2, T3) and e-commerce change (ECC1, ECC2, ECC3) fully converge the factors described in the study-scale summary.

Table 2. EFA discovery factor analysis results (Source: Processing results using SmartPLS 4.0)		
Factor	**Variables**	**Outer loadings**
Marketing Strategy (MS)	MS1	0.897
	MS2	0.885
	MS3	0.902
Customer Behavior (CB)	CB1	0.904
	CB2	0.837
	CB3	0.865
Digital Platform (DP)	DP1	0.897
	DP2	0.885
	DP3	0.909
Risk (R)	R1	0.879
	R2	0.881
	R3	0.775
Trust (T)	T1	0.924
	T2	0.916
	T3	0.899
E-Commerce Change (ECC)	ECC1	0.902
	ECC2	0.912
	ECC3	0.901

SEM model

Measurement model testing

To assess customer confidence at the scale, the team conducted this study with a global confidence factor, the total variance for AVE extraction, and a single-factor load factor (external load). Of those coefficients, the combined reliability must be greater Than 0.7 and the external load factor must be greater than 0.4 to achieve the highest reliability value (Hair et al., 2014). Furthermore, according to Fornell and Larker (1981), the sum of the sample variances must be greater than 0.5, that is, confirm the reliability and convergence value of the scale. The results of the calculation of total reliability, load factor and exact variance of the subscales show that the conceptual scales all meet the given reliability and convergence value requirements.

To assess the degree of differentiation of the scale, the study used the malformation-monosexual ratio. Research results. The table shows that the differentiation of the scale is very good, showing that all Heterotrait – Monotrait (HTMT) values are much lower than the threshold value of 0.85. Therefore, these elements all meet the requirements of the distinguishing value.

Table 3: Measurement model inspection table (Source: SV processing data with SmartPLS 4.0)				
	Cronbach's Alpha	**Composite reliability (rho_a)**	**Composite reliability (rho_c)**	**Total Variance (AVE)**
MS	0.876	0.878	0.924	0.801
CB	0.838	0.839	0.903	0.756
DP	0.879	0.881	0.925	0.805
R	0.800	0.808	0.883	0.717
T	0.900	0.901	0.938	0.834
ECC	0.889	0.892	0.931	0.818

Table 4: Correlation matrix between Heterotrait – Monotrait (HTMT) conceptual structures (Source: Data processing results using SmartPLS 4.0)						
	MS	**CB**	**DP**	**R**	**T**	**ECC**
MS						
CB	0.798					
DP	0.807	0.812				
R	0.470	0.399	0.443			
T	0.783	0.780	0.705	0.527		
ECC	0.651	0.756	0.731	0.399	0.692	

Table 5: Correlation matrix between conceptual structures according to the Fornell–Larcker standard table (**Source:** Data processing results using SmartPLS 4.0)						
	MS	**CB**	**DP**	**R**	**T**	**ECC**
MS	0.895					
CB	0.684	0.869				
DP	0.709	0.697	0.897			
R	-0.394	-0.328	-0.372	0.846		
T	0.695	0.678	0.627	-0.447	0.913	
ECC	0.578	0.654	0.647	-0.341	0.623	0.905

Structural model inspection

To evaluate the structural model, the team used the R square, f square and SRMR indicators along with the path coefficient. The path coefficient is estimated based on the regression of each dependent variable and the predicted variable (Hair et al., 2014). If multiline

Phenomena occur among independent variables, path coefficients are not guaranteed. If the VIF value is within the accepted range VIF = 1,600 – 2,700 < 5, then the association between the predictors does not violate the assumption of multicollinearity, so the model does not violate this phenomenon.

Table 6: Table of VIF coefficients (Source: Processing results using SmartPLS 4.0)	
Bright	
MS1	2.405
MS2	2.308
MS3	2.416
CB1	2.451
CB2	1.764
CB3	2.046
DP1	2.454
DP2	2.286
DP3	2.551
R1	2.083
R2	2.168
R3	1.429
T1	3.286
T2	3.003
T3	2.454
ECC1	2.384
ECC2	2.865
ECC3	2.632

Table 7: Table R Square and R Square Adjusted		
	R-square	R-square adjusted
CB	0.547	0.544
ECC	0.530	0.523

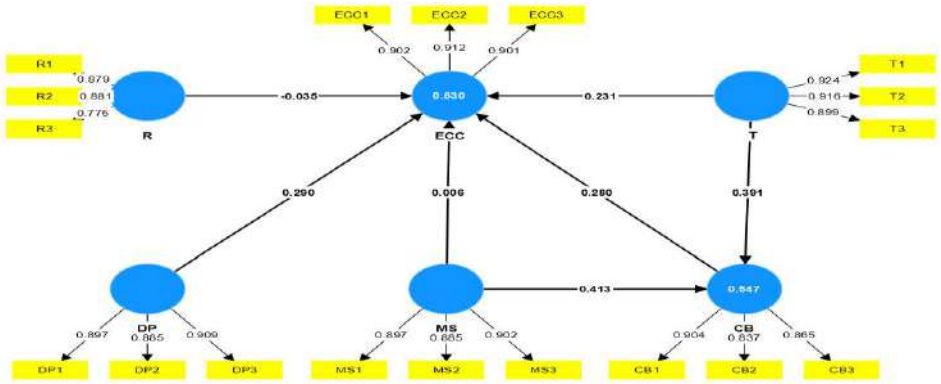
An R2 value of 0.25 represents a weak endogenous structure, a relative 0.5, and a high 0.75 (Hair et al., 2014). The results of the analysis showed that the adjusted R2 value of the Behavior model was 0.547 and the adjusted R2 value of the trust model was 0.530.Thus, customer behavior explains 54.4% of the variation in dependency. The remaining 45.6% unexplained is due to other factors that have not yet been included in the model. The change in e-commerce explains 52.3% of the variation in dependency. The rest of the unexplained 47.7% is due to other factors that have not yet been included in the model.

Table 8: Group f Square					
MS	CB	DP	R	T	ECC
MS	0.194				0.000
CB					0.066
DP					0.072
R					0.002
T	0.174				0.046
ECC					

Source: Processing results using Smart PLS 4.0

At the same time, using the impact factor f (influence size index) of 2 Cohen (1988). The results of relationships reaching meaningful f2 values between 0.000 and 0.194 showed that the strength of these relationships has a small to large impact**.**

Model results diagram



Source: Processing results using Smart PLS 4.0

Auditing with Bootstrapping

Since the data analyzed in PLS is assumed to be non-standard distribution, parametric significance testing in regression analysis cannot be used to test the meaning of coefficients such as path coefficients. Instead, PLS relies on nonparametric guidance analysis to test the meaning of the coefficient. To Check whether the path coefficient differs from 0 significantly, the t-values are calculated by booting. In this study, the nonparametric start-up technique was performed with 310 observations and repeated 5000 times to ensure the requirement to test the linear structure model.

Table 9: Bootstrapping results structure model

	Original sample (O)	Sample mean (M)	2.5%	97.5%
MS → CB	0.413	0.411	0.299	0.522
MS → ECC	0.006	0.006	-0.140	0.143
CB → ECC	0.280	0.279	0.148	0.411
DP → ECC	0.290	0.289	0.149	0.418
R → ECC	-0.035	-0.039	-0.127	0.044
T → CB	0.391	0.392	0.283	0.496
T → ECC	0.231	0.231	0.098	0.368

Source: Processing results using Smart PLS 4.0

Table 10: Direct impact results of relationships

		Original sample (O)	Sample mean (M)	Standard deviation (STDEV)	T statistics (| O/STDEV|)	P Values	Result
H1	T → ECC	0.340	0.340	0.065	5.261	0.000	Accepted
H2	T → CB	0.391	0.392	0.054	7.238	0.000	Accepted
H3	CB → ECC	0.280	0.279	0.067	4.203	0.000	Accepted
H4	MS → ECC	0.122	0.121	0.076	1.596	0.110	Rejected
H5	MS → CB	0.413	0.411	0.057	7.265	0.000	Accepted
H6	DP → ECC	0.290	0.289	0.069	4.201	0.000	Accepted
H7	R → ECC	-0.035	-0.039	0.044	0.808	0.419	Rejected

Source: Processing results using Smart PLS 4.0

Through the data processing tables using SmartPLS 4.0, we can clearly see the impact of these variables on the intention to buy food products after the Covid-19 pandemic in the following order:

Trust, customer behavior, marketing strategy and digital platforms, and e-commerce change have a direct and indirect impact on consumers' intent to buy food. In particular, trust also indirectly affects passenger behavior to promote the change of e-commerce; on the other hand, marketing strategy does not directly affect the change of e-commerce but indirectly affects through customer behavior.

Among those factors, the digital platform factor is the factor that positively impacts the change of e-commerce. That is, the higher the digital platform factor, the greater the change in e-commerce.

Result

From table 10, comparing the impact of these variables on intent and trust variables in descending order is as follows: we see that marketing strategy variable has the strongest impact on customer behavior (β = 0.413), followed by trust variable that affects customer behavior (β = 0.391); trust variable impacts the e-commerce change (β = 0.340), digital platform variable impacts e-commerce change (β = 0.290); customer behavior variables impacting e-commerce change (β = 0.280); marketing strategy variables impacting e-commerce change (β = 0.122); risk variable impact e-commerce change (β = -0.035). Thus, hypotheses H1, H2, H3, H5 and H6 are all accepted as having a positive effect on ECC dependent variable while the 2 hypothesis rejected are MS → ECC because P = 0.110 > 0.05 and R → ECC because P = 0.419 > 0.05.

In this study, we also looked at the relationship between customer behavior (intermediate variables) and e-commerce change through the impact of marketing strategies and showed that the relationship is strongly correlated and has the same relationship as follows:

CB → ECC through the impact of MS with an impact factor (β = 0.280).

Thus, the results of linear structure analysis and tests show that all assumptions H1, H2, H3, H5 and H6 are accepted. That is, trust, customer behavior, and digital platforms have a direct positive influence on e-commerce change. Moreover, marketing strategy, trust has an indirect influence on customer behavior to influence e-commerce change.

Discussion

The team conducted this study with the aim of reviewing and evaluating the factors affecting Vietnam's food industry after the Covid-19 pandemic. The findings and evidence of the implementation team have shown that there are 4 factors that positively affect the food industry: Trust, customer behavior, digital platforms and e-commerce change. In addition, risk factors and marketing strategies also have certain impacts on consumers' food buying behavior but only to a low level; to be more in-depth, the study takes time to implement. The main objective of the study was to look at the influence of various factors on the intention to buy food products in Vietnam on e-commerce channels that use the internet as an intermediary.

The findings demonstrate that four factors that have a beneficial influence on variables depend on: digital platforms, e-commerce change, customer behavior and trust; these are the factors that combine the times and health. The society is growing, the higher the needs of consumers, besides, the need for health care and protection is the most important thing. The higher these demands, the more consumers tend to buy healthcare items. One study has suggested that health considerations positively influence consumer intention to buy online (Xin and Angelika, 2021). Subjective norms in e-commerce refer to the post-pandemic effects of society, consumers buying food products for the purpose of protecting their health and family; fast-growing e-commerce helps them buy electronic goods, minimizing time and preventing the spread of disease. And, what businesses need to do right now is to create trust in the hearts of customers to maximize the amount of food sold in the market. The form of a communication sales system at markets and stores is fighting being replaced by an e-commerce platform. Businesses need to sell products with "ENOUGH - RIGHT - ADDITIONAL" information to reach more customers.

To do that, businesses and business people need to: Apply marketing forms into business such as:

● Create promotions - discounts to attract customers.

● Run ads: traditional and electronic (run ads) to reach customers, increase traffic and shop purchases/follows.

On the other hand, it is necessary to diversify payment methods, delivery methods, etc. Therefore, issues of transaction terms, payment invoices, and delivery and exchange methods must also be fully paid and transparent to specified user name. Now is the 4.0 era, let technology help you get rich. Home, people are using the internet - people's homes are using social networks. Therefore, forms of internet marketing such as social advertising will help customers trust your food more. Consumers always have the habit of reading reviews before buying, so build yourself factors such as: images, detailed information: manufacturer, expiry date, ingredients, processing, ... most clearly; it will help customers understand and come to themselves.

Conclusion

After the team collects, evaluates and discusses the collected data; the authors found that the influence of factors on purchase intent is listed in order: digital platform, action micro-customers, marketing strategies and beliefs. Through in the study, the authors came to the conclusion that: All 4 factors have a positive influence on food purchase intentions at restaurants. E-commerce platform today. However, the marketing strategy variable does not directly impact the purchase intent, but it does impact customer behavior; leading to an increase in purchase intent. Based on the data and research model, the authors proposed governance implications for the current food industry. Now through 4 main elements: Digital platform, customer behavior, marketing strategy and trust. The purpose of introducing these variables is to help businesses and entrepreneurs, establish - propose models/ specific, solid business solutions to be able to attract more customers to increase revenue and succeed in time with the great internet.

After the Covid-19 pandemic, the health of Vietnamese people in particular is greatly affected and vulnerable to the environment and diet. People are increasingly interested in diet and the percentage of nutrition present in food. In addition, they will prioritize supplementing nutrients, functional foods, multivitamins to protect health; strengthening resistance as well as preserving the perfect physique for the body. Although food is a matter of great concern to consumers, how do they learn about those products and making purchasing decisions is still a difficult problem to solve. Therefore, companies must also improve various characteristics such as beliefs, attitudes and subjective standards. To enable these features, it is necessary to continue and step up social media activities. In today's age of technology, almost everyone uses social media to study, work, and communicate and more. That's it. As of February 2022, the average online time of Vietnamese people is 6 hours and 38 minutes. In particular, mobile phones accounted for the highest position of 53.2% equivalent to 3 hours and 32 minutes, while desktop and tablet were 3 hours and 6 minutes.

According to the research model that the authors carried out, the digital platform variable is having the strongest impact (research model), combined with the development of technology, companies/business people need higher attention to the presence of consumers. That includes page load performance, user interface, customer experience, and product information optimization. Businesses need to regularly update news/changes of digital transformation. Add/ update your product information as well as the characteristics of products traded on the e-commerce platform as a source origin, composition, description, instructions for use, price, storage and repair/warranty regime, ... (if applicable). This helps customers get the most useful information about the product and saves time searching/selecting goods. Policies/procedures for buying and selling including: payment information, refund policy, customer care, shipping transfer, it also needs to be transparent and easy to understand to increase credibility and greater brand recognition. Through that, the reputation of the shop/product will increase, customers will have trust and return to create a source of loyal customers.

On the other hand, consumer behavior is very special, positively affecting the intention to buy food at e-commerce platforms but also being affected. Enjoyed by 2 variables: digital platform and marketing strategy. Therefore, businesses need to have an effective intra-floor marketing and off-the-shelf marketing strategy to direct products to customer segments and expand the implementation of the strategy applied to each platform. Other social networks include Shopee, Lazada, Facebook, Instagram, YouTube, TikTok, and more. Business will definitely have competition, to limit and prevent that businesses must choose the business qualities separately, personality or measure the large number of advertising campaigns operating on these platforms. With this topic, the authors tried to research and give the best customer comments and reviews. However, this study, like many others, has a backlog of flaws. The team did not conduct a thorough evaluation of the stated criteria because the study and survey time was quite urgent. The group has not yet counted all the studies related to the topic. The author's analysis lacks finality because it only considers the food purchase intentions of users of the vast majority of ages. From 15-25 years old, this group has unstable income and the scope of research is mainly concentrated in Ho Chi Minh City and neighboring provinces. Therefore, the team proposes that the following studies can expand the research area to come up with solutions and tissues. The figure is more appropriate, since this is a common problem of the world. This scientific topic has aroused many curiosities. Finally, future studies should consider additional factors that may affect the tendency to buy goods customers' online food, according to the authors.

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The Customer Trust Factor Affects Buying Behavior on E-Commerce Websites in Ho Chi Minh City: Food Products

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Abstract

Information technology, which has also developed worldwide, drives the Internet and the digital economy. Many new technologies help businesses promote and enhance product images through websites. Therefore, attracting consumers to change from traditional methods to online shopping. Therefore, many food businesses participate in online shopping. How customers access the site into users will depend on many different factors. Knowing the reasons, the authors chose the following influencing factors: awareness of usefulness, perception of risk, awareness of ease of use, social influence, customer trust, perception of enjoyment, and expected efficiency have an impact on the behavior of buying food customers' e-commerce websites. This study combines two methods of qualitative research and quantitative methods. The survey was 369 people; the subjects were Ho Chi Minh City consumers. After collecting feedback patterns, the data is analyzed using SPSS software. The study also found that the trust factor significantly impacted food-buying behavior on e-commerce websites. From the research results, the proposal implies appropriate governance for food businesses after the Covid-19 pandemic.

Keywords: Customer trust, behavior, e-commerce website, and food products.

Introduction

In early 2022, according to e-commerce market research by Metric.vn Data Research Company, Vietnam is becoming the second largest market in Southeast Asia with 36%, second only to Indonesia at 41% (Yến, 2022). In the first quarter of 2020, traffic to Shoppe Vietnam increased by 5.2 million visits/month compared to the end of 2019, of 43.16 million visits/month. Shopee is the e-commerce platform with the largest market share, accounting for 72% of the market share in 6 months from November 2021. Ranked 2nd is Lazada, with a market share of 20.9% (Anh, 2021). According to the Department of E-commerce and Digital Economy - Ministry of Industry and Trade (2020), it is estimated that in 2020, the number of consumers shopping online is about 49.3 million people, with an average shopping value of about 240 USD per person.

The breakthrough growth of the e-commerce market has made Vietnam one of the most potential markets in the ASEAN region. This issue is more urgent for online businesses and websites in particular. The characteristic of online food products business is that sellers are not in direct contact with buyers, so they are limited to capturing their age, gender, personality, and cultural characteristics. This significantly gains customer satisfaction and retains them for a long time. Therefore, to gain an advantage in a highly competitive industry with a strong level of rejection due to the impact of rapid technological innovation, online businesses need to find ways to understand the trust factor and predict consumer behavior towards food. This topic aims to learn about trust in customer behavior when shopping for food products on e-commerce websites. From there, give a suitable model to consumers based on the premise and data analysis results. Propose solutions to improve service quality in shopping on e-commerce websites.

Theoretical basis

Theory of Reasoned Action (TRA)

The Theory of Reasoned Action (TRA) was formulated by Ajzen and Fishbein in 1967 and expanded over the years. From the TRA model, it is seen that consumer trends are the best predictor of consumer behavior. Also, from the TRA model, attitude is measured by the perception of the product's attributes. In the Theory of Reasoned Action, each person's beliefs about the brand or product will influence behavior-oriented attitude, and behavior-oriented attitude would affect the buying trend, not directly affecting the buying behavior (Al-Suqiri. 2015).

Technology Acceptance Model (TAM)

Inheriting the Theory of Reasonable Action (TRA), Davis (1989) built the Technology Acceptance Model (TAM) intending to predict a consumer's ability to adopt a technological system. The TAM model indicates that, as users interact with new technology, key factors can influence their decisions about using it (Al-Suqri, 2015)

E-commerce Adoption Model (ECAM)

Authors Joongho Ahn, Jinsoo Park, and Dongwon Lee (2001) developed an E-CAM acceptance model by combining the TAM model with risk perception theory. This research has added knowledge about the factors that influence the conversion of internet visitors into potential customers. Perceived ease of use and perceived usefulness must be raised, while the perception of risk related to products and services and

the perception of risk related to online trading must be reduced (Lee et al., 2001).

1. Perceived usefulness

Perceived usefulness is the level at which a person believes that using a specific system will enhance their performance (Davis, 1989). Studies by Ta Van Thanh and Dang Xuan On (Thành and On, 2021). Ha Nam Khanh Giao and Be Thanh Tra (2018) (Giao and Trà, 2020). Perceived usefulness is a factor in the TAM model and is widely researched in the new age of technology. Pham Van Kien (Pham et al., 2020) have found that perceived usefulness positively influences the intention to use e-commerce.

H1: Perceived usefulness has a positive impact (+) on food buying behavior on e-commerce websites

2. Perception of enjoyment

According to Mohsen Manzari (2008), perception of enjoyment is "the perceived enjoyment of a person during the use of a purchase computer that is not related to the results of performing that action" (Manzari, 2008). The results of Sun and Zhang's study confirmed that the effects of perceived enjoyment on perceived ease of use were of higher value than the other way around (Sun and Zhang, 2006). At the same time, mobile applications' flexibility and convenience create excitement, thereby raising user awareness of the product's usefulness (Alalwan et al. 2018 Reference of this citation is missing in reference section) (Pousttchi and Goeke, 2011).

H2: Perception of enjoyment has a positive (+) impact on food buying behavior on e-commerce websites.

H3: Perception of enjoyment has a positive impact (+) on Perceived Usefulness

3. Perceived ease of use

The convenience of online shopping, or in other words, the ease of using online shopping services, has a positive impact on consumers' intention to choose this method of shopping, which has been given in many previous studies (Bhatti et al., 2018; Lee et al., 2011; Yan & Dai, 2009, Katawetawaraks and Wang, 2011). Research by Seok Kang, Jaemin Jung (2014), Huang (2007) has shown a positive effect of perceived ease of use on perceived usefulness (Kang and Jung, 2014).

H4: Perceived ease of use has a positive impact (+) on Food behavior on e-commerce websites.

H5: Perceived ease of use has a positive impact (+) on the Perception of Usefulness

4. Expected efficiency

Kang and Namkung (2018) have highlighted that consumers find that performance and efficiency positively affect customer confidence when using online food delivery services (Kang and Namkung, 2018). The expected efficiency of e-commerce implies convenience, choice of products and services, rich information. The effect "is the expected result of using e-commerce systems" (Al-dalahmeh and Saleh, 2007).

H6: Expected efficiency positively impacts (+) food buying behavior on e-commerce websites.

H7: Expected efficiency has a positive impact (+) on Customer Trust

5. Customer trust

Customer trust in e-commerce is the level of trust that indicates the intentions and actions of a consumer in the transaction process (Wijoseno & Ariyanti, 2017). Research by El-Masri and Tarhini in 2017 (El-Masri and Tarhini, 2017), Carvajal-Trujillo and Escobar-Rodríguez (2014) has demonstrated that trust is the strongest predictor of online purchase intention (Escobar-Rodríguez and Carvajal-Trujillo, 2014).

H8: Customer trust has a positive (+) impact on Food buying behavior on the EC website

6. Social influence

Venkatesh (2003) added social influence to the TAM model, which is defined as "an individual's level of awareness of the importance of others thinking the individual should use a technology" (Venkatesh et al., 2003). Research by Singh, Alryalat, Alzubi, and Sarma (2017) has shown that social influences impact consumers' trust when intending to buy online (Singh et al., 2017).

H9: Social influence has a positive (+) impact on Food buying behavior on e-commerce websites.

H10: Social influences have a positive (+) impact on Customer Trust

7. Perception of risk

Although the benefits of online shopping are undeniable, product risks (low-quality products, payment safety, returns) are also an issue consumers are concerned about. According to Lin et al. (2010), "the risk of losing money, not being delivered, and miss-delivering hurts online shopping behavior intent" (Chen et al., 2010).

H11: Perception of risk has a negative impact (-) on Food buying behavior on e-commerce websites.

Source: General Author

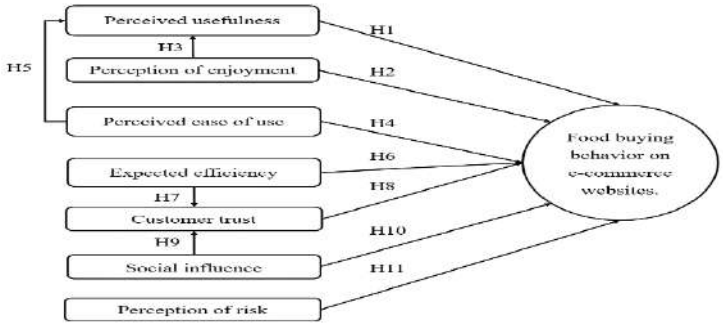

Figure 1. Recommended author model

Research methodology

The research process is conducted with 2 methods: quantitative and qualitative. Qualitative research methods: the author proceeds to collect material from various sources. Use a qualitative approach with a group discussion technique focusing on the trust factor influencing consumer food buying behavior in Ho Chi Minh City. After that, complete the construction for the draft model. Quantitative research methodology: Conduct extensive surveys for a broad audience, collect and statistics the opinions of most consumers on e-commerce websites through quantitative questionnaires designed with a 5-degree Likert scale to measure the importance of factors drawn from qualitative research. The total number of samples participating in the survey was 439, and after the filtering and cleaning phase, 369 samples were satisfactory. Finally, the author uses SPSS software to analyze data: descriptive statistics, Cronbach's Alpha scale test, EFA discovery factor analysis, and linear regression analysis.

Results

1. Research sample characteristics

Regarding gender, the percentage of males participating was 56.86% higher than that of females. Meanwhile, the proportion of women accounts for only 43.4%. About age under 18 years of age, 55 customers accounted for the lowest rate with 14.9%, the age from 18 to 22 years of participation in the survey accounted for the highest rate with 56.4%, the age from 22 to 35 years of participation in the survey accounted for 28.7%. In terms of occupation, the proportion of survey participants who are students, accounting for nearly 51.8% of the study sample; office workers also accounted for 18.2% compared to other professions; workers account for about 8.7% compared to other industries; teachers surveyed accounted for nearly 3.3%; Freelance business accounted for 13%, while other industries surveyed accounted for nearly 5.1%. In terms of income, less than VND 3 million accounts for 40.1% (the highest rate). Then there is the income level below 3 million is 35.8%, the income level from 3 million to 15 million is 59.3%, and finally, the income level above 15 million is 4.9%, respectively - the lowest rate compared to other income levels.

2. Cronbach's Alpha Test

Cronbach Alpha coefficient testing aims to determine which observational variables contribute to the conceptual measurement of the study content. Analyzing the coefficient of confidence eliminates variables observed with insufficient reliability (variable correlation index – total < 0.3). The scale is good, with a Cronbach Alpha coefficient of 0.8 to 1. However, in cases where the measurement concept is new or new to respondents, a Cronbach Alpha coefficient of 0.6 or higher is acceptable (Hoàng and Chu, 2008). Finally, we conclude that the proposed scales, such as PU, EE, PE, PR, PEU, SI, CT, and FBB are qualified and meaningful in statistics. Details are provided in Table 1.

Table 1. Cronbach's Alpha Analysis of Survey Data

STT	Scale	Number of observed variables	Cronbach's Alph
1	Perceived usefulness (hereafter PU)	4	0.853
2	Expected efficiency (hereafter EE)	5	0.868
3	Perception of enjoyment (hereafter PE)	5	0.889
4	Perception of risk (hereafter PR)	4	0.853
5	Perceived ease of use (hereafter PEU)	3	0.829
6	Social influence (hereafter SI)	4	0.840
7	Customer trust (hereafter CT)	4	0.840
8	Food buying behavior (hereafter FBB)	3	0.841

3. Explore factor analysis (EFA)

EFA discovery factor analysis aims to shorten a set k that turns the observation into a set F (F<k) of more significant factors. In factor analysis, the Principal Components extraction method is accompanied by Varimax rotation. After EFA analysis for 7 independent variables, KMO equal to 0.940 (greater than 0.5 and less than 1) and Sig = 0.00 <0.05 showed relevant data for factor analysis. The variance was 69.711% (>50%), and 7 factors extracted explained 69.711% of the volatility of the observed data. The coefficient of Eigenvalues is 1.047; the extraction coefficient has good information. The observed variables all have a > load factor of 0.5, so the observed variables are statistically significant. (Results are presented in appendix 2)

EFA analysis for KMO value dependencies equal to 0.726 (greater than 0.5 and less than 1), and Sig=0.00 <0.05 indicates that the data is suitable for using factor analysis. The extraction variance reached 75.946% (>50%), showing that the 7 factors extracted explained 75.946% of the data volatility. The coefficient of Eigenvalues is 2.278 > 1; the extraction coefficient has good informational significance. Thus, after conducting the EFA analysis, 7 independent factors were analyzed, suitable with the model and theoretical basis.

4. Pearson correlation analysis

Before regression analysis, correlation analysis should be carried out to examine the linear relationship between dependent variables and independent variables and the linear relationship between independent variables through the Pearson Correlation Coefficient (Nguyễn, 2012). The correlation coefficient matrix results of Table 2 have a statistical significance level of Sig.< 0.05, and the

correlation coefficients between the variables show that the 7 independent variables have a relatively strong linear correlation with the dependent variable, the behavior of buying food on the e-commerce website. Thus, the model's scale of research concepts achieves a differentiating value, meaning that all scales in this study that have measured different research concepts should be included in the regression analysis.

Table 2. Correlation coefficient matrix

		FBB	PU	EE	PE	PR	PEU	SI	CT
FBB	Pearson Correlation	1	.582**	.548**	.565**	-.532**	.534**	.508**	.583**
	Sig. (2-tailed)		.000	.000	.000	.000	.000	.000	.000
PU	Pearson Correlation	.582**	1	.521**	.543**	-.447**	.579**	.446**	.448**
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000	.000
EE	Pearson Correlation	.548**	.521**	1	.467**	-.458**	.444**	.418**	.505**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000	.000
PE	Pearson Correlation	.565**	.543**	.467**	1	-.543**	.526**	.462**	.518**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000	.000
PR	Pearson Correlation	-.532**	-.447**	-.458**	-.543**	1	-.432**	-.449**	-.539**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000	.000
PEU	Pearson Correlation	.534**	.579**	.444**	.526**	-.432**	1	.426**	.463**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000	.000
SI	Pearson Correlation	.508**	.446**	.418**	.462**	-.449**	.426**	1	.422**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000		.000
CT	Pearson Correlation	.583**	.448**	.505**	.518**	-.539**	.463**	.422**	1
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000	

**. Correlation is significant at the 0.01 level (2-tailed).

5. Regression analysis

With the hypothetical model, the team of authors began to analyze the regression in turn according to the following 3 steps: First, conduct regression that tests the influence of independent variables (perceived usefulness, perception of risk, perceived ease of use, social influence, customer trust, perception of enjoyment, and expected efficiency) with variables dependent on food buying behavior on EC websites.

Second, performing regression to test the effects of two independent variables is perceived ease of use and perception of enjoyment, with the dependent variable being perceived usefulness?

Third, regression tests the impact of two independent variables, social influence, and expected efficiency, with the dependent variable, customer trust.

5.1. 1st regression for FBB variable

Level of interpretation of the model:

To test the research hypothesis about the relationship between independent factors (PU, PR, PEU, SI, CT, PE, EE) and dependent variables (FBB) – Food buying behavior on e-commerce websites. After obtaining the results through linear regression and presented in the tables:

Table 3. Evaluation of the relevance of the multivariate linear regression model

Model	R	R^2	$Radj^2$	Std. Error of the Estimate	Durbin-Watson
1	0.743[a]	0.551	0.543	0.64671	1.975
a. Predictors: (Constant), CT, SI, PEU, EE, PR, PU, PE					
b. Dependent Variable: Y					

The correlation coefficient value is 0.743 > 0.5. Therefore, the appropriate model to use to evaluate the relationship between independent variables and dependent variables. 2. Table 3, the regression results show that the coefficient $R^2 = 0.551$ (≠0) i.e., 55.1% of this indicator means that the variation of FBB variation is explained by the variability of 7 factors (CT, SI, PEU, EE, PR, PU, PE), the remaining 44.9% belongs to error and other random factors. In this model, the Radj² index (R squared correction) = 0.543 (54.3%), helping to determine the model's suitability would be more accurate and safe. The value of Durbin - Watson is 1.975 in the range of 1-3, so the data does not have the phenomenon of first-order correlation; the data is satisfactory. Table 4 shows F = 63,383 and significant levels Sig. = 0.000 (Sig. ≤ 0.05), meaning that the regression model matched the data collected.

Table 4. ANOVA factors affecting the behavior of buying food on e-commerce websites

Model suitability test	Sum of Squares	Df	Mean Square	F	Sig.
Regression	185.562	7	26.509	63.383	.000[b]
External	150.981	361	0.418		
Total	336.543	368			

b. Predictors: (Constant) CT, SI, PEU, EE, PR, PU, PE

Table 5. Regression weighting factors that impact food buying behavior (Multivariate linear regression model test)

Model	Unnormalized coefficient		Standardized Coefficients	t	Sig.	Collinearity Statistics		Hypothesis	Result
	B	Std.Error	Beta			Tolerance	VIF		
(Const)	.527	.325		1.625	.105				
PU	.193	.051	.185	3.808	.000	.526	1.901	H1	Accept
EE	.159	.051	.143	3.143	.002	.601	1.665	H6	Accept
PE	.116	.048	.118	2.429	.016	.524	1.909	H2	Accept
PR	-.103	.046	-.103	-2.225	.027	.574	1.741	H11	Accept
PEU	.101	.045	.105	2.235	.026	.567	1.763	H4	Accept
SI	.142	.046	.133	3.090	.002	.669	1.496	H10	Accept
CT	.221	.050	.206	4.403	.000	.569	1.756	H8	Accept

As shown in Table 5, the VIF coefficient is less than 2 (the largest is 1.901), and the Tolerance coefficient is > 0.5 (as small as 0.524), indicating that no multi-linear phenomenon occurs (Hoàng and Chu, 2008). The Sig. index of all variables PU, EE, PE, PR, PEU, SI, and CT is less than 0.05, so the above variables affect food buying behavior on e-commerce websites. The variables PU, EE, PE, PEU, SI, and CT have the same impact on the variable depending on the behavior of buying food on e-commerce websites due to the positive Beta coefficient. And the PR variable has an inverse effect on the variable depending on the behavior of buying food on e-commerce websites due to the negative Beta coefficient. Thus, the regression model is:

FBB = CT * 0.206 + PU * 0.185 + EE * 0.143 + SI * 0.133 + PE * 0.118 + PEU * 0.105 – PR * 0.103 + ε

4.5.2. 2nd regression for the variable PU

To test the research hypothesis about the relationships between PEU, PE, and PU variables. The team performed linear regression, the results of which are presented in the table below:

Table 6. ANOVA PE, PEU factors impact PU

Model		Total squares	Df	Mean square	F	Sig.
1	Regression	128.812	2	64.406	129.477	.000[b]
	Residual	182.060	366	0.497		
	Sum	310.872	368			

a. Dependent Variable: PU

b. Predictors: (Constant), PE, PEU

Table 6 shows F = 129.477 and meaningful levels Sig. = 0.000 (Sig. ≤ 0.05), meaning that the regression model matches the data collected. As shown in table 7, VIF = 1,382 < 2. We can therefore show that the multivariate phenomenon in this model is small. The Sig. index of PEU and PE variables is less than 0.05, so it is concluded that the above variables affect PU. β normalization of the variables PEU = 0.406 and PE = 0.330 shows that both are positive. So these two variables have the same impact on the PU-dependent variable. 3rd regression for CT variable: To test the hypothesis of the relationships between EE and SI factors with CT variables. The team performed linear regression, the results are synthesized and presented as follows:

Table 8 shows F = 81,799 and the mean level Sig. = 0.000 (Sig. ≤ 0.05), which means that the regression model matches the data collected

Table 7. Regression weighted table of PEU, PE factors impacting PU

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. error	Beta			Tolerance	VIF
1	(Constant)	1.349	.166		8.112	.000		
	PEU	.377	.044	.406	8.634	.000	.724	1.382
	PE	.310	.044	.330	7.016	.000	.724	1.382

a. Dependent variable: PU

Table 8. ANOVA factors SI, EE impact CT

Model		Sum of squares	Df	Mean squared	F	Sig.
1	Regression	90.067	2	45.033	81.799	.000[b]
	Residual	201.497	366	.551		
	Total	291.563	368			

a. Dependent Variable: CT

b. Predictors: (Constant), SI, EE

As shown in table 9, VIF = 1,212 < 2. The Sig. index of the EE and SI variables is less than 0.05, so the above variables affect CT. β normalization of the variables EE = 0.399, SI = 0.255 so the EE and SI variables act in the same direction on the CT dependent variable. We can therefore show that the multivariate phenomenon is negligible.

Hypothesis testing

The hypothesis is characterized by 11 hypotheses. Following the table 5, the hypotheses (H1, H2, H4, H6, H8, H10, H11) all have Sig. <0.05, so the hypotheses H1, H2, H4, H6, H8, H10, H11 are accepted. The H3, H5 hypothesis tested at the second regression presented in table 7 shows that the Sig. of the variables PEU (H5) and PE (H3) is 0.00, so H3 and H5 are accepted. The H7, and H9 hypothesis tested at the second regression presented in table 9, shows that the Sig. of the variables EE (H7) and SI (H9) is equal to 0.00, so H7 and H9 are accepted.

Discussion

Based on the analysis results above, the research team offers a topic to discuss the influence of customer trust factors on food buying behavior on e-commerce websites in Ho Chi Minh City.

According to the linear regression equation, we can conclude that the behavior of buying food on e-commerce websites of consumers in Ho Chi Minh City is influenced by 7 main factors: perception of usefulness, perception of enjoyment, perception of ease of use, expected efficiency, customer trust, social influences, and risk perceptions. In particular, the risk perception factor hurts the behavior of buying food on e-commerce websites; the higher this factor, the lower the behavior of buying food on e-commerce websites. The factors of awareness of usefulness, awareness of enjoyment, awareness of the ease of use, practical expectations, customer trust, and social influences positively impact the behavior of buying food on e-commerce websites of consumers in Ho Chi Minh City. The higher this factor, the higher the behavior toward buying food on e-commerce websites. In particular, customer trust has the most significant impact; the second is the perception of usefulness, the third is the expected effect, the fourth is social influence, the fifth is the perception of enjoyment, and finally, the perception of ease of use. As a result, the linear regression equation accurately reflects the correlation between independent and dependent variables in the study model.

The study of El-Masri and Tarhini (El-Masri and Tarhini, 2017) also suggests that trust is the most influential factor in e-commerce purchases. This research paper suggests that businesses and retailers need to focus on and improve more than the value of trust in customers.

The perception of usefulness positively impacts the behavior of buying food on e-commerce websites. This result is similar to the research results of Pham

Table 9. Regression-weighted table of EE and SI factors affecting CT

Model		Unstandardized coefficient		Standardized coefficient	t	Sig.	Collinearity Statistcs	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	1.356	.209		6.481	.000		
	EE	.413	.050	.399	8.334	.000	.825	1.212
	SI	.252	.047	.255	5.326	.000	.825	1.212

a. Dependent variable: CT

Van Kien (Pham et al., 2020), Ta Van Thanh, and Dang Xuan Thanks (Thành and Ơn, 2021). It is also thought that Ease of Use awareness positively impacts buying behavior on e-commerce websites. Previous research (Christian Brand, Tim Schwanen, Jillian Anable, 2020) also gave similar results, so this factor needs to be considered, creating the ease of consumer purchases on e-commerce websites (Brand et al., 2020). Perceptions of enjoyment, expected performance, and social influence positively impact food-buying behavior on e-commerce websites. This finding is consistent with the past research findings of Mohsen Manzari (Manzari, 2008). Past research by Mahmoud Al-dalahmeh and Ali Salman Saleh (Al-dalahmeh and Saleh, 2007) suggests that customers' risk aversion would negatively affect e-commerce purchases. This result is entirely similar to the results of the research paper. Organizations and individuals need to take measures to limit the most likely risks for consumers to have a more positive view of buying food online. In addition, the perception of ease of use and enjoyment influences the perception of usefulness. In which the perception of ease of use has the most significant impact. This finding is consistent with past studies (Kang and Jung, 2014). The results of the second perception of enjoyment are implied by the study of Pousttchi and Goeke (Pousttchi and Goeke, 2011). In the last regression, variables such as expected performance and social influence also positively affected customer trust. In particular, the expected effect has the most substantial impact. This is consistent with the studies of Mahmoud Al-dalahmeh and Ali Salman Saleh (Al-dalahmeh and Saleh, 2007). Next comes the less impactful social influence factor. This is consistent with the findings of Singh, Alryalat, Alzubi, and Sarma (Singh et al., 2017).

Conclusions

Based on the analysis and research discussion results, the authors came to the following conclusions. The order of factors that influence a guest's intent to buy food is trust, perception of usefulness, expected efficiency, social influence, perception of enjoyment, perception of ease of use, and perception of risk. In particular, the risk perception factor hurts ntd's food-buying behavior. Therefore, setting out marketing strategies and business, helps to improve business efficiency and increase revenue. Based on research results, "proposing solutions suitable for food business on e-commerce websites."

Trust is the critical factor that can help consumers overcome risk pressure in shopping transactions (Pappas, 2016). Therefore, online businesses can increase the value of customer trust by consolidating information coming from official sources in the following forms:

Businesses must allow consumers who have bought food to share their reviews and experiences on the sales website and encourage customers to exchange product information by effectively creating and managing fan pages or information exchange forums.

Enterprises should provide information about food products and present information about purchasing policies straightforwardly and clearly. This is an essential thing for customers to trust in the transparency of the website's operation. In addition, if food is imported, it is necessary to have a warranty certificate of standard and quality goods to the ntd. Besides, businesses need to offer transportation costs, delivery methods, and service price comparison tables so that consumers can feel secure and make faster food-buying decisions.

In addition, expected efficiency and social influence also impact customer trust. Online food retailers can also increase customer satisfaction and trust by perfecting exchange, return, and refund policies. Since the characteristics are not viewed directly, there may always be situations that require a refund from the customer. Therefore, businesses present the return policy and guide customers on the return process. This not only ensures the interests of businesses when the goods received still retain the original quality but also leaves a good impression in customers' hearts about the prestige and attentive care of the business. Therefore, businesses need to focus on caring for those who have bought food with incentives and promotions when introducing new users to buy goods. Businesses need to change their marketing strategies to maintain their market, especially using influencer advertising (IM).

Due to the limited research time, funding, and experience of the author is not much; the research paper still has limitations and shortcomings. The scope of the study was conducted in HCMC, so the results are not representative of the behavior of Vietnamese consumers. If it is possible to expand the scope of research subjects throughout Vietnam, the

study can bring a more general and comprehensive view of Vietnamese consumers. Second, because the research sample was conducted using a conventional methodology, it is possible that the findings were not highly reliable. So similar studies must be conducted by selecting a more diverse and random sample; the study results will be more objective and accurate. Finally, the authors suggest future studies should explore additional factors or attributes that may influence the decision to buy food online.

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Appendix 1. Synthesis of observed variables

Variable observation	Source
Perceived usefulness (PU)	
Shopping on e-commerce websites makes it easier and faster for me to find product information. **(PU1)**	Tunsakul (2020), Lin et al. (2010)
Easier and more economical payment when using direct payment by bank card or e-wallets **(PU2)**	
Shopping on e-commerce websites saves me more time **(PU3)**	
Shopping on e-commerce websites increases my product search productivity **(PU4)**	
Expected Efficiency (EE)	
I find that buying on e-commerce websites saves me time. **(EE1)**	Tunsakul(2020), Lin et al(2010), PaulaBajdor (2021)
I see buying on e-commerce websites that help me buy anytime, anywhere. **(EE2)**	
I can buy all kinds of products through shopping on e-commerce websites. **(EE3)**	
I find that when buying on e-commerce websites, it is easier to compare products. **(EE4)**	
I want to buy on e-commerce websites to enjoy many incentives and promotions. **(EE5)**	
Perception of Enjoyment (PE)	
I find it very interesting to see the goods on the e-commerce website. **(PE1)**	Goldsmith (2002); Mohsen Manzari (2008)
I like the convenience of buying on e-commerce websites. **(PE2)**	
I find buying on e-commerce websites more interesting than buying in stores. **(PE3)**	
I like buying on e-commerce websites because there are many choices. **(PE4)**	
I like to see reviews of customers who have bought products on e-commerce websites **(PE5)**	
Perception of Risk (PR)	
I find it very difficult to assess product quality accurately when buying online. (PR1)	P.Bulsara, PratiksinhS. Vaghela (2020); Hoang Quoc Cuong (2010); Ngoc Duc (2008)
I am worried about the safety of the payment before receiving the goods, I may lose money with inappropriate products. (PR2)	
I'm worried the product is lost during delivery (PR3)	
I am afraid that personal information will be disclosed to other partners that I do not want (PR4)	
Perceived Ease of Use (PEU)	
I find it quite easy to use e-commerce websites. **(PEU1)**	Venkatesh et al. (2003)
I am very proficient in buying on e-commerce websites. **(PEU2)**	
I had no trouble paying online. **(PEU3)**	
Social Influence (SI)	
I often pay attention to products that are rated multi-star by buyers. **(SI1)**	Pham Van Tuan (2020), Ha Nam Khanh Giao and Be Thanh Tra (2018)
I will often buy recommended products from friends and relatives or celebrities (KOLs, influencers). **(SI2)**	
I've found that positive comments on social media influence purchasing decisions on my e-commerce websites. **(SI3)**	
I have read a lot of information and reviews on e-commerce sites, on forums, social networks before shopping on e-commerce websites. **(SI4)**	
Customer Trust (CT)	
I trust the reviews of customers who have bought products on e-commerce websites. **(CT1)**	Mahmoud Al dalahmeh and-Ali SalmanSaleh (2007)
I believe that customers' personal information is confidential when buying goods on e-commerce websites. **(CT2)**	
I believe in buying at e-commerce websites because the information and products that e-commerce sites provide are specific and clear. **(CT3)**	
I believe in buying at e-commerce websites because many of my acquaintances have bought and felt fine. **(CT4)**	
Food buying behavior (FBB)	
I have been buying food on e-commerce websites. **(FBB1)**	Hoang Quoc Cuong (2010)
I will continue to buy food on e-commerce websites. **(FBB2)**	
I would opt to buy e-commerce food instead of traditional purchases. **(FBB3)**	

Appendix 2: EFA table for 7 independent variables

Rotated Component Matrix[a]

	Components						
	1	2	3	4	5	6	7
PE3	.794						
PE2	.755						
PE5	.739						
PE4	.711						
PE1	.692						
EE3		.803					
EE1		.740					
EE5		.740					
EE2		.724					
EE4		.684					
SI3			.788				
SI4			.781				
SI1			.740				
SI2			.726				
PU2				.748			
PU3				.744			
PU1				.732			
PU4				.698			
PR2					-.764		
PR4					-.764		
PR1					-.755		
PR3					-.682		
CT1						.770	
CT4						.744	
CT2						.712	
CT3						.692	
PEU1							.782
PEU3							.738
PEU2							.728
KMO			0.940				
Bartlett's (Sig.)			0.00				
Eigenvalues			1.047				

EFA table for FBB dependent variable

Component Matrix[a]

	Components
	1
FBB2	.883
FBB1	.874
FBB3	.858
KMO	.726
Bartlett's (Sig.)	0.00
Eigenvalues	2.278

Factors Affecting Student's Repurchase Intention to buy Food on Shopee

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Abstract
During the strong broadcast of the COVID-19 pandemic, online shopping has significantly impacted users. Users are starting to switch to online shopping more than traditional shopping as before. Users tend to focus on commodity equipment elements, including products. Therefore, the study was conducted to determine the factors that affect the student's purchase of products on shopee. The online survey and the results collected from 300 survey respondents were included in SmartPLS software to evaluate the reliability of Cronbach's Alpha scale, Fornell-Lacker's intervariable value analysis and SEM analysis linear. The result for satisfaction, trust, usefulness, ease of use, and convenient features directly influences students' intention to repurchase products on shopee. The research results recommend that businesses and individuals selling online develop effective sales strategies.

Keywords: intention, repurchase intention, shopee, satisfaction

Introduction

The remarkable development of e-commerce in recent years has brought positive changes to Vietnam's consumption trends. According to a survey on online shopping in Vietnam, participation increased from 77% in 2019 to 88% in 2020. According to a report on the Southeast Asian digital economy by google, Bain & company, temasek conducted research for the results in 2021, saying: Vietnam has 41% of new internet users, accounting for the highest percentage in Southeast Asia region. More than half, up to 94% of people in this proportion, intends to continue using those e-services. E-commerce brings many benefits to individuals and businesses. Consumers can have more choices, access information, and shop conveniently anytime, anywhere. For businesses to expand development opportunities, expand the volume of customers. Vietnam has many e-commerce platforms that develop various products to meet consumers' needs, including major e-commerce platforms such as lazada, tiki, sendo, and shopee. According to iPrice group's statistics in Q4/2021 Shopee is the most popular platform, with about 89 million monthly visits. This traffic is many times larger than other major electronic exchanges such as lazada's 20.6 million, tiki's 17.8 million, and sendo's 5 million visits. This shows that Shopee is a leading e-commerce platform in the Vietnamese market, which businesses pay the most attention.

Shopee is an e-commerce platform with a wide range of products and many preferential prices, and it has attracted many customers, which is impossible, not to mention the customers who are students. Students are a young customer group with high shopping needs and are sensitive to technology. Therefore, it has brought businesses that know how to exploit this group of objects high revenue and profit.

Businesses always want to create and maintain consumer relationships, so customer retention is a top concern for online retailers. What factors influence customer repurchase behavior? What needs to be determined to encourage consumers to buy again from the online store? Therefore, the authors have chosen the topic: “Factors Affecting Student's Repurchase Intention to buy Food on Shopee". This topic explores and identifies the factors affecting the repurchase behavior of student customers on the e-commerce platform shopee. From there, based on theories and analyzed data to create a customer research model. They are helping businesses better understand customer intent and offer appropriate directions to retain and attract more customers.

Objectives of the study

Learn and determine the factors affecting the repurchase intention of the student group of customers on the Shopee e-commerce platform. Evaluate the influence of these factors. Helping businesses understand the long-term relationship between student customers and shopee shopping websites, offering appropriate business solutions.

Subjects and scope of research

The study aims to identify the factors affecting the buying behavior of students on shopee. Based on the theory of students' repurchase intention, the topic focuses on the relationship and impact of independent factors on dependent factors. From there, we draw conclusions based on this relationship. Survey of customers who are university students who meet the following conditions: are those who use the internet to shop on e-commerce site shopee and are 18 years old or older. The research scope is limited to shopee purchases, and the study period is about five months, from August 2022 to November 2022.

Research Methods

The research process was carried out with two methods: qualitative and quantitative.

- Qualitative research: collected through relevant research documents from relevant articles and online journals. Research papers related to shopee repurchase behavior such as:

Effect of e-satisfaction on repurchase intention in Shopee user students (Pandiangan, et al., 2021). Exploring factors of consumer repurchase intention in online shopping (Dutta, 2016)

Factors affecting the online purchase intention of Shopee consumers in Malaysia (Ru et al., 2021)

Factors affecting shopping decisions on Shopee marketplace during the Covid-19 Pandemic *(*Anggraeni et al., 2022*).*

- Quantitative research: Collecting information by surveying internet users by survey interview questionnaire.
- Respondents

The surveyed object is 300 university students because these people often use the internet to shop online on e-commerce platforms.

- How to proceed?

Surveys are carried out mainly by Google Forms and Facebook tools

The subject has used many data analysis tools using SmartPLS software to perform descriptive statistical analysis.

1. Theory of planned behavior *(TPB)*

The theory of planned behavior *(TPB) (*Azjen, 1991*)* is an extension of the theory of reasoned action (TRA) *(*Azjen and Fishbein, 1980*).* The theory of reasoned action (TRA) is that an individual's performance of a particular behavior is determined by their intention to perform that behavior. Recently, TRA and TPB have also been the basis for several studies on online buying behavior *(George, 2002;* Khalifa and Limayem, *2003).* TPB is a reputable framework "for conceptualizing, measuring, and empirically identifying the determinants of behavior and behavioral intentions" *(*Vermeir and Verbeke, 2008). The theory of planned behavior (TPB) deals with the factors influencing consumer behavioral intentions and behaviors, including attitudes, subjective norms, and behavioral control awareness. In which, Behavioral Intent refers to the expression generated in the decision process; usually indicates whether certain behavior will be acceptable. Behavioral intention is a necessary process in any form of behavioral expression; it is a decision made before an actual act is adopted.

TPB model (Excerpt from Ajzen 1991*)*

Perceived behavioral control refers to people's perception of how easy or difficult it is, and perceived behavioral control can vary with different situations and actions. Attitude towards the behavior refers to the extent to which a person has favorable or unfavorable evaluations or ratings of the behavior. Perspectives reflect how users are influenced by perceptions of their behavior by significant references, for example, friends or colleagues, among others *(*Fishbein and Ajzen, 1973; Schofield, 1974*).* According to Ajzen 1991, the more favorable the attitudes and subjective norms toward behavior and the greater the perceived ability to control the behavior, the stronger should be an individual's intention to perform the behavior is under review

2. TAM technology acceptance model

The Triple Technology Acceptance Model is a theoretical information system in the form of a model that guides users to use the technology and accept its use. The actual use of the system is the final stage in which the user uses the technology. One of the factors that make people use technology is habitual behavior. This habit is influenced by attitude and repetition every day. The TAM technology acceptance model was invented by Davis *(1986)* based on the theory of rational action *(TRA for short).* This model is developed based on the technology acceptance model, which is directly related to the problem of predicting the acceptability of an information system or a particular computer network. The triple technology acceptance model was born to predict the acceptability of a tool type and specify the modifications that must be introduced into the system. Only then can it be accepted and trusted by users. This model also shows that the acceptability of an information system is determined by two fundamental factors: perceived usefulness and perceived ease of use.

3. Loyalty

Loyalty is the factor that shows the return of customers. Loyalty is divided into brand loyalty and electronics loyalty. Brand loyalty is defined as "a favorable attitude towards a brand that leads to the purchase of that brand consistently over time" and electronic loyalty as "attitudes towards brand loyalty" customer convenience towards e-retailers leads to repeat buying behavior" (Srinivasan, et al., 2002*).* According to Oliver, consumer loyalty is defined as "a deeply held promise to repurchase or reuse a favorite product or service repeatedly in the future, thereby causing repeat purchases of the same brand, regardless of the situation, the effects and possible marketing efforts that lead to behavior conversion." This loyalty helps significantly in the business because of the flow of old customers; there are also new customers from the marketing of old customers. Consumer buying patterns in repurchase intention are related to customer loyalty; consumers frequently purchase, spreading positive reviews to others who can assist with sales (Lagita and Briliana, 2018*).*

H1: *Consumer loyalty positively affects consumer repurchase intention.*

4. Satisfaction

The customer's confidence in the purchase decision represents the degree to which they have confidence in their product choice decision. It is not enough to rely on perceived usefulness and simplicity of use to raise customer repurchase intention because they can only contribute to a limited level, but trust and confidence are also insufficient. Pleasure can contribute higher to customer repurchase intention (Sadiki and Dwiyanti, 2021). Trust significantly affects satisfaction and intention of customer acquisition (Juniwati and Sumiyati, 2020*)*. The essential factor in shopping is satisfaction, satisfaction with products, services, and customer care. No customer will come back to shop when that place does not give them the satisfaction they want. E-satisfaction is "the state of the consumer with affection towards the website as a result of the evaluation of all aspects that make up the consumer relationship" (Flavián and Guinalíu, 2006). Consumer satisfaction is a fundamental assumption for consumer loyalty; as consumer satisfaction rises, so will repurchases (Dutta, 2016).

H2: *E-satisfaction has a positive effect on e-loyalty.*
H3: *Consumer satisfaction*

5. Confidence

To increase customer repurchase intention, relying on perceived usefulness and ease of use is not enough as they can only contribute to a certain extent. The customer's confidence in the purchase decision represents the degree to which they have confidence in their product choice decision. However, trust and confidence are not enough, and happiness may contribute higher to customer repurchase intention (Sadiki and Dwiyanti, 2021). Trust significantly affects satisfaction and intention. Customers repurchase *(*Juniwati and Sumiyati, 2020). Trust plays an essential role in creating customer loyalty (*Chaturvedi, et al., 2016)*

H4: *Trust enjoys a significant amount of satisfaction*
H5: *Trust affects repurchase intention*

6. Usefulness

Perceived usefulness is the degree to which consumers believe online shopping may improve their online shopping performance. Davis first developed perceived usefulness in 1985 in a theoretical acceptance model, namely how users believe that a user's attitude towards a given system would increase their job performance when using a particular system. (Sadiki and Dwiyanti, 2021). Perceived usefulness is the extent to which users believe that online shopping efficiency would be improved. Usefulness and ease of use have a significant relationship (Ru et al., 2021). Usefulness positively affects online trust *(*Dutta, 2016*)*. According to Bireswar Dutta, perceived usefulness is positively related to repurchase intention.

H6: *Usefulness affects trust*
H7: *Perceived usefulness affects repurchase intention*

7. Ease of use

Perceived ease of use is when one feels confident that working using a particular system would not be difficult and one system is easier to use than another would be more acceptable to the users (Davis, 1989; Wafiyyah et al., 2021). Ease of use of technology leads to people's perception of usefulness; perceived ease of use is also measured as a factor of post-purchase expectation and is predicted to affect repurchasing intention (Dutta, 2016) positively. Ease of use affects consumers' online purchase intention when using Shopee (Ching et al., 2021*)*. Consumers perceive ease of use to have a positive and significant Impact on repurchase intention on Shopee (Wafiyyah et al., 2021)

H8: *Ease of use affects the usefulness*
H9: *Ease of use affects repurchase intention*

8. Convenience

Convenience is estimated by three factors: time spent, location to shop, and purchase process. It can effectively influence consumers to shop online (Ching et al., 2021). Convenience also affects customer satisfaction and repurchase intention towards the market (Juniwati and Sumiyati, 2020). Consumers' repurchase intention is influenced by the benefits that the buyer perceives when shopping online

H10: *Convenience has a relative influence on customer satisfaction*
H11: *Convenience has an effect Relative influence on customer's intention to repurchase on the market*

Result

Analysis

This research is done through two steps to evaluate the data monitoring. First, study the parser using Cronbach's Alpha test to determine the dependability of the scale, analyzing the discriminant value between the variables by Fornell-Lacker to see the difference of one structure when compared with the other structure architecture in the model, next by the entire computer structure (SEM) to verify the data model is output matches the original research title.

Cronbach's Alpha test

In the proposed theoretical model, to study the antecedent factors affecting the customers repurchase behavior and its results, the author has proposed seven concepts that need to be measured, which are: (1) loyalty; (2) satisfaction; (3) trust; (4) usefulness; (5)

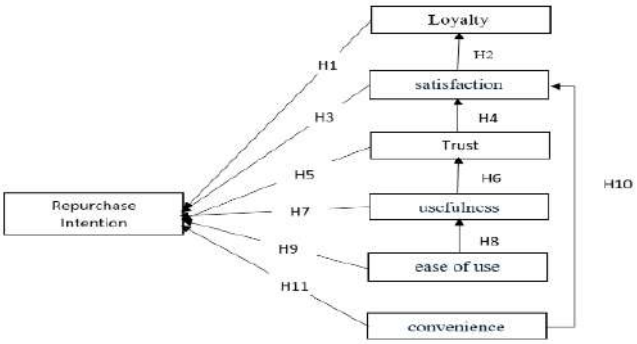

Figure 1: Model of the author's proposed group

ease of use; (6) convenience; (7) intention to repurchase. In analyzing the scale's reliability, Cronbach's alpha system is used quite commonly (it is recommended to have at least 3 observed variables). This coefficient is evaluated using the internal consistency method. Many researchers suggest Alpha levels as follows: greater than 0.8 is a good scale; from 0.7 to 0.8 are usable; from 0.6 and above can be used in case the research concept is new or new in the research context *(Nguyen Dinh Tho, 2014)* before being included in EFA exploratory factor analysis. Therefore, it can be concluded that the proposed scales, such as LTT, HL, TC, HI, DSD, TT, and YDML meet the standards and are shown in Table B.1

Table 1. Cronbach's Alpha results for 7 scales

No.	The scale	Number of observed variables	Coefficient Cronbach's Alpha
1	Loyalty (LTT)	2	0.748
2	Satisfaction (HL)	3	0.673
3	Trust (TC)	3	0.895
4	Usefulness (HI)	3	0.811
5	Ease of use (DSD)	3	0.817
6	Convenience (TT)	2	0.800
7	Repurchase intention (YDML)	4	0.859

Discriminant Valuation Analysis by Fornell-Lacker

The discriminant value indicates the distinctiveness of a structure when compared to other structures in the model. The traditional approach to assessing discriminants uses the square root index AVE proposed by Fornell and Larcker (1981). This method compares the square root coefficient AVE (SQRT (AVE)) of the observed variable

Scale used to measure a latent variable with the correlation coefficients between that latent variable and the latent variables other hidden. AVE (average variance extracted) is the average explanation of the latent variable to its observed variables. If the SQRT (AVE) coefficient is larger than the remaining correlation coefficients, then the scale ensures discrimination.

Table 2: Results of the discriminant analysis by Fornell-Lacker

	DSD	HI	HL	LTT	TC	TT	YDML
DSD	0.856						
HI	0.618	0.852					
HL	0.589	0.658	0.776				
LTT	0.610	0.646	0.566	0.894			
TC	0.602	0.726	0.766	0.610	0.786		
TT	0.743	0.613	0.522	0.594	0.558	0.913	
YDML	0.765	0.712	0.656	0.625	0.687	0.701	0.838

Result

SQRT(AVE) of DSD > the correlation coefficient between DSD and HI, HL, LTT, TC, TT, YDM, so the discriminability of the DSD scale ensures the discriminant

SQRT(AVE) of HI > the correlation coefficient between HI with DSD, HL, LTT, TC, TT, YDML, so the discriminability of the HI scale ensures the discriminant

- SQRT(AVE) of HL > the correlation coefficient between HL and DSD, HI, LTT, TC, TT, YDML, so the discriminant HL scale ensures the discriminant
- SQRT(AVE) of LTT > the correlation coefficient between LTT and DSD, HI, HL, TC, TT, YDML; therefore, calculating the difference of the LTT scale ensures the discriminant
- SQRT(AVE) of the TC > the correlation coefficient between TC and DSD, HI, HL, LTT, TT, YDML distinction
- SQRTAVE) of TT > correlation coefficient between TT and DSD, HI, HL, LTT, TC, YDM L, so the discriminatory of the TT scale ensures the discriminant
- SQRT(AVE) of YDML > the correlation coefficient between YDML and DSD, HI, HL, LTT, TC, TT; therefore, the discriminability of the YDML is guaranteed. Discriminability

Conclusion: The discriminant values between the variables in the proposal are all guaranteed to be discriminatory, so the proposed model of the author group is appropriate.

PLS-SEM

Measurement Model Evaluation

This study uses a quantitative research method by applying linear structural modeling (SEM) on SmartPLS software. The concepts in the measurement model include 7 concepts and 21 observed variables. This study uses composite reliability (CR), which must be higher than 0.6; the extracted mean-variance (AVE) must be higher than 0.5, and Outer Loadings must be higher than 0.7 for the measurement model to be accepted. The results show that these values satisfy the requirements for repurchase intention. Table 1 also shows a high Cronbach's Alpha value, indicating that the scales have high reliability.

Table 3. Reliability results and convergence value of the scale (Source: Processing group survey results)

	Cronbach's Alpha	Composite reliability (rho_A)	Composite reliability (rho_c)	Average Variance Extracted (AVE)
Loyalty (LTT)	0.748	0.749	0.888	0.789
Satisfaction (HL)	0.673	0.686	0.820	0.603
Trust (TC)	0.793	0.797	0.866	0.618
Utility (HI)	0.811	0.817	0.888	0.726
Convenience (TT)	0.800	0.813	0.909	0.833
Ease of use (DSD)	0.817	0.818	0.891	0.733
Repurchase Intention (YDML)	0.859	0.860	0.904	0.703

Table 4: Outer loadings. Results (Source: Processing survey results of the research team)

	DSD	HI	HL	LTT	TC	TT	YDML
DSD1	0.841						
DSD2	0,863						
DSD3	0,864						
HI1		0,83					
HI2		0,897					
HI3		0,827					
HL1			0,796				
HL2			0,725				
HL3			0,806				
LTT1				0,888			
LTT2				0,899			
TC1					0,783		
TC2					0,836		
TC3					0,721		
TC4					0,799		
TT1						0,899	
TT2						0,926	
YDML1							0,834
YDML2							0,815
YDML3							0,867
YDML4							0,837

The authors conducted a discriminant test (assessed through Fornel-Lacker criteria) to test the measurement model when comparing the relationship between variables. They are presented in Table 3 and guarantee the segregation of the measurement factors. Processing results from the group survey and multicollinearity test for VIF < 5, so it is concluded that the model does not violate this phenomenon. The test results of the linear structural model presented in Figure 1 shows that the measurement indexes in this model have satisfied all the eligibility requirements to be included in the SEM analysis to test the hypotheses in the measurement model. The research results are presented through Bootstrap analysis in PLS, confirming the reliability of the research model. This study tested the bootstrapping technique with 5000 observations to test the research hypotheses shown in Table 5.

Table 5: The results of the analysis of discriminant validity by Fornel-Lacker (Source: Processing survey results of the research team)

	DSD	HI	HL	LTT	TC	TT	YDML
DSD	0.856						
HI	0.618	0.852					
HL	0.589	0.658	0.776				
LTT	0.610	0.646	0.566	0.894			
TC	0.602	0.726	0.766	0.610	0.786		
TT	0.743	0.613	0.522	0.594	0.558	0.913	
YDML	0.765	0.712	0.656	0.625	0.687	0.701	0.838

Table 6. Results of analysis of VIF coefficient according to observed variables (Survey source: Processing YDML4 results of the research team)

	DSD1	DSD2	DSD3	HI1	HI2	HI3	HL1	HL2	HL3	LTT1
VIF	1, 718	1, 872	1, 878	1, 909	2, 281	1, 584	1, 374	1, 293	1, 282	1, 555
	LTT2	TC1	TC2	TC3	TC4	TT1	TT2	YDML1	YDML2	YDML3
VIF	1, 555	1, 664	1, 825	1, 423	1, 664	1, 801	1, 801	2, 003	1, 858	2, 317

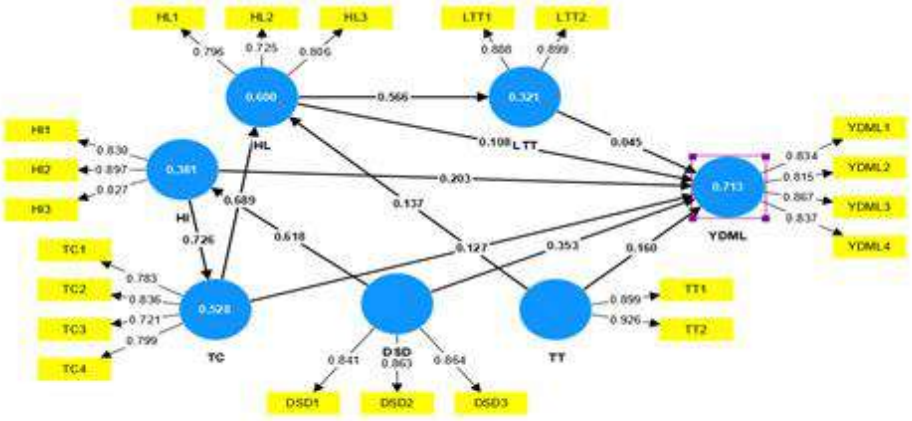

Figure 2: PLS-SEM results for the measurement model (Source: Processing survey results of the research team)

Table B.5 shows that the Loyalty Hypothesis (LTT) has an impact to repurchase intention (YDML) and was rejected because the coefficient P values > 0.05. Remaining hypotheses in the model can be concluded to be reliable.

Test the model's hypotheses.

The initial research objective is to find out and identify factors related to convenience, trust, and satisfaction through which customer loyalty. The authors have conducted to identify the factors affecting the repurchase behavior, thereby giving the necessary factors.

The results described the statistical values for testing the hypotheses from the Bootstrapping method in Table 5. From that, it shows that the hypothesis H1 is rejected and the remaining 10 hypotheses H2, H3, H4, H5, H6, H7, H8, H9, H10 and H11 were all accepted because of their statistical P-value <5%.

Table 7. Bootstrapping structural model results (Source: Processing survey results of the research team)

Relationships	Original Sample (O)	Sample mean (M)	Standrad deviation (STDEV)	T Statistics (|O/STDEV|)	P Values
LTT -> YDML	0,045	0.048	0.052	0.860	0.390
HL -> LTT	0.566	0.567	0.050	11.293	0.000
HL -> YDML	0.133	0.135	0.063	2.115	0.034
TC -> HL	0.689	0.692	0.042	16.246	0.000
TC -> YDML	0.219	0.220	0.060	3.638	0.000
HI -> TC	0.726	0.728	0.037	19.558	0.000
HI -> YDML	0.362	0.363	0.048	7.546	0.000
DSD -> HI	0.618	0.618	0.052	11.932	0.000
DSD -> YDML	0.577	0.577	0.061	9.497	0.000
TT -> HL	0.137	0.135	0.048	2.859	0.004
TT -> YDML	0.078	0.177	0.057	2.708	0.007
LTT -> YDML	0.048	0.045	0.052	0.860	0.390
HL -> LTT	0.566	0.567	0.050	11, 293	0.000
HL -> YDML	0.063	0.135	0.133	2.115	0.034
TC -> HL	0.689	0.692	0.042	16, 246	0.000
TC -> YDML 0.219	0.220	0.060	3, 638	0.000	HI
-> TC	0.726	0.728	0.037	19, 558	0.000
HI -> YDML	0.363	0.048	0.362	7, 546	0.000
DSD -> HI	0.618	0.618	0.000	0.052 11, 932	DSD
-> YDML	0.577	0.577	0.000	0.061 9.497	TT
-> HL	0.137	0.135	0.048	2, 859	0.004
TT -> YDML	0.078	0.177	0.057	2, 708	0.007

Table 8. Hypothesis test results (Source: Processing survey results of the research team research)

Hypothessis	Relationships	Original Sample (O)	T Statistics (|O/STDEV|)	P Values	Result
H1	LTT -> YDML	0,045	0.860	0.390	Reject
H2	HL -> LTT	0.566	11.293	0.000	Accept
H3	HL -> YDML	0.133	2.115	0.034	Accept
H4	TC -> HL	0.689	16.246	0.000	Accept
H5	TC -> YDML	0.219	3.638	0.000	Accept
H6	HI -> TC	0.726	19.558	0.000	Accept
H7	HI -> YDML	0.362	7.546	0.000	Accept
H8	DSD -> HI	0.618	11.932	0.000	Accept
H9	DSD -> YDML	0.577	9.497	0.000	Accept
H10	TT -> HL	0.137	2.859	0.004	Accept
H11	TT -> YDML	0.078	2.708	0.007	Accept

Discussion

Based on the above analysis results, the group discussed the factors influencing students' willingness to repurchase food products in the Shopee e-commerce channel. After analyzing PLS-SEM, it was concluded that students' willingness to buy food on the Shopee channel is influenced by 5 main factors: satisfaction, trust, usefulness, ease of use, and convenience. These factors positively impacted student's willingness to buy food from Shopee. The higher these factors, the higher the willingness to buy again. Among them, ease of use has the most significant impact, followed by the usefulness factor, third trust factor, fourth satisfaction factor, and finally, convenience factor.

The study results are consistent with previous studies. According to Bireswar Dutta, usefulness and ease of use affect individual's attitudes toward internet use and repurchase intention. Ahn, Ryu, and Han also found that usefulness considerably affects online repurchase intention.

Trust positively and significantly affects online repurchase intentions (Weisberg, et al., 2011). According to Bireswar Dutta, trust positively influences online repurchase intention. Therefore, if online retailers can make consumers more secure and satisfied, consumers are more likely to intend to shop from online shopping sites again.

When consumers are satisfied with the services provided, they may return to the same online retailer to get the same kind of positive experience again (Dutta et al., 2016). When customers feel satisfied with their purchased product, they may repurchase it and even recommend it to others. On the contrary, if they feel unsatisfied, they will not repurchase it (Santoso, et al., 2020). Consumers emphasize the importance of convenience through the interface design of shopping platforms and search engines, convenience improves satisfaction, and consumer purchase intentions return to the market. Convenience increases customer satisfaction and willingness to buy from the marketplace again *(*Juniwati and Sumiyati, 2020*).*

Conclusion

From the research results, the group concluded that the factors affecting students' intention to buy food in a shop are sorted by importance: ease of use, usefulness, trust, satisfaction, and convenience. Based on the research results "proposing suitable solutions for shopee food business". To research to develop new business intentions and marketing directions that are more effective and more suitable for the needs of students and all consumers in general. For shopee to develop a more favorable development strategy, this research has provided managerial implications on factors such as convenience, ease of use, usefulness, repurchase intention, trust, and satisfaction

Use is the most influential factor in students' intention to buy food in the shop. This says that e-commerce businesses should pay more attention to the actual shopping process of customers. The study results showed that many consumers want to save more time on shopping, they want easy ordering and payment, and customers want their online purchases to be fast and safe. Entrepreneurs must provide business directions, distribute rich and diverse products and services, and provide customers with accurate, detailed, and fast information about products and services. In addition, usefulness also affects the ease of use. Therefore, the usefulness of customer's shopee's products and services is also a factor that should be considered. With the unpredictable nature of increasingly advanced Internet technology, customers are concerned that "someone" may threaten their property or reveal personal information, even endanger their lives *(Hoffman).* Therefore, electronic suppliers should enhance consumers' online purchase intention by providing precise and complete rules and regulations on account and security when transacting online, enhancing customers' trust.

Research results also show that products and services must target the right audience. Administrators need to focus on product information posted and improve quality and advertising to increase traffic to help customers shop easily. The product must be aimed at the right audience; customer needs. The study on students' intention to buy food in a shopee has achieved specific results, but with limited research time, it does not avoid some limitations. However, the research team also offers solutions for businesses to improve product quality and attract interest, increasing the intention to buy food for students and all customers. Ultimately, the team hopes that its research can help businesses and consumers make informed choices when buying online across all e-commerce products.

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The Impact of Attitudes and Personal Characteristics on Vegetarian Food Consumption Behavior Intentions

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Abstract

Vegetarian foods have had evidence of positive health effects, which are plant-based and provide essential nutrients and a lower percentage of fat than animal-derived foods. Animal foods are recommended to replace plant-based supplies but should be approached in terms of awareness among vegetarian food consumers. The fundamental theories of customer behavior have guided individual attitudes and characteristics that influence behavioral intentions for vegetarian food consumption. This study will apply planned behavior theory to examine associations in plant-based food consumption behavior, which will help people better understand the perception and health protection of plant-based or vegetarian food choices in life. This study was designed according to the model of testing hypotheses, conducting an online survey of 350 consumers on the social networking platform Zalo Vietnam. The study's results are analyzed according to the model of linear structural equations. The study results show that perceived public attitudes have a direct positive effect on vegetarian intentions. Emotional awareness factors, health awareness, nutrition awareness, and environmental awareness indirectly positively influence vegetarian intentions. Research findings and health-protecting dietary needs impact green, organically sourced, and innovative food needs that can be applied to prevent several nutrition-related diseases. According to global trends, this result contributes to people's choices about safe, organically sourced foods. This is one of the studies that focus on the impact of human eating trends and behaviors through the perception of community and individual attitudes to the intention to choose consumption according to organic food trends.

K**eywords**: Vegetarianism, trends, cuisine, health, green consumption

Introduction

Choosing food for vegetarianism is one of the beliefs of many people worldwide, including Vietnamese people. Whatever the purpose, vegetarianism has been and is welcomed by many people as meaningful and humane work. In general, most consumers choose safe food, food of organic origin. Họ has an attitude to living on a diet consisting of cereals, legumes, seeds, vegetables, fruits, mushrooms, algae, or other non-animal foods such as salt and honey. In recent years, consumers tend to choose clean products, some studies on green label product consumer behavior have found such as (Pham Thi Lan Huong, 2014; Dung, V. A., 2012) The attitude of choosing to be vegetarian is a personal characteristic of eating associated with thoughts, work, and life. Human life cannot exist and cannot be enjoyed without food. Eating and drinking symbolize the holistic life that expresses the culture and awareness of people, people.

People can choose the behavior of consuming vegetarian foods for many different reasons, the unexpected effects of vegetarianism include losing weight; many people who want to improve their weight index will prefer to use natural vegetables and tubers over protein-rich foods, protein or fat. Therefore, scientists recommend that consumers choose plant-based foods to prevent and reduce the risk of obesity. The purpose of fasting is to lower blood pressure; Obesity is one of the clinical manifestations of risk factors for hypertension. Therefore, vegetarians often have less risk of high blood pressure. Vegetarian food users avoid the risk of coronary artery disease, statistical research has shown, and shows that vegetarian food users have a very low incidence and death from cardiovascular disease. When building a vegetarian diet menu, they limit the use of fats, low cholesterol, and rich in fiber. Therefore, for vegetarians, the concentration of bad cholesterol in the blood is deficient, minimizing the risk of leading to atherosclerosis and embolism; vegetarian food users reduce the risk of cancer, with plant ingredients containing antioxidants, rich in fiber and low in soluble fats, can avoid the risk of leading to many dangerous cancers, especially throat cancer, colon cancer.

Vegetarian cuisine is increasingly valued and chosen by people as a trend to protect the environment, health, and living beings. People with vegetarian attitudes have influenced the intention to consume vegetarian food, simply hobbies, want to change their lifestyle, train their bodies and keep a calm, peaceful mind and positive life concept. In addition to the field of vegetarian food consumption research from

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theoretical and practical perspectives, this study examined the impact of attitudes and individual characteristics on the behavioral intentions of vegetarian food consumption. In particular, the study explains the influence of factors affecting human eating trends and intention behaviors through consumer choice according to organic food trends.

Literature review

In this study, the background theory is applied mainly based on Theory of Planned Behavior (TPB). This is a theory that has been applied by many behavioral studies and clearly evidenced in reports on customer behavior. Areas expanding from tourism, dining (McDermott et al., 2015), Diet behavior (McEachan et al., 2011), Augmented products, functional foods (Menozzi et al., 2017), and green food consumption (Zhu et al., 2013). Previous researches have reported the results positive attitudes, subjective norms, and perceived behavioral control directly affects the intention to consume plant-based alternatives and that intention affects the behavior of using plant-based products (Olfert & Wattick, 2018). TPB's tested hypotheses, this study applies attitudes and personal cognitive behavior control to the intention to consume plant-based products to vegetarians.

These vegetarian populations claimed to have different perceptions of attitudes towards environmental benefits, health and food safety, which changed consumption behavior and resulted in a significant change from traditional lifestyles to modern life in a healthy, ethical, and natural environment (Greenebaum, 2018). Knowing consumer attitudes and behaviors toward the consumption of vegan products can clarify consumers' decisions about plant-based foods (Ajzen, 1991).

Predictions of vegetarian behavior, issues related to the individual's choice of vegetarian behavior, and the influence of various relevant aspects of perception of animals, health, nutrition, and environment. Recognizing positive attitudes that influence positive behavior, people who choose vegetarian foods are more likely to have animal concerns, and measurements of animal interest have documented scientific evidence (Rollin, 1993). Recent studies have also found that people with vegetarian views are associated with animal emotions and vegetarian decisions (Rosenfeld, 2019).

H1: *The level of animal interest has a positive effect on vegetarian intentions*

There are many other views on health related to vegetarian food, and the concept of health awareness has identified risk factors affecting the health of individuals (Gould, 1990). When consumers discover the poor nutritional value of certain foods, it will impact attitude changes and abandonment of certain foods (Szakály et al., 2012). Studies have found that health-conscious consumers buy their favorite foods, flavors, and nutrition (Shen and Chen, 2020). Customers with better health perceived also intend to choose foods of clear origin, and customers who also choose vegetarian brands related to calorie awareness, frequency of consumption, and nutritional value (Besson et al., 2020). Perceived health is a measure of green consumer attitudes and behavior, and this perception is related to health information that greatly influences individual attitudes toward using plant-based foods (Ma and Chang, 2022).

H2: *Health perceived has a positive influence on vegetarian intention*

H3: *Nutritional perceived has a positive influence on vegetarian intention*

More recently, climate change has affected environmental change, and environmental influences have altered eating behaviors and plant-based food choices (Chai et al., 2019). A sustainable diet has little impact on the environment, protects and respects biodiversity and ecosystems, ensures nutritional value, and is safe, healthy, culturally acceptable, and affordable (Aleksandrowicz et al., 2016).

H4: *The level of environmental awareness has a positive effect on vegetarian intentions*

Other side, personal characteristics also have a particular influence on consumption intentions. The personality aspects of pleasurable, extroverted, conscientious, and neurotic expressions correlate with interpersonal behavioral aspects of integration, empathy, and control (Forrester et al., 2016). Related studies on vegetarian food choice behavior also addressed individual characteristics, among which are concerned with vegetarian-oriented attitudes and social conformity (Hopwood et al., 2020).

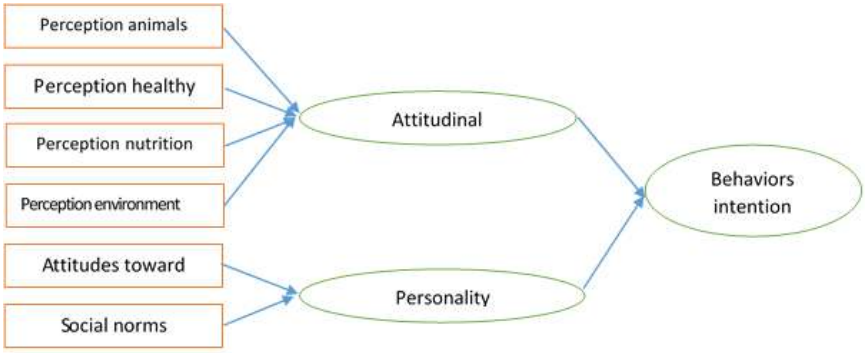
Recent research findings show that vegetarian-oriented attitudes are significantly correlated with nutritional preferences. The relationship between nutritional attainment and vegetarianism is interrelated. Enhanced nutritional knowledge can lead to a positive attitude towards vegetarian orientation and else (Pribis et al., 2010).

H5: *Orientation attitudes have a positive effect on vegetarian intentions*

Moreover, perceptions of the social environment also affect individual personalities (Holler et al., 2021). People who receive vegetarian food from social culture, community culture, and social environments also impact the behavior of choosing plant-based foods (Chen and Antonelli, 2020). In particular, for vegetarians oriented to persistent food preferences, choose according to a diet often to consider personal preference, social fit, and the potential to influence values, attitudes, beliefs, and well-being. Vegetarians have a greater perceived pro-social intention than omnivores and have a more liberal political perspective (Nezlek & Forestell, 2020).

H6: *Perceptions from social norms have a positive effect on vegetarian intentions*

In addition, socio-demographic scores, age (Lea et al., 2006), gender, income (Bryant et al., 2019), and education (Li et al., 2010) are related to the choice of a plant-based diet. From the studies cited, the research model is proposed below



The proposed research model

Research methodology

1. Data collection

An online survey based on questionnaires has been designed to assess consumer attitudes and knowledgeable knowledge about plant-based products or vegan products. Consumer expectations are based on perceived value and intent to choose plant-based vegetarian foods. The questionnaire is designed with a total of 39 variables and the questionnaire structure has two parts. The first part includes information base socio-demographic characteristics (age, gender, education, employment, income).The second part covers the proposed theoretical model structures and categories. The details apply the results from previous studies and the proposed measurement on apply Likert scale with 5 levels, of which from level 1 ("strongly disagree") to level 5 ("strongly agree").

2. Analysis methods

This study used descriptive statistical methods and frequency analysis for demographic measurement variables. Using the Cronbach Alpha scale test method to assess the reliability of the observed variables, the results were applied when the Cronbach Alpha coefficient was more significant than 0.7. Cronbach's alpha and synthetic reliability (CR) are used to assess structural reliability (Santos, 1999), and the average value extraction point (AVE) is selected for convergence validity assessment (Chau & Hu, 2001). The correlation coefficient between the structures and the square root of AVE was estimated to determine the distinguishing validity of structures. Moreover, the relationship between theoretical model structures is proposed to apply the method of analyzing the model of structural equations (SEM). The model fit was reported by chi-square, the GFI (Goodness of Fit Index), TLI (Tucker Lewis Index), CFI (Comparative Fit Index) and RMSEA (Root Mean Square Error of Approximation).

Research results and discussions

Demographic characteristics

Table 1. Results of frequency analysis of information about survey sample characteristics

Character	Content	Frequency	Percent
Gender	Male	110	33.6
	Female	217	66.4
Age	18 - 25	15	4.6
	26 -30	140	42.8
	31 - 35	111	33.9
	36 -40	27	8.3
	41 - 45	34	10.4
	Up 45	15	4.6
Education	College	44	13.5
	University	174	53.2
	Master	60	18.3
	Ph. D	35	10.7
	Other	14	4.3
Income	Less than 7	30	9.2
	From 7 – 12	139	42.5
	From 12 - 17	105	32.1
	Up 17	53	16.2

From table 1, for gender characteristics, the proportion of participants who answered was female. 4%, the proportion of men accounted for 33.6%. Most respondents were 18 – 25, accounting for 4.6%, and ages 26-30 account for 42. 8%, about age 31-35 accounted for 33.8%, aged 36-40 accounted for 8.3%, aged 41-45 accounted for 10.4%, and over 45 years old accounted for 4.6%. Respondents in this survey had college degrees accounting for 13. 5% and university accounted for 53. 2%, master's degree 18.3%, doctoral degree 10. 7% and other 4. 3%. Regarding income level, respondents said that the income level below 7 million VND is estimated at 9.2% and the group with income from 7 million to less than 12 million VND accounts for 42.5%.

Table 2. The results of the analysis are consistent with the data

Code	Items	Mean	SD	Loadings	CR	AVE
CA	**Perception animals**	**4.1**	**0.81**		**0.88**	**0.66**
PA1	Like humans, animals also have emotions.	4.12	1.048	.779		
PA2	The level of support for the right to life of animal	4.15	.944	.788		
PA3	The animals are the same as the hard animals you keep.	4.17	.974	.720		
PA4	The government should take care to protect all animals.	4.16	.987	.718		
PH	**Perception healthy**	**3.97**	**0.91**		**0.92**	**0.76**
PH1	Cardiovascular Disease	3.95	.975	.857		
PH2	Fasting reduces obesity	4.01	.950	.797		
PH3	Vegetarian diabetes prevention	3.98	.965	.861		
PH4	Vegetarian prevention of osteoporosis	3.94	.975	.823		
PN	**Perception nutitrion**	**3.70**	**0.98**		**0.90**	**0.70**
PN1	Vegetarianism, as well as non-vegetarianism, needs to provide nutritional value but is of plant origin, so it feels safer	3.68	1.043	.820		
PN2	Vegetarianism provides nutritional value but is low in fat	3.79	1.049	.840		
PN3	Vegetarianism is also a major source of vitamins	3.72	1.071	.797		
PN4	Vegetarianism provides natural minerals	3.70	1.109			
PE	**Perception environment**	**4.08**	**0.74**		**0.88**	**0.65**
PE1	Vegetarianism reduces the amount of carbon in nature due to energy-intensive meat processing	4.04	1.064	.731		
PE2	Vegetarianism saves more water than eating meat due to the multi-stage processing of meat	4.16	.986	.751		
PE3	Raising cattle requires more energy costs than growing plants.	4.06	1.000	.765		
PE4	Vegetarianism is worth the image of environmental protection	4.08	1.033	.749		
AT	**Attitudes toward vegetarians**	**4.10**	**0.89**		**0.92**	0.69
AT1	When choosing food, preference is given to a higher percentage of plants than meat.	4.10	.880	.810		
AT2	Having favorable conditions in the meal, choose to eat vegetarian more than eat meat	4.17	.854	.786		
AT3	Favorite meals with plant foods	4.09	.888	.779		
AT4	To be vegetarian, you must have a strong personality	4.12	.907	.752		
SO	**Social norms**	**4.00**	**0.92**		**0.91**	**0.67**
SO1	I was caught not participating in the meals because there is attending a local festival	3.98	1.057	.711		
SO2	I was called a monk for being a vegetarian	4.03	.995	.782		
SO3	I have often faced harassment when eating out	4.01	1.023	.814		
SO4	I have been ridiculed when eating with my friend	4.02	1.088	.770		
SO5	I have been picked for being a vegan	3.97	1.004	.792		
BI	**Behaviors intention vegetarian**	**4.14**	**0.84**		**0.92**	**0.75**
BI1	Your choice is to buy vegan food.	4.24	.878	.867		
BI2	You choose to buy products from brands that avoid animal testing.	4.23	.873	.814		
BI3	You always buy cruelty-free products	4.20	.892	.836		
BI4	When you need to buy anything, you do not choose to buy any items that are made from animal	3.92	.961	.774		

Another group of respondents has an income of 12-17 million VND (32.1%) and the other group has an income of over 17 million VND, estimated at 16.2%. Summary, the demographic characteristics of this research have a higher proportion of women than men; the majority group age from 26 to 35 years old, the education level is mainly university, and the income level is 7-12 million VND.

4.2 Confirmatory factor analysis (CFA)

In the study, the analytical method of linear structural model was used (Hair Jr et al., 2017). The results are presented in detail in Table 2.

Note: SD = standard deviation, CR = composite reliability, AVE = average variance extracted

Before applying the structural measurement analysis model, all variables and the loading coefficients of the factors are tested for the appropriateness of the data. Measured variables are tested, variables with load factor less than 0.40 will be excluded. Furthermore, the mean extracted variance (AVE) index ensures that each construct must be greater than 0.50, and the correlation between the variables is also less than 0.85 (T. J. B. Kline, 2005). This study tested the reliability of the measurement structure by an aggregate reliability value (CR) higher than 0.70 Hair (2017). According to (Shook et al., 2004), CR and AVE metrics must be verified on the obtained data set before using the SEM method.

From table 2, the results obtained from the real data audit met the appropriate data standards (R. B. Kline, 2015), Index value CR greater than 0.7, and value of AVE greater than 0.5, with these results, the data obtained are consistent with recent studies that have suggested (Raza et al., 2020).

Table 3. The Fornell-Larcker criterion of discriminate validity

	PA	PH	PN	PE	AT	SO	BI
PA	0.78						
PH	0.23	0.77					
PN	0.26	0.27	0.78				
PE	0.22	0.23	0.26	0.71			
AT	0.17	0.18	0.20	0.17	0.58		
SO	0.26	0.27	0.30	0.26	0.12	0.64	
BI	0.29	0.31	0.35	0.29	0.21	0.32	0.65

Note: PA = perception animals, PH = perception healthy, PN = perception nutrition, PE = perception environment, AT = attitudes toward vegetarians, SO = social norms, BI = Behaviors intention vegetarian

The analysis of the appropriate differential value of the various latent variable structures, the application of the Fornell-Larcker criterion, and the results are presented in detail in Table 3. Fornell-Larcker criteria are reliably met of the data obtained.This ensures credibility for the application of statistical analysis models.

Assessment of structural model and hypothesis testing

This study is designed to include measurement variables such as: animal awareness, health awareness, nutrition awareness, environmental awareness, personal orientation, social norms, and vegetarian intentions. The purpose of the study is to explore the relationship between latent variables according to the linear structural model and test the appropriateness of the data set. The results are described in Table 4.

Table 4. Estimated results in the linear structural model

Hypotheses	Estimate	Std.Err	P(>|z|)	Decision
AF =~CA	1.000	0.153	0.000	Accepted
AF =~CH	1.049	0.158	0.000	Accepted
AF =~CN	1.181	0.171	0.000	Accepted
AF =~CE	1.003	0.157	0.000	Accepted
PF =~AT	1.000	0.233	0.000	Accepted
PF =~SO	1.513	0.256	0.000	Accepted
Bi~ AF	1.119	0.278	0.000	Accepted
Bi~PF	0.272	0.369	0.461	Not accepted

Note: PA = perception animals, PH = perception healthy, PN = perception nutrition, PE = perception environment, AT = attitudes toward vegetarians, SO = social norms, BI = Behaviors intention vegetarian

From table 4, the relationships in the study model were validated and statistically significant with 95% confidence.The relationship of the impact of attitude perception on vegetarian behavior intentions is measured by variables PA = perception of animals, PH = perception of healthy, PN = perception of nutrition, and PE = perception of environment has

the same effect as vegetarian behavior. Thus, hypotheses of animal perception, health, nutritional value, and environment influence the intention of vegetarian behavior to be accepted. Personal cognitive relationships demonstrate positivity directly to the problem AT = attitudes toward vegetarians, SO = social norms, but there is no indirect relation to vegetarian intentions. Notably, in this study, which looked at the association between attitude perception and vegetarian intentions with an influencing factor of 1.11 and was statistically significant, this meant that when positive vegetarian attitude perception increased by 1 unit, vegetarian intentions increased to 1.11 and these results contributed to greater clarification of the positive. Positive in vegetarian behavior towards the planned behavior theory framework. In addition, this study looked at the relationship between individual cognition and unrecorded vegetarian behavior intentions; the results obtained were not statistically significant. So, the hypothesis of individual cognition that influences vegetarian intentions is unacceptable. This fact is most common for people to cite vegetarianism as less persuasive for others to eat with and may help explain why there are relatively fewer vegetarians eating meat and why those are motivated by social awareness; these results are also consistent with those of other subjects (Hoffman et al., 2013; Radnitz et al., 2015) without personal compliance with a vegetarian diet (Ruby, 2012).

Conclusions and implications

This study tests a model based on a planned behavioral theory zone to predict consumers' vegetarian intentions or choices to use plant-based foods. The results suggest that animal attitudes, health perceptions, nutritional value perceptions, and environmental awareness positively affect vegetarian intentions or plant-based food use. The results of this study confirm the relevance of attitude perceptions to vegetarian intentions but were unable to record individual attitudes related to vegetarian orientation or social norm issues affecting vegetarian intentions. In addition, the results revealed that citizens with a positive perception of animals, a positive perception of health, a perception of nutrition, and a positive awareness of the environment are interested in vegetarianism. However, groups driven primarily by personal motivation are unlikely to respond to vegetarian intentions. One explanation for these results is that most people are interested in animals, health, nutrition, and the environment, but people don't connect individual cognition and general perception. In addition, the demographic characteristics of the description that recorded the result had a higher proportion of females than males, the age at which it was recorded from 26 to 35 years old, the education level is forcus university, and the income level is 7-12 million VND. The findings of this study are significant because they explain Thai perceptions associated with vegetarian intentions in social communities. This proposes policy implications related to animals, public health, and education on nutritional values, environmental protection advocacy, and the use of resources to promote food choices. Plant origins are increasingly replaced, minimizing animal-related meat processing. The report can be used to improve the effectiveness of public health projects focused on promoting the adoption of plant-based diets and reducing meat consumption in the future.

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Effect of Moisture Absorber and Modified Atmosphere on Quality of Packaged Sweet Cherry

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Abstract

This work was aimed to investigate the effect of moisture absorber and perforation packaging on cherries during storage. Cherries were packed in 1 or 3 perforations packaging together with FruitPad30 (30 % fructose), FruitPad90 (90 % fructose), or without FruitPad. All samples were stored at 10 ± 0.5 °C for 9 d and an additional 2 d for shelf-life at ambient temperature. There was no significant difference between FruitPad30, FruitPad90, and without FruitPadin headspace gas concentration, visual evaluation, and decay. One perforation on packages resulted in more depletion of O_2 during shelf-life compared to three perforations. It was observed that perforation packaging could maintain the quality of sweet cherries during storage compared to control. In addition, the visual appearance of packed samples was also better than the control.

Keywords: moisture absorber, perforation, packaging, fructose, storage

Introduction

Sweet cherry is considered to be one of the most favorite fruit due to its attractive color, taste, and nutrition (Serrano et al., 2005). However, this product easily deteriorates after harvest with symptoms including surface pitting, stem browning, and rot (Bernalte et al., 2003). The fungal decay occurring commonly throughout postharvest storage causes a high loss of value on the market (Martínez-Romero et al., 2006). Therefore, maintaining the quality of fruit including nutrition, microbial safety, and specific characteristics during storage is necessary. There have been many approaches limiting the negative changes after harvest. To meet the market requirements, many technologies have been applied to prolong the postharvest life of produce including hot air treatment (Wang et al., 2015), chemical treatment (Ma et al., 2019), edible coating (Dam et al., 2020, Pham et al., 2022), 1-MCP treatment (Nguyen et al., 2016), optimized storage temperature (Zhao et al., 2019), ethylene absorber and controlled or modified atmosphere (Kappel et al., 2002). Modified atmosphere packaging (MAP) has been applied successfully to preserve fresh attributes for strawberry (Rux et al., 2016), cherry (Wani A.A. et al., 2014), slice carrot (Dawange et al., 2016), fresh cut carrot (Dam et al., 2020), asparagus (Siomoset al., 2000). However, in some cases, moisture condensation occurs inside the package due to the respiration and transpiration of fruit and vegetable easily leading to microbial growth (Nguyen et al., 2020). Thus, controlling the moisture inside the package is crucial to prolong the postharvest life of fruit and vegetables. This work was aimed to investigate the effect of moisture absorbers and perforation packaging on the quality of cherry during storage at 10 °C and shelf-life at ambient temperature.

Materials and methods

1. Cherry

Sweet cherries (*Prunus avium L.*) were harvested at the commercial maturity stage from an orchard. Samples were transported to the laboratory within an hour. Cherries (around 24 mm in diameter) free from damage, greenish stems, and uniform were used for the experiment. The fruits were cooled to 10 °C before packaging.

2. FruitPad

FruitPad was used in this study as a moisture absorber. These FruitPads (FruitPad00) were incorporated with two concentrations of fructose (30 and 90 %, henceforth called FruitPad30 and FruitPad90, respectively in the manuscript). In the middle layer using the commercial production facilities of McAirlaid'sVliesstoffe GmbH.

3. Packaging and storage

The packaging procedure was followed (Nguyen et al. 2020). FruitPad (10.3 × 7.5 cm) was placed at the bottom of each polypropylene tray (16 cm length × 11 cm width × 5 cm depth) for groups stored with FruitPad. Around 200 ± 5 g of cherries were packed in the polypropylene tray with FruitPad30, FruitPad90, or without FruitPad. These trays were covered with polypropylene films (25 µm thickness). The lid film was perforated with 1 or 3 micro-perforations using a needle. The diameter of the perforation is 0.7 mm. The

unsealed trays served as control. The samples were divided into 7 groups as follows:

a) Open tray (control)
b) 1 perforation
c) 3 perforations
d) 1 perforation + FruitPad30
e) 3 perforations + FruitPad30
f) 1 perforation + FruitPad90
g) 3 perforations + FruitPad90

4. Storage

Samples were stored in the chamber at 10 ± 0.5 °C, RH 90 ± 1 % for 9 d, and an additional 2 d for shelf-life at ambient temperature. Three replicates of each group were performed. The measurements were carried out on days 0, 3, 6, 9, and 11.

The gas concentration was measured at 10 °C during the cold storage period and at 20 °C for shelf-life. Visual evaluation and decay were determined at each interval at 20 °C.

Measurement

1. Gas concentration

Three trays per group were randomly taken out of storage and the headspace gas concentration of O_2 and CO_2 in the package were measured before the lid film opened. The needle was pierced into the package, then the gases were pumped out with the needle and injected into a gas analyzer (CheckMate3, Dansensor, Denmark) for measuring. The oxygen concentration value was displayed on the digital panel of the instrument. The results were expressed as the percentage of O_2 and CO_2 inside the package (Nguyen et al., 2020).

2. Visual evaluation

Stem quality

The stem of the cherry was assessed based on how the surface color turned brown with a scale of 1-5 (where 5=0 % browning, 4=1-25 %, 3=25-50 %, 2=50-75 %, 1=75-100 %) (Serrano et al., 2005).

Fruit appearance

The appearance of fruit was assessed based on surface pitting with a four-point scale (where 4 = unblemished fruit, 3 = slight defect, 2 = moderate defect, 1 = severe defect) (Kappel et al., 2002).

3. Decay incidence

Cherry was examined for mold growth on the fruit surface or stem during the storage period of 11 d. The decay incidence was calculated as a percentage of the total number of fruits. (Baranyai et al., 2020, Nguyen et al., 2022).

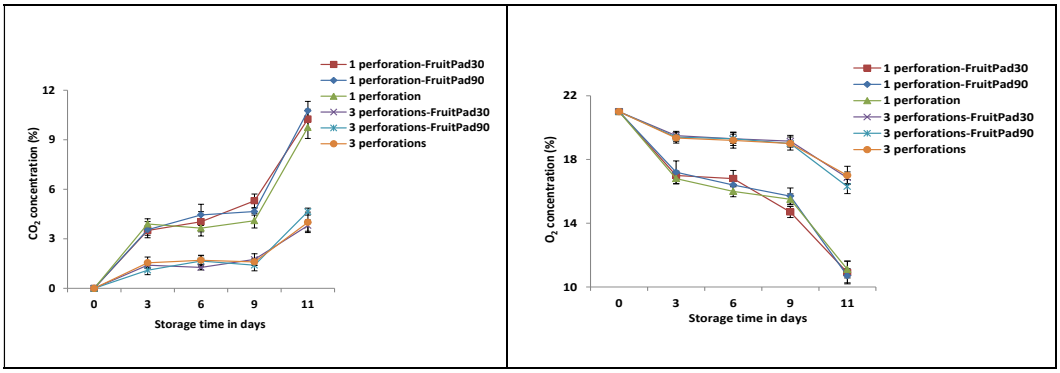
Statistical analysis

All data were subjected to statistical analysis with SPSS version 22 (SPSS Inc, USA) using analysis of variance (ANOVA). The effect of the factors of FruitPad, number of perforations on packages, and storage time were evaluated. The ANOVA F value was used to compare effects to the natural variability of readings. Tukey's method was used as a posthoc test to compare groups with $p<0.05$. The results are reported on charts with mean and standard deviation.

Results and discussions

1. Headspace gas

Figure 1 showed the changes in oxygen concentration in the packages with different numbers of perforations over the whole storage. In the case of three perforations packaging, the oxygen concentration in packages reduced to around 19 % at the first 3 d and retained in a similar range throughout 9 d of cold storage at 10 °C, after that decreased to 16.8 % during shelf-life. Meanwhile, the oxygen concentration in one perforation pack reduced to less than 17.2 % in the first three days, then to approximately 15.5 % after 9 d of cold storage, and around 10 % after shelf-life.



(a) (b)

Fig 1. Gas evolution inside the packages, O_2 (a) and CO_2 (b) during 9 d storage at 10 °C and subsequent 2 d of shelf-life at 20 °C

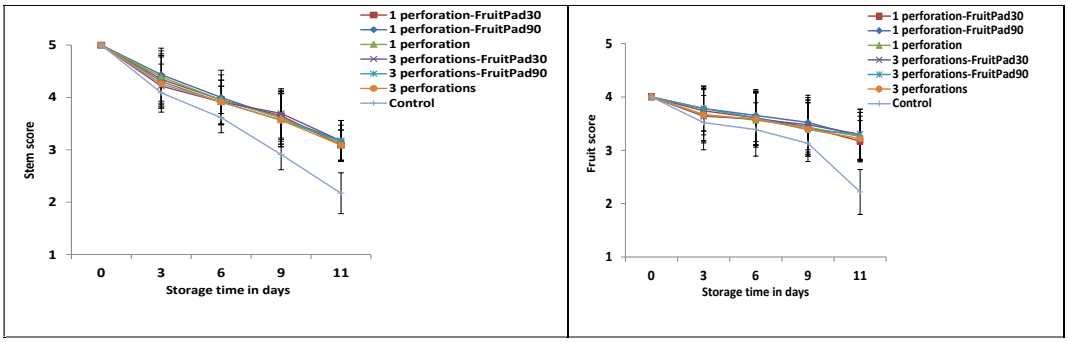
The CO_2 concentration inside the packages changed in relation to oxygen consumption (Fig. 1). During cold storage, the CO_2 concentration of all three perforation packages increased to approximately 1.4 % on the first 3 d, and maintained steadily till the 9th day, then sharp increase after shelf-life. Corresponding values for one perforation pack were higher, around 3.6 % at the first 3 d and nearly 10 % at the end of shelf-life. A similar result was found for carrots stored under MAP at 0 °C during 7 d (Khan and Patel, 2010). Those authors reported that the headspace gas was 11.85 % for O_2 and 13.38 % for CO_2.

The evolution of the headspace gas composition in the packages depends on packaging material, film surface area, storage temperature, the number of perforations, and the respiration rate of produce (Klaiber et al., 2005; Dawange et al., 2016).In our study, the changes in oxygen concentration were different between cold storage and shelf-life for all packs. The oxygen concentration inside the packages decreased rapidly when transferred to 20 °C. It was in agreement with the previous study for cherry (Serrano et al., 2005). Besides, three perforations on the lid film provided a higher gas exchange rate than one perforation on packages. Our results were in agreement with other reports for carrots (Dawange et al., 2016). The lower number of perforations resulted in more oxygen depletion due to the higher barrier attributes of packs. There was no significant difference in oxygen and carbon dioxide concentration in the packages among FruitPad90, FruitPad30, and without FruitPad.

2. Visual appearance

Sweet cherry is mainly consumed as fresh produce, however, it has a short postharvest life due to moderate respiration rate and susceptibility to microbial decay (Alonso &Alique, 2006). The quality of cherry decreased rapidly and lost marketability when stored at 20 °C within one or two days. In this work, perforation packaging preserved cherry fruit quality compared to the control.

Fig. 2 shows the change of visual quality in different packaging conditions for cherry. The browning process of the stem occurred gradually during storage for all fruit, however, the browning intensity of the stem was much higher in the control sample than in the packed cherry. Perforation packaging obtained positive results in delaying stem browning. At the end of the experiment, the stem of the control fruit had nearly 75 %of the browning surface, whereas, the packed cherry was less than 50 % of the browning area.



(a) (b)

Fig 2. Scores for the visual aspect of stems (a) and fruit (b) during 9 d storage at 10 °C and subsequent 2 d of shelf-life at 20 °C

(scale of 1-5 for stem, where 5=0 % browning, 4=1-25 %, 3=25-50 %, 2=50-75 %, 1=75-100 %); (scale of 1-4 for fruit, where 4 = unblemished fruit, 3 = slight defect, 2 = moderate defect, 1 = severe defect)

Similarly, the packed fruit was better in appearance than the control sample at the end of storage. There was no difference between FruitPad90, FruitPad30, and without FruitPad as well as the number of perforations. Visual attributes of cherries are important factors impacting consumer acceptability (Nguyen et al., 2021). Our results showed that perforation packaging maintained the visual appearance of the cherry during storage. Similar results were also reported for fresh-cut fruit and vegetables (Ma et al., 2017).

Decay incidence

No sign of decay was detected for all samples during 9 d of cold storage at 10 °C. However, after 2 days of shelf-life at 20°C, the percentage of decay was 1.3% for packed samples and 8 % for control samples.

Conclusion and Future Perspectives

The results of this experiment provided information about the effect of packaging with the different numbers of perforations (1 and 3) and FruitPad on the quality of cherry during storage. There was no significant difference in quality changes among perforated packages as well as moisture absorbers (FruitPad). Packages used in this study could maintain cherry quality for cold storage and shelf-life. FruitPad can be used as a moisture absorber in cherry packaging to control the humidity. However, achieving a modified atmosphere depends on the quantity of product in the particular size of the pack,

as well as the number of perforations. Experiments optimizing the amount of commodity, number of perforations, and the fructose concentration embedded in the FruitPad are suggested for further research.

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Section C: Food Biotechnology and Food Waste

Genetically Modified Fish and Seafoods: Pros and Cons

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Abstract

Transgenics have relatively recently started being used in aquaculture to increase production load and decrease running costs. Genetic modification can be used to increase the growth rate of fish or render them more resistant to exposure to adverse environmental conditions. Direct consequences of these benefits are a lower impact on the environment from overfishing and the protection of natural resources. Nevertheless, any effects on the ecosystem, as well as the health of the consumers, must be thoroughly examined and communicated. Measures should be put in place in facilities handling genetically modified organisms to prevent any escapes to the environment and adherence to any law requirements (e.g., GMO food labeling) should be strictly enforced. Moreover, consumer awareness and education are necessary to enhance the marketability and acceptance of these products especially when new markets are considered.

K**eywords**: Genetic modification, fish, seafood, pros and cons

1) Introduction

Commercialization of genetically modified organisms (GMOs) such as fish and seafood products has raised a debate among the scientific community and consumers because apart from their apparent benefits for both food producers and consumers, there are also critical concerns about the presence of potential biomedical risks and environmental deterioration. Consumers' opposition and skepticism toward GM fish and seafood products were related to potential short- and long-lasting health side effects that might be posed due to the use of such state-of-the-art biotechnology (Zhang et al., 2016). Nowadays, a substantial market share of food products either consists of totally GM food or at least one of their ingredients is produced using gene modification technology. GMOs could bring a bunch of benefits to consumers, such as enhanced nutritive quality combined with extended product shelf-life and organoleptic quality. Moreover, GMOs might present higher resistance to diseases or viruses and environmental stress. In contrast, potential disadvantages of GMOs could be threatening genetic diversity by contributing to unfair competition between organic and traditional suppliers. Additionally, by increasing GMO production, the food industry could be dominated only by a few major biotechnology companies promoting a biopiracy phenomenon (Ozkok, 2015). Specifically, in China, consumers are becoming more concerned about GM food mainly due to their declining trust in the government, biotechnology scientific community, press media, and food companies. From Chinese consumers' point of view, there is a low level of GM food product knowledge leading to confusion about the pros and cons of GM food. Potential health risks associated with GM food are critical for consumers' attitudes and behavior. This fact indicates that the commercialization of GM food in China could be underpinned by the dissemination of objective product knowledge to consumers accompanied by the strengthening of appropriate food safety supervision practices (Xu et al., 2020).

Recently, the use of transgenics in fish has broadened their potential benefits for aquaculture. Nevertheless, there are still critical concerns about the environmental deterioration provoked due to the unintentional escape of transgenic fish and their unfair competition with wild populations (Maclean and Laight, 2001). Despite this fact, due to their remarkably higher growth rate in comparison with conventional ones, transgenic fish could increase substantially aquaculture production in developing countries, increasing in parallel the income of relevant stakeholders, such as farmers and industry. For instance, under lab-scale trials, the growth rate of transgenic fish reached a higher than a 10-fold increase. Unimproved fish species response seems to be higher, thus being more beneficial for the majority of developing countries. The successful implementation of transgenics in aquaculture could potentially increase both the total production and production efficiency relieving the effects of habitat loss used for food production, and eliminating pressure from wild populations' overfishing and introduction of exotic species. The recent inaugural production of transgenic fish in aquaculture could expand within the next few years. Nevertheless, market entry strategies for transgenic fish should include as a prerequisite consumer education and awareness about any environmental risks and food safety issues. The currently limited environmental risk data are not able to verify the hypothesis that recombinant DNA technology applied in genetically improved fish in comparison with traditional selective breeding does not potentially increase the level of environmental risk. Therefore, the necessity for further research concerning environmental risk data

on a case-by-case basis seems to be imperative to clarify the ecological risk of transgenic fish and their future perspectives in aquaculture (Dunham, 1999).

During the last decade, a substantial number of fish (finfish) species have been genetically modified. Nevertheless, these efforts have only recently become market-oriented, including species such as Atlantic salmon (Salmo salar), coho salmon (Oncorhynchus kisutch), common carp (Cyprinus carpio), tilapia (Oreochromis niloticus), and channel catfish (Ictalurus punctartus). Initially, research was focused on the growth rate, but gradually additional traits including disease resistance, cold tolerance, and sterility were incorporated (Maclean, 2003). Decision-making strategies concerning aquatic GMOs can pose significant challenges but can achieve long-term sustainable development with no additional cost. This approach can lead to economic development enhancing both the consumer nutrition potential and environmental protection (Curieux-Belfond et al., 2009). The decision-making process should incorporate transparency throughout projects, procedures, and obtained results. Additionally, the monitoring of potential long-term adverse health effects from a third-party independent expert audit could increase transgenic fish product credibility to consumers. Apart from aquatic GMOs, an independent evaluation should include an assessment of alternative technologies comparing their benefits /costs and the level of consumer awareness (Curieux-Belfond et al., 2009).

2. Pros and Cons of Commercially Important GMO Fish Species

Growth rate comprises one of the most desirable traits for modification in domesticated agricultural animals. Recent scientific efforts focused on the transgenesis of growth factor genes such as primarily growth hormone (GH) have been stimulating the performance of agricultural species. Particularly, it should be stressed that GH gene transgenesis in several fish species seemed to have a positive impact on their growth rates in comparison with a relatively neutral impact during the implementation of genetic engineering in domestic mammalian livestock. In addition, wild strains without or with little selection for enhanced growth showed the most remarkable response to GH transgenesis. On the contrary, in livestock, genetic modification was applied in highly domesticated strains with a higher level of growth rate. Thus, this type of rapid-growth domesticated strain might be more resistant to additional enhancement applied by both state-of-the-art genetic methodologies (Devlin et al., 2009).

2.1. Atlantic Salmon

In novel seafood products, the impact of GM technology on consumer behavior in relation to their awareness of the production process, labeling, and other information was examined by Weir et al. (2021). According to this study, an online choice experiment took place among 1,043 participants/consumers of seafood products in the USA. Specifically, the obtained results showed that consumers with a lack of available GM information expressed a will to pay a premium for Verified Non-GM and Organic fillets in contrast to Fed-GM and GM fillets where they claim a reasonable discount. The willingness to pay (WTP) for Organic, Non-GM, and GM fillets was reduced when positive information was available in contrast to negative information, which resulted in significantly increased WTP for Organic fillets and reduced WTP for GM fillets, respectively. Moreover, WTP for Organic and Verified Non-GM salmon was reduced when balanced information was available. Similarly, balanced information provoked a WTP decrease for GM-Fed and GM salmon.

According to Hedrick (2001), transgenic growth hormone genes seemed to affect significantly the size and fitness-related traits of salmon and other species. This study used a deterministic model to show that in the case of a transgene male-mating advantage and a decreased viability rate, their invasion in wild populations could be still quite successful. For instance, an increased frequency of transgene was observed concerning 66.7% of potential mating combinations and viability parameters. In addition, fixation was observed for 50% of combinations. Transgene's increased frequency provoked a decrease in the wild population viability contributing to their higher extinction probability. According to the obtained results, the concerns about the adverse effects of transgenic organisms on wild populations were confirmed, particularly in the case of wild salmon populations.

During the pre-smolt growing phase (8-55g), a comparison of several traits such as growth rate, feed conversion and digestibility, and body composition of transgenic Atlantic salmon F_2 generation presenting enhanced growth with non-GM salmon was conducted in the study of Cook et al. (2000a). This comparison showed that transgenic Atlantic salmon presented a 2.62- to 2.85-fold higher growth rate than non-GM salmon. As far as the daily feed consumption is concerned, it should be stressed that it was 2.14- to 2.62-fold higher for transgenic Atlantic salmon in comparison with non-GM individuals. On the other hand, transgenesis seemed not to be involved in protein and energy digestion presenting quite a similar range of digestibility coefficients such as 88% and 81% in comparison to 90% and 84% respectively, regarding non-GM salmon. Nevertheless, it should be noted that a 10% increase in gross feed conversion efficiency was observed in transgenic salmon individuals. In contrast, the body composition attributes were significantly lower for transgenic salmon compared to non-GM ones apart from significantly higher moisture. The transgenic salmon individuals that were used in this study presented adequate physiological plasticity to accelerate their growth beyond normal range levels contributing to a few effects apart from a higher appetite and a leaner body.

Similarly, Cook et al. (2000b) study assessed the impact of food deprivation on the oxygen consumption

rate and the energy reserves utilization rate during the pre-smolt phase (8-55g), by conducting a comparison between the F_2 generation of transgenic Atlantic salmon with enhanced growth rate and the non-GM salmon individuals. The eight-week food deprivation period revealed that transgenic Atlantic salmon presented a higher oxygen consumption in comparison to non-GM ones. However, a remarkably rapid decrease in oxygen consumption was observed as food deprivation progressed (8 weeks) for transgenic fish.

Therefore, according to the initial weight and duration of food deprivation, there was a decrease in the oxygen consumption rate of transgenic fish in contrast to non-GM fish, which presented a higher oxygen consumption rate. Moreover, the body protein, lipid, dry matter, and energy depletion were lower in non-GM fish compared to transgenics. In addition, it was observed that lipids' catabolism was faster than proteins' ones for both non-GM and transgenic fish. Apart from the fact that food-deprived transgenic fish were able to decrease similarly to the non-GM salmon their metabolic rate, the capability of transgenics to maintain higher metabolic rate and their lower initial endogenous energy reserves implies that beyond intensive aquaculture transgenic salmon individuals with enhanced-growth traits might have the even lower ability from non-GM salmon to achieve their survival or maximum growth level. Moreover, according to Cook et al. (2000c), the oxygen consumption rates of transgenic Atlantic salmons with enhanced growth during the pre-smolt phase (8-55g) were compared with non-GM salmons in order to detect if transgenics present a higher metabolic rate. Specifically, oxygen consumption rates (mg O_2/h) of transgenic fish were 1.54- to 1.70-fold higher than non-GM ones. It should be stressed that transgenic salmon individuals presented 42% less total oxygen consumption related to non-GM salmons during the time interval from the first feeding to smolt size. After 24h starvation period, transgenic fish presented oxygen consumption rates that were 1.58- to 2.30-fold higher than non-GM individuals. This fact should be taken into account by smolt producers as the additional cost to provide more water or oxygen to maintain the higher metabolic rate of enhanced-growth fish should be compared to the benefits arising from the decrease in smolt production time.

Furthermore, Deitch et al. (2006) evaluated the cardiorespiratory physiology of GH transgenic salmon using a stable line of similar size GH Atlantic salmon (*Salmo salar*) which were reared simultaneously with controls in a shared tank at 10ºC for 9 months approximately. Particularly, the GH transgenic salmon presented a 3.6-fold higher growth rate, 21% higher mass-specific routine oxygen consumption, and 25% higher standard oxygen consumption (M_{O2}) than control individuals. Nevertheless, it should be stressed that the maximum $\dot{M}_{O2}$ was not increased simultaneously, thus presenting a significantly lower metabolic range (18%) and a decreased critical swimming speed (9%). Consequently, this lower metabolic range resulted in a 29% larger heart and an increased (18%) mass-specific maximum in situ cardiac output for transgenic fish. In addition, transgenics seemed to present higher post-stress blood hemoglobin concentration (14%) and substantially increased values of catecholamine regarding resting and post-stress, two-fold and 1.7-fold, respectively. Moreover, an increase (5-10%) in red muscle and heart aerobic enzymes, such as citrate synthase and cytochrome oxidase was observed. It was remarkable the fact that solely gill surface area was not positively affected and according to the obtained data, there was potentially a limited gill oxygen transfer. Specifically, this study revealed that GH transgenesis in Atlantic salmon leads to substantial metabolic costs and confirmed the enhancement of cardiac function. In contrast to symmorphosis, it seemed that there was a lack of a universal upregulation in cardiorespiratory physiology during post-smolt GH transgenic salmon. Last but not least, this study suggested that diffusion-limited processes should be enhanced to accomplish better metabolic and swimming performance.

2.2. Coho Salmon

The study of Devlin et al. (2009) showed that both GH transgenesis and domestication become involved in similar genetic pathways. This fact was confirmed via a comparison between GH transgenic, wild, and domestic populations' gene expression. Particularly, according to the gene expression results, a modified expression of GH transgenic and domestic strains was seen in comparison to the wild populations. Thus, growth regulation genes such as IGF1, IGF-II, GHR, and THR were consistent regarding their regulation in both GH transgenic and domestic populations presenting increased values of circulating IGF1. As far as the GH muscle expression in non-transgenic strains is concerned, it seemed that was higher in domesticated than wild individuals. Therefore, the obtained results could be used for the genetic improvement of domesticated populations as well as for the regulation and risk assessment of transgenic stocks.

Moreover, in the study of Devlin et al. (1999) where salmonids were examined, their traits such as appetite, growth, and foraging were related to growth hormone (GH). Specifically, this study examined the impact of higher GH levels in GH-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum) on the increased foraging competition by providing feeding stimulation. The transgenic salmon strain was comprised of sockeye metallothionein-B promoter in the type 1 growth gene coding region. According to the experimental design, there were six feeding trials comparing non-transgenic coho salmon with transgenic ones (F_1 individuals) of a mean size of 250g. Feed-contested pellets were provided *ad libitum* until three consecutive pellets were not consumed by both fish types. The results revealed that transgenic coho salmons presented 2.5-fold higher consumption of contested pellets in comparison to non-transgenics, implying the increased foraging ability of transgenics due to GH transgenesis. In addition, transgenics showed in total a 2.9-fold higher pellet consumption than non-transgenic individuals, hence transgenics presented a higher feeding motivation. Therefore, both

GH transgenesis and GH treatments provoke analogous alterations in salmonids' feeding behavior. Thus, GH transgenic escapees might dominate wild fish populations due to their enhanced feeding motivation. Another study by Devlin et al. (2004) revealed that there was a higher growth rate and feeding ability in the case of reared GH-transgenic coho salmon (*Oncorhynchus kisutch*), corresponding to a significantly (>7-fold) larger body size compared to non-transgenic individuals. Nevertheless, in wild populations' genotype foraging ability could be critical concerning its survival. In general terms, in the case of simultaneous cohabitation of transgenic with non-transgenic salmons, transgenics seemed to take advantage of dominating non-transgenic individuals affecting negatively their growth except for high food availability. In the case of low food availability, the dominant transgenic individuals showed high levels of agonistic and cannibalistic behavior to cohorts. Particularly, transgenic salmon individuals presented extremely low survival, in contrast to non-transgenic ones that presented 72.0± 4.3% SE survival, increasing their population biomass simultaneously. Therefore, food availability and population structure seemed to be critical factors regarding the impact of GH-transgenic salmon individuals on non-transgenic cohorts. Despite the fact that the obtained results provide significant information regarding the potential impact of transgenics on wild populations, these might underscore genotype by environment interactions to risk assessment for GMOs implying that data for species such as salmon should be applied with considerable caution.

Moreover, the study of Devlin et al. (2012) focused on the growth acceleration impact on eye development in GM coho salmon. Specifically, growth hormone (GH) transgenic *Oncorhynchus kisutch* presented an increased body growth and eye size. Nevertheless, relative eye growth was more isometric instead of negatively allometric. Hence, transgenic individuals showed smaller eyes than non-transgenic ones implying that this is an indirect effect of growth rate modification instead of GH overexpression. Additionally, total brain size had analogous growth to the eye. In contrast, other organs such as the liver, heart, and spleen seemed to be constant in size between both groups. Similarly, Kotrschal et al. (2012) conducted measurements to determine brain and body size between reared transgenic and wild coho salmon *Oncorhynchus kisutch* under both aquaculture and natural habitat stream conditions. The obtained results revealed that reared individuals presented higher growth and larger brain size. Moreover, wild individuals under both aquaculture and natural habitat stream conditions presented relatively larger brain sizes in comparison with transgenics. Furthermore, the transgenics showed a smaller cerebellum under natural habitat stream conditions, and both transgenics and wild individuals had a larger optic tectum under hatchery conditions. On the contrary, the wild population under hatchery conditions presented a significantly smaller telencephalon. Consequently, despite the impact of environmental cues, genetic factors seemed to play a pivotal role in growth rate influencing both brain and body size.

2.3. Common carp (*Cyprinus carpio* L.)

Transgenic fish with enhanced traits could dominate wild populations provoking adverse effects in aqueous ecosystems. Therefore, Guan et al. (2008) examined the potential differences in the depletion of energy stores between growth hormone (GH) transgenic and non-transgenic common carp individuals using their metabolic rates during both routine and food deprivation intervals. Initially, GH-transgenic individuals presented 2.12-fold higher mean daily feed intake than non-transgenics for a four days feeding period. After 96h of food deprivation, GH-transgenics showed 1.32-fold higher average oxygen uptake in comparison with non-transgenic individuals. Nevertheless, between a 96-144 h intervals of food deprivation, no significant difference was observed and GH transgenics did not seem to deplete faster energy stores than non-transgenics. It was therefore proposed that elevated oxygen uptake in GH transgenic common carp versus the control fish might be a result of feeding, rather than caused due to basal metabolism increase. GH-transgenic fish did not differ from the controls in regulating their metabolism to allow the normal distribution of energy during starvation (Guan et al., 2008).

Chen et al. (1993) examined a transgenic common carp, Cyprinus carpio, that possessed a long terminal repeat (LTR) sequence of avian Rous Sarcoma Virus (RSV) fused to the rainbow trout (rt) growth hormone (GH1) complementary DNA (cDNA). It was initially observed that the transgenic common carp allowed the transmission of the foreign DNA to a major fraction of their progeny in three of four crosses of transgenic males with control females. The growth of these progeny was found to be 20 to 40% quicker than that of their non-transgenic animals. Further studies were carried out allowing inheritance and expression of the foreign GH gene in transgenic common carp to be evaluated, as well as monitoring how these transgenic fish grew. A total of 4 P1 (parental generation produced by microinjection) x nontransgenic controls, 4 P1 x P1, and one P1 x F1 mating were made, with the rates of transgenic progeny that resulted from them being 0, 32, 42, 100 (4 progeny only), 21, 21, 31, 30, and 23%, respectively. All crosses, apart from 1 control x P1 mating, demonstrated progeny ratios that did not exceed 50 or 75% transgenic. It was therefore shown that the majority of the transgenic P1 had the foreign gene in their germ line but were mosaics, while at least one transgenic animal had no RSVLTR-rtGH1 cDNA in its gonads. Production of trout growth hormone mRNA and polypeptide was seen in both P1 and F1 transgenic fish, while the effect of rtGH1 cDNA on growth was highly variable in F1 transgenic individuals.

In the study of Chatakondi et al. (1995), a comparison was made between F_1 transgenic common carp found to contain rainbow trout growth hormone

gene, pRSVrtGH1 cDNA, and non-transgenic individuals to understand if differences in body composition existed. Significantly ($P < 0.05$) more protein was found in transgenic animals compared to controls (19.5 vs. 18.1), while significantly less fat ($P < 0.05$) was found in transgenic individuals (3.3), compared to controls, (3.8). Significantly lower ($P < 0.05$) moisture content was found in transgenic animals versus the controls (70.9% versus 75.8%). Moreover, a total of 18 amino acids were examined. It was found that the transgenic genotype body composition was significantly ($P < 0.05$) richer in aspartic acid, cystine, glutamic acid, histidine, lysine, and threonine. The body composition of transgenic individuals was generally richer in all amino acids apart from glycine, alanine, proline, and tryptophan. Comparing the ratios amongst the amino acids, it was demonstrated that were steady in most cases but increased ($P < 0.05$) in the cases of glutamic acid and histidine from 15.4 to 15.8%, and 2.7 to 3.0%, respectively, while decreasing in the case of glycine from 6.6 to 4.9%, for transgenic individuals versus the controls. Lysine's and histidine's ratios were higher ($P < 0.05$) in transgenic in comparison to control fish body composition, while the most common fatty acids found in both groups tested were C18:1.

Li et al. (2013) reported that there is a strong connection between gut microbiota and several essential functions of an organism, including metabolism, immunity, the use of energy, and general well-being. To better understand whether this argument is valid, a rapidly-growing transgenic common carp (*C. carpio*) was used to evaluate how its body size is affected by its gut microbiota. The use of metagenome-based fingerprinting and high-throughput sequencing on bacterial 16S rRNA genes demonstrated that the principal microorganisms present in the gut of the fish examined were Proteobacteria, Fusobacteria, Bacteroidetes, and Firmicutes, while their populations differed significantly between transgenic and wild fish. Biochemical and histological tests showed that the body of transgenic fish exhibited higher carbohydrate and lower lipid metabolism rates. Moreover, transgenic fish had a considerably lower Bacteroidetes: Firmicutes ratio in comparison to wild individuals, thus suggesting that gut microbiota has a great effect on the growth of fast-growing transgenic fish, and the relative abundance of Firmicutes over Bacteroidetes might be one of the most important factors affecting this trend. It was noted that as the large body size of transgenic fish is proportional to body growth (a trend that is not followed in the case of human obesity), the relation between obesity and gut microbiota cannot be followed by simply calculating the Bacteroidetes: Firmicutes ratio change.

In another study, measurements of the food consumed, the number of movements, and the feeding hierarchy of transgenic *C. carpio*, as well as non-transgenic individuals (of similar sizes) were taken while feeding the fish with small amounts of feed. It was observed that transgenic fish were moving 73·3% more and had a higher feeding order, but also needed 1.86 times more pellets compared to non-transgenic. The researchers, therefore, concluded that these transgenic fish are very competitive when found in an environment of scarce feed supply, which could be advantageous if escaped from the aquaculture facilities. Nevertheless, other factors that limit this potential, such as the abundance of predators and food availability, should also be taken into consideration (Duan et al., 2009).

In the study of Li et al. (2009), a comparison was made between swimming speeds (U_{crit}) and morphological characteristics between the F4 generation of GH-transgenic carp and non-transgenic individuals. It was shown that the mean absolute U_{crit} value of transgenic fish was 22·3% lower in comparison to that of the non-transgenics. Principal component analysis was used to detect differences in body characteristics. It was revealed that transgenic fish had considerably deeper heads, bigger caudal lengths of the dorsal region, longer standard length and shallower body and caudal region, and shorter caudal length of the ventral region compared to non-transgenics. Swimming speeds were found to be significantly affected by the development of a deeper body and caudal region, longer caudal length, shorter head depth, and caudal length of the dorsal region and standard length. It was therefore indicated that differences in such characteristics which lead to maximum thrust and minimum drag in GH-transgenic *C. carpio* may greatly affect how well the animals can swim.

Moreover, Cao et al. (2014) used a grass carp (*Ctenopharyngodon idellus*) growth hormone transgene incorporated into transgenic common carp (*C. carpio*) lines to investigate the potential interaction between their growth and reproduction. According to the obtained results, a delayed gonadal development was observed in GH-transgenics in comparison with non-transgenic individuals. Therefore, in this study, the monitoring of gonad development concerning body growth and any reproduction alterations regarding genes and hormones lasted two years. Specifically, it seemed that in GH-transgenic common carp, there was a decrease of luteinizing hormone (LH) in pituitary and serum level, respectively. Additionally, an over-expression of GH was observed which increased peripheral GH transcription and serum GH levels. Particularly, in GH-transgenics there was an inhibition of pituitary lhβ and supplementary in vitro analyses revealed an inhibition of lhβ expression by GH. Moreover, a potential direct regulation of GH on gonadotrophs was supported by the localization of ghr with LH. Furthermore, a pituitary sensitivity decrease to salmon gonadotropin-releasing hormone (GnRH) agonist and dopamine antagonist was observed. Consequently, the results of this study implied that the delayed reproductive development in GH transgenic common carp individuals was due to the LH production decrease.

2.4. Tilapia (*Oreochromis niloticus*)

The remarkable worldwide aquaculture expansion of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) rendered this species an important globally traded commodity. Initially, the natural habitat

of tilapia was solely in Africa, but most of its reared populations could also be found in Asia. The majority of used aquaculture broodstocks regarding Nile tilapia are derived from a narrow genetic base. Moreover, the wild populations of Tilapia are yet to be adequately genetically characterized and several wild populations were among the critically endangered species of Africa (Eknath and Hulata, 2009).

In the study of El-Zaeem et al. (2010), an effort was made to create a genetically modified Nile tilapia, *O. niloticus* with enhanced salinity tolerance. The goal was achieved using either Sea bream, *Sparus aurata*, or Artemia, *Artemia salina* fragmented purified DNA introduction into gonads. According to the obtained results, the GM-tilapia individuals with DNA derived from Sea bream showed a significant enhancement in growth rate and feeding utilization ($P \leq 0.05$) under various salinity levels ($\leq$ 16 ppt)in comparison to both non-GM and GM-tilapia treated with DNA derived from Artemia. In contrast, it should be stressed that at higher salinity levels (32 ppt) the GM-tilapia individuals with DNA derived from Artemia showed remarkably enhanced traits in comparison with both other groups ($P \leq 0.05$). Additionally, the phenotype of GM-tilapia individuals using DNA derived from Sea bream was characterized by a silver color throughout their body and the absence of any dark vertical bands. The application of random amplified polymorphic DNA (RAPD) fingerprinting using random primers revealed high genetic polymer-phism among fish populations with foreign DNA (35.95%). The obtained results of this study implied that GM-tilapia fish populations with both enhanced growth rate and salinity tolerance could be produced promptly using fragmented purified DNA introduction into gonads.

Similarly, in the study of El-Zaeem (2011) an introduction of a fragmented purified DNA derived from either Blue tilapia *Oreochromis aureus* or Nile tilapia *O. niloticus* into parent *O. niloticus* or *O. aureus* gonads took place, using interspecific hybridization and GM-breeding, producing GM-tilapia individuals. According to this study, a comparison and evaluation regarding the productive performance traits of F0 generation between both groups were conducted. The obtained results implied a significant improvement in several traits of GM-tilapia individuals such as growth rate, feed utilization, and body composition ($P \leq 0.05$) in comparison with interspecific hybridization and pure breeding individuals. Moreover, a parsimony tree was created using RAPD analysis, considering the various genotypes. In addition, according to RAPD fingerprinting hierarchical cluster analysis, the genotypes were discriminated in two main groups. The first main group showed that the purebred *O. Niloticus* and *O. Aureus* and their reciprocal hybrid were grouped quite closely. In contrast, the second main group showed that the *O.* aureus using DNA derived from *O. niloticus* were more genetically dissimilar in comparison with the *O. niloticus* using DNA derived from *O. aureus*. Consequently, similarly to previous studies, the obtained results implied that GM-tilapia individuals of both species *O.* niloticus and *O. aureus* presenting enhanced growth rates could be produced promptly using fragmented purified DNA introduction into gonads.

In addition, Nguyen et al (2007) focused on genetic correlations among trait expressions regarding both sexes and body size parameters such as body length, weight, depth, and width to estimate genetic parameters of genetically improved reared Tilapia populations. Specifically, restricted maximum likelihood methods were used for the analysis of 12,038 individuals, progeny of 232 sires, and 340 dams, respectively. Moreover, sexual dimorphism genetic variation was estimated by treating trait expressions in both sexes like different traits. The results of this study revealed a high similarity level for heritability and both maternal and common environment effects in both sexes and for all traits. In particular, the fact that for all traits the genetic correlations between both sexes ranged from 0.91 to 0.96 implied the absence of sex by genotype interaction. Furthermore, the heritability of body size parameters that were treated as single traits ranged from 0.20 to 0.35 ($\pm$ 0.04 to 0.05SE).On the other hand, the genetic correlations among all body size parameters were highly positive ranging from 0.94 to 0.99 implying a potential low level or lack of genetic variation. Therefore, according to the obtained results, during the genetic improvement programs, the trait expressions in both sexes were not necessary to be treated as different traits.

Finally, in the study ofGarduño-Lugo et al. (2003), a comparison of several productive traits between wild Stirling Nile tilapia *O. niloticus* and Florida red hybrid tilapia was conducted for 98 days. Particularly, the initial weights were 139.0g and 207.3g regarding wild Stirling *O. niloticus* and Florida red hybrid (N = 22 males in triplicates), respectively. According to the feed composition, crude protein was approximately 36.8%. The wild Stirling Nile tilapia *O. niloticus* reached a final body weight of 473.0g and the Florida red hybrid tilapia 348.8g. Nevertheless, both species presented a similar fillet yield, namely 32.0% and 33.4%, respectively. It should be stressed that the lipid content in fresh fillets of *O. niloticus* was remarkably higher (>1.74%) than the lipid content in Florida red hybrid fresh fillets.

3. Conclusions

Transgenics are a valuable tool that can be used in aquaculture to increase the production of food while limiting any impact on the environment from overfishing and the depletion of natural resources. It is however of paramount importance that, as with any novel technology, any effects on the health of the consumers and the environment (e.g. biodiversity) are considered and thoroughly studied. The enforcement of measures that guarantee the safe use of such technologies can only be done via legislation, and therefore robust legal frameworks across the globe are necessary. Particular attention should be paid to potential escapees from aquaculture facilities and their negative impact on wild populations via interbreeding and unfair competition adverse effects. Therefore, the aquaculture sector has to make efforts to adopt all the

adequate measures to prevent such incidents. Additionally, both the scientific community and public authorities in cooperation with pertinent stakeholders should enhance consumer awareness about the commercialization of GM fish and seafood products by providing objective product knowledge and ensuring their appropriate food safety supervision.

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The Raw 264.7 Cell Membranes and DNA Protection of The Extracts from *Isaria tenuipes* VHI-2 Biomass Isolated in Vietnam

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Abstract

In previous studies, we cultured the fungus *Isaria tenuipes* VHI-2 isolated in the mountains of Liangbiang, Lam Dong province (Vietnam), and collected their biomass to investigate in vitro antioxidant capacity. Accordingly, the ethyl acetate extract had the highest antioxidant activity among the tested extracts. The target of this study was to evaluate and compare the ability to protect DNA as well as protein and lipid membranes of RAW 264.7 cells of extracts from *I. tenuipes* VHI-2 biomass. These results showed that ethyl acetate extract could protect cell membranes from damage by oxidizing agents better than ethanol extract, with IC50 = 83.112 ± 5,137 µg/mL (membrane protein protection survey); IC50 = 40.179 ± 0.837 µg/mL (protection of cell lipid membranes) and protected 97,454 ± 0.308 % DNA of RAW 264.7 cells at a concentration of 100 µg/ mL. From the obtained results, the ethyl acetate fractional extract from *I. tenuipes* VHI-2 biomass was an extract containing secondary compounds that could potentially protect DNA and cell membrane damage from oxidizing agents and be a potential extract for separation for antioxidant studies in animal and human clinical models.

Keywords: *Isaria tenuipes*, biomass, protein-membrane, lipid membrane, DNA, RAW 264.7 cells.

Introduction

Isaria tenuipes of the family Cordycipitaceae, genus Isaria (Sung et al., 2007) is a species of parasitic fungus on insects, asexually of *Cordyceps takaomontana. I. tenuipes* has a relatively high degree of metabolism, parasitizing only the pupae of scaly worms. The scale wing worm species, when pupating, are usually located in leaves, branches, or the soil. After being infected by the fungus *I. tenuipes*, biomass appeared with a characteristic white growth on the pupae. *I. tenuipes* species are rich in shape and often found in vegetation or soil layers 1-2 cm deep. In Vietnam, *I. tenuipes* was found in Hoang Lien Son National Park and quite common in Tam Dao National Park (Vinh Phuc), Bidoup - Nui Ba National Park and recorded in Pu Mat National Park (Nghe An) (Thúy et al., 2017). This fungus was a biological product to prevent pests and diseases in agriculture and fishery. In addition, the fruiting body and biomass of *I. tenuipes* are used as traditional medicine, lowering blood sugar, lowering blood fat, anti-tumor, antibacterial, anti-depressant, and immuno-modulating (Zhang et al., 2019). The active ingredients in *I.tenuipes* have been studied and applied in many countries worldwide. Potential compounds isolated from fungal biomass such as Paecilomycin A, B, C, ergosterol peroxide, acetoxyscirpenediol, N6-(2-hydroxyethyl) adenosine (HEA) and polysaccharides, flavonoid, phenolic compounds, etc. It has been shown to have antioxidant and inhibitory effects on human cancer cell lines such as gastric tumors, liver cancer, and colon cancer (Kikuchi et al., 2004). However, in Vietnam and worldwide, there is still very little research data on the effects of compounds extracted from *I. tenuipes* mushroom biomass on cell models. In previous studies, we cultured the fungus *I. tenuipes* VHI-2 isolated in the mountains of Liangbiang, Lam Dong province, and collected their biomass. After investigating the antioxidant activity of the extracts and selecting the potential extract as ethyl acetate (EA) extract, in this study, we continue to investigate the ability to protect DNA as well as proteins and lipids of cell membranes by oxidative stress on RAW cells. From there, we have more data on the potential activity of *Isaria tenuipes* VHI-2 biomass extracts for further studies in animal cell models.

Materials and Methods

2.1 Materials Mushroom strain *I. tenuipes* VHI-2 was collected in the mountains of Liangbiang, Lam Dong province, Vietnam. The biomass of *I. tenuipes* VHI-2 fungus was collected by static liquid culture method at the Biochemistry laboratory of the University of Natural Sciences - Vietnam National University, Ho Chi Minh City. 2.2 Chemicals Ethanol 96% (Vietnam), ethyl acetate (Vietnam), phosphate buffered saline pH 7.4 (PBS) (India), DMEM culture (USA), thiazolyl blue

tetrazolium bromide (MTT) (Germany), dimethyl sulfoxide (DMSO) (Germany), NaCl (China), Triton X-100 (Germany), Tris-HCl (India), ethylene-diamine-tetraacetic acid (EDTA) (China), 2,4-dinitrophenyl hydrazine (USA), guanidine hydrochloride (USA), H2O2 (China), FeSO4 (China), thiobarbituric acid (TBA) (Germany), KCl (China), KH2PO4 (China), RNAase (USA), proteinase K (USA), Sodium dodecyl sulfate (SDS) (China), phenol (China), chloroform (China), isoamyl alcohol (China), Ethanol 100% (Germany), CH_3COONa; $3H_2O$ (China), glacial acetic acid (China), TRIS-base (India), agarose (Germany), gel red (USA), were used.

2.3 Extraction

Extraction procedures were performed according to the extraction method of Nguyen Kim Phi Phung (2007) (Phung and N, 2007) with adjustments to fit laboratory conditions.*I. tenuipes* VHI-2 biomass was dried to constant weight and then ground. Biomass was extracted completely in ethanol 96% (48 hr), then evaporated the extract at 50^0C to obtain EtOH (total extract). Continue extracting fractions from the EtOH extract, including Ethyl acetate (EA), and evaporating the solvent to obtain the EA fraction. The collected samples will be stored in the dark at 4^0C for experiments.

2.4. Cell culture

The RAW 264.7 cell line was obtained from the Korean cell line collection (KCLB, Seoul, Korea) and the cell line collection of the Department of Biochemistry, University of Science, Vietnam National University, Ho Chi Minh City. Cells were maintained in RPMI medium supplement with 10% fetal bovine serum and 1% streptomycin/penicillin. Cell cultures were grown in a humidified atmosphere of 5% carbon dioxide (CO_2) at 37°C. After 10-15 passages, the cells were no longer used for the assays.

2.5. Cell viability assay

The procedure was performed according to (Taciak et al., 2018) with adjustments to fit laboratory conditions. Incubate RAW 264.7 cells on a 96-well plate until a density of 180 µL/10^4 cells/well is reached. Added 20 µL of the test sample and incubated for 48 hr. Then, the cells were added with 100 µL of PBS, add 50 µL of 1X MTT (MTT/DMEM), and incubate for 4 hr. Added 100 µL of DMSO to dissolve the formazan salt and incubate overnight. Specimen optically measured at 562 nm using a Microplate Reader (Perkin Elmer). The cytotoxicity or cell viability degree was calculated and compared with control cells. The cell viability was calculated as follows:

$$Cell\ viability\ (\%) = \frac{OD\ sample}{OD\ control} \times 100\%$$

$$Cell\ viability\ (\%) = \frac{OD\ sample}{OD\ control} \times 100\% \quad \textbf{(Eq 1.)}$$

Where OD is the optical density.

2.6. Cell membrane proteins oxidation assay

The experimental process was as previously described (Levine et al., 1990) with adjustments to fit the laboratory conditions. RAW 264.7 cells were cultured on phosphate-buffered saline (PBS) medium until the cell density reached 5×10^3 cells/mL. Then cells were washed three times with PBS medium and cell lysates in buffer medium (components included: Tris-HCl 25 mM pH 7.8, EDTA 2mM, NaCl 180 mM, Triton X-100 1%)— cells were incubated with 5 µL of sample solution at 37°C for 30 min. Then, 10 µL of $FeSO_4$ solution (400 µM), 20 µL of H_2O_2 solution (0 – 1 mM), and 5 µL of distilled water were added and incubated at 37ºC. After 1h of incubation, 400 µL of 20% TCA was added and the whole mass was centrifuged. The supernatant was discarded, and the solid was suspended in 150 µL of 2,4-dinitrophenyl hydrazine solution mixed in 2M HCl, the mixture was kept at room temperature for 40 min, and vortexed evenly. The protein was precipitated with 20% TCA, and the precipitate was washed 3 times with EtOH: EA (1:1) solution, and then dissolved in 500 µL of 6M guanidine hydrochloride. The incubation was continued at 37ºC for 15 min, then centrifuged at 6000 RPM for 5 min. Finally, the optical density of the supernatant was measured at λ = 370 nm. Simultaneously, a non-parallel test sample was prepared similar to the survey sample, but only for 2M HCl instead of the 2, 4-dinitrophenyl hydrazine solution.

2.7. Cell membrane lipid oxidation assay

Obtain cell culture in the presence of EA extract and then measure the optical density at λ = 528 nm as described by (Kikuzaki and Nakatani, 1993). RAW 264.7 cells were cultured on PBS medium until the cell density reached 5×10^3 cells/mL. Wash cells with FBS and add 5 µL of survey sample solution to the mixture. Add 10 µL of $FeSO_4$ solution (400 µM) and 20 µL of H_2O_2 and continue incubation at 37ºC for 1 hour. Then add 10% TCA solution (1:2) (v/v), and centrifuge to collect the supernatant. Continue to add 1% TBA solution (1:1) and incubate at 90ºC for 30 min. Cool and centrifuge the reaction mixture, measuring the optical density of the mixture at λ = 528 nm.

2.8. Determination of radical-mediated DNA damage

The experiment, according to the description of Milne et al. (1993) (Milne et al., 1993), includes 2 steps:

- DNA acquisition from RAW 264.7 cells: RAW 264.7 cells were washed twice with PBS, added 5 mL of trypsin, and incubated for 15 min. Add 1 mL of PBS containing 5 mM EDTA, centrifuge at 1000 RPM for 10 min at 25°C, and discard the supernatant (replicate 2 times). Add 25 µL RNAase (0.5 mg/mL), 10 µL proteinase K (10 mg/mL), 25 µL 10% SDS, and 350 µL CH_3COONa solution (0.2 M), respectively. Gently invert and incubate at 37ºC for 30 min and 55ºC for 55 min. Add to the mixture of phenol: chloroform: isoamyl alcohol (25:24:1) in the ratio (1:1), gently invert, and centrifuge at 12000 RPM for 5 min at 4ºC (repeated twice). Collect supernatant, add cold 70% ethanol at a 1:1 ratio, and centrifuge at 12000 RPM for 5 min. Discard the solution; the solid was dissolved with 50 µL of TE buffer (pH 8.0).
- Electrophoresis on agarose gel and read results: prepare a reaction mixture consisting of 4 µL DNA, 1 µL distilled water, 1 µL $FeSO_4$, and 1 µL H_2O_2, respectively. Incubate the mixture at room temperature for 10 min. Add 1 µL of EDTA (0.5M) and conduct electrophoresis on 1% agarose gel for 20 min with 100V current. Gel results are projected under a UV lamp projector with integrated VisionCapt gel image analysis software.
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2.9. Statistical analysis

The results are expressed as MEAN ± SD. All experiments were repeated 3 times on the same sample, and the resulting data were processed using GraphPad Prism statistical software (ver 8.4.3). The statistics used are ANOVA One-way and ANOVA two-way. The test results reached statistical significance $P < 0.05$.

Results and Discussion

3.1. Cell viability assay

To investigate the toxicity or viability of RAW 264.7 cells under the effect of samples to evaluate the activity of compounds in the sample. The results of RAW 264.7 cells after treatment with EtOH (control) and EA fractions of *I. tenuipes* biomass are shown in the following Table 1:

Conc (µg/mL)	0	25	50	100	200
EtOH	100	81.61 ± 8.75^{a}	81.59 ± 5.39^{a}	76.42 ± 4.65^{ab}	60.41 ± 1.49^{b}
EA	100	102.10 ± 1.05^{a}	96.44 ± 1.04^{b}	91.64 ± 2.89^{b}	68.64 ± 4.77^{c}

Table 1 shows that RAW 264.7 cells have a survival rate of 76.42% at **an EtOH** extraction concentration of 100 µg/mL and 91.64% at an EA extraction concentration of 100 µg/mL. Therefore, we chose the extract concentration to treat RAW 264.7 cells from 0 to 100 µg/mL for the following experiments.

3.2. Cell membrane proteins oxidation assay

The destruction of proteins by free radicals causes aging and other diseases. This can be produced by electron loss, metal ion-dependent reactions, and protein oxidation. In this investigation, the ability to protect membrane proteins was based on the product of protein oxidation, reactive carbonyl radical's guanidine hydrochloride, and determined through optical density (Oliver et al., 1987). The survey results are represented by the IC_{50} value (minimum sample concentration capable of protecting membrane proteins) presented in Table 2.

Sample	EtOH	EA	BHA
IC_{50} (µg/mL)	146.089 ± 7.965^{a}	83.112 ± 5.137^{b}	9.511 ± 0.574^{c}

Table 2 shows that EA extraction has IC_{50} values lower than EtOH extraction, with IC_{50} are 83.112 ± 5.137 µg/mL and 146.089 ± 7.965 µg/mL, respectively. Accordingly, EA extraction can better protect membrane protein in RAW 264.7 cells than EtOH extraction. The reason for these results is that EA extraction is a fraction extract that has a lot of secondary metabolites and showed antioxidant activity. And EA extraction maybe not contain some secondary metabolites that can inhibit binding in protein on cells membrane like EtOH extraction. In addition, EA extraction maybe contains some phytochemicals like flavonoids, polyphenols, etc. which has a strong effect on the antioxidant activity of mushroom (Yadav et al., 2014).

3.3. Cell membrane lipid oxidation assay

The main consequence of free radical formation is oxidative damage to cellular components, including cell membranes made of lipids. It causes so many different pathologies in humans. Therefore, the inhibition of lipid peroxidation (lipid oxidation) is considered a potentially important antioxidant activity. In this experiment, the lipid peroxidative products bind Thiobarbituric acid (TBA) and appear in color. Its color of it was determined photometrically. The experimental results are shown by the IC_{50} values (minimum sample concentration capable of protecting cell membrane) in Table 3

Sample	EtOH	EA	BHA
IC_{50} (µg/mL)	140.202 ± 7.444^{a}	40.179 ± 0.837^{b}	11.59 ± 0.17^{c}

Table 3 shows that EA extraction has an IC_{50} value lower than EtOH extraction, which is 40.179 ± 0.837

µg/mL and 140.202 ± 7.444 µg/mL, respectively. Therefore, EA fraction extraction is more effective in inhibiting lipid peroxidation than EtOH extraction. In addition, some plant metabolites separated according to solvent polarity showed lipid peroxidation inhibitory better than metabolites from total extraction.

3.4. Determination of radical-mediated DNA damage

The free radicals, oxidative stress, and oxidizing agents can destroy DNA molecules, affecting human health. In this survey, the sample extraction shows the ability to capture free OH radicals according to Fenton's reaction, which is followed by Milne's procedure (Milne et al., 1993). The results are shown in Fig. 1 and Fig. 2. The results of agarose gel electrophoresis for DNA of RAW 264.7 (Fig 1) showed that the brightness of DNA bands gradually darkened with the increase of extract concentrations.

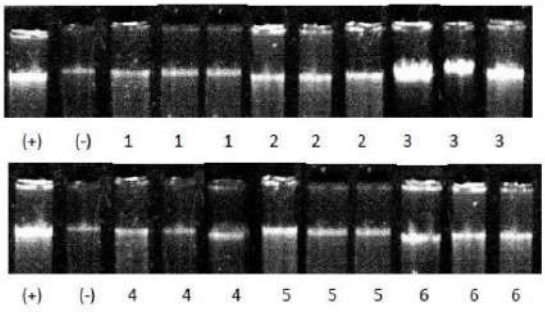

Fig. 1: Agarose gel electrophoresis for DNA of RAW 264.7 after treatment with *Isaria tenuipes* biomass extracts[2]. Lane (1) to (3) are EA extract in 25, 50, 100 µg/mL, (4) to (6) are EtOH extract in 25, 50, 100 µg/mL, (+) is blank sample and (-) is negative

Therefore, the structure of DNA molecules is not destroyed when the extract concentration increases gradually from 25 to 100 µg/mL. At 100 µg/mL, DNA of RAW 264.7 after treating EA extract had the clearest band (Fig 1), and EA extract protected 97.454 ± 0.308% of DNA (Fig 2). It increased by 59.632% compared to the negative control, respectively. EtOH extraction only protected 67.104 ± 0.308% of DNA, which means that only increased by 39.282% compared to the negative control, respectively.

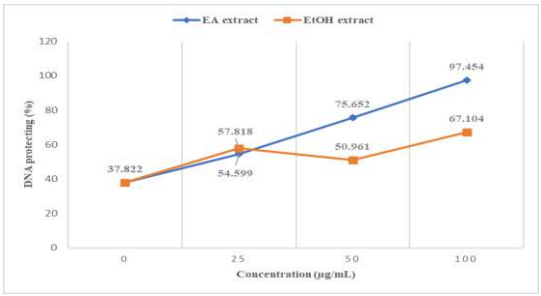

Fig. 2: Percentage of protecting DNA RAW 264.7 cells after treatment with *Isaria tenuipes* biomass extracts in concentration from 25 to 100 µg/mL[11]. Blue line is EA extract and orange line is EtOH extract.

When evaluating the DNA of RAW 264.7 cells protection while increasing the sample concentration (Fig 2), it showed that only the EA extract has a percentage (%) of DNA protection that increases steadily with the gradual increase of extract concentrations. In the EtOH extract, the DNA protection rate tended to decrease while increasing concentration from 25 to 50 µg/mL, but it rose again when the concentration was up to 100 µg/mL. These results show that the DNA protection rate is only dependent on the concentration of EA extracts and independent of EtOH extracts. The reason may be that the EA extract contains many secondary metabolites that have bioactivities, and it maybe doesn't contain some inhibitory compounds like EtOH extract. According to Chhetri (2020), *I. tenuipes* biomass contains many polyphenols (Chhetri et al., 2020), and following (Zhang et al., 2019) that EA extract from *I. tenuipes* biomass contains N6-(2-hydroxyethyl) adenosine (Zheng et al., 2008). These compounds have been to increase the stimulation of antioxidant enzymes, reduce lipid peroxidation damage and protect DNA from damage (Yadav et al., 2014; Wang et al., 2019). In addition, *I. tenuipes* biomass contains some phytochemicals showing potential bioactivities on apoptosis or the cytotoxicity of cancer cell lines. For example, Chhetri et al. (2020) showed that *I. tenuipes* mycelium extract has a good effect on the cytotoxicity of HeLa cells (43.45 µg/mL), PC3 cells (119.33 µg/mL) and HepG2 cells (125.55 µg/mL) by MTT assay, respectively (Chhetri et al., 2020).

Conclusion

The results of the evaluation of protecting RAW 264.7 cell membrane after treatment with some extracts from *Isaria tenuipes* VHI-2 biomass showed that the ethyl acetate (EA) fraction extract could protect the cell membrane higher than the ethanol (EtOH) total extract. Therefore, EA extract from *I. tenuipes* VHI-2 biomass is a potential extract containing many secondary metabolites with many bioactivities. Based on these results and the purpose of clarifying some other bioactivities of *I. tenuipes* biomass, we need to quantify the content of compounds present in the EA extract and carry out experiments on clinical.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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Papain Hydrolysates of Rice Bran and Soybean Flour Promote Bioethanol Fermentation

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Abstract

The usage of biofuel as a potential alternative source for fossil fuels has gained significant importance in the last two decades. Bio-ethanol, an ideal bio-fuel could be used to reduce the total contribution of greenhouse gas from the atmosphere. Agricultural byproducts can be used for the production of bioethanol. In this study, rice bran and soybean flour were used as fermentation accelerators to produce ethanol. This study demonstrated that pre-hydrolysis of rice bran or soybean flour with papain promotes alcoholic fermentation. The alcohol fermentation-promoting effect was proportional to the free amino acid concentration due to digestion by papain. It was shown that the amount of these hydrolysates added was limited to 2%. Since rice bran had the same effect as soybean flour, effective use of rice bran is expected. These effects were greater than the effects of adding magnesium and phosphoric acid.

Keywords: Rice bran, soybean flour, papain, bioethanol,

1. Introduction

Bioethanol is expected as one of the sustainable energy sources in the future. Therefore, the research is progressing on using the industrial waste of agricultural products as a raw material for bioethanol instead of using edible agricultural products (Moodley et al., 2023). However, 94% of the total bioethanol production in the United States was based on corn starch in 2019. Brazil, on the other hand, mainly obtains bioethanol (99% of the total production) from sugarcane (Ray and Ramachandran, 2018). In the EU, bioethanol is produced from different feedstock including wheat (18.7%), maize (19.6%), sugar beet (57.9%), barley (1.9%), and rye (1.9%) (Duque et al., 2021).

Bioethanol production should be readily available, low-cost, and environment-friendly. The materials used for the production of bioethanol are desirable to contain both the sugars that are converted to ethanol and the nutrient sources necessary for the growth of yeast. Molasses meets the above requirements and is used in alcoholic fermentation all over the world. On the other hand, the sugars supplied by acid hydrolysis and the enzymatic decomposition of lignocellulosic wastes such as rice straw, wheat straw, corn straw, and bagasse (Mohanty, and Abdullahi, 2016; Dragone, et al., 2004; Binod, et al.,2010; Talebnia, et al., 2010 and Li et al., 2010) have a little amount of nutrients required for yeast cell growth, and additional nutrients are required for alcoholic fermentation. For example, rice bran was evaluated as a nutrient source or energy source for alcoholic fermentation of low sugar (Moreira et al., 2019) or lignocellulosic waste such as rice straw (Mochidzuki et al., 2015).

Rice cultivation is popular in Bangladesh, and rice bran is used as feed for livestock. In this experiment, the effect of rice bran and the hydrolyzate of rice bran on alcohol fermentation was investigated. As a comparison, soybean flour was used. The reason is that soybean peptone, which is a hydrolyzate of soybean, has been widely used as a component of the microbial medium.

2. Biomass and Microorganisms for Bioethanol Production

Researchers are always looking to produce a high yield of bioethanol in a cost-effective manner using a low-cost substrate to meet global demands. Ethanol production is currently being carried out using grain crops- maize, corn, sugar crops: sugarcane, sugar beet(Ray and Ramachandran, 2018), lignocellulosic hydrolysate of plants (Ray, 2021), and agricultural residues (Moodley et al., 2023) or using certain industrial byproducts such as molasses (Behera and Ray 2012), wine substrates, whey, waste sulfite liquor (Ayas, 2018, Rudolf et al., 2009).

Based on the feedstock and processing techniques the biofuel productions are classified into 1st to 4th generations (Mohapatra et al., 2018). In 1st generation, biofuels are produced from edible sugar and starch-containing food crops. In the production of first-generation biofuel, the sugar-containing feedstocks are treated with washing, crushing, and extraction of sugar juice before going through the fermentation process by microbes. The feedstock composed of starch such as corn or wheat requires an additional step, enzymatic hydrolysis and saccharification to yield fermentable glucose (Pascault and Fuertes, 2012). In 2nd generation, of bioethanol production different lingo-cellulosic substrates such as cereal

straw, sugarcane bagasse, and forest residues are used (Behera et al., 2012). These feedstocks require physical, chemical, and biological pretreatment before fermentation. In 3rd and 4th generation bioethanol, algae and other microbes are used as substrates (Dutta et al. 2014). Liquid first-generation ethanol production from food crops is limited due to increased world food demand and high production and processing costs (Ray and Ramachandran, 2018). Moreover, only traditional crops are unable to meet the global demand for bioethanol (Gupta and Verma, 2015). To mitigate these challenges, research should be focused on the production of bioethanol from waste biomass (Behera et al., 2019).

Food loss and food waste biomass are rich sources of carbon, nitrogen, and minerals (Bancal and Ray, 2022; Ray, 2022). Thus these can be used for the production of bioethanol. Agricultural wastes such as rice straw, rice husk, corn stove, leaves, roots, stem, and lost agricultural crops can be used as the substrate for the value-added production of bioethanol. Generally, agricultural residues are composed of lignin, cellulose, hemicellulose, and starch, but they contain vitamins, bioactive compounds, and minerals also. Sometimes the industrial process of agricultural products produces sugar-rich byproducts. For example, molasses and watermelon juice waste are produced by the sugar and juice industries, respectively. Molasses is a highly rich source of sucrose, fructose, and glucose and it is the most prominent agro-industrial residue for the production of bioethanol (Behera and Ray, 2012). Despite of high sugar concentration molasses is low in nitrogen content (Salakkam et al., 2017). The watermelon juice waste also contains fermentable sugars and free amino acids and it could be used as an additive to the molasses in ethanol fermentation (Bibra et al., 2022).

Recently, studies are focused on the technical advantages of bioconversion of agricultural residues derived from cereal crops to bioethanol (Jeevan Kumar et al., 2020). Among the agricultural residues, rice husk is produced in huge amounts in rice-cultivating countries like China, India, Bangladesh, Brazil, the US, Cambodia, Vietnam, Myanmar, and South East Asia (Pode, 2016). Tentatively 10 million tons of rice husk is produced annually in Bangladesh (Ahiduzzaman and Islam, 2016), but this large amount of rice husk is not converted into value-added products. This rice husk is composed of lignocellulosic materials and can be evaluated for the production of bioethanol (Madu et al., 2018).

Bio-ethanol productions from the lignocellulosic materials have several challenges and limitations such as biomass transport and handling and efficient pretreatment for removing the lignin. These pretreatments- physical (Moodley and Trois, 2021), chemical (de Jong and Gosselink, 2014), physicochemical (Jamaldheen et al., 2022) and biological pretreatments have been used for lignin degradation and to make the complex polymer into fermentable sugars. In biological pretreatments, either lignolytic microorganisms or enzymes are used. Lignin removal is conducted by growing microbes into the lignocellulosic biomass or using mixed enzymes. Biological pretreatment requires longer incubation time which could be overcome using potent microbes (Ummalyma et al., 2019). White rot fungi are extensively studied for delignification (Jeevan Kumar et al., 2020) and enhanced degradation of lignin could be achieved by a consortium of white rot fungi cultivation (Cui et al., 2021).

After pretreatment, saccharification is mandatory in which step the cellulolytic microorganisms or enzymes convert the lignin-free cellulosic materials into fermentable sugars. The cellulolytic enzymes are produced by some bacteria such as *Bacillus* spp. (Orencio-Trejo et al., 2016) , *Clostridium thermocellum* (An et al., 2021), and *Cellulomonas* spp. *(*González-Bautista et al., 2017)and fungi such as *Rhizopus oligosporus*, *Penicillium funiculosum, Trichoderma viride, T. reesei, Aspergillus niger etc.* Several studies reported simultaneous saccharification and fermentation for the production of high-yield bioethanol (Jeevan Kumar et al., 2020). The glucose produced from the saccharification of cellulosic, hemicellulosic, and starch materials is fermented into ethanol by the metabolic activity of *Saccharomyces cerevisiae*, *Zymomonas mobilis*, or *Mucoralean. S. cerevisiae* can utilize different forms of fermentable sugar as the carbon source under a strict anaerobic environment (Kannah et al., 2020).

Recently, researchers studied for selection of potential microbes and genetically modified them for a higher yield of bio-ethanol. Some special or genetically engineered microbes are required to ferment complex carbohydrate sources. Advanced technologies such as metabolic engineering or genome editing can proceed with economic bioprocess for the production of bioethanol. Researchers have been successful in the development of genetically engineered *Z. mobilis*, *Escherichia coli*, and Klebsiella *oxytoca*. *Z. mobilis* can ferment glucose and fructose to produce a high amount of bioethanol (Dien et al., 2003; Behera et al., 2010).

3. Bioethanol Fermentation Using Rice Bran and Soybean Flour as Organic Nitrogen Source

Production of ethanol from agro-industrial residues is not always economically sustainable due to lower yield and the requirement of treatments which in turn make the production process expensive. On the other hand, chemical treatment may contribute to environmental pollution. Therefore, researchers always prefer biological treatments with microbes or enzymes. Fermentable biomass requires additional nitrogen sources such as yeast extract and ammonium sulfate, but this makes the production process more expensive. Therefore, a readily available and inexpensive nitrogen source is very essential for the economic production of bioethanol (Salakkam et al., 2017). Food waste with high protein ingredients can be used as an alternative to expensive nitrogen sources in the bioprocessing of ethanol (Bibra et al., 2022). In the present study, rice bran and soybean flour were evaluated as organic nitrogen sources for the yeast

Saccharomyces cerevisiae for the production of bioethanol.

3.1 Materials and Methods

3.1.1. Yeast strain and growth conditions

S.cerevisiae ATCC26422 (Kyokai No.7)was grown in Yeast extract Peptone Dextrose (YPD) medium (2% glucose, 1% peptone, and 0.5% yeast extract) at 30°C. Yeast cells were washed twice with water by centrifugation after 24 h cultivation. The pellet of yeast cells was suspended in water, and the cell density was adjusted to wet weight 100mg/ml. The suspended yeast cells were used for alcoholic fermentation.

3.1.2 Alcoholic fermentation

Sucrose, yeast extracts, rice bran, or soy flour were mixed with water, and the volume and pH were adjusted to 30ml and 6.0, respectively. A 30 ml culture solution composed of sucrose, rice bran, or soy flour contained in a 50 ml Erlenmyer flask was inoculated with the yeast cells and allowed to stand at 30°C. This culture solution was lightly agitated every 2 h.

Preparation of rice bran and soy flour hydrolyzed with papain

The pH of the 20% (w/v) suspension of rice bran or soy flour in water was adjusted to 6.7 with NaOH. Protein hydrolysis was performed by the addition of 0.2% papain (w/v) at 50°C for 2 h, and the mixture was boiled for 15 min. The hydrolyzed rice bran or soy flour was used at 1% or 2% (w/v) for alcoholic fermentation.

3.1.3. Determination of peptides(Moore, 1968)

After centrifugation of 20% rice bran or soy flour suspension at 10,000g for 3 min, the supernatant was used for the determination of peptides by ninhydrin reaction as follows. The supernatant was diluted 1000 folds with pure water, and 0.5 ml of the diluted solution was mixed with 0.375 ml of ninhydrin solution composed of 2% ninhydrin and 0.3% hydrindantin in 75% dimethyl sulfoxide and 25% 4M acetate buffer (pH 5.2). The mixture was heated at 80°C for 30 min. After cooling the mixture, 0.5 ml of 50% ethanol was added, and the absorbance at 570 nm was determined. The concentrations of free amino acids and peptides were calculated based on glycine.

3.1.4. Determination of ethanol (Yamashoji et al., 2020)

The alcohol concentration of the fermentation broth was measured by an enzymatic reaction. Each fermentation broth was diluted 1000 folds with water, and 0.5 ml of the diluted solution was mixed with 0.5ml buffer, 0.1 ml of an aqueous solution of NAD (6mg/ml), 0.1 ml of 0.1% thiazolyl blue tetrazolium bromide (MTT) and 0.001% 1-methoxy-5-methylphenazinium methyl-sulfate (1-methoxy PMS) solution and 0.01ml of 1% Bovine serum albumin and alcohol dehydrogenase (300U/ml) in 0.1 M potassium phosphate buffer (pH 7). The buffer contained 10g $Na_2P_2O_2.10H_2O$, and 2.5g semicarbazide. HCl, and 0.5g glycine in 300 ml water. After the incubation of the mixture at 30°C for 15 min, the absorbance at 570 nm was determined. The concentration of ethanol in the diluted solution was calculated based on the absorbance of the standard ethanol solution from 0 to 0.0 2%.

The absorbance of blank and 0.02% ethanol was approximately 0.3 and 1.3, respectively. From the blank to 0.02% ethanol, the absorbance increased linearly.

3.1.5. Chemicals and reagents

All the chemicals were purchased from FUJIFILM Wako Pure Chemical Corporation, Japan.

3.1.6. Statistical analysis

The mean and the standard deviation were calculated by using Microsoft Office Excel.

3.2. Results

Fig. 1 shows the progress of ethanol fermentation for 5 days. In the presence of 2% yeast extract, 14% alcohol was produced after 5 days. In the case of 2% rice bran, 11% alcohol was produced. The addition of 0.15% $(NH_4)_2SO_4$, 0.05% $MgSO_4$, and 0.05% KH_2PO_4, to 2% rice bran did not show any further increase in alcohol production.

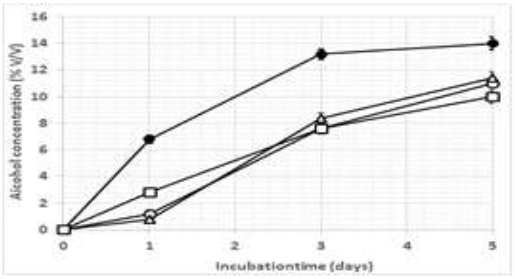

Fig. 1. Effect of rice bran on alcoholic fermentation. ● 2% yeast extract, ○ 2% rice bran, □ 2% rice bran, 0.05% $MgSO_4$ and 0.05% KH_2PO_4, □ 2% rice bran, 0.15% $(NH_4)_2SO_4$, 0.05% $MgSO_4$ and 0.05% KH_2PO_4. Each value is the mean of three different determinations, and the small bars represent the standard deviation.

On the other hand, the addition of 2% soy flour produced only 2.4% alcohol after 5 days as shown in Fig.2. The additional addition of 0.05% $MgSO_4$ and 0.05% KH_2PO_4 produced 3% alcohol production. The additional addition of 0.05% $MgSO_4$, 0.05% KH_2PO_4, and 0.15% $(NH_4)_2SO_4$ produced 12% alcohol. These facts suggest that $MgSO_4$ and KH_2PO_4have little effect on alcohol fermentation in the presence of rice bran or soy flour and that $(NH_4)_2SO_4$ supplements the nitrogen source of soybean to promote alcohol fermentation.

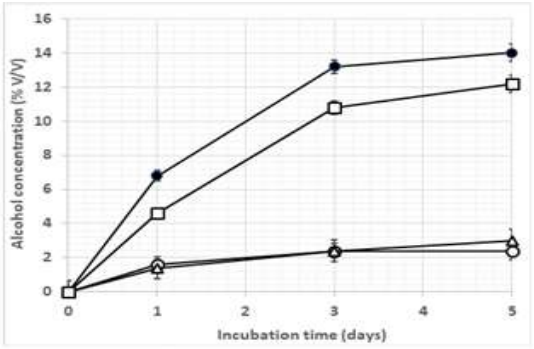

Fig. 2. Effect of soy flour on alcoholic fermentation. ● 2% yeast extract, ○2% soy flour, △2% soy flour, 0.05% MgSO4 and 0.05% KH2PO4, □ 2% soy flour, 0.15% (NH4)2SO4, 0.05% MgSO4 and 0.05% KH2PO4.. Each value is the mean of three different determinations, and the small bars represent the standard deviation.

Fig.3 shows that 2% rice bran and 2% soy flour hydrolyzed with papain promote alcohol fermentation. The hydrolysate of 2% rice bran induced a 3.2% higher alcohol concentration than 2% rice bran. On the other hand, the 2% soybean flour hydrolysate induced an 11.6% higher alcohol concentration than 2% soybean flour. In each case, the maximum alcohol concentration after 5 days was about 13% to 14%. Since it was difficult for the addition of 2% or more to bring about a higher alcohol concentration (data not shown), the addition concentration of 2% is considered to be appropriate.

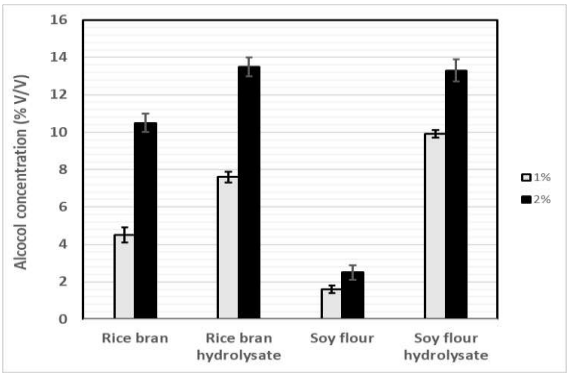

Fig.3. Alcoholic fermentation promoted by papain-hydrolyzed rice bran and soy flour. Large bars and small bars represent the mean and the standard deviation, respectively. Each value is the mean of three different determinations.

Fig.4 shows the concentration of ninhydrin-positive substances based on glycine before and after hydrolysis by papain. Ninhydrin-positive substances in 1% rice bran suspension increased 3 folds after the hydrolysis by papain, and those in 1% soy flour increased 6 folds. As ninhydrin reaction-positive substances include the substance having an amino group in addition to peptides, the concentration of peptides produced by papain digestion was estimated to be approximately 2 mM for 1% rice bran and 1.5 mM for 1% soy flour. On the other hand, 1% yeast extract was estimated to be 32.5 mM (data not shown).

Comparing Figs. 3 and 4, there seems to be a correlation between the concentration of ninhydrin-positive substances and the alcohol concentration, but there was no proportional relationship. This suggests that when the ninhydrin-positive substances reach some extent, alcohol fermentation reaches the upper limit, or substances other than ninhydrin-positive substances control alcohol fermentation.

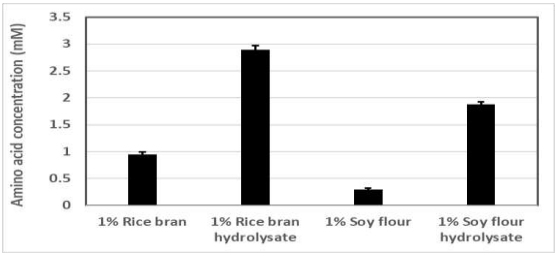

Fig.4. Ninhydrin-positive substances of rice bran and soy flour hydrolyzed with papain. Large bars and small bars represent the mean and the standard deviation, respectively. Each value is the mean of three different determinations.

3.3. Discussion

Magnesium, phosphorus, and ammonium sulfate have been used for the alcoholic fermentation of molasses, but the role and effect of these compounds are not clarified (Fadel, et al., 2013; Gu, et al., 2001 and Darvishi et al., 2019). In this study, it was demonstrated that the addition of magnesium and phosphorus had little effect on alcoholic fermentation in the presence of rice bran or soy flour. Magnesium and phosphorus contained in rice bran or soybean flour might contribute to the promotion of alcoholic fermentation as rice bran contains 0.85% $MgSO_4$ and 2% KH_2PO_4, and soy flour contains 0.3% $MgSO_4$and 0.7% KH_2PO_4 (Gancedo and Serrano, 1989; Boulton, et al., 2013; Pretorius et al., 2003; Blackwell et al., 1997 and Walker, 1994). *S. cerevisiae* utilizes amino acids and peptides (Duc, 2020) as nitrogen source. A recent study revealed that the greater consumption of glutamate-rich peptides by Fot1-2 entailed a remodeling of the central carbon and nitrogen metabolism pathways (Marsit et al., 2016). Free aspartic acid and glutamic acid were also reported to promote biomass production and the exponential growth rate and to shorten the lag phase and the fermentation time in alcoholic fermentation (Fairbairn et al., 2017). Aspartic acid, glutamic acid, and glutamate-rich peptides (Becerra-Rodríguez et al., 2020) are contained in papain-hydrolyzed soy flour. For example, soybean meal contains 6% aspartic acid and 9.45% glutamic acid. On the other hand, rice bran contains 1.25% aspartic acid and 1.97% glutamic acid (Kaufmann et al., 2005). These reports might support the fact that 1% soy flour hydrolysate promotes alcohol fermentation more effectively than 1% rice bran hydrolysate despite having less ninhydrin-positive substances in soybean flour hydrolysates than rice bran hydrolysates as shown in Figs. 3 and 4. Since the 2% rice bran hydrolysate induces a similar alcohol fermentation-promoting effect as the 2% soybean flour hydrolysate, it is preferable to use rice bran, which is a livestock feed, rather than edible soybean flour. Since Bangladesh is a major rice-producing area, the effective use of rice bran is an important issue, and for this reason, the use of rice bran for the

alcoholic fermentation industry is expected to contribute to a sustainable local industry.

Declaration

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Computer Simulated Molecular Docking Model: Anticancer, Anti-Sarcovid-19, Glucosidase Enzyme Inhibiton, And Admet Properties of 1,3-Thiazole Derivatives

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Abstract
The analogue 1,3-thiazole compounds synthesis showed high cytotoxicity against human breast, lung, and colon cancer cell lines. As their high cytotoxicity properties *in vitro*, we continuously performed in *silico* molecular docking models such as anticancer, anti-Sarcovid-19, and glucosidase enzyme inhibition activities of entries 3a-3j. The calculation results indicated that **3d** is the best candidate among the synthesized chemicals according to its strong inhibition against cancer cell lines *via* the enzyme inhibition mechanism, topoisomerase I (top I), ***1T8I****: PDB*. For antivirus *in silico*, all entries 3a-3j were calculated for their docking characteristics and entry **3f** strongly inhibited the crystal structure of COVID-19 main protease, **6LU7.** With α-glucosidase enzyme **4J5T** for anti-diabetic activity *in silico*, compound **3g** inhibited well from this enzyme among entries 3a-3j. Protein-ligand (position no.81) was observed to have the highest (negative) binding energy against Human DNA Topoisomerase (PDB: 1T8I) and was subjected to molecular dynamics (MD) simulation assessment. A 100 ns MD simulation was also undertaken to optimize the interaction, forecast complex flexibility, and assess the stability of the protein-ligand (**3g**) complexin real environment by Desmond sofware in LInux, which is stable.

Keywords: 1,3-thiazole, in *silico* molecular docking model**,** anticancer, Coronavirus, α-glucosidase enzyme

1. Introduction

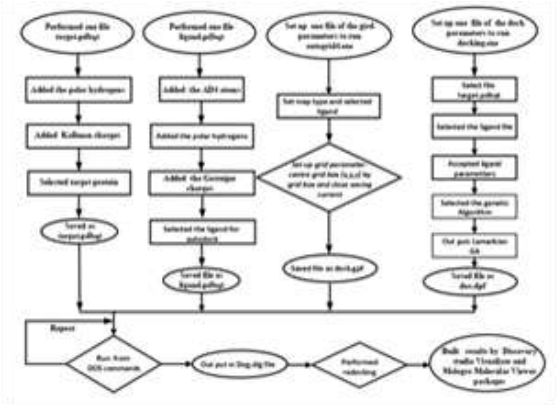
Heterocyclic compounds are famous for their notable uses in several industries, namely pharmaceuticals, agrochemicals, fine and bulk chemicals, additionally, ligands for catalysis (De Siqueira et al., 2019; Ayati et al., 2019; Scarim et al., 2019). Thiazole is the class of 5-membered heterocyclic compounds that contained sulfur and nitrogen atoms in their structures, they play a significant role in most biochemistry activities and are used to apply in clinical anticancer drugs, for instance, dasatinib, dabrafenib, ixabepilone, patellamide A and epothilone. They highly inhibit polymerase and affect the cell cycle, proving their potential anticancer activity and toxicity reduction (Sharma et al., 2020). The typical activities of the nucleus in thiazole were reported in the literature for its antibacterial activity (Bondock et al., 2013; Wagle et al., 2008; Liaras et al., 2011), anticancer (El-Subbagh and Al-Obaid, 1996; Dawood et al., 2013; Rabow et al., 2002; Mura et al., 2010), antifungal activity (An et al., 2019; Schmidt et al., 2012; Cukurovali et al., 2006; Wang et al., 2010), anticonvulsant (Siddiqui and Ahsan, 2010; Harish et al., 2013; Pandeya et al., 1998), antimycobacterial activity and in *silico* docking model (Hublikar et al., 2020), recently, antioxidant activity and in silico docking model (Hossan., 2020). Although they have been reported in a small number of studies, they have been shown to bind the -glucosidase enzyme or vascular adhesion protein-1 (VAP-1) inhibitor in the treatment of diabetes (Inoue et al., 2013). The synthesis methods of these heterocyclic substituted- thiazoles had been performed former in an easy approach, as known as Hantzschthiazole synthesis (Wang., 2010) and based on Hantzsch strategies such as (Elkanzi., 2018; An and Lee, 2013; Das et al., 2006; Bono and Vedula., 2016; Zavozin et al., 2013; Salem et al., 2019; Helal et al., 2013; Wagle et al., 2008; Giri et al., 2009). These bioactivities of substituted thiazoles were considered to be outstanding among others, but the predictable models explain to Quantitative Structure Activity Relationship (QSAR) of substituted thiazole compounds, which proved potential FabH inhibitors to account for antibacterial activity in *silico* docking models (Li et al., 2014), only appeared in a few articles for this group. Some typical QSAR modelings predicted its well-antifungal activity by conducting Hologram QSAR (HQSAR), Comparative molecular field analysis (CoMFA), Molecular similarity indices in a comparative analysis (CoMSIA), and Receiver operating characteristic (ROC) model to verify the predictability of models (Lino et al., 2018). In *silico* docking model and pharmaceutically relevant properties (ADMET) were expected to have anticancer and antifungal activities driven by substituted thiazoles (Sangshetti et al., 2014; Park et al., 2011). Many powerful molecular docking packages were approached to discover drugs based on their structures (Park et al.,

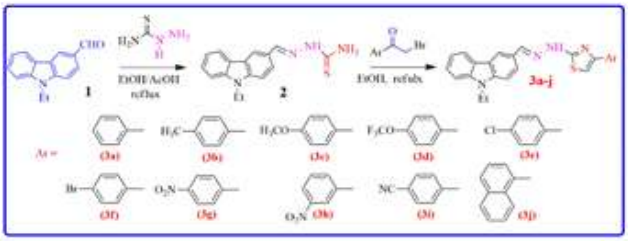
2011; Meng et al., 2011; Kostal, 2016). Among strategic solutions, the Auto Dock package had been recognized as a significant tool to explain why ligand shows high biochemical activity in *silico*. Additionally, it uses used the Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function that give the prediction for the bound conformations of flexible ligands to macromolecular targets. It is designed and tested in connection with a new scoring function that anticipated the change in free energy upon binding (Hachem et al., 2016; Huey et al., 2012; Morris., 2010) and as a result, it has been successfully employed in explaining highly bioactive molecules using an in silico docking model (Thuy et al., 2020; My et al., 2020; Dhameja and Gupta, 2019; Sudileti et al., 2019; Nhan et al., 2017; Gomha et al., 2016; Brancolini et al., 2012; Revie et al., 2018). In *silico* many docking, models have performed their bioactivities perfectly such as mushroom tyrosinase of thiosemicarbazone for melanogenesis inhibition (Hałdys et al., 2018), antioxidant, anti-inflammatory, and antibacterial that affect the human peroxiredoxin 5 (PDB code: 1HD2), phosphodiesterase 4 (PDE4) (4WCU), and Gluco-samine-6-phosphate synthase (2VF5) (Eze et al., 2019), α-glucosidase enzyme inhibition (Wang et al., 2017; Trang et al., 2021), tubulin polymerization (Salehi et al., 2013) or Human topoisomerase Iinhibition (Staker et al., 2005) for anticancer, and FabH inhibitors for antibacterial activity (Uwabagira et al., 2019). Due to the interesting anticancer activity of substituted-1,3-thiazole heterocyclic compounds that being mentioned in some of our prior researches (Kumar et al., 2015; An et al., 2020; An et al., 2014) we had concluded that the derivative thiazoles showed high anticancer activity (De Siqueira et al., 2019) and many interesting mechanism types (Ayati et al., 2019; Shirai et al., 2021), *in vitro*, which leads us to aim the in *silico* docking model to account for anticancer, antioxidant activities based on mechanism and predicted antivirus and glucosidase enzyme inhibition.

2. Materials and Methods

The carbazole analogue 1,3-thiazole molecules were synthesised, tested for bioactivity, and described in our earlier publication (An et al., 2015). In addition, in *silico* docking model was investigated and illustrated in Fig. 1. The target compounds of 1,3-thiazole heterocyclic compounds, **3d**-**3g** were synthesized and indicated the highest anticancer *in vitro* have been continuously implemented *in silico* molecular docking model. The AutoDockTools–1.5.6rc3 package was used to predict dockings of one ligand to target its protein or enzyme. For supporting information, the crystal structures of receptors were collected from the Protein data bank and validated. The active centers of enzyme or protein targets had been identified in the article (Khan et al., 2020), additionally, ligand or pose anchored to one target protein by the same grid parameter that assessed the validation of the used method via RMSD values (Bell and Zhang, 2019). For anticancer property analysis, the crystal structure of topoisomerase I (top 1), code 1T8I: PDB was used to process docking and explain anticancer activity via top1 enzyme inhibition mechanism in *silico* docking and the grid parameter were detected as X=22.439, Y= 0.576, Z = 30.278, grid point spacing of 0.375 Å, and the number of user-specified grid Points of (X= 60, Y= 60, and Z=60) (Staker et al., 2005; Shirai et al., 2021; Warren et al., 2006). Additionally, the crystal structures of the COVID-19 main protease, code 6LU7 represent antivirus characteristics, they were applied and installed the grid parameters as X= -26.075, Y=12.576, Z= 59.145, grid point spacing of 0.375 Å, and the number of user-specified grid Points of (X= 60, Y= 60, and Z=60). For α-glucosidase enzyme inhibition, the crystal structure of the enzyme, 4J5T was used and detected for their central grid box in the values of (X= -18.524, Y=-20.732and Z= 8.202), grid point spacing of 0.5 Å, and the number of user-specified grid Points of (X= 60, Y= 60, and Z=60) (Ngoc et al., 2021). In *silico*, a receptor (PDB file) deleted small molecules, for instance, water molecules, small ligands, and heteroatoms, the crystal structure were then saved in a format file (receptor. pdb), which were conducted by Discovery Studio 2019 Client (DSC) package. For ligand, it optimized conformation energy by conducting the Molecular Mechanics & Force Fields (MMFF94) method, performed by the Avogadro package. The optimal conformation of the ligand was selected for docking calculation. AutoDock 4.2 package had the purpose of calculating the target (receptor) and ligand (entries, active thiazole compounds *in vitro*). For target protein/enzyme preparation, the polarized hydrogens and Kollman charges were added to all atoms. For docking pose or ligand, it was filled with polarized hydrogens, computed Gasteiger charges, merged non-polarized hydrogens, and then saved in pdbqt format. One ligand's conformation is linked to a target or receptor by a Lamarckian genetic algorithm method. The maximum negative value of free energy of binding was selected corresponding to the most stable conformation ligand (pose) after 2500000 energy evaluations and run 200 times for dockings. Discovery Studio 2021 Client and Molegro (MMV) packages had taken part to visualize and present the results in the forms of tables and Figs. Molecular dynamics (MD) simulation of the best-docked pose of protein-ligand (pose no#81) was executed using GROMOS96 43a1 forcefield (Chiu et al., 2009; Van Der Spoel et al., 2005). PRODRG tool (Schüttelkopf and Van Aalten, 2004) was used to get the compound (**3g**) topology. For the best-docked pose of the protein-ligand complex, the simple point charge (SPC) was chosen as a solvent model (tri-clinical water box size 50*75*70Å). The system set for MD simulation was neutralized with sodium or chlorine ions based on the total charges. The steepest descent algorithm (5000 steps) was used to minimize the system before conducting the MD simulation. The MD simulations were then run with 0.15 M NaCl in the presence of a constant temperature

(300 K) and pressure (1.0 bar). The number of frames in each simulation was estimated to be around 5000. 100 ns-MD simulation was chosen as the simulation time. The simulation trajectories obtained from MD simulation were used to calculating some parameters such as the root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), hydrogen bonds, solvent-accessible surface area (SASA), and radius of gyration (Rg) values to achieve a better understanding about the stability of the protein-ligand complex.





Scheme 2. The synthesis 2,4-subtituted -1,3-thiazole (**3a-3j**).

Scheme 1. The general procedure performed docking of an optimal ligand to a receptor.

3. Results and Discussions

In vitro cytotoxicity by MTT assay: As seen in Fig. 1, entries **3e-3g** inhibited highly against human lung cancer cell line A549 at concentrations of 5 or 10 µM. Among them, entry **3f** exposed the highest activity against human lung cancer cell lines at both 5 and 10 µM, meanwhile, other entries showed the least inhibition at most of the concentrations. As shown in Fig. 2, entries **3d**, **3f-3g** indicated high inhibitions against human breast cancer cell line MCF-7 among test entries 3a-3j. Among active entries, entry **3g** has the greatest inhibition against MCF-7 at both concentrations, 5 and 10 µM. Whilst, others revealed less inhibition against human cancer cell line MC7-7.

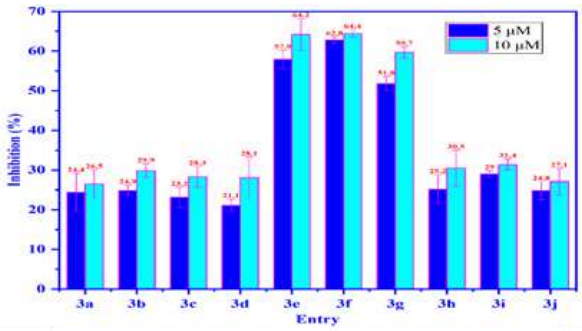


Fig. 1. The ability of entries **3a-3j inhibited** against human lung cancer cell line A549 by MTT assay.

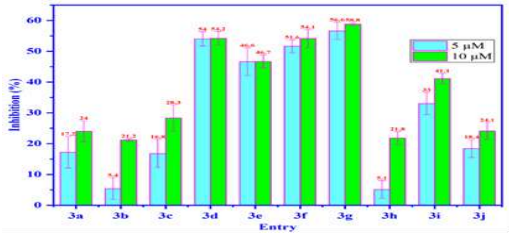


Fig. 2. The ability of entries **3a-3j inhibited** against human breast cancer cell line MCF-7 by MTT

As indicated in Fig. 3, entries **3e-3f** showed high cytotoxicity against breast cancer cell line, HT29 at both 5 and 10 µM, and entry **3g** indicated high HT29 at 10µM (An et al., 2015).

In *silico* molecular docking model

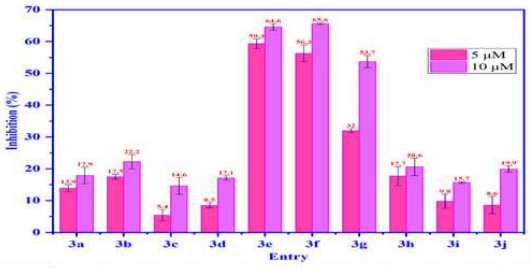


Fig. 3. The ability of entries **3a-3j inhibited** against human breast cancer cell line HT29 by MTT (Indicate their place in the text)

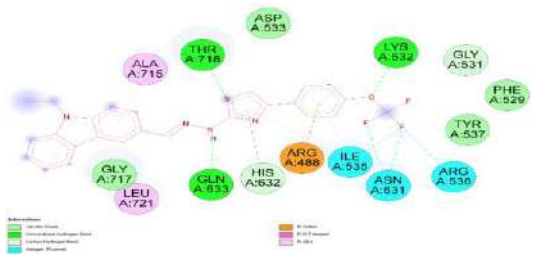


Fig. 4. The significant ligand interactions between **pose 166**, ligand **3d**, and crystal structure of enzyme code 1T8I, human topoisomerase I (top 1).

Table 1. The fundamental results in *silico* performed between docking pose **3d**, **3f,** and **3g** and *crystal structure of enzyme human topoisomerase I (Top 1), code 1T8I.*

Entry	Docking pose	ΔG^{o} [a]	K_i[b]	Hydrogen bond [c]	Property and bond length[d]
3d	166/200	-8.18	1.01	2	A:Lys532:N–pose166:O (3.02 Å) Pose166:H– A:Gln633:O (1.99 Å)
3e	186/200	-9.18	0.19	1	A:Arg488:N–pose186:N (2.69 Å)
3f	109/200	-9.04	0.24	0	-
3g	81/200	-9.34	0.15	1	Pose81:H–A:Gln633:O (2.46 Å)
Drug[e]	16/200	-8.24	0.91	4	A:Arg488:N–Drug:N (2.40 Å) A:Lys532:N–Drug:N (2.51 Å) A:Gln633:N–Drug:O (2.81 Å) Drug:H–A:Asp533:O (1.78 Å)

[a], [b]. They performed from AutoDockTools-1.5.6rc3 in the unit of Kcal.mol^{-1} and µM, respectively. [c], [d]. They built from Discovery Studio 2021 Client. [e], the Drug was used Camptothecin.

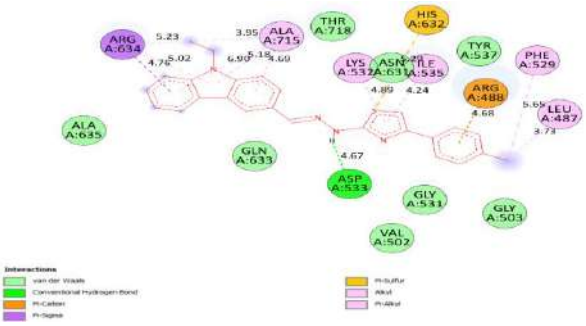


Fig. 5. The significant ligand interactions between **pose 186**, ligand **3e,** and crystal structure of enzyme code 1T8I, human topoisomerase I (top 1).

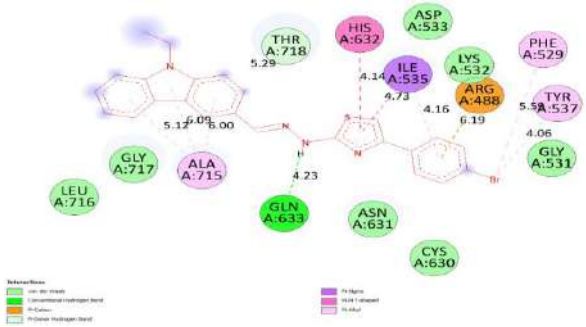


Fig. 6. The significant ligand interactions between **pose 109**, ligand **3f,** and crystal structure of enzyme code 1T8I, human topoisomerase I (top 1).

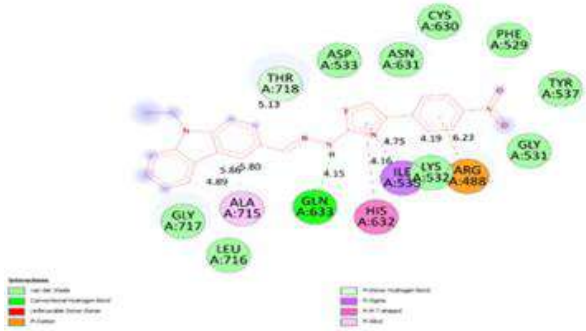


Fig. 7. The significant ligand interactions between pose 81, ligand 3g, and crystal structure of enzyme code 1T8I, human topoisomerase I (top 1).

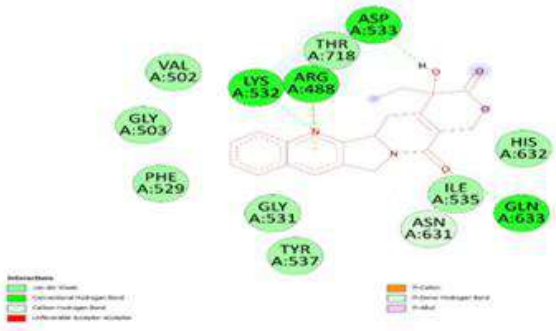


Fig. 8. The significant ligand interactions between pose reference, ligand Camptothecin and crystal structure of enzyme code 1T8I, human topoisomerase I (top 1).

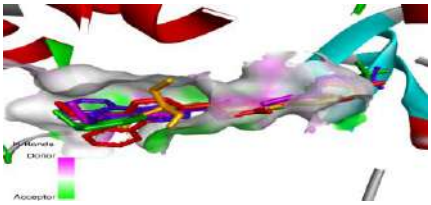


Fig. 9. Pose186 (3e, red color), pose 81 (3g, blue), pose166 (3d, green), pose109 (3f, pink), and pose16 (ligand Camptothecin, yellow) docked to the crystal structure of human topoisomerase I enzyme, code 1T8I at the same cavity of enzyme

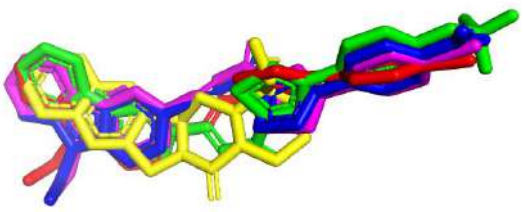


Fig. 10. The validation of the molecular docking model was proved by the values of RMSD and calculated by PyMOL version *2.5.2*.

Anticancer: highest anticancer activities of entries 3d-3g *in vitro* were selected and continuously proved in *silico* anticancer test based on enzyme inhibition mechanism via human topoisomerase I (top I) enzyme, code 1T8I. It represented the molecular target of synthesis compounds such as camptothecins, indolo-carbazoles, and indeno isoquinolines. The most conformation ligand impeded the target enzyme inhibition that was corresponding to prevent the cancer cell line. Ligand that formed the covalent complex with Top1 (1T8I) was poisonous because it transferred a basic enzyme into DNA damaging agent. The poisonous top I was available in S-phase. When cells are divided, the DNA replication fork collides with the top 1-DNA complex and broke the double strand that led to the death of cells via apoptotic (Staker et al., 2005). The significant ligand interactions of active entries 3d-3g and target enzyme, 1T8I are presented in Table 1; Figs. 4, 5, 6, 7 and 8. As shown in Table 1, at the thermodynamic site, the docking ability of entries 3d-3g and drug Camptothecinwith the same crystal structure of the enzyme, 1T8I, are detected in expression 3g> 3e> 3f> Drug> 3d– due to their inhibitor constants – as determined in the equation, $K_i = e^{\frac{\Delta G^o}{RT}}$ (Ingersoll et al., 1976). The best docking pose166/200 of ligand 3d well docked to target enzyme, 1T8I with the values of ΔG^o and K_I of -8.18 Kcal.mol^{-1} and 1.01 μM, as respectively shown in Table 1. Pose166 has anchored to enzyme 1T8I because its three parts detected well protein. The connecting unit of pose166 identified protein of enzyme via pi -alkyl ligand interaction from Ala715 and Leu721 to pi systems of carbazolyl ring, pi -T shaped from His632 to the pi system of thiazole ring, pi -alkyl from Ile535 to thiazole ring. The linker part or hydrophobic linker interacted with protein via an electrostatic interaction or pi-cation from Arg488, Lys532, and Ile535 to pi electrons of the phenyl ring. Finally, the bonding groups formed the hydrophilic interactions from Thr718 to sulfur of the thiazole ring, Gln633 to hydrogen atom of the hydrazine group, and Lys532 to the oxygen atom of trifluoro-methoxy. The hydrophilic interactions were halogen interactions, which linked from Ile535, Asn631, and Arg536 to two fluorine atoms of OCF_3. Other weak Van der Waals interactions performed around pose166 such as Gly717, Asp533, Gly531, Phe529, Tyr537, and His632. In this model, ligand 3d or pose166 well inhibited amino acids Arg488 (pi-cation) and Arg536 (Fluorine). They are the essential amino acids that prevent cells from dividing. In silico docking model, ligand (3d) indicating active groups (interactions with enzyme) were thiazole rings, hydrazine, and trifluoromethoxy groups. The pose186/200, the most stable conformation ligand 3e docked to the crystal structure of enzyme 1T8I with the values of free Gibbs energy and inhibition constant of -8.18 Kcal.mol^{-1} and 1.01 μM, respectively, as shown in Table 1. One hydrogen bond, A: Arg488:N–pose186: N (2.69 Å) formed from Arg488 of A chain of amino acid to the nitrogen atom in pose186 (3e) with a bond length of 2.69 Å. As shown in Fig. 5, one 2D diagram indicated the significant ligand interactions between pose186 (3e) and enzyme 1T8I. Pose186 interacted well with the enzyme because its three parts included the capping group (Cap), hydrophobic group, connecting unit (CU) or hydrophobic linker, and binding group (BG) that fully identified enzyme 1T8I (Giannini et al., 2012). The cap group of pose 186 detected enzyme via pi-sima from Arg634: A chain to the phenyl ring, alkyl or pi-alkyl from Arg634 to five heterocyclics, from Ala715 to phenyl rings and five heterocyclic, alkyl or pi-alkyl from Lys532, Ile535 to pi electron of thiazole ring, pi-sulfur from His632 to the sulfur atom (S) of thiazole ring, and pi cation from Arg488 to the pi electron of phenyl. The connecting unit identified 1T8I by pi-alkyl or alkyl from Phe529 and Leu487 to the methyl group of pose 186 or Arg634 and Ala715 to the ethyl group of pose 186. Finally, the binding group of this pose was found via a hydrogen bond, hydrophilic interaction from Asp533 to the hydrogen atom of the hydrazine group in pose 186 (3e). Ligand3e or pose 186 inhibited well this enzyme by interaction assignments and specially inhibited Arg634 (pi-sigma) and Arg488 (pi-cation) of the enzyme that prevented cells to divide. Pose 109/200 was the most stable conformation ligand of ligand 3f anchored to enzyme 1T8I with the values of ΔG^o and K_i of -9.04 Kcal.mol^{-1} and 0.24 μM, respectively as seen in Table 1. Pose 109 was evaluated good ligand interactions with 1T8I because three parts of ligand 3f were identified well via ligand interactions and weak Van der Waals interactions as shown in Fig. 7 in the 2D diagram. The capping group detected pi-alkyl from Ala715 to aromatic rings. The connecting unit was found via pi-cation interaction from Arg488 to pi electrons of the aromatic ring and pi-sigma interaction from Ile535 to pi electrons of the aromatic ring. The binding groups detected via interactions such as pi-sigma from Ile535 to pi electrons of the aromatic ring, pi -pi T shaped from His632 to pi electrons of the aromatic ring, pi-alkyl interactions from Phe529 and Tyr537 to bromo atom in this pose, and hydrogen bond from Gln633 to hydrogen atom of hydrazine in the pose. The ligand interaction between Arg488 and pose 109 was the most significant because pose 109 interacted with Arg488 of enzyme 1T8I which took the role to divide the cell.Pose 81/200 was corresponding to ligand 3gas good ligand interactions because three parts of pose 81 well interacted with the crystal structure of enzyme 1T8I. Pose81anchored to enzyme 1T8I with the values of ΔG^o and K_i of -9.34 Kcal.mol^{-1} and 0.15μM, respectively as seen in Table 1. The lowest constant of pose81 of ligand 3 demonstrated that it linked the strongest interactions among the most stable conformation ligands at the thermodynamic site. As shown in Fig. 7, the capping group of pose 81 was identified via pi-alkyl ligand interactions from Ala715 to aromatic rings. The connecting unit of pose 81 was detected via one pi-cation interaction from Arg488 to pi electrons of the aromatic ring and one pi-sigma interaction from Ile535 to pi-electrons of the aromatic

ring. The functional group was identified via one hydrogen bond from Gln633 to hydrogen atom of the hydrazonyl group, one pi-sigma interaction from Ile535 to pi electrons of thiazole ring, and one pi-pi T-shaped from His632 to pi electrons of thiazole ring. In this model, one pi-cation interaction that formed from Arg488 to the aromatic ring was the fundamental ligand interaction and the nitro functional group was without interaction with active sites in enzyme 1T8I.Pose 16/200, ligand Camptothecin interacted well with enzyme 1T8I by capping group from Arg488 to pi electrons of the aromatic ring via one pi-alkyl interaction, connecting unit from Arg488 to pi electrons of the aromatic ring via one pi-cation interaction and Lys532 to the carbon atom of heterocyclic five members via one pi-donor. The functional group of pose 16 formed hydrogen bonds from Lys532 and Arg488 to atom nitrogen of heterocyclic six members, Gln633 to the oxygen atom of the carbonyl group, and Asp533 to hydrogen atom of the alcohol group.Analytical interactions and thermodynamic site indicated that pose 166 (3d), pose 186 (3e), pose 109 (3f), and pose 81 (3g) interacted well with enzyme 1T8I and pose 166, ligand 3d was the best docking pose among them in *silico* docking model. The active groups in ligands found were the thiazole ring, hydrazine group, OCF_3 (3d), Clo (3e), and Br (3f). The NO_2 group in ligand 3g showed interactions with the target enzyme, 1T8I.

Docking validation in silico anticancer

As shown in Figs. 9 and 10, the ranked docking poses re-docked to the same crystal structure of enzyme 1T8I at pocket enzyme such as *p*ose186 (3e, red color), pose 81 (3g, blue), and pose166 (3d, green), pose109 (3f, pink), and pose16 (ligand Camptothecin, yellow). Pose 166 (3d) was the reference ligand and the values of RMSD between poses after calculations were 1.566 Å (pose 81 and 166), 0.290 Å (pose186 and 166), 0.954 Å (pose109 and pose166), and 2.408 Å (pose16, pose166). The values of RMSD proved the docking parameters corrected and this model would be thereby recognized and validated through interaction, conformation, and reproducing orientation (Hidalgo-Figueroa et al., 2021).

Table 2. The significant results in *silico* between the best conformation ligand/pose and crystal structure of COVID-19 main protease, code 6LU7

Entry	ΔG[a]	K_i[b]	The number of hydrogen bonds [c]	Property and bond length[d]
3a, pose145/200	-7.82	1.84	1	Pose145:H - A:ARG105:O (1.92 Å)
3b, pose82/200	-7.83	1.83	1	Pose82:H - A:ARG105:O (1.97 Å)
3c, pose169/200	-7.55	2.93	1	Pose169:H - A:ARG105:O (1.87 Å)
3d, pose139/200	-6.75	11.20	0	-
3e, pose 108/200	-7.76	2.05	1	Pose 108:H - A:Arg105:O (2.17 Å)
3f, pose 149/200	-7.84	1.79	1	Pose149:H - A:Arg105:O (1.87 Å)
3g, pose50/200	-7.66	2.44	0	-
3h, pose96/200	-7.97	1.44	0	-
3i, pose 184/200	-7.82	1.85	1	Pose184:H - A:Arg105:O
3j, pose86/200	-8.42	0.70	1	Pose86:H - A:Arg105:O
Remdesivir, Pose55/200	-4.37	622	5	Pose55:H - A:Lys137:O (2.17 Å) Pose55:H - A:Glu290:O(2.00 Å) Pose55:H - A:Glu290:O(2.37 Å) Pose55:H - A:Gln127:O (2.20 Å) Pose55:H - A:Cys128:S(2.18 Å)

[a], [b]. They performed from AutoDockTools-1.5.6rc3 in the unit of Kcal.mol-1 and μM, respectively. [c], [d]. They built from Discovery Studio 2021 Client.

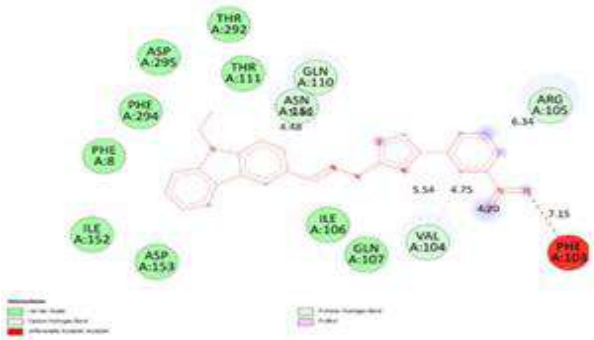


Fig. 11. Pose 96/200, entry **3h** anchored to crystal structure of COVID-19 main protease, code 6LU7

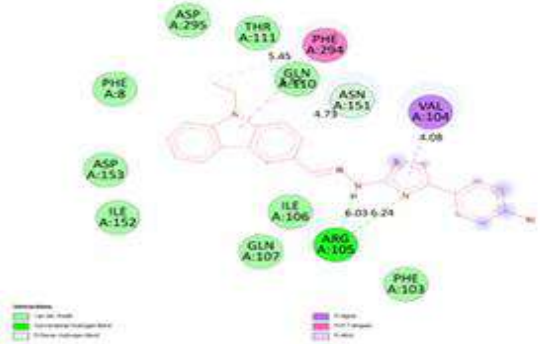


Fig. 12. Pose 149, entry **3f** anchored to crystal structure of COVID-19 main protease, code 6LU7

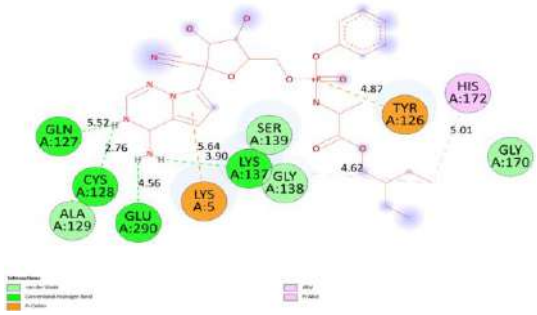


Fig. 13. Pose*55, ligand **Remdesivir** bond to crystal structure of COVID-19 main protease, code 6LU7.*

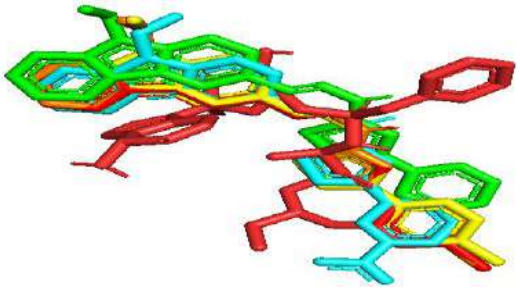


Fig. 14. The best docking poses of ligand **3a-3j**, ligand **Remdesivir**, pose 55 aligned to pose 149, ligand 3f, and performed by PyMOL

***Table 3. The values of RMSD calculated based on reference pose 3f via** PyMOL package in silico docking poses against* COVID-19 main protease, code 6LU7

RMSD (Å)	3a Pose145	3b Pose82	3c Pose169	3d Pose39	3e Pose108	3g Pose50	3h Pose96	3i Pose184	3j Pose86	Remdesivir Pose55
3f, Ref, pose149	0.162	1.024	0.878	0.348	0.186	0.566	0.586	1.018	1.091	5.502

In *silico* docking model for anti-virus

For antivirus, the crystal structures of COVID-19 main protease, code 6LU7 was selected to predict thiazole derivatives (**3a-j),** showing antivirus in silico comparable to Remdesivir– one drug that recently supported the treatment for Coronavirus (CoV). The results of docking (**3a-3j**) and Remdesivir drug are indicated in Table 2-3, Figs. 11, 12 and 13, and Figs. S1, S2, S3, S4, S5, S6, S7 and S8 supporting the information file. As shown in Table 2, the docking abilities of the best stable conformation ligands or ranked poses to the crystal structure of virus 6LU7, based on the thermodynamic site, were determined as pose 149 (ligand **3j**) > pose 96(**3h**) > pose 149 (**3f**) > pose 82 (**3b**) > pose 145 (**3a**) > pose 184 (**3i**) > pose 108 (**3e**) > pose 50 (**3g**) > pose 169 (**3c**) > pose 139 (**3d**) > pose 55 (**drug, Remdesivir**).

Pose 139 (**3d**) and pose 50 (**3g**) did not interact well with the main protease, 6LU7, one enzyme structure because they did not show full ligand interactions at three parts of poses, as seen in Figs. S4 and S6, respectively. As shown in Fig. 11, pose 96 (**3h**has no hydrogen bond or hydrophilic interaction and it indicated one unfavorable aceptor-aceptor interaction, which linked from Phe 103 to the oxygen atom of NO_2 group in pose 96. This unfavorable interaction increased the interaction energy between pose 96 and 6LU7. It exposed pose 96 which did not interact well with 6LU7. As shown in Fig. 12 and Figs. S1, S2, S3, S4, S5, S6, S7 and S8, the ligand interactions between pose 149 (**3f**), pose 145**(3a**), pose 82 (**3b**), pose 169 (**3c**), pose 108 (**3e**), pose 184 (**3i**), and pose 86 (**3j**) and one target enzyme 6LU7:PDB, COVID-19 main protease were presented in 2D diagrams, respectively. Active poses were found as pose 145 **(3a**), pose 82 (**3b**), pose 169 (**3c**), pose 108 (**3e**), pose 149 (**3f**), and pose 184 (**3i**). Pose 145 (**3a**), pose 82 (**3b**), pose 169 (**3c**), and pose 184 (**3i**) detected ligand interactions, which are similar and relative to residual amino acids of target enzymes such as Phe 294: pi-pi T-shaped and pi-alkyl interactions, Val 104: pi-sigma interactions, and Arg105: hydrogen bonds or hydrophilic interactions. The capping group of those active poses was identified by pi-pi T shaped from Phe 294 to pi electrons of the carbazole ring and pi-sigma from Val 104 to pi electrons of the thiazole ring. The connecting unit was detected by pi-alkyl from Phe 294 to the carbon atom of the ethyl group. The functional group was exposed by two hydrogen bonds from Arg105 to the hydrogen atom of the hydrazine group and the nitrogen atom of thiazole. With pose 108 (**3e**), amino acid Phe 294 disappeared**,** pi-pi T shaped interaction and replayed by one amide pi stacked interaction from amino acid Asn 151 to pi electrons of carbazole ring. For pose 86 (**3i**), amino acid Phe 294 showed without pi-pi T- shaped and pi-alkyl interactions with pose 86. Amino acid Asn 151 presented pi-donor hydrogen bond interaction with this pose and Arg105 indicated pi-pi alkyl interaction. Residual amino acids that interacted with active sites in poses were Arg 105 (binding group), Phe 294, Val 104, and Asn151 (capping group or connecting unit). Among active poses, ligand 3f or pose 149 was observed as the best docking pose in silico docking. Pose 149 (3f) inhibited well crystal structures of COVID-19 main protease, code 6LU7 via Phe 294 (pi-pi T shaped or pi-alkyl), Val 104 (pi-sigma), and Arg 105 (hydrogen bonds). Pose 55, ligand Remdesivir formed five hydrogen bonds with receptor 6LU7 that proved ligand more hydrophilic character, as seen in Table 2. As shown in Fig. 13, the capping group of Remdesivirwas identified by one pi-cation interaction from Lys 5 to heterocyclic nitrogen of five members and connecting

unit detected by alkyl or pi-alkyl interaction from His 172 and Lys 137 to the carbon atom of 2-ethyl butyl group. The binding group of this pose formed 4 hydrogen bonds from Gln 127 and Cys 128 to hydrogen atoms of the secondary amino group in the pose and from Glu 290 and Lys 137 to hydrogen atoms of the primary amine group in the pose. Another binding group was one pi-cation interaction from Tyr 126 tophenoxyphosphoryl amino in pose. Pose 55 indicated full ligand interactions with enzyme 6LU7 via 3 parts of the conformation ligand.

Docking validation in *silico* antivirus

Redocked poses to the same crystal structure of target enzyme 6LU7 were shown in Table 3 and Fig. 14. Pose 149, ligand **3f** selected reference pose, and other comparable poses. As seen in Table 3, the values of RMSD calculated change from 0.162 Å (ligand 3a, ligand 3f) to 1.091 (ligand 3j, ligand 3f). They indicated the worthy model in docking parameters, interactions and conformation, and reproducing orientation. As shown in Fig. 14, these were overlaps or superimposes that led to the fit RMSD. For Remdesivir drug, *p*ose55 (red color), the value of RMSD was 5.502 Å and this was explained by ligand 3f and ligand Remdesivir drug was not superimposed.

Table 4. The fundamental results in *silico* between the best docking pose of ligands and crystal structure of α-glucosidase enzyme, code 4J5T.

Entry	ΔG[a]	K_i[b]	The number of hydrogen bond [c]	Property and bond length[d]
3a, pose126	-9.46	0.12	2	A:Arg428:N–pose126:N (3.04 Å) Pose126:H– A:Glu429:O (1.89 Å)
3b, pose85	-9.72	0.07	1	Pose85:H–A:Glu429:O (2.29 Å)
3c, pose125	-9.49	0.11	1	Pose125:H–A:Glu29:O (2.25 Å)
3d, pose4	-8.79	0.36	1	A:Arg387:N–pose 4: N (3.02 Å)
3e, pose5	-9.81	0.07	1	Pose5:H–A:Glu429:O (2.18 Å)
3f, pose68	-9.88	0.06	2	A:Arg387:N – pose68:N (3.04 Å) Pose68:H–A:Glu429:O (2.06 Å)
3g, pose12	-9.55	0.10	1	A:Lys439:N–pose12:O (3.09 Å)
3h, pose37	-9.80	0.07	2	A:Lys439:N–pose37:O (3.14 Å) A:Lys439:N–pose37:O (2.64 Å)
3i, pose150	-9.68	0.08	2	A:Asn448:N–pose:N (2.97 Å) Pose150:H–A:Glu429:O (2.38 Å)
3j, pose124	-9.73	0.07	1	A:Arg387:N–pose124:N (3.02 Å)
Acarbose, pose103	-5.74	61.78	10	A:Trp391:N–pose103:N (2.93 Å) A:Arg428:N–pose103:O (2.77 Å) A:Asn453:N–pose103:O (2.94 Å) A:Asn453:N–pose103:O (2.94 Å) A:Tyr709:O - pose103:O (2.97 Å) Pose103:H–A:Leu563:O (2.38 Å) Pose103:H–A:Asp392:O (2.16 Å) Pose103:H–A:Glu429:O (2.40 Å) Pose103:H–A:Glu429:O (2.19 Å) Pose103:H–A:Glu771:O (1.81 Å)

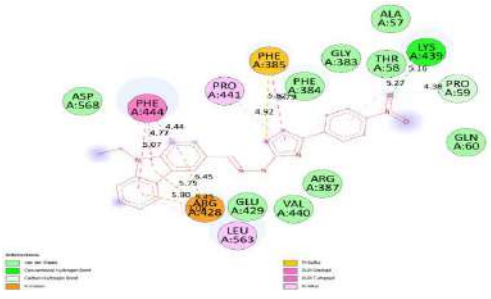


Fig. 15. Pose 12, entry 3g docked to the crystal structure of α-glucosidase enzyme, code 4J5T in the 2D diagram

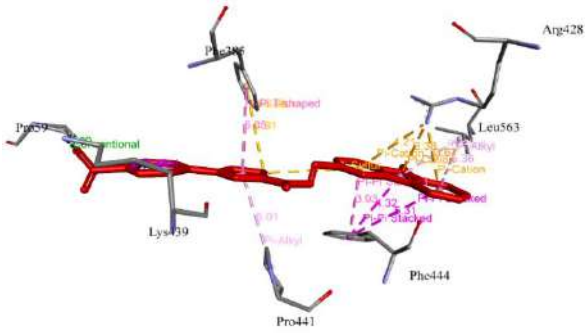


Fig. 16. *Pose 12, entry* ***3g*** *docked to the* crystal structure of α-glucosidase enzyme, code 4J5T in a 3D image.

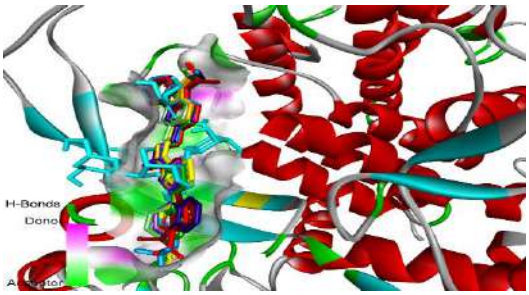


Fig. 17. The best docking poses of ligands **3a-3j** and Acarbose docked to the same enzyme pocket of*α-glucosidase enzyme, code 4J5T*

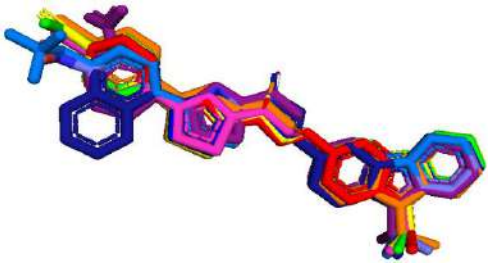


Fig. 18. The best docking poses of ligand **3a-3j** aligned to reference pose, pose12, ligand 3g to calculate RMSD in silico docking *of α-glucosidase enzyme, code 4J5T and* perform by PyMOL software.

Table 5. The values of RMSD were calculated based on reference pose 3g via PyMOLpackage in silico docking *between the best docking poses **3a-3j**and crystal structure of α-glucosidase enzyme, code 4J5T.*

RMSD (Å)	3a, pose126	3b, pose85	3c, pose125	3d, pose4	3e, pose5	3f, pose68	3h, pose37	3i, pose150	3j, pose124
3g, pose12	*0.865*	*0.631*	0.704	*0.151*	*0.590*	*0.468*	*0.23*	*0.806*	*0.168*

In silico docking model of inhibition α-glucosidase enzyme:

The docking and redocking calculation results of ligand 3a-3j and Acarbose are illustrated in Figs. 15, 16, 17 and 18, Figs. S9, S10, S11, S12, S13, S14, S15 and S16, Table 4, and Table 5. As seen in Table 4, based on their inhibition constants, the ability of docking poses was arranged in order as **3f > 3b = 3e = 3h > 3i > 3g > 3e > 3a > 3d >Acarbose.** Among docking poses of conformation ligands, ligand **3f** is the most stabilized in thermodynamics, because its free Gibbs's energy obtains the lowest value. Insight ligand interactions between one ranked pose and crystal structure of α-glucosidase enzyme, code 4J5T did not interact well with enzyme as ligand **3h, 3f,** and **Acarbose**, detailed information is indicated in Figs. S14 and S15 and Fig. S18, respectively. As seen in Fig. 12, one unfavorable donor–donor ligand interaction between Glu429 and the nitrogen atom of the thiazole ring, is displayed as the red line. As shown in Fig. S15, one unfavorable donor–donor ligand interaction, illustrated by red color, was formed from Arg 387: A chain to the nitrogen atom of the hydrazine group in pose 37. This ligand interaction was made to increase the system's energy of ligand interactions between pose 37 (3g) and 4J5T. As shown in Fig. S18, it has similarity with ligand Acarbose, additionally, one unfavorable donor-donor ligand interaction appeared between Asn 453 of receptor 4J5T and hydrogen atom of hydroxy group in pose 103 or ligand Acarbose. Pose 85, ligand 3b has a weak interaction with the enzyme at 3 parts of this pose, as illustrated in Fig. S10.The capping group was detected via one pi-pi alkyl interaction ligand that stemmed from Pro 441 and connected to electron pi of the aromatic ring of the carbazole group. Connecting unit (CU) of this pose was found via one pi-cation from Arg 428 to pi electrons of the aromatic ring. In addition, the Functional group of this pose was determined via one hydrogen bond from Glu 429 to hydrogen atom of the hydrazine group, one pi-cation from Arg 428 to pi electrons of thiazole ring, one pi-pi stacked from Phe 444 to pi electrons of thiazole ring, and one pi-cation interaction from Phe 444 to the sulfur atom of thiazole ring. In general, pose 85 (**3b**) interacted with potential ligand interactions. Pose 5, ligand 3e which was illustrated in Fig. S13 has fully indicated the interaction between ligands, thus, giving the conclusion that it has similar mentioned interactions with pose 85 (3b) to enzyme 4J5T. These interaction ligands were determined between Pro 441, one pi-pi alkyl interaction, Arg 428, one pi-pi cation, Phe 444, pi-pi stacked, and Glu 429, hydrogen bond. The distance from atoms of pose 5 (3e) to residual amino acids of the enzyme was longer compared to the mentioned distance of pose 85 (3b). it is proved that ligand 3e has weaker interaction with enzymes than ligand 3b. Pose 150, ligand 3i, as exposed in Fig. S16, ligand 3b (interacted full ligand interactions) fully interacted with the enzyme at three parts of pose 150. The capping group of pose 150 was identified by two pi-pi stacked ligand interactions from Phe 385 to pi electrons of the carbazole ring. The connecting unit of this pose was determined as one pi-cation and one pi-alkyl interaction from Arg 428 and Leu 563 to pi electrons of the aromatic ring, respectively. The functional group was detected by hydrogen bonds from Glu 429 and Asn 448, and additionally, the hydrogen atom of hydrazine and the nitrogen atom of

nitrile groups. Other ligand interactions such as pi-sulfur and pi-pi stacked/pi-pi T-shaped interactions from Phe 444 to the sulfur atom or pi electrons of the thiazole ring were also considered to be the functional groups of this pose. Pose 12, ligand 3g, was concluded as the best docking pose among highly active poses such as **3b**, **3e,** and **3i.** As provided in Figs. 15 and 16, the fundamental ligand interactions between pose 12 and enzyme 4J5T were presented as a 2D or 3D diagram. As shown in Fig. 15, the capping group of pose 12 was indicated as pi-pi stacked/pi-pi T shaped, pi-cation, and pi-alkyl interactions from Phe 444, Arg 428, and Leu 563 to pi electrons of three aromatic rings of carbazole group, respectively, meanwhile, the connecting units were one pi-alkyl from Lys 439 to pi electrons of the aromatic ring. Within pose 12, the found functional groups were one hydrogen bond and one carbon-hydrogen bond interaction from Lys 449 and Pro 59 to one oxygen atom of the nitro group. Additionally, another functional group of this pose was the thiazole ring that had been detected by one pi-pi stacked/pi-pi T shaped, pi-sulfur, and pi-alkyl interactions from Phe 385, Phe 385, and Pro 441 to pi electrons, a sulfur atom, and pi electrons of thiazole ring. Pose 12 was identified as fully ligand interactions at three parts of this pose and was the best candidate ligand in silico docking against α-glucosidase enzyme 4J5T. These active poses, 3b, 3e, 3i, and 3g indicated similar ligand interactions with the enzyme via amino acids of the enzyme as Pro 441, Phe 444, Arg 428. Amino acids such as Phe 444 and Arg 428were the essential amino acids in ligand interactions that were concluded to be similar to Phe 444 and Arg 428in our former article at ligand 11, the ligand that was identified to be the best candidate in silico docking model (Ngoc et al., 2021). The docking abilities of ligands or docking poses were evaluated in order**3g> 3i>3b = 3e.**

Docking validation in silico antivirus

The redocking model provided the values of the docking model reproducing orientation, conformation, and ligand interactions those results of ligand **3a-3j** are presented in Figs. 17 and 18, and Table 5; Fig. 17 illustrated the ranked docking poses of ligands that had docked to the same cavity of pocket enzyme 4J5T. As shown in Fig. 18, these poses aligned to the reference pose 12 of ligand 3g and was performed by PyMOLsoftware. The values of RMSD between ranked pose and reference pose 12 changed from 0.151 to 0.865 Å, as performed in Table 5, and were less than 2 Å, hence, they are proves that are good active compounds (Hidalgo-Figueroa et al., 2021).

Prediction of physiochemical and ADMET properties

The physiochemical and pharmacokinetic properties of compound (**3a-3j**) were predicted and presented in Table 6-7, using the basis of the article (Yang et al., 2018). As seen in Table 6, the parameters such as logarithmic lipo-hydro partition coefficient, H-bond acceptors, H-bond donors, and the number of comfortable bonds were permitted ranges. Furthermore, in Table 7, the values of BBB, Caco-2 permeability, HIA, Ames mutagenesis, and Carcinogenicity were determined to be in the standard ranges, additionally, these values represent the absorption and distribution characteristics of the given drugs; meanwhile, Ames mutagenesis and Carcinogenesis were genomic toxicity of the drug. The HIA is known as oral absorption and is the most important property of the ADME model; while The BBB is the microvascular endothelial cell layer of the brain and gives a major role in the separation between the brain and blood. Genotoxicity is a significant factor in testing the pre-clinical toxicity of the drug design (Wang et al., 2015).

Table 6. *Physicochemical properties of compound (**3a-3j**)*

Entry	A.Log P[a]	HBA[b]	HBD[c]	N Rotatable[d]
3a	6.38	5	1	5
3b	6.69	5	1	5
3c	6.39	6	1	6
3d	7.28	6	1	6
3e	7.04	5	1	5
3f	5.48	5	1	4
3g	6.29	7	1	6
3h	6.29	7	1	6
3i	6.26	6	1	5
3j	7.54	5	1	5

[a]. Logarithmic lipo-hydro partition coefficient, 2 ≤ALog P ≤10. [b]. The number of H-Bond acceptors, the value ≤ 10**.** [c]**.** The number of H-Bond Donors, the value ≤ 5. [d]. The number of Rotatable Bonds ≤5.

Table 7. The pharmacokinetic properties predicted by ADMET–SAR Tool.

Entry	Molecular weight[a]	Blood-Brain barrier (BBB)[b]	Caco-2 Permeability ($Caco^{2+}$)[c]	Human intestinal absorption (HIA)[d]	Ames mutagenesis [e]	Carcinogenicity[f]
3a	396.52	0.9817	-0.5872	0.9583	0.7100	-0.7714
3b	410.55	0.9819	-0.5788	0.9583	0.6500	-0.7714
3c	426.55	0.9785	-0.5970	0.9670	0.7400	-0.8429
3d	480.52	0.9769	-0.7497	0.9434	0.6400	-0.7857
3e	430.96	0.9818	-0.6617	0.9653	0.6300	-0.7571
3f	399.32	0.9818	-0.6516	0.9342	0.7100	-0.7857
3g	441.52	0.9760	-0.7415	0.9073	0.9200	-0.7429
3h	441.52	0.9760	-0.7001	0.9073	0.9100	-0.7429
3i	421.53	0.9817	-0.7135	0.9426	0.6800	-0.7714
3j	446.58	0.9817	-0.6877	0.9583	0.7100	-0.7714

[a]: Molecular weight is less than 500. [b]. Admet SAR, the positive value. [c]. Admet SAR, the negative value. [d]. Admet SAR, the positive value.[e]. Admet SAR, the positive value. [f]. Admet SAR, the negative value.

Molecular dynamics (MD) simulations analysis

Molecular dynamics is utilized to examine the ligand's binding effectiveness toward the protein over time at an atomic level. Several characteristics, including RMSD, RMSF, and the radius of gyration, volume, density, and hydrogen bonds play a major role in providing information about the binding pattern. As a result, a 100 ns MD simulation study was done to compare with the native Human DNA Topoisomerase to evaluate the overall stability and binding effectiveness of chemical (3g) with Human DNA Topoisomerase (Fig. 19a). RMSD parameter analysis provides detailed structural information for understanding the conformational stability of the system. Thus, RMSD studies for the protein (1T8I.pdb) in the presence of compound (3g) were performed. The analysis revealed that the protein-compound 3g complex is highly equilibrated and stable; yet, after 95 ns simulation (Fig. 19b), the ligand-complex was slightly fluctuating within its limit, indicating that the protein-compound (3g) complex is stable overall. The root means square fluctuation (RMSF) method examines the flexibility of protein residues in the presence of a compound (3g). As shown in Fig. 19c, the protein-compound (3g) complex exhibited a nearly identical fluctuation pattern, confirming the constrained motions over the 100 ns simulation. However, some residues namely Lys321, Glu322, Leu325, and Met675 that were not directly involved in the catalytic site had a high level of mobility with fluctuations ranging from 0.2 to 0.85 nm, indicating non-significant, whereas residues involved in the catalytic site formed the best pose with compound (3g) had significant dynamic behavior that led to a stable protein-compound (3g) complex. In addition, the complex-ligand radius of gyration (Rg) was measured (3g). The radius of gyration indicates the protein's compactness with protein folding and unfolding structures via the thermodynamics effect during the Molecular dynamics simulation's 100 ns. Fig. 19d depicts Rg values in nm forprotein–ligand complexes. The Rg values of the best docking pose under MD simulation initiated at 2.90 nm, and the structure gradually increased the Rg value until it reached 2.975 nm at 10 ns (10000 ps). The Rg value was immediately reduced to 2.85 at 20 ns, followed by equilibrium at 90 ns and a modest rise under the limit, indicating that the receptor ligand (3g) was stable and firmly packed (Fig. 19d).

To count the number of H-bonds involved in the MD simulation, the Hydrogen bonding utility based on Gromacs was peromed. The number of H bonds formed between the ligand (3g) and the protein was determined using 100 ns simulation trajectories, illustrated in Fig. 19e. Compound (3g) demonstrated a continuous maximum of 3–4 H-bonds, hence, this output assists the ligand to be stabilized with no changes in RMSD value during the simulation period. Aside from these findings, density/volume analysis (Fig. 19f) supported theprotein–ligand complex in its stability by demonstrating the continuous stable equilibrium during simulation.

Conclusions

Compounds 3d or pose 186 inhibited well against human topoisomerase enzyme I by inhibition mechanism explanation in silico anticancer docking model. Two essential amino acids of 1T8I determined Arg488 (pi-cation interaction) and Arg536 (Fluorine interaction), which were related to good inhibition and functionally active groups in 3d, which controlled interaction between ligands and thiazole ring, hydrazine, and OCF¬ group. With antivirus COVID-19 main protease, code 6LU7, compound 3 was the best candidate for enzyme inhibition 6LU7via fundamental amino acids, Phe 294, Val 104, and Arg 105. For α-glucosidase enzyme inhibition4J5T or antidiabetic

activity in silico, compound 3g interacted well with this enzyme via Pro 441, Phe 444, Arg 428, Phe 385, Pro 59, and Lys 439 of the enzyme. The values of RMSD in silico docking models for anticancer, antivirus, and α-glucosidase enzyme inhibition were obtained to predict validations about interaction ligands, conformation, and reproducing orientation with compounds 3d, 3f, and 3g. Furthermore, Rg, RMSF, RMSD, RMSF, hydrogen bond, and volume/density parameters were used to analyze the simulation for a better understanding of backbone fluctuations and complex stability. The results demonstrated that the complex remained stable for the course of the simulation.

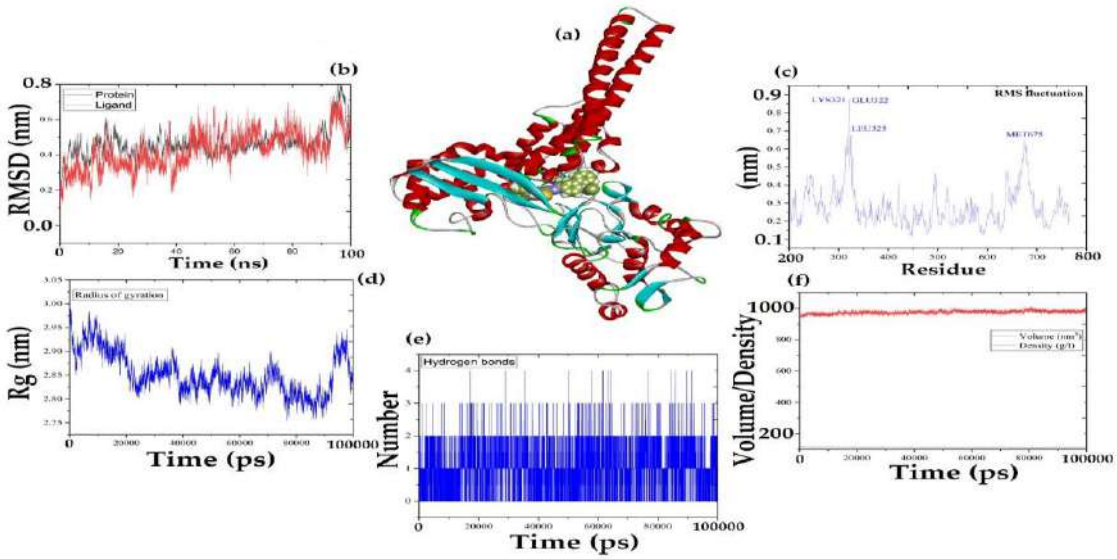


Fig. 19. (a) Molecular dynamics simulation analysis of best pose (pose no#81), (b) RMSD of the ligand (3g) and protein backbone calculated using 100 ns MD simulation, (c) Cα atoms RMSF as a function of amino acid complex, (d) Radius of gyration (Rg) plot, (e) The number of hydrogen bonds formed between residues of protein- compound is replay by ' complex of pose 81 (3g) and 1T8I during the simulation and (f) density/volume vs time (ps) during simulation.

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In silico identification of de novo and Conserved MicroRNAs of Model Moss Physcomitrella patens

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Abstract
During the past three decades, the moss *Physcomitrella patens* has transformed from an unfamiliar bryophyte to a model organism. *Physcomitrella patens* has exceptional tolerance to abiotic stresses. This moss was quickly chosen for genome sequencing because of its phylogenetic placement as a member of the sister lineage to vascular plants. Identification of numerous miRNAs, both novel and conserved, have presented an exceptional chance to deepen our understanding of the role of miRNAs in the evolution of land plants. By binding to reverse complementary regions and producing cleavage or translational inhibition of the target RNA, miRNAs create precursor RNAs with the specific hairpin structure that regulate the expression of relevant target genes. Here, we summarise our current understanding of moss' miRNAs and propose techniques for examining how they operate in *Physcomitrella patens*. Expressed Sequence Tags (ESTs) based homology searches between 383,068 non-redundant ESTs from the *Physcomitrella patens'* genome and 3514 known miRNAs from flowering plants were permitted, which results the prediction of 5 possible miRNA candidates from five different miRNA families. The putative miRNAs were made up of 22 nucleotides with (A + U) content in their pre-miRNAs ranging from 39% to 61% and MFEI values between -0.52 and -0.95. The study used a detailed computational methodology to find and characterize the preserved miRNAs in the *Physcomitrella patens*' genome, including genetic analysis of target genes.

Keywords: Physcomitrella patens, miRNAs, EST

Introduction

A moss (bryophyte) called *Physcomitrium patens*, often known as the spreading earthmoss, is employed as a model organism for research on the physiology, development, and evolution of plants. Over the past 80 years, the moss *Physcomitrella patens* has been utilized as an experimental organism. Its usage as a model to investigate plant functions has significantly increased during the past 15 years [1]. Since 2010, *Physcomitrella patens* has been one of the United States Department of Energy's (US DOE) flagship genomes, which choose a set of crucial species that enable us to concentrate our computational and experimental efforts to go beyond sequence to function and to offer the most immediate benefit to mission science [2]. Due to its phylogenetic position, propensity for gene targeting via homologous recombination, relatively simple morphology, and the dominant haploid phase that is the defining characteristic of all bryophytes, *Physcomitrella patens* makes an excellent moss and model organisms for evolutionary developmental biology (evo-devo) studies [3]. As it is simple to cultivate and spends the most of its life cycle in the haploid state, *Physcomitrella patens* makes it possible to apply experimental methods that are comparable to those used with bacteria and yeasts. Its growth is quite straightforward, and it produces few tissues with a small variety of cell types. Despite lacking genuine roots, stems, leaves, flowers, and seeds, mosses nonetheless possess many of the angiosperm-specific signaling pathways. For instance, the photo morphogenic pigments phytochrome and cryptochrome, as well as the phytohormones auxin, cytokinin, and abscisic acid, are all woven into separate but overlapping pathways and connected to various developmental phenotypes [4]. According to an investigation of histone changes, protonemal tissue is epigenetically prepared for the drought stress response, which most likely influences the gametophore stage and may be an adaptation to the lifestyle of the *Physcomitrella patens* organism [5]. The discovery of moss genes may have consequences for the safe manufacture of complex biopharma-ceuticals in moss bioreactors, crop development, or improvements to human health. Practically, around one-quarter of the moss genome comprises genes with no known function based on sequence characteristics, enhancing the likelihood that efforts to discover novel and inventive gene functions will be successful [6,7].

Small, single-stranded, non-coding RNAs known as microRNAs (miRNAs) play significant roles in controlling gene expression [8]. They were discovered in plants for the first time less than 20 years ago, but research has since demonstrated that they are essential regulators of developmental processes including leaf morphogenesis, the transition from the vegetative to the blooming phase, and the response to abiotic stressors. Numerous ancient miRNAs control genes that are essential for the growth of terrestrial plants, such as highly conserved transcription factors or other regulatory genes [9]. By targeting mRNAs for cleavage or suppressing translation, miRNAs play significant roles in the regulation of plant post-transcriptional genes. Plant growth, signal transduction, protein breakdown, response to

environmental stress and pathogen invasion, and regulation of their own synthesis are all impacted by miRNAs [10].

In many plant species, the computational analysis of expressed sequence tags for miRNA identification has been extensively used. Even non-model species can benefit from investigations on structural and functional genomics because of EST sequencing's labor and financial efficiency. As a result, ESTs are an effective resource since they have a high level of functional information and frequently correlate to known or anticipated hypothetical functions [11]. This has led to the development of many methods for miRNA detection in plants employing expressed sequence tags (ESTs) [12]. We performed an EST-based homology search and a number of computational and analytical procedures to uncover conserved miRNAs in *Physcomitrella patens* and their functional annotation in the *Physcomitrella* genome using publicly accessible ESTs from the NCBI Genbank database.

Methods

2.1. Acquisition of EST sequences and reference miRNAs

The genome based BLAST search of *physcometrella patens* (GenBank Assembly Accession: GCA_ 000002425.2) suggested available ESTs of the NCBI database. The suggested ESTs of *physcometrella patens* were retrieved from the EST database of NCBI. Moreover, to search potential miRNAs in *physcometrella patens*, all previously known mature miRNA sequences of plant dicotyledons were extracted from miRBase database (http://www.mirbase.org/). These miRNAs were employed as the reference set with the retrieved ESTs of *physcometrella patens* genome for identifying conserved miRNAs family in p. patens. To avoid redundant or overlapping miRNAs and ESTs, the repeated sequences of miRNAs and ESTs were removed by CD-HIT (http://weizhongli-lab.org/cd-hit/) and only the single ones were retained for further study.

2.2. Search for potential miRNAs in the jute genome

The reference miRNAs retrieved from miRBase were used as the query templates for homology search against the ESTs of *physcomitrella patens* by using BLASTn of NCBI database considering all default parameters. For each miRNA search, only the top EST result was used. Again, CD-HIT performed a redundancy check on all of the ESTs from the BALSTn search.

2.3. Screening for the non-coding miRNA candidates

As the miRNAs are non-protein coding genes, the precursor miRNAs should also be non-protein coding. After redundancy check, the top hits from BLASTn were subjected to BLASTx analysis for removing the protein-coding sequence. A number of non-protein coding EST sequences were screened. By investing - ating the miRNA precursor determinant properties, these miRNA candidates were allowed for proper testing to finalize the pre-miRNAs.

2.4. Identification of putative miRNAs with reported criteria

Various criteria were used to screen precursor miRNAs based on statistical comparative genomics approaches developed by Zhang et al. in 2006, which had already been applied in other EST-targeted miRNA discovery and characterization investigations [13, 14]. Here, only candidate ESTs that exhibited the traits listed below were given priority as important miRNAs in P. patens: (1) The length of the finalized putative mature miRNAs must be kept within the range of 18 nucleotides; (2) No more than three mismatches between the putative miRNAs and all previously reported putative miRNAs of plant species were allowed; (3) The pre-miRNA sequence must fold into an accurate hairpin stem-loop secondary structure, and the identified miRNAs should be placed in one arm of the corresponding hairpin stem-loop. (4) the mature miRNAs should allow less than 3 mismatches with the opposite miRNA strand in the other arm; (5) the percentage of (A + U) content of pre-miRNA should be kept within the range of 30%–70%; and (6) the secondary structure of mature miRNAs must have lower minimal (highly negative) folding free energy (MFE) and minimal free energy index (MFEI) value.

2.5. Prediction of pre-miRNA and hair-loop secondary structure of mature miRNAs

To identify the potential pre-miRNA candidates, the ESTs for putative miRNA prediction were submitted to miRNAFold (https://tanuki.ibisc.univ-evry.fr/ evryrna/ mirnafold/mirnafold_form). The pre-miRNA was set to have a minimum length of 85 nucleotides, and the Mfold online server's Zuker folding algorithm was used to predict secondary structures in these precursor sequences of functional miRNA homologs (http://www.unafold.org/mfold/applications/rnafolding -form.php). As the predicted secondary structure should have the higher minimal negative value of minimal folding free energy index (MFEI) and minimal folding free energy (MFE), only the best fitted premiRNA candidates were obtained considering all parameters [15-17]. The MFEI was calculated using the following equation; Adjusted MFE (AMFE) represented the MFE of 100 nucleotides. AMFE = (MFE / length of RNA sequence) * 100; MFEI = AMFE / (G + C) %; MFEI = [(MFE/length of the RNA sequence) *100] / (G + C) %

2.6. Nomenclature and family annotation of predicted microRNAs

The putative miRNAs were used as a query sequence for local blast in miRBase to reconfirm the family of the putative mature miRNAs. The predicted microRNAs were named by a nomenclature followed by miRBase (http://www.mirbase.org/). Sequence alignment of homologous members of predicted miRNA family was investigated in the miRBase search output and rechecked by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The percentage of each nucleotide base of putative mature miRNAs and their respective homologue miRNAs from miRBase were also calculated and analyzed.

2.7. Prediction and functional analysis of putative miRNA targets

Due to the unavailability of *physcomitrella patens* cDNA library for miRNA target prediction, *Arabidopsis thaliana* was considered as the reference organism for prediction and functional annotation of the putative newly detected mature miRNAs in *p. patens* genome. The widely used miRNA target prediction tool psRNATarget database (https://www.zhaolab.org/psRNATarget/analysis) was used here, and the mature miRNAs were employed as query against the *P. patens* considering the following parameters: (1) maximum expectation value should be 5; (2) complementarity scoring (HSP) size should be kept within the length of 18; (2) target sites' multiplicity must be allowed to 2; (3) range of central mismatch for translational inhibition should be in the range of 10–11 nucleotide; (4) highest level of mismatches at the complementary site 4 except any gaps.The psRNATarget ensures reverse complem - entary matching between target transcripts and respective miRNAs, leading to the target site accessibility by calculating unpaired energy (UPE) needed for beginning the secondary structure around the miRNA target site [18]. The schematic outline of the total methodology was illustrated in Figure 1.

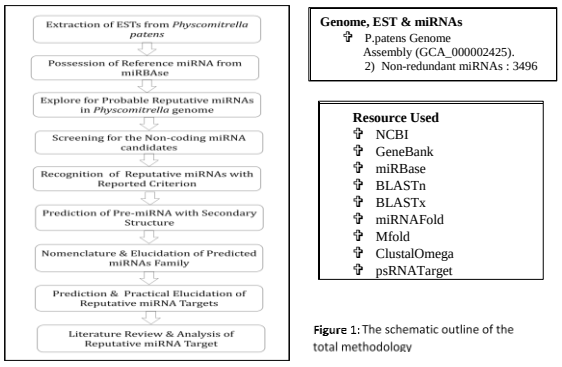

Figure 1: The schematic outline of the total methodology

Results

1.1. Search of potential miRNAs in genome of P. patens

To identify and characterize the conserved microRNA of *Physcomitrella patens*, a comprehensive EST based study was employed. There were differences *in silico* steps where a huge volume of preliminary ESTs of *P. patens* were screened to finalize the putative miRNAs with specific gene targets involved in the biological processes of *Physcomitrella patens*. The summary of details processes with the numerical data was presented in Table 1. About 3514 previously known mature miRNAs under the dicotyledons were extracted from the microRNA database. 383068 *Physcomitrella patens* ESTs were searched against 3496 non-redundant mature miRNA sequences. After the removal of redundancy by CD-HIT, around 2969 ESTs were selected as potential miRNA sequences for *Physcomitrella patens*. Moreover, it had been found that around 377 ESTs were non-coding which could be the potential candidates of miRNA homologs.

3.2. Prediction of pre-miRNAs and hair-loop secondary structure of mature miRNAs

The putative miRNA precursors were looked into in relation to the 377 non-coding ESTs. To exclude the potential miRNAs from Physcomitrella patens, the various predefined criteria have to be properly adhered to in each phase. Here, five ESTs were carefully analyzed and found to be pre-miRNA, whereas the remaining ESTs were discarded since they did not meet the prerequisites outlined before. Using the Zuker folding algorithm of the MFOLD program, the final five precursor sequences of putative miRNAs were examined to see whether they could really form secondary structures. All five pre-miRNAs were found to be capable of folding themselves into the proper hairpin form that mirEval had predicted. Additionally, the miRNAs' hairpin architectures barely included loops (Figure 2).

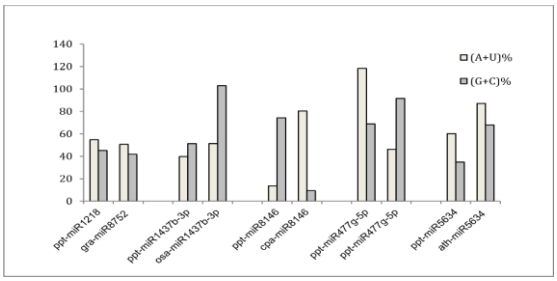

Figure 2: Overall nucleotide compositions (%) of putative miRNA from Physcomitrella patens compared with miRNA homologs

For the pre-miRNAs, the MFEI (minimum free energy index) values vary from -0.53 to -0.95. The secondary structure of the matching sequences is more thermodynamically stable the lower the MFE. The pre-miRNAs' minimal folding free energies (MFE, Dg in kcal/mol) ranged from -12.7 to -61.9, while the precursors' percentages of A + U content ranged from 39% to 61% (Table 2).

Table 2: Putative miRNA in the genome of Physcomitrella patens with the suggested criteria

miRNAs of miRBase	Length of miRNA	Number of Mismatches	(A + U)%	MFE	MFEI
ppt-miR1218	21	0	55	-48.2	-0.72
osa-miR1437b-3p	18	2	39	-58.4	-0.68
cpa-mirR8146	18	2	61	-12.7	-0.53
ppt-mir477g-5p	21	0	56	-61.9	-0.95
ath-miR5634	19	3	56	-37.7	-0.61

1.2. Nomenclature and family annotation of predicted microRNAs

The miRBase database was used to search out and address the microRNAs family of the predicted of the five putative miRNAs. The five miRNAs of *Physcomitrella patens* belong to five different miRNA families (miR1218, miR1437b-3p, miR8146, miR477g-5p, miR5634) (Figure 3).

Figure 3: Secondary structure of final putative miRNA by using the Zuker folding algorithm of MFOLD software; Here, (a) ppt-miR5634, (b) ppt-miR8146, (c) ppt-miR477g, (d) pptmiR1437b, (e) ppt-miR1218

1.3. Prediction and functional analysis of putative miRNA targets

The five novel *Physcomitrella patents*' miRNAs were found to be involved with a total of 328 targets as predicted by the psRNATarget. Comprehensive literature studies were employed to find out the involvement of predicted gene targets in different biological processes. There were few gene targets which were found to be directly or indirectly involved with the plant growth, development and defense responses. These studies have revealed a wealth of miRNAs, including novel and conserved ones, creating a unique opportunity to broaden our understanding of miRNA functions in land plants and their contribution to the later evolutions.

Discussion

Endogenous non-coding RNA molecules known as microRNAs (miRNAs) exist. These miRNAs can impede protein translation or promote mRNA degradation by binding to the 3'-untranslated region (3'-UTR) of target genes to control gene expression post-transcriptionally [19]. As a result, they are crucial for several biological processes, including defenses, cell division and proliferation, and embryo development [20, 21]. In this study, we discovered five unique microRNAs in the *Physcomitrella patens* genome (miR1218, miR1437b-3p, miR8146, miR477g-5p, and miR5634), and we named the predicting putative miRNAs ppt-miR1218, pptmiR1437b-3p, ppt-miR8146, ppt-miR477g-5p. The putative miRNAs of *Physcomitrella patens* were shown to be highly conserved in other organisms by multiple sequence alignment and comparative nucleotide composition investigations, supporting the evolutionary convergence of higher plant species [22]. The research found that putative miRNA of *Physcomitrella patens* associated with the coding genes for histone-like protein, harpin-induced protein, lightharvesting chlorophyll a/b-binding (LHCB) proteins, and mitogen-activated protein kinase were exclusively involved in the plant's adaptation to abiotic stresses like salinity, draught, diseases, etc. A key component of plant defense mechanisms is the histone-like protein, which increases plant disease resistance by triggering PAMP-induced immunity. The apoproteins of the lightharvesting complex of photosystem II (PSII), which are often linked to chlorophyll and xanthophylls and act as the antenna complex, are known as light-harvesting chlorophyll a/bbinding (LHCB) proteins. This protein plays an important role in plant adaptation to environmental stresses [23]. The extraordinarily efficient function of mitogen-activated protein kinase in plant growth, development, and defensive responses. Here, it was discovered that the putative potential miRNAs target critical genes involved in diverse cellular processes and abiotic stress response in plants. The putative miRNA of *Physcomitrella patens* may display regulatory activities over the significant cellular expression through in vivo and in vitro validation as miRNA focused regulation over the 63 expression has previously been described in other plant species [24–26]. Since the regulatory profile of targeted miRNAs may have a major influence on improved crop production as well as the desired traits of agricultural plants, EST-based miRNA prediction is becoming more and more important in many crops [27, 28]. In order to explore the phenotypic effects in transgenic plants, a gene-replacement construct that can be built to replace the original target gene sequence with a mutant miRNA-resistant variant may be developed. For this reason, the aforementioned study may be of considerable significance.

Conclusion

Physcomitrella patens is an unusually efficient gene targetter among terrestrial plants. It offers a rare opportunity to comprehend the functions played by each miRNA in a terrestrial plant. It is anticipated that by analyzing the Physcomitrella patens miRNA using the aforementioned methods, these regulators' unique and conserved functions would be revealed. Some of these activities ought to shed light on the beginnings of terrestrial plants and their early land adaptations. This discovery will be very helpful to future researchers looking to locate conserved miRNAs in the dicotyledone family using *in silico* approaches and will speed up their ability to find more conserved miRNAs.

Table 1: Steps involved in the characterization of putative miRNAs from ESTs of *Physcomitrella patens*

Serial No.	Steps	EST Number
1	Retrieval of EST sequences from the genome *Physcomitrella patens*	383068
2	Retrieval of previously known mature miRNA sequences from miRBase	3514
3	Non-redundant miRNA sequences	3496
4	Search for potential miRNAs in *P. patens* genome by BLASTn	2969
5	Candidate ESTs with non-protein coding sequences after BLASTx	377
6	Putative pre-miRNAsequences with hair-loop secondary structure	267
7	Identification of newly recognized miRNA family	5

Table 3: Putative miRNA targets in the crucial cellular procrss of Physcomitrella patens

Putative miRNA	Target protein	Target accession	Biological function
ppt-miR1218	Mitogen-activated protein kinase	CF450807	Involved in the abscisic acid signalling pathway in the context of abiotic stresses(e.g. drought)
ppt-miR1437B3P	Histone-like protein	TC3910	Defense responses like • Antibacterial humoral response • Response to gram positive bacterium • Innate immune response in mucosa
ppt-miR8146	Harpin-induced protein 1 containing protein	CF441427	• Response to osmotic stress • Response to drought • Response to salt stress • Response to UV-B • Response to cold
ppt-miR8146	Membrane protein-like	TC7188	• Resistance to environmental stress
ppt-miR8146	Senescence-associated protein 5	TC4242	• Response to toxic substances
ath-miR5634	Latex-abundant protein	TC4665	• Plant defence

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Conflicts of Interest

The author declares no conflict of interest.

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Section D: Food Safety, Food Security and Climate Change

Sustainable Resource Management for Food Security in Rural Asia

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Abstract

Intensifying human activities such as deforestation, increased human settlement, intensive agricultural activities, accompanied with high economic development, have often caused serious ecological risks such as flooding, soil erosion, and water pollution in many Southeast Asian countries. Such food risks result in inadequate food supply as well as poor-quality and contaminated food, which significantly contribute to endangered public health risks. Based on our empirical research conducted in Sekampung River watersheds, Lampung Province in Indonesia and Laguna de Bay areas, Philippines, we have identified various negative impacts of poor resource management on expanding disaster risks and declining soil and water resource capacity. Furthermore, such negative impacts have adversely affected farm and fishery households' income and livelihood. Because sustainable resource management with good agricultural and forestry practices would provide significant ecosystem services to local people and regional economy, the concept of "Green Infrastructure" (GI, in short) has been paid greater attention. GI could provide invaluable functions to protect or reduce natural disaster risks such as flooding, tsunami, soil erosion or even drought. GI would also provide sustainable fishery, aquaculture, forestry and agricultural resources. Well-managed ecosystems, such as wetlands, forests and coastal systems, would act as natural infrastructure, reducing physical exposure to many hazards and increasing socio- economic resilience of people and communities.

Keywords: Ecological risk, Food security, Community-based watershed management, Payment for Ecosystem Services, Sustainable Resource Management;

1. Introduction

Asia is facing serious environmental degradation, mainly caused by forest losses and land use changes. Intensifying human activities such as deforestation, increased human settlement, intensive agricultural activities, and other industrial development have often caused serious ecological risks such as flooding, soil erosion, and water pollution in many Asian countries. Food security, closely linked with such environmental problems, has become one of the most important contemporary issues in Asia. Especially in recent decades, ecological degradation such as sedimentation, water quality degradation and frequent flood occurrence, many of which are enhanced by climate change impacts, is expanding in Indonesia and many other Southeast Asian countries and affecting adversely the quantity and safety of foods. The basic issue here is whether and how Asian agriculture and fisheries can supply sufficient amount and quality of food to meet the requirements of a rapidly growing population, without jeopardizing the natural and environmental resources. The main objective of this paper is to examine the two basic questions. Namely, (1) How environmental conservation and productivity enhancement are compatible? and (2) How community oriented and watershed-based approaches are effective? As a typical case study, we have conducted a long-term, watershed-based empirical research in and Sekampung River, Lampung Province in Indonesia and Laguna de Bay, Philippines. We have identified various negative impacts such as declining soil and water resources. Such negative impacts are widespread and appear on declining agricultural productivity and negative health impacts, and appear to adversely affect farm and fishery households' income and livelihood.

2. Examination of Sustainability of Smallholder Farmers in Sekampung Watersheds, Sumatra

The main objective of our collaborative research has been to determine the impact of natural resource degradation on the income of smallholder coffee farmers and the sustainability of eco-certification among smallholder coffee farmers in Sekampung Watersheds, Sumatra-Indonesia. An empirical research was conducted in the Upper Sekampung watershed in Lampung, Indonesia, involving a household survey of 408 coffee farmers in the watershed. Many of the farmers have been adopting an agroforestry system since the 1980s, especially in coffee production centers located in the degradation-prone watersheds. This system included the planting of shade trees, fruit trees, timber, and other multi-purpose tree species. Although Sekampung is the main watershed in Lampung Province, serving as a major food basket for such commodities as rice, maize, and other crops, it also produces major export agricultural commodities such as coffee, cocoa, palm oil, coconut, etc. The alternative land-use systems in the study sites were analyzed and compared using the BWS (best-worst scaling) method. Examined also were the management of land-use system and the effectiveness of eco-certification for the coffee agroforestry system in the catchment area. Results show that coffee eco-certification provide significant

positive financial and environmental benefits from reduction in the use of chemical fertilizers. Also, participation in the Rainforest Alliance (RFA) certification provides higher financial and environmental benefits than participating in the Common Code for the Coffee Community (4C) certification.

3. Economic and Ecological Impacts of Adopting Coffee-Agroforestry System

The economic and ecological impacts of coffee agroforestry system in Upper Sekampung Watershed that serve as conservation strategy in the catchment area was first examined by Ismono et al. (2016). Simple criteria for the agroforestry system was applied in the study, i.e. where farmers grow at least 100 shade trees per hectare in their coffee farms, taking consideration that one tree could provide some functional shade (light, nutrient etc.) for about four coffee trees. The findings showed that adoption of agroforestry systems have no significant negative impacts on the farm income of farmers. Additional analysis also focused on the effect of joining coffee eco- certification on the performance of farmers. It is found that coffee eco-certificates provide significant positive economic benefits from higher farm income and environmental benefits from reduction in the use of chemical fertilizers. Also, participation in the Rainforest Alliance (RFA) certification provides higher economic and environmental benefits than participating in the Common Code for the Coffee Community (4C) certification. The major consideration however of farmers in their production decisions is the market price rather than environmental risks.

Coffee eco-certification scheme has emerged from growing concerns over global environmental governance, as an alternative new vehicle of corporate control over global food production, trade, and consumption. Eco-certification generally connects consumers and businesses in the so- called developed countries with producers and small farmers in developing countries. In the face of this development, the Southern actors from business, civil society, and government need to redefine their position and the supply chain of export commodities to change the structure of commodity value chains. The coffee eco-certification generally requires establishment of farmers' organizations and locally adopted conduits. However, many of these standards provide no guarantee that direct benefits, particularly price premiums, would reach farm laborers or local communities. The social and economic effects of eco-certification in agricultural commodity chains are still to be analyzed and examined more extensively.

Other important findings are as follows: (a) farmers have adopted coffee agroforestry system since the 1980s using shaded fruit trees and multi- strata coffee system to secure their own household income and contribute to conservation practices in the catchments area of the watershed; (b) Average coffee production in Upper Sekampung is 394 kg/ha, which is way below the national average of 645 kg/ha, mostly due to the continuing use of traditional farming practices and simple processing techniques; (c) Coffee crops remain profitable in the watershed, but ample opportunities could be explored to improve the coffee yield and quality to meet the increasing demand for coffee; and (d) Agroforestry system has provided additional income sources, mostly from tree products and by-products, as shown by a significantly higher B/C ratio than the coffee farming system alone.

4. Yamang ng Lawa: A Fish Garden Sanctuary Technology Based on Local Knowledge at Laguna de Bay, Philippines

Laguna de Bay is a shared water body among the 25 rapidly urbanizing lakeshore municipalities in Laguna Province that is characterized by uncontrolled waste generation and pollution. This is adversely affecting the quality and quantity of inland biodiversity, particularly, open food fishes, support micro-organisms, and plant communities. The problem facing the lake is a common pool resources issue referred to as the tragedy of the commons that leads to the over harvesting of fishes. In addition, there are problems related to water pollution, a common problem faced by open access resources. The Yaman ng Lawa (YNL), or "Blessing of the Wealth in the Lake", is a social action research with the aim of demonstrating the scientific basis of traditional knowledge. The Project is a component grassroots action research of the LakeHEAD Program with participation of the leaders of 10 fishing villages, agri-staff of Calamba City, and Laguna Lake Development Authority. Our collaborative study was conducted upon the request of a group of fishermen who did their own challenge by use of Kamatsile (*Pithecello - biumdulce*) branches to establish a fish sanctuary that attract more community of fishes to live, seek refuge, and breed. The project used the "Trans-sectoral Approach" that involves the exchange of local and scientific knowledge of the lake ecosystem risks between fishing communities and scientists from the University of the Philippine and Research Institute for Humanities and Nature in Kyoto, Japan. For this purpose, the local fishermen are trained in the actual analyses of water and lake sediments for Digestible Oxygen and Oxidation Reduction Potential of the Yankaw Fish Garden lake waters. The project has two significant contributions to social action research, namely, exchange of local knowledge and science on lake water management and revival of the local community "Bayanihan Spirit," or "helping each other".

5. Significance and Impacts of the "Yaman ng Lawa" Project on the Local Government

The very noticeable improvement in fish catch has convinced the local fishermen on the importance of their Kamatsile-based Yankaw as a fish sanctuary for inducing the breeding, aggregation and reproduction, and eventual restoration of the local fishes in the lake. The Yankaw is becoming the new home, refuge, and breeding place for the community

of fishes that share a new and common habitat. While there are still a number of local fishes spread thinly across the Fishing Zones of Calamba City and nearby towns and Cities, it is interesting to note that during the few months of establishment of the Yankaw, the local fishes living inside the Yankaw were much better in physical appearance and eating quality. The result of the study shows a strong possibility for implementing the Yankaw method in nearby towns and cities along Laguna de Bay. This will provide Laguna de Bay a very good opportunity for recovering it's lost image as the life-support system for lakeshore communities and enjoy once more the Yaman ng Lawa or blessings of the natural wealth of the Lake. Yankaw is significant for a couple of reasons. First, it showcases the traditional Filipino "Bayanihan" spirit, a collective volunteerism where local communities help each other in all communal activities. It is both an economic as well as a social event. In the past, members joined hands and helped each other in clearing a field of timber or constructing a barn that needed many workers. Jobs like corn husking or threshing could be done as a group to allow socializing during an otherwise tedious chore. In such gatherings, refreshments were served and entertainment was provided. Secondly, for the first time in the history of Agriculture and Fisheries, Calamba City became a recipient of the prestigious national recognition award during the National Search of TOFARM 2013 for Outstanding Farmers of the Year. This was due to their implementation of Lake HEAD Yaman ng Lawa Projects. The Calamba City Barangay Fishery and Agriculture Fishery Council and the Yaman ng Lawa Yankaw Fish Garden Sanctuary Project received the "National Silver Award" or second place for the" Best Agriculture Initiative" category. In fact, the result of our study is recently showing a strong possibility for implementing this Yankaw method in nearby towns and cities along Laguna de Bay area.

6. Summary and Conclusion

The incidence of food insecurity can be viewed as the ultimate effect of several inter-related biophysical and socio-economic factors that served as main hazards and risks. These include environmental degradation, the interaction between man and his physical resources, and naturally occurring hazards. To address this issue of food insecurity, it will be necessary to adopt a systems approach to the development of short- and long-term planning and management of an integrated watershed. With these evidences in mind, some alternative institutional arrangement is desirable which internalizes the value of ecosystem services provided by adopting environmentally friendly techniques and other sustainable agriculture or fisheries. Recently, the practice of payment for ecosystem services (PES) has been gaining increasing attention. In agricultural contexts, a PES as well as eco-certification programs can provide good incentive for farmers who switch to conservation practices that generate greater ecosystem services.

Based on our long-term, watershed-based empirical research conducted in Laguna de Bay, Philippines and Sekampung River, Lampung Province in Indonesia, as typical case studies, we have identified various negative impacts such as declining soil and water resources. Such negative impacts would be widespread and appear on declining agricultural productivity and negative health impacts, and appear to adversely affect farm and fishery households' income and livelihood. The incidence of food insecurity can be viewed as the ultimate effect of several inter-related biophysical and socio-economic factors that served as main hazards and risks. These include environmental degradation, the interaction between man and his physical resources, and naturally occurring hazards. To address this issue of food insecurity, it will be necessary to adopt a systems approach to the development of short- and long-term planning and management of an integrated watershed. With these evidences in mind, some alternative institutional arrangement is desirable as for the next steps which internalizes the value of ecosystem services provided by adopting environmentally friendly techniques and other sustainable agriculture or fisheries. Recently, the practice of payment for ecosystem services (PES) has been gaining increasing attention. In agricultural contexts, a PES as well as eco-certification programs can provide good incentive for farmers who switch to conservation practices that generate greater ecosystem services.

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Rational use of antibiotics as agricultural chemicals to ensure food security.

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Abstract

Antibiotics have significantly contributed to human life, particularly in medical treatment and agricultural output. In 1954, the USDA stated that medical-use antibiotics were used as pesticides in crop agriculture. Streptomycin and oxytetracycline are common agricultural drugs used in vegetable and fruit production. Without antimicrobial pesticides, modern agricultural yields would be impossible to achieve. However, extensive use in the medical field and animal husbandry, aquaculture, and agriculture may contribute to the antimicrobial resistance (AMR) dilemma. The World Health Organization issued a global AMR warning, and the World Health Assembly endorsed a "Global Action Plan on AMR" in 2015. As a result, it must comprehend the current state of AMR in the agricultural environment. As a result, since 2017, we have been investigating AMR in agricultural specimens, irrigation water, soil, and crops. Based on culture-dependent methods at 37^0C, we discovered that resistant bacteria to penicillin, streptomycin, and oxytetracycline are widely distributed in agricultural contexts such as soil or irrigation water, even when antimicrobial pesticides are not used. On the contrary, the population level of oxytetracycline-resistant bacteria in the edible part of lettuce grown with oxytetracycline application under legally managed conditions was below the detection limit. So far, antibiotics derived from metabolites of common environmental microorganisms have been created. The natural environment can be considered a zone where antibiotic producers and resisters coexist. However, eliminating AMR contamination of fresh fruit with antimicrobial pesticides may be doable.

Keywords: Antibiotics, agriculture chemicals, rational use, and food security

1. Introduction

The discovery of antibiotics has contributed significantly to human life, especially for treating bacterial infections. Scottish physician and microbiologist Sir Alexander Fleming found a unique compound with antiseptic activity in the crude extract of *Penicillium* mold in 1928 (Wainwright M., 1993). It was the first isolation of an antibacterial agent from a microbe named "Penicillin" (Fleming A., 1929). Clinical trials were conducted in the 1930s (Chain E. *et al*., 1940, Fleming A., 1943), leading to practical application and commercial production began in 1946. Treatment of synthetic antimicrobial agents for infectious disease was standard then, but Fleming and his colleagues focused on naturally occurring substances produced by microbes. Penicillin inhibits specific enzymes in bacterial cell wall synthesis but does not affect human enzymes. The discovery of penicillin began a new era in infectious disease treatment. In the 1940s and the 1950s, various compounds from microbes or their culture base that were effective in treating infectious diseases were discovered one after another. And semi-synthesized derivatives were produced, and side effects were reduced compared to the primary compound. Fleming discovered penicillin in 1928 and analyzed the function of it for more than ten years. He also found resistance to penicillin by penicillin-sensitive bacteria early on whenever too little amount of penicillin was used or when it was used for too short term. He shared the Nobel Prize in Physiology or Medicine with Howard Florey and Ernst Boris Chain for discovery and practical application to humans in 1945. In his Nobel lecture, he talked as follows.

The time may come when anyone in the shops can buy penicillin, then there is the danger that the ignorant man may easily under dose himself and expose his microbes to non-lethal quantities of the drug, making them resistant (Fleming A., 1945).

It is impossible to purchase antibiotics without a prescription at a drug store in Japan. But the range of applications has expanded. It is to control pathogens of humans and animals and promote growth in food animals or horticultural crops. World Health Organization, WHO warned about antimicrobial resistance, AMR as a global crisis, and World Health Assembly adopted Global Action Plan on AMR in 2015. Following this action, the Japanese government proposed National Action Plan on AMR in 2016 and completed it. Now, the government is preparing a new action plan for AMR. So, there is an urgent need to understand the actual state of AMR in all fields we are involved in. To grasp the actual situation of AMR in the agricultural environment, we have been focusing

on agricultural specimens such as soil, irrigation water, and plant to observe the population of resistant microbes since 2017. Here we show resistance to β-lactam and quinolone antibiotics, frequently used in the medical field, streptomycin, and oxytetracycline, which are often used in the agricultural field in Japanese agricultural specimens.

1.1. Antimicrobial agent and antimicrobial resistance in the agricultural environment

In 1954, a unique concept demonstrated the potential of antimicrobial agents as agricultural chemicals to control plant pathogens (USDA, 1954). Several bactericides or fungicides had already been discovered and applied to humans then. In this report, USDA focused on the potential use of streptomycin, tetracycline, and actidione as a pesticide. Streptomycin was discovered from *Streptomyces griseus* as a bactericide by Albert Schatz and his thesis adviser Selman Abraham Waksman at Rutgers University with financial support by Merck and Co. (Schatz, *et al.,* 1944). Mass production began a year after its discovery, functioning as a specific medicine for tuberculosis. Now it is on the World Health Organization's List of Essential Medicines (WHO 2021a). In 1952 Waksman was awarded the Nobel Prize in Physiology or Medicine for his discovery of streptomycin, the first antibiotic effective against tuberculosis. In agriculture, it is a vital compound to control bacterial plant pathogens, such as *Pseudomonas* spp., *Streptomyces* spp., and *Erwinia* spp. in vegetable or fruit production. In addition, streptomycin treatment promotes seedlessness and the enlargement of grape berries, like gibberellic acid treatment.

Tetracyclines are a group of broad-spectrum antibacterial agents isolated directly from several *Streptomyces* bacteria or produced semi-synthetically from isolated compounds. Oxytetracycline is the second to be discovered in the tetracycline group and is used to control bacterial plant pathogens in agricultural fields. It was isolated from the soil actinomycete, *Streptomyces rimosus,* by a research group at Pfizer (Finlay, *et al.,* 1950), patented in 1949, and commercialized in 1950 under the trade name "terramycin". This discovery was a significant advance in tetracycline research and paved the way for the discovery of doxycycline, an oxytetracycline derivative, one of the most applied antibiotics today. It is on the World Health Organization's List of Essential Medicines (WHOb, 2021) as an alternative to tetracycline.

Actidione, also known as "cycloheximide" was discovered by Alma Joslyn Whiffen-Barksdale at the pharmaceutical company named “Upjohn Company” (Whiffen, *et al.,* 1946). It was isolated as a metabolite of *Streptomyces griseus* that also produced streptomycin and showed fungicidal activity but did not have bactericidal activity. It was used as an agricultural chemical to control fungal pathogens in vegetables and larch from 1959 to 1981 in Japan. Actidione is a crucial experimental tool in molecular biology or microbial ecology. However, due to its potent cytotoxicity, it is not used as a human medication.

Penicillin and cephalosporin are members of β-lactam compounds and are widely used as human medicine to cure bacterial infection. Cephalosporin was discovered in the culture medium of *Cephalosporium acremonium* (now known as *Acremonium*) from sewage samples by the Italian pharmacologist Giuseppe Brotzu in the 1940s (Bo G., 2000) and characterized in the UK (Newton and Abraham, 1955; Newton and Abraham, 1956, Abraham and Newton, 1961). Mass production began in 1964 by the American pharmaceutical company Eli Lilly and Company. Benzylpenicillin, the parent compound of penicillin, is adequate for Gram-positive bacteria. Since many bacterial plant pathogens are Gram-negative, semi-synthetic derivatives may act on pathogenic bacteria in agriculture. Another β-lactam compound, cephalosporin, is available to control both Gram-positive and Gram-negative bacteria. However, β-lactam compounds have never been applied to the agricultural field.

Nalidixic acid (Lesher, *et al.,* 1962) is quinolones' first synthetic antimicrobial agent and parent compound. Quinolone is used as a first-line drug, such as ciprofloxacin (Wise, *et al.,* 1983) for humans, and applied to both animal husbandry and agricultural production, such as oxolinic acid (Turner, *et al.,* 1967). The emergence of resistance to β-lactam and quinolone leads to higher risks, especially in the medical field since many of these are used as first-line drugs in the treatment of infectious diseases.

Development of antimicrobial agents as agricultural chemicals in Japan

After World War II, malnutrition was a serious problem, and it was an urgent task to increase crop yields in Japan. Controlling plant pathogens by pesticide application is thought to be one way, and the application of chemical pesticides began in the agricultural environment. Applying chemical pesticides such as organo-mercury compounds dramatically improved crop productivity and saved Japanese citizens from starvation. But further research on these compounds unveiled high persistence in rice grains and substantial toxicity to humans. Developing next-generation pesticides with low environmental impact, toxicity to humans and animals, and high specificity to plant pathogens became necessary. The

research group in The University of Tokyo, RIKEN, and the National Institute of Agricultural Science tried to examine several microbial culture substances to inhibit rice pathogen activity in the 1950s. Kazuo Fukunaga, National Institute of Agricultural Science, isolated *Streptomyces griseochromogenes* Fukunaga that can produce a fungicidal compound to blast fungus of rice (Fukunaga, *et al*., 1955). Hiroshi Yonehara and Yusuke Sumiki analyzed the function of this substance and named it "Blasticidin S" (Takeuchi, *et al*., 1958). This compound was first discovered antimicrobial agent by microbial fermentation specialized in agriculture. Commercial production of Blasticidin S began in 1961 and contributed to the peak productivity of rice in 1967.

Later, several Japanese researchers tried to find microbes that could produce beneficial compounds from soil samples all over Japan. Kasugamycin, bactericide, and fungicide from *Streptomyces kasugaensis* (Umezawa, *et al.,* 1965), Validamycin, bactericide, and fungicide from *Streptomyces hygroscopicus* (Iwasa, *et al.,* 1970) and Polyoxins, fungicide from *Streptomyces cacaoi* (Suzuki, *et al.,* 1965) were discovered and applied to rice paddy or vegetable field. Japanese chemical company Sumitomo Chemical developed the synthetic compound oxolinic acid as agrichemical and applied it in the agricultural environment. Undoubtedly, these compounds were saviors for Japanese citizens of that time.

AMR in agricultural specimens, our attempt

Since the discovery of penicillin by Fleming in 1928, humankind has discovered various antimicrobial agents to fight against infectious diseases. Especially from the 1940s to the 1950s, antimicrobial agents' discovery and practical application progressed rapidly, and they are still helpful today. On the contrary, finding new substances to control pathogens is very rare. So, we must fight with drugs discovered in the middle of the 20th century if we encounter new types of resistant microorganisms. Antimicrobial agents are used not only for medical purposes but also for producing meat, fish, and crops. Improper use of antimicrobial agents can lead to the development of antimicrobial resistance, AMR. Current methods for antimicrobial agents in agricultural fields are based on the control effect of plant pathogens or produce yield but do not concern the emergence of AMR. Some types of antimicrobial agents are used executively in agriculture, but some have applications in other areas. We have been focusing on a few types of fresh produce and its cultivation field to understand the current situation of antibiotic resistance in the agricultural environment since 2017.

β-lactam resistance and quinolone resistance

β-lactam and quinolone are primary weapons to fight against bacterial infection. So, the emergence of resistance to these antibiotics causes severe problems in the medical scene. As a first step of understanding the resistance to b-lactam and quinolone in agricultural environments, we observed microbial resistance to aminobenzylpenicillin and ciprofloxacin in agricultural specimens. Benzyl penicillin, the parent compound of penicillin, can affect Gram-positive bacteria, and a semi-synthetic derivative, amino benzyl penicillin, can inhibit the growth of Gram-positive and Gram-negative bacteria. Ciprofloxacin is 2nd second-generation quinolone antibiotic that can be applied to Gram-positive and Gram-negative bacteria.

To observe microbial resistance to these substances, we collected soil samples from strawberry fields and irrigation water from water streams near berry fields. Collected samples, potting soil, and irrigation water were diluted in PBS (pH 7.4) and spread on agar plate R2A agar plate. To identify resistance of β-lactam and fluoroquinolone, aminobenzylpenicillin (40 µg/L) or ciprofloxacin (40 µg/L) were added to R2A agar plate. The colony-forming unit (CFU) on each agar plate was counted to confirm bacterial population after incubation at 37^{0}C for 48 h. In irrigation water, the population of amino benzyl-penicillin-resistant bacteria was 3.39 Log CFU/ml, almost 10 % of total bacteria (4.67 Log CFU/g). Ciprofloxacin-resistant bacteria were found below the detection limit (Fig. 1).

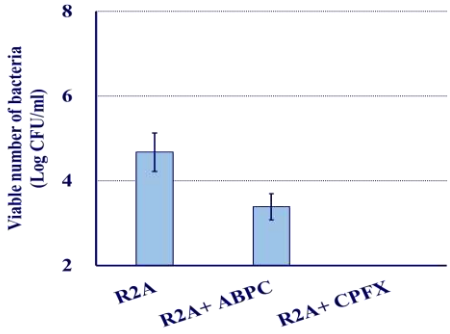

Fig. 1. Population size of resistant bacteria to amino benzylpenicillin and ciprofloxacin in irrigation water

The population size of resistant bacteria was decided by counting colony forming unit, CFU on R2A agar plate with 40 µg/L of aminobenzylpenicillin, ABPC, or 40 µg/L of ciprofloxacin, CPFX as a selective agent after incubation at 37 oC for 48 hours. Asterisk indicates below detection limit without applying this substance, there was a higher level of resistant bacteria to amino benzylpenicillin in the water stream. The total bacteria population on the R2A agar plate in

potting soil was 7.18 Log CFU/g. Those of resistant bacteria to aminobenzylpenicillin and ciprofloxacin were 6.85 Log CFU/g and 4.45 Log CFU/g, respectively. It was almost the same population size as total bacteria and resistant bacteria to aminobenzylpenicillin (Fig. 2).

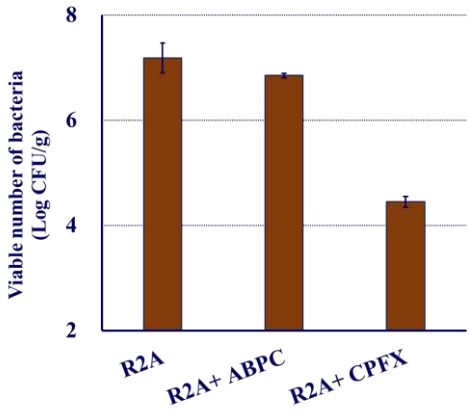

Fig. 2. Population size of resistant bacteria to amino benzylpenicillin and ciprofloxacin in strawberry potting soil

The population size of resistant bacteria was decided by counting colony forming unit, CFU on R2A agar plate with 40 µg/L of aminobenzylpenicillin, ABPC, or 40 µg/L of ciprofloxacin, CPFX as a selective agent after incubation at 37oC for 48 hours. The resistant bacteria to amino benzylpenicillin and ciprofloxacin were present in potting soil without applying these substances. Almost all isolated bacteria showed resistance to aminobenzylpenicillin in numerical data. These results suggest that resistant bacteria to amino benzylpenicillin or ciprofloxacin were present as natural flora in potting soil. The basic structure of amino benzylpenicillin, a semi-synthetic compound, is the same as the naturally occurring compound benzylpenicillin. If there is a benzylpenicillin producer, it may be one of the reasons why it acquires resistance to β-lactam antibiotics in the soil environment. On the contrary, ciprofloxacin is categorized in 2nd generation quinolone and a most common quinolone antibiotic as medicine. Oxolinic acid, 1st generation quinolone antibiotic, is available to apply as a control agent for bacterial pathogens in the agricultural field. However, it was not to be applied to strawberries in our experiments. It was true that there were resistant bacteria to ciprofloxacin, but at this stage, we do not have any answer why they were in the natural environment.

Streptomycin and Oxytetracycline resistance, direct interaction of pesticide

Currently, various antibiotics, streptomycin, oxytetracycline, kasugamycin, and oxolinic acid, are used as agricultural chemicals to grow crops in Japan. Among these, streptomycin and oxytetracycline are common drugs in treating infectious diseases in the medical field. And streptomycin and oxytetracycline are essential substances as agricultural chemicals to control bacterial plant pathogens, Xanthomonas spp., *Pseudomonas* spp., *Pectobacterium* spp., and *Erwinia* spp. in vegetables or fruits production since 1956 in Japan. Applying plant hormones is an important method for improving the quality and quantity of horticultural crops. Streptomycin, isolated as an "antibacterial agent" in 1944, also has a unique function that promotes seedlessness in grapes and the enlargement of grape berries like gibberellic acid. In 1976 the researcher at the Fruit Tree Experiment Station of Hiroshima Prefecture, Shizuo Ogasawara, found this phenomenon (Ogasawara, 1985), and recently the method has spread to vineyards across the country. And the application of streptomycin to grapes is allowed to promote seedless berries but not as a bactericide in Japan. Streptomycin affects to small subunit of ribosomal RNA (30S), inhibits protein synthesis in bacteria, and leads to the death of bacterial cells. The function of streptomycin in bacterial cells is already known, but there are many unknown points in the above function in grapes, although practical steps have been identified. To understand the effect of antibiotics application on the development of resistance in agricultural fields, we observed bacterial properties on baby onion surfaces, several soil samples for streptomycin resistance, and lettuce leaves and onion field soil for oxytetracycline resistance.

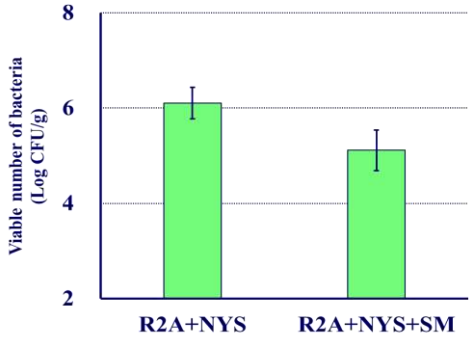

Fig. 3. Population size of streptomycin-resistant bacteria on baby onion surface. Population size was decided by counting colony forming unit, CFU on R2A agar plate with 50 µg/L of nystatin, NYS as a fungicide, and 40 µg/L of streptomycin, SM as a selective agent after incubation at 37^{0}C for 48 hours.

Onions grown for about two months after sowing seeds in the ground were harvested to observe resistance to streptomycin. The harvested baby onion appeared tiny; the bulb was not enlarged yet and looked like "leek". Collected whole baby onion plants were suspended and diluted in PBS (pH 7.4), and spread on the agar plate, R2A agar plate with streptomycin (40 µg/L) as a selective agent and with

nystatin (50 µg/L) as fungicide if necessary. There were four soil samples with different cultivation histories, three from conventional farming systems and one from organic farming systems, to observe streptomycin resistance. The soil samples were suspended in four volumes of PBS (pH 7.4), diluted in the same buffer, and spread on an agar plate with streptomycin (40 µg/L) as a selective agent and cycloheximide (50 µg/L) as a fungicide. Lettuces grown in both conventional and organic cultivation systems and soil from onion fields were collected to observe oxytetracycline resistance. The outermost three leaves or soil samples were suspended and diluted in PBS (pH 7.4) and spread on an agar plate, R2A agar plate with oxytetracycline (40 µg/L) as selective agent and nystatin (50 µg/L) as a fungicide. To decide the population size of resistant bacteria to streptomycin or oxytetracycline, the number of colony-forming units, CFU, was counted after incubation of agar plates at 37^0C for 48 hours. The population of streptomycin-resistant bacteria on the surface of baby onion was 5.11 Log CFU/g and was equivalent to nearly 10 % of total isolated bacteria (6.10 Log CFU/g) (Fig. 3). The application of streptomycin to onion is allowed five times in the entire cultivation period and is mainly applied to growing plants such as young onions or adult onions but not to baby onions. Observed baby onions were cultivated under conventional conditions but have never been exposed to streptomycin since they were collected before the first exposure. However, there was a large population of streptomycin-resistant bacteria on the surface of the baby onion. This result may suggest that resistant bacteria to streptomycin exist in onion cultivation environments as part of natural microbial flora, even without streptomycin treatment in agricultural activity. Soil samples were collected at four different places in Japan, three from conventional farming systems and one from organic farming systems. The population of streptomycin-resistant bacteria was from 4.60 to 5.60 Log CFU/g in conventional farm soil and 5.41 Log CFU/g in organic farm soil (Table 1).

Table 1. The population size of streptomycin-resistant bacteria in agricultural soil

Sampling Place Prefecture	Soil Property	Cultivation History	SM Resistant Bacteria (Log CFU/g)
Hokkaido	Brown soil	Onion Conventional	4.67
Fukushima	Brown lowland soil	Peach Conventional	4.60
Wakayama	Black soil	Japanese apricot conventional	5.60
Hiroshima	Brown lowland soil	Lettuce organic	5.41

Soil samples were collected at four places with different cultivation histories, three from conventional farming systems and one from organic farming systems. The population size of streptomycin, SM-resistant bacteria was decided by counting colony forming unit, CFU on R2A agar plate supplemented with cycloheximide (50 µg/L) as a fungicide, and streptomycin, SM (40 µg/L) after incubation at 37 ºC for 48 hours. Even in organic farms managed for more than 20 years, there was no significant difference in the population size of streptomycin resistant bacteria between conventional and organic farm soil. This result suggests that resistant bacteria to streptomycin exist in agricultural soil environments as natural microbial flora even without using streptomycin as an agricultural chemical. Oxytetracycline, the trade name terramycin, is a member of tetracycline and a broad range of antibiotics to treat bacterial infections. It was discovered from soil samples, *Streptomyces rimosus,* and have been applied as a pesticide since 1956 in Japan. Conventional and organic cultivation farms obtained lettuce samples and three outermost leaves to detect oxytetracycline-resistant bacteria. On the surface of lettuce leaves from conventional farm population size of total isolated bacteria was 4.87 Log CFU/g, and that of oxytetracycline-resistant bacteria was below the detection limit. While in samples from organic farms, the total bacteria population was 5.99 Log CFU/g, and that of oxytetracycline-resistant bacteria was 2.30 Log CFU/g (Fig. 4). The population level of total bacteria on organic lettuce leaves was higher than on conventional samples. A small population of oxytetracycline-resistant bacteria was present on lettuce leaves from organic farming systems (Fig. 4b) but not from conventional farming systems (Fig. 4a). The soil sample was collected from an onion field managed as a conventional farm to detect resistant bacteria to oxytetracycline. The population size of total bacteria was 6.91 Log CFU/g, and that of oxytetracycline-resistant bacteria was 4.24 Log CFU/g. The application procedure of streptomycin or oxytetracycline as agricultural chemicals are managed by law and the application of amino benzylpenicillin or ciprofloxacin is prohibited in Japan. A large population of resistant bacteria to concerned antibiotics were found in observed specimens, irrigation water, potting soil, fresh

produce, and agricultural field soil, even without the application of antibiotics as agricultural chemicals. The resistant bacteria to concerned antibiotics in agricultural specimens may have existed initially in each sample and were not directly affected by pesticide application in the agricultural field.

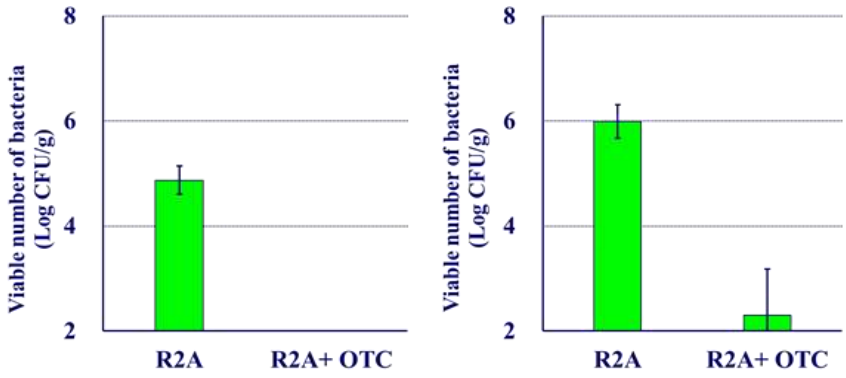

Fig. 4: Population size of oxytetracycline-resistant bacteria on lettuce leaves. Bacteria were isolated from lettuce leaves cultivated in conventional farms (a) and in organic farms (b). The population size of oxytetracycline, OTC-resistant bacteria was decided by counting colony forming unit, CFU on R2A agar plate with oxytetracycline, OTC (40 µg/L) after incubation at 37 °C for 48 hours.

Most antibiotics are produced by soil microbes as their metabolite and used for us as beneficial compounds. If the observed samples contain numerous microorganisms capable of producing antimicrobial agents, microbes should possess the ability of resistant to various antimicrobial agents in these circumstances. It may be one reason why even without applying resistance to concerned substances were present in the agricultural environment. As a following step observation, it is necessary to observe what kind of bacteria have resistance and what types of genetic element bacteria have.

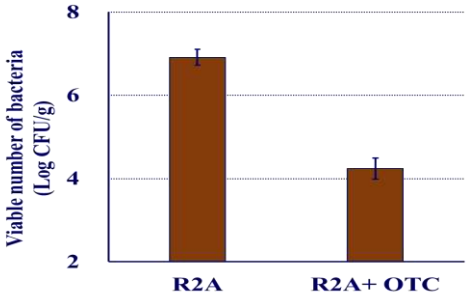

Fig. 5: Population size of oxytetracycline-resistant bacteria in onion field soil. The population size of oxytetracycline, OTC-resistant bacteria was decided by counting colony forming unit, CFU on R2A agar plate with oxytetracycline, OTC (40 µg/L) as a selective agent after incubation at 37°C for 48 hours.

Conclusions

Here, we discussed the resistance to four antibiotics, amino benzylpenicillin, ciprofloxacin, streptomycin, and oxytetracycline, in agricultural specimens. These substances are important for medical purposes and valuable for food production. Although amino benzylpenicillin and ciprofloxacin are not allowed as agricultural chemicals, there were resistant bacteria to aminobenzylpenicillin in soil and water and ciprofloxacin in soil. Streptomycin and oxytetracycline are used as agricultural chemicals to produce vegetables or fruits in Japan. Despite applying these substances, resistant bacteria to streptomycin or oxytetracycline were present in fresh produce and its cultivation soil. These results suggest that resistant bacteria to observed substances exist in agricultural environments as natural microbial flora.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

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Comparative Analysis of Food Safety Systems between Europe and Developing Countries

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Abstract

National food safety authorities can be found in the world's wealthiest countries but are often lacking in developing countries. However, there are several intriguing stories of countries taking the risk of establishing food safety authorities or systems. This article examines food safety standards in Europe and other developing countries by classifying countries into four groups: those with laws and a food safety agency, such as the European Union and its food law regulation 178/2002, which has been in effect since 2005. This regulation 178/2002 describes some of its articles and phrases. Terms such as food definition, food traceability, trust in the food system, expert independence, risks and hazards, insurance, rapid alert system, food crisis, precautionary principle, and Codex Alimentarius are defined, and examples are provided. The author also defines three other classifying countries where producers set their food safety system, consumers manage safety, and countries without food safety. These latter countries are frequently in conflict or suffering from natural disasters.

Keywords: food safety system, developing countries, expertise, risks, hazards, regulations

1. Introduction

Food safety is understood very differently depending on the level of development between rich and developing countries. The increased complexity of food chains, the impact of contaminated food on human health, and the economic well-being of the agri-food industry are very different and depend on the country's-richness. It is, therefore, necessary to assess the risks associated with food hazards from production to consumption, including transformation systems, transportation, and conservation systems, considering the new context of climate change. At the consumer level, preparation conditions and consumption modalities are also required. The definition of hazard is relatively straightforward. Chemical dangers (pesticides, certain additives, toxins, hormones...), biological hazards (pathogenic bacteria, toxigenic molds, viruses, prions...), and heavy metals (mercury, lead, cadmium, copper, sulfur...) all exist in the food chain. The risk is more difficult to comprehend because it is the likelihood of encountering a threat. If the danger is absent, the risk is zero; if the hazard is highly present, the risk is proportional to the hazard's magnitude (Montet, 2019). A complex interaction between the doses and effects of a pathogenic agent or toxic substances and the duration of exposure determines toxicity. The exposed population, which includes youngsters, the elderly, and the sick, is also an important factor to consider. Obtaining the effects of food risks is still tricky in high-income nations because people who are not highly sick treat themselves, and some medical doctors do not disclose data on foodborne diseases to relevant institutions. Statistical agencies collect data on deaths, causes of death, and hospitalizations. On the contrary, many low- and middle-income nations lack a statistical institute, therefore, the number of deaths and sick individuals from certain causes must be estimated based on insufficient data (Montet et al., 2019). Statistics on the causes of deaths, illnesses, and hospitalizations could assist food and health authorities develop public health policy. Sickness and hospitalizations are exceedingly expensive for public healthcare systems in wealthy countries, even with health insurance. We should also examine the second effect of foodborne infections in low-income countries on tourism, trade, and families without access to health care.

Risk management is very poor in low- and middle-income nations, and the population is vulnerable to several food hazards. In industrialized countries, hazards are adequately handled by industry, therefore, it is common to work on risks to assess the population's protection from hazards. It is critical to understand that a working food safety system is quite costly. First, the country must pass laws and incorporate them into its legal system. As a result, the government must have lawyers skilled in these numerous international regulations and give sanctions. Second, a sanitary police force with national control capabilities is required. Only if the country has official control laboratories can this policy be effective. The country needs experts in every aspect of food [microbiology, water, toxins, chemicals, animal diseases, plant diseases, and GMOs (Genetically

Modified Organisms)], to comprehend the investigation's findings. They must then be able to punish offenders without falling under corruption. In the extreme case of public poisoning, the government must be able to stop the production of the offending product by stopping food chains or refusing food at borders. The simple fact of recovering food products already dispersed on the national market requires the intervention of many inspectors, especially a very dangerous media dissemination for the offending industrialist (see example below). This needs assessment demonstrates the difficulty of establishing a national food safety system unless the national leadership is convinced of its fundamental necessity.

We must add that human sensibilities are not universal. I'll share a few experiences from my time in Thailand working at an international center called the Asian Institute of Technology in Bangkok. I met a Belgian chocolate producer who claimed he was no longer selling chocolate... Belgian chocolate. The explanation stemmed from perfectly accurate information broadcast on local television, which announced that amounts of dioxin had been discovered in Belgian milk. Thai consumers rapidly realized the connection between Belgian milk, dioxin, and Belgian chocolate! Let me reassure Thai consumers that Belgian chocolates in Thailand are made with milk from Australia, New Zealand, or even Thailand.

The insurance connection distinguishes developing countries from the richest countries. People in wealthy countries must get insurance for all areas of life and death. This mandatory insurance adds significant value to items. Death, for example, does not have the same societal cost in different countries. There are only a few industries where insurance is not required. One exception is some poor farmers who cannot afford to donate sufficiently. Governments force insurers to make efforts over time and on the sums paid during natural disasters. Climate insurance will emerge fast, raising the overall cost. At COP 27 (2022), international political leaders established a fund to cover climate disasters in developing nations. It is too bad they did not fare so well in food disasters. It is critical to understand what a food crisis is. A crisis is, by definition, a complex issue that arises from a real sanitary problem occurring globally and is immediately transmitted to the rest of the world via media. According to the DGCCRF (France's Directorate-General for Competition, Consumer Affairs, and Fraud Control), a crisis is "a situation of real or perceived risk that can cause collective concern." For example, the enormous spread of avian influenza has resulted in a global problem. The virus affected cattle ranches in underdeveloped countries like Vietnam and wealthier ones like Taiwan. Due to the lack of insurance, producers were forced to sell contaminated animals. This swiftly erodes the consumer surveillance system's credibility.

Companies are required to attend crisis management training sessions. A single corporation implicated in one area puts the entire sector at risk. To forecast and manage a crisis, it is consequently vital for producing companies in the same industry to band together. Government sensitivity to food safety is often low, not because they neglect this research, but because their government has many more essential things to deal with, such as hospital development or food security (agricultural production).

Consumer sensitivity varies not just depending on the country's wealth but also with time. Consumers in the least developing countries will have to trust their local sellers not to make them sick, but wealthy consumers can choose their food based on other characteristics. I'll give you a few examples that can be psychological or highly genuine. Some are about health, some about religion, and still others about food safety:

- What is the danger of GMOs to health?
- What to eat to improve my sports performance?
- Is there pork or alcohol in my food?
- Are short supply chains good for health, but the consumer rarely asks himself the question of the safety of an uncontrollable product in some cases?
- The consumer considers organic products suitable for health because they are free of chemicals while they contain, for the most part, so-called natural fungicides (copper sulfate, sulfur…) and other molecules (insecticides)? Problems with energy usage are beginning to influence agri-food products, and life cycle assessment (LCA) is gaining traction and getting more sophisticated. LCA is a defined framework for assessing the environmental implications of a product or system across its entire life cycle, from raw material extraction to end-of-life or recycling (ISO 14040 and 14044). It is now required to quantify food carbon printing and its environmental impact.
- How can developed countries reduce food waste? These, too, vary in terms of wealth. In general, losses in impoverished countries are tied to agricultural productivity, whereas losses in rich countries are primarily related to consumer losses, as consumers can toss extra food in the trash.
- Can food plants (oilseeds, starch) be transformed into energy while still necessary for some people's nutrition?

Since 2013, a complex database built according to the LCA methodology called Agribalyse (Ademe, France) has provided reference data on the environmental impacts of agricultural and food products. This database includes all stages in product manufacturing (production, processing, distribution, consumption) while considering various environmental issues (climate, water, air, soil, etc.).

Consumer questions lead scientists to study complicated issues such as endocrine disruptors (Jondeau-Cabaton et al., 2013), often confused with toxic compounds. Guignard et al. (2022) controlled the impact of the induction of region-specific changes in brain metabolomic fingerprints by bisphenol A during sheep's gestation.

Food safety has a relatively long history in rich countries because the word first originated in the 1990s, driven by the European mad cow epidemic (1996), giving politicians and lawyers' time to establish the most important legislation of the twenty-first century. This European Regulation, known as Food Law 178/2002, went into effect on January 1, 2005, and the main articles are discussed in this text.
This European Regulation gave legislators and food chain stakeholders' options to procure and deliver healthy food to European consumers (Article 1, Regulation 178/2002). The term "healthy" was coined by jurists who may not have realized what it concealed scientifically. The lawyers wanted all food consumed, imported, or exported from the EU to be safe for the consumers.

Regulation 178/2002 was a global legal revolution. It explained the definition of food, the level of safety that European consumers could expect from it, the concept of traceability and responsibility, and the establishment of the European Food Safety Authority (EFSA). This traceability law is particularly intriguing because it is not detectable by analytical materials (Guyon et al., 2020). Only administrative records can guarantee food traceability throughout the food chain. However, which publications describe DNA molecular methods (El Sheikha et al., 2009), isotopic methods (Camin et al., 2016, 2017), or metabolomic approaches (Guyon et al., 2020) to investigate geographical origin? The barcode is the most extensively used technology for tracing food goods worldwide. The principles governing food and feed safety at the community and national levels are also outlined in Regulation 178/2002. It sets shared principles and obligations (Article 17, Regulation 178/2002) and the food crisis management principle. The development of Internet media in 1993 prompted LDC countries to focus on agricultural production (food security) and food safety, but these two factors evolved with time in wealthier countries. As a result, we see questions raised by the internet in countries where production is insufficient to feed the people. GMOs are one such example. Indeed, rich countries investigate the hazards linked with GMOs in their country, and most expert evaluations see GMOs as safe. Even in the LDC countries, where there are no public experts to examine the situation, studying the danger causes a sense of unease.
Based on the state of healthcare systems, the food safety ranking of countries is proposed as given below:

- Countries with an uncontrolled food safety status
- Countries with a population-controlled food safety status
- Countries with an industrialist-controlled food safety status
- Countries with a regulatory-controlled food safety status

1. Countries With a Sanitary Status Controlled by Regulations

The countries with sanitary status controlled by regulation are the most difficult to describe. Europe is the continent that has created the most recent law, which was drafted in collaboration with 27 countries. Furthermore, this paragraph emphasizes that food, whether imported or manufactured locally, must be nutritious for European consumers (Article 11, Regulation 178/2002). This text requires European manufacturers to export nutritious food to all other countries. As a result, practically every country on the earth is affected by this passage.

American countries such as the United States and Canada have distinct ways of thinking. The world's first food safety law was written by Canada shortly after the country was founded when they were creating their constitution. Safe Food for Canadians (Canadians Regulations, Act, SC 2012) governs food safety. The United States, for example, has included the concept of traceability in its terrorist legislation (The Bioterrorism Act, 2001; Montet, 2021) as well as its Food Modernization and Safety Act (USA-FDA FSMA 2011). In Europe, European Regulation No. 178/2002 is the legally binding regulation adopted by the 27 European countries. It was published in 2002 and has been in force since January 1, 2005. It is located on the web server of the European Union with free access to all the languages of the community (http://eur-lex.europa.eu).

It is worth noting that this rule specifies food for the first time (Article 2, regulation 178/2002). According to the regulation, "food" refers to any substance or product, whether processed, partially processed, or unprocessed, that is meant to be consumed or is reasonably expected to be ingested by humans. This word also includes beverages, chewing gum, and any item, including water, that is purposefully mixed into food during its manufacture, preparation, or processing. It should also be mentioned that water became a food in Europe in 2005, having previously been classified as a separate thing.
Lawyers made an intriguing choice in determining what is and is not food. According to European regulations, the term "Food" does not include Feeding stuff.

- Live animals
- Pre-harvest plants
- Medicines
- Cosmetics
- Tobacco and derivatives
- Narcotics or psychotropics
- Residues and contaminants

The general population understands that food is a substance or mixture of components consumed by mouth. However, the legislation states that living animals and plants are not food. Eating a cow straight from the pasture is not food but an agricultural product subject to another set of rules. The same is true for salads, which must be consumed immediately after harvesting. We can see an oversight with shellfish; however, the legislator has remedied this error in the European microbiological rule (EC regulation No 2073/2005). Cosmetics, tobacco, medicines, and drugs are not food, even if taken by mouth. Food that

is injected into you intravenously is not considered food but medicine and therefore falls under other medical regulations.

All of this is straightforward for consumers; however, the final line, "Residues and contaminants," clarifies that we should not find chemical residues (EC Regulation No 470/2009) such as herbicides and pesticides from agricultural treatments (EC Regulation No 1107/2009), nor contaminants from, for example, microbial contamination (EC Regulation No 2073/2005) that could produce toxins and mycotoxins (Montet et al., 2021 a, b). Of course, this is not always the case. Bacteria, yeasts, and molds can be present in fresh food.

The European Food Safety Agency (EFSA) publishes an annual report on pesticide residue levels in foods sold on the European market. According to the 2019 report, 96.1% of the total 96,302 samples evaluated fell below the maximum residue level (MRL), while 3.9% surpassed this threshold. According to these findings, the level of residues is unlikely to harm consumer health (EFSA Report on Pesticides 2019).

To comply with this article of law, it is required to resort to the set of norms that establish the limit beyond which a dangerous substance cannot be included in food, rendering it unfit for marketing. The most widely used norm system is Codex Alimentarius. Conflict of interest regulations governs the independence of specialists in Europe. Article 6 of Regulation 178/2002 states that the risk assessment is based on "available scientific evidence and shall be conducted in an independent, objective, and transparent manner." Such texts are uncommon in impoverished countries. Many countries have food safety specialists; however, they are frequently strongly associated with the government.

One of the most notable recent cases exemplifies this brilliantly. If veterinarians in South-East Asia, who are quite skilled, could freely communicate with the international community, the avian influenza virus would have travelled considerably more slowly from South-East Asia to Europe. They are usually civil officials in their country and hence have a close relationship with the government, some of whose members are connected to significant chicken producers! This is not illegal, but this personal and professional relationship must be governed by law. A scientist can only rely on peer-reviewed literature or collective knowledge. Media reports do not provide scientific proof. Most individuals do not see the "available evidence" that a scientist does.

Collective expertise is a technique national food safety authorities use to reflect on food safety concerns and create opinions for political leaders who are accountable to the people who elected them (Montet et al. a2019, 2020). The author (Montet et al., 2019) tested the use of Collective Expertise in the context of the Erasmus+ project (EU) DAfrAli titled "Societal Challenges and Governance of African Universities: The Case of ALIments in Morocco, the Democratic Republic of the Congo, and Senegal." This initiative aims to develop African Higher Education Institutions' governance capacity to mobilize their resources to respond to important societal crises involving external stakeholders.

The findings of this project's Collective Expertise pilot exercises are documented in a second article titled: Collective Expertise was used to analyze societal difficulties in food safety in Morocco, the Democratic Republic of the Congo, and Senegal. (Montet et al., 2020).

The following topics were studied and published in this paper as the possible creation of a food safety expertise in the three countries with different levels of policies, testing the food safety hazards per country by collective expertise. Food safety was analyzed in the three low- and middle-income countries. The conclusion was translating these health challenges into state, society, local authorities, and academic-level mitigation actions.

Another section of Regulation 178/2002 is extremely significant and novel. This is known as the "precautionary principle" in Article 7. According to this article, when a consumer threat is discovered, it is essential to contact the manufacturer. It must respond proportionally. If the risk is significant and the danger is immediate, the maker must remove the food from the food chain as soon as feasible. If the threat is unknown, but experts believe it is feasible, the maker must research to determine the real risk to the consumer.

Three examples are given below:

- A glass jar shatters a baby food chain. The risk is significant, it is known, and the manufacturer must eliminate it promptly. After that, the traceability system will be used. The European rule requires that the customer be informed through the media as soon as possible, especially if the violating lots have been placed on the market. It entails going on television and saying that your organization is unprepared to deal with hazards. This will ultimately result in legal issues, which may result in administrative closure. The company must be prepared for crisis management to avert this type of catastrophe. Some courses are already available.
- A confectionery producer is concerned about the safety of caramel, which is manufactured by carbonizing sucrose. This reaction could result in the formation of a carcinogenic chemical. This scenario could not exist in Europe because traditionally consumed products are not often risk-assessed. If this question were posed, the industrialist would be asked to do research with qualified experts and produce a report on the safety of caramel.
- The case of GMO authorization in Europe is instructive. In contrast to the United States, 80% of European consumers are concerned about GMOs. Some customers accuse them of being hazardous to human health, whereas experts believe and demonstrate the reverse! Do we have the technological means to determine the long-term impacts of GMO consumption?

Regrettably, no! Can we forecast toxicity in the next 50 years?

Experts are unsure, but they are also unsure about other plants derived from traditional crosses. Scientists, on the other hand, have developed standardized tests to quantify existing risks. They are exceptionally knowledgeable about the toxicity or allergenicity of foods. As a precautionary measure, Europeans have chosen to research the dangers of GMO plants on a case-by-case basis with the help of their agencies' specialists. When so many anti-GMO organizations dominate the media, consumers cannot form an opinion on the hazard.

2. Countries with a State of Food Safety Status Mastered By Industrialists

When a unified body of food safety regulation is lacking, countries may rely on Codex Alimentarius. Codex Alimentarius aims to provide people with "safe and good food for everyone - everywhere," particularly in the current environment of worldwide food distribution and consumption (Article 14, Regulation 178/2002). The Codex is a collection of international food standards, guidelines, and codes of practice that help to ensure the safety, quality, and fairness of international food trade. Codex standards are developed by worldwide expert groups and are based on independent international risk assessments organized by FAO and WHO, allowing them to be trusted (Codex Alimentarius).

Countries with a state of health managed by manufacturers are those in which the government has not accepted responsibility for food safety for various reasons. The two most significant are a lack of comprehension of the situation and financial resources. Many countries still lack regulations or old writings that are no longer useful. On the other hand, some of these developing countries have enterprises that sell to Europe or other developed countries and are thus required to apply the international safety and quality system, including food safety rules. The use of media such as television, radio, or social networks is a cost-effective way to instruct actors in the food chain. For example, consider the Pangasius sector in Vietnam, which has been operating at a high level of sanitation for many years, or Thailand, which recognized the need to train street vendors through the media more than 20 years ago.

Universities are common in this group of countries. Most of their teachers or researchers have completed graduate studies, post-doctoral fellowships, or advanced training studies in their own countries or abroad. They could examine local problems precisely using their international scientific contacts, scientific publications, and an internet connection as data sources. When the government lacks a food safety agency, food analysis could be performed by public or private laboratories and then analyzed by those researchers/teachers, resulting in data that can be used to inform political decisions. This is referred to as Collective Expertise. This technique will transfer knowledge to authorities involved in food safety management. It has the potential to be a robust tool for addressing problems and addressing the challenges of food safety and security. However, this pooled competence must be recognized by the government.

3. Countries whose Food safety Status is Under Control by the Population

This category comprises countries whose governments and manufacturers do not take food safety seriously. The actors thus assure the food safety of street shops or other self-sufficient small companies. Some countries may have received insufficient public training due to media coverage or dissemination by training actors. Few examples are provided that do not necessarily represent national food safety systems. Some food business person covers their meat with fabric in various African towns. These butchers believe it will protect their meat from various microorganisms, particularly harmful bacteria. The use of clay jars to filter water from rivers or streams with questionable hygiene was witnessed. The large size of some countries and the country's spread over several islands is a concern. The number of food safety officials would thus be too large and prohibitively expensive. Official analysis laboratories might sometimes be placed far from food production and customers. We may speak of short supply chains in many developing nations because food makers are frequently close to customers. We frequently observe the cook making meals directly on the site, which is contaminated by several clouds of dust that can contain mold and germs that could impair the consumers' health.

It is worth noting that short-supply chains are gaining popularity in industrialized countries as a symbol of trust and product quality. The consumer believes that a product that is sent directly from the manufacturer to the consumer does not include dangerous additives and is hence not ultra-processed. As we now know, ultra-processing can cause a variety of ailments in consumers.

For example, in France, more than 35% of food sold in supermarkets is deemed processed; it is self-evident that a public food safety system cannot supervise sanitary quality quickly, no matter how sophisticated. The biggest problem is that these countries can be targeted by ill-intentioned exporters who can sell them more or less contaminated food that countries with an efficient health system would refuse.

4. Countries With Uncontrolled Food Safety Status

Countries generally at war or have experienced big disasters, such as earthquakes or major floods, are classified in this group. A flooded area can no longer maintain the cold chain required for food preservation. Among these, cholera epidemics are major in these countries, where consumers cannot obtain drinking water due to climatic or tectonic dangers. The water quickly becomes contaminated, and these countries either lack a purification system or have equipment that the catastrophe has damaged. These

circumstances also encourage the growth of harmful bacteria.

Cholera is caused by the bacteria *Vibrio cholerae*, which is common in many underdeveloped nations. It will develop if climatic conditions allow during a significant climate change. For example, it is usual in Africa to see vendors selling bottles without labels along the roadside, and with experience, we quickly recognize that these bottles do not contain fruit juice or wine, as one might expect, but rather gasoline for cars. This fuel is derived from unreported transportation, and there is no insurance coverage for the use of these goods. It is also reasonably usual to come across bottles containing peanuts or other nuts on the roadside vendor. These nuts are commonly found in new-born formulae (Waré et al., 2017; N'zi et al., 2023), and it is difficult to determine a professional on the most severe threat between malnutrition and carcinogenic mycotoxins in young children. As Manizan et al. (2018) demonstrated in Ivory Coast, it is impossible to know the contamination by multi-mycotoxins.

5. Conclusions

This section depicts the problems that all countries face while developing or operating a national food safety system. When a country has a national system like this, it is expensive since it necessitates the following: Sample collectors (national inspectors), certified analysis laboratories, national experts for interpretation of results and crisis management (national agency), a political reaction chain operational 24 hours a day in the event of a proven food safety crisis, and police to remove offending batches from the market. A national system that involves numerous ministries without placing them in competition is ideal. The following system is proposed:

- Ministry of Commerce: a controlled food collection and laboratory analysis of food
- The Ministry of Agriculture manages the national food safety agency, recruits and appoints national experts, and manages food safety crises.
- The Ministry of Health manages the statistics agency and suggests treatments in cases of consumer intoxication.
- Consumer associations must be given the legal power to question expert panels, which the government and expert committees already have.
- In small countries, a simplified system with at least public control laboratories, a group of specialists who can recommend governments, and an established government reaction chain is possible.
- In any event, involving specific media to remove foods from the market that may harm consumers' health is beneficial. In this instance, it is critical to develop communication skills to avoid destroying industries while just one industrialist is implicated.
- Insurance against losses caused by diseases such as avian influenza is typically required for adequate contamination management. When a farmer is not insured against losses, he is more likely to sell contaminated commodities.
- We need much political will to construct a national food safety system.

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Non-Chlorine Sanitizer Enhances the Food Safety of Common Fresh Salad Vegetables

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Abstract

Various reasons, including use, abuse, and excess using sanitizers ineffectively or at low doses, endanger vegetable food safety. People have become ill after eating raw vegetables, and pathogen-caused disease outbreaks on fresh vegetables have become more common worldwide, are widely covered in the media, and affect the profitability of producing vegetable crops. Postharvest horticultural produce environments can reduce food safety risks by limiting microbial development and preventing cross-contamination. In the food safety arsenal, sanitizers were used for various reasons, including eliminating pathogens and reducing lingering microorganisms, cleaning hands, tools, and vegetable contact surfaces, and extending produce shelf life. Choosing a suitable sanitizer for all vegetables is challenging because there is a lack of information on which sanitizers are best for the various types of vegetables grown on farms in various environmental conditions. Although chlorine-based sanitizers, such as sodium or calcium hypochlorite, have been widely used for the past 50 years, recent research has revealed that chlorine reacts with an organic compound in fresh vegetables to create trihalomethane, a carcinogen precursor, and thus, many countries have banned the use of chlorine in all foods. As a result, horticulture study groups worldwide are looking for non-chlorine and environmentally friendly sanitizers for the vegetable industry. They also want to learn more about the current practices for using alternative sanitizers in the vegetable industry and their efficacy and potential risks to the food safety of fresh salad vegetables. This review-cum-research paper provided comprehensive information on non-chlorine sanitizers, including their efficacy, benefits, drawbacks, regulatory requirements, and the need for additional research to reduce the risk of salad vegetable food safety.

Keywords: Non-chlorine sanitizer, Food safety, Common fresh salad vegetables

1. Introduction

Consumers today prefer safer, fresher, and healthier options in their daily diets, and vegetables are becoming a more important "healthy" group than animal proteins, complex carbohydrates, or sweeter alternatives like fruits (Mir et al., 2018; Akoachere et al., 2018; Sagoo et al., 2003). Fresh vegetables, including cucumber, tomato, carrot, capsicum, lettuce, green chili, and onion, are frequently used as salad items in Asian countries and are an essential part of a healthy diet. The demand for ready-to-eat or ready-to-cook foods also means that cutting, peeling, and dicing vegetables is an increasingly common practice, significantly raising food safety risks. Bacteria (such as *Aeromonas spp., Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Escherichia coli* (pathogenic and non-pathogenic), *Listeria monocytogenes*, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus,* and *Yersinia enterocolitica*), virus (such as Hepatitis A, Rotavirus and Norovirus) and parasites (such as *Cryptosporidium parvum, Cyclospora cayetanensis, Giardia lamblia,* and *Toxoplasma gondii*) are the food safety concern microorganisms. Table 1 provides the lists of microorganisms responsible and specific vegetables that contributed to the foodborne outbreaks. Plant pathogenic fungi are mainly responsible for the spoilage of vegetables, known as quality deterioration. Table 2 summarizes the microorganisms that cause postharvest spoilage of selected fresh vegetables. All of these vegetables have the potential to pick up pathogenic microorganisms while being harvested on the field, handled afterward, processed and packaged in the pack house, or transported and distributed to stores. The agroecology of the geographical locations, genetic diversity, agronomic practices, and environmental responses in different farm production stages all influence the level of microbial contamination. Following the postharvest process, human activity and the environmental responses of the vegetable-packing plants can also increase the risk of contamination. The vast majority of studies reported in the published literature claimed that contamination happens mostly before harvesting, either through contaminated manure, sewage, irrigation water, and wastewater from livestock operations or directly from wild and domestic

animals, or during harvesting, transport, processing, distribution, at the pack house, and marketing, or even at home (Eraky et al.2014; Rahman et al. 2014; Pagadala et al. 2015; Maffei et al. 2016). Vegetable food safety risks in connection to agroecology during farm production and the environment of vegetable-packaging facilities during postharvest operations can be significant, and this field of study is just beginning. As mentioned in the literature, vegetables can also become infected at the retail or consumer levels, and this can happen through direct contamination, contact with contaminated soil or water, symptomatic and asymptomatic employees, or cross-contamination with other foods (Lambertini et al., 2016). This risk, however, is completely determined by regional food safety culture, which is also a new area of study. Figure 1 depicts the typical annual average microbiological quality and safety indicator microorganisms discovered in different salad vegetables in Bangladesh from 2011 to 2022.

Table 1. Selected vegetable pathogens associated with outbreaks

Bacteria	Selected Vegetables	References
Clostridium botulinum	carrots	Marshall et al., 2014
Shiga-Toxigenic *Escherichia coli*	Lettuce, Tomato	Mazaheri et al., 2014; Tahir et al., 2022
Listeria monocytogenes	Lettuce, tomato	Shenoy et al., 2017; Honjoh et al., 2016
Salmonella spp.	Lettuce, tomato	Ceuppens et al., 2014; Bartz et al., 2015
Shigella spp.	Lettuce, salad vegetables	Guchi et al., 2010
Staphylococcus aureus	Lettuce, tomato, carrot	Wei et al., 2016; Colombari et al., 2007; Denayer et al., 2017
Yersinia enterocolitica	Carrots, cucumber, lettuce, tomatoes	Rimhanen et al., 2009;Islam et al., 2015; MacDonald et al., 2012
Viruses		
Hepatitis A and Norovirus	Lettuce	Coudray-Meunier et al., 2015; Donnann et al., 2012
Protozoa *Cryptosporidium* spp. and *Cyclospora* spp.	Lettuce	Utaaker et al., 2017; Buss et al., 2016

Table 2: Summary of microorganisms that cause postharvest spoilage of selected fresh produce.

Vegetable crop	Scientific name	Type of microorganism
Lettuce	*Aeromonas* and *Pectobacterium* spp. (bacterial soft rot)	Bacteria
Tomato	Lactic acid bacteria	Bacteria
Lettuce and Tomato	*Xanthomonas*	Bacteria
Carrot, Lettuce, and Tomato	*Pseudomonas* (bacterial spot)	Bacteria
Carrot, Cucumber, Lettuce, and Onion	*Erwinia* (soft rot),	Bacteria
Cucumber, Onion, and Tomato,	*Bacillus*	Bacteria
Carrot	*Thielaviopsis basicola* (black root rot)	Fungi
Cucumber	*Pythium* (cottony rot)	Fungi
Tomato	*Phytophthora*	Fungi
Cucumber, and Tomato	*Penicillium* (blue mold) and *Rhizopus*	Fungi
Onion, and Tomato	*Aspergillus niger* (black rot)	Fungi
Carrot, Lettuce, and Tomato	*Sclerotinia* (white rot, white mold)	Fungi
Carrot, Lettuce, Onion, Tomato	*Geotrichum*	Fungi
Capsicum, Cucumber, Onion, and Tomato	*Collectotrichum* (Anthracnose)	Fungi
Capsicum, Carrot, Cucumber, and Tomato	*Rhizopus* spp. (storage rot, rhizopus rot)	Fungi
Carrot, Cucumber, Lettuce, Onion, and Tomato	*Botrytis* spp. (neck rot, grey mold)	Fungi
Capsicum, Carrot, Cucumber, Onion, Tomato	*Fusarium* (soft rot, dry rot) and *Alternaria* spp. (black rot)	Fungi

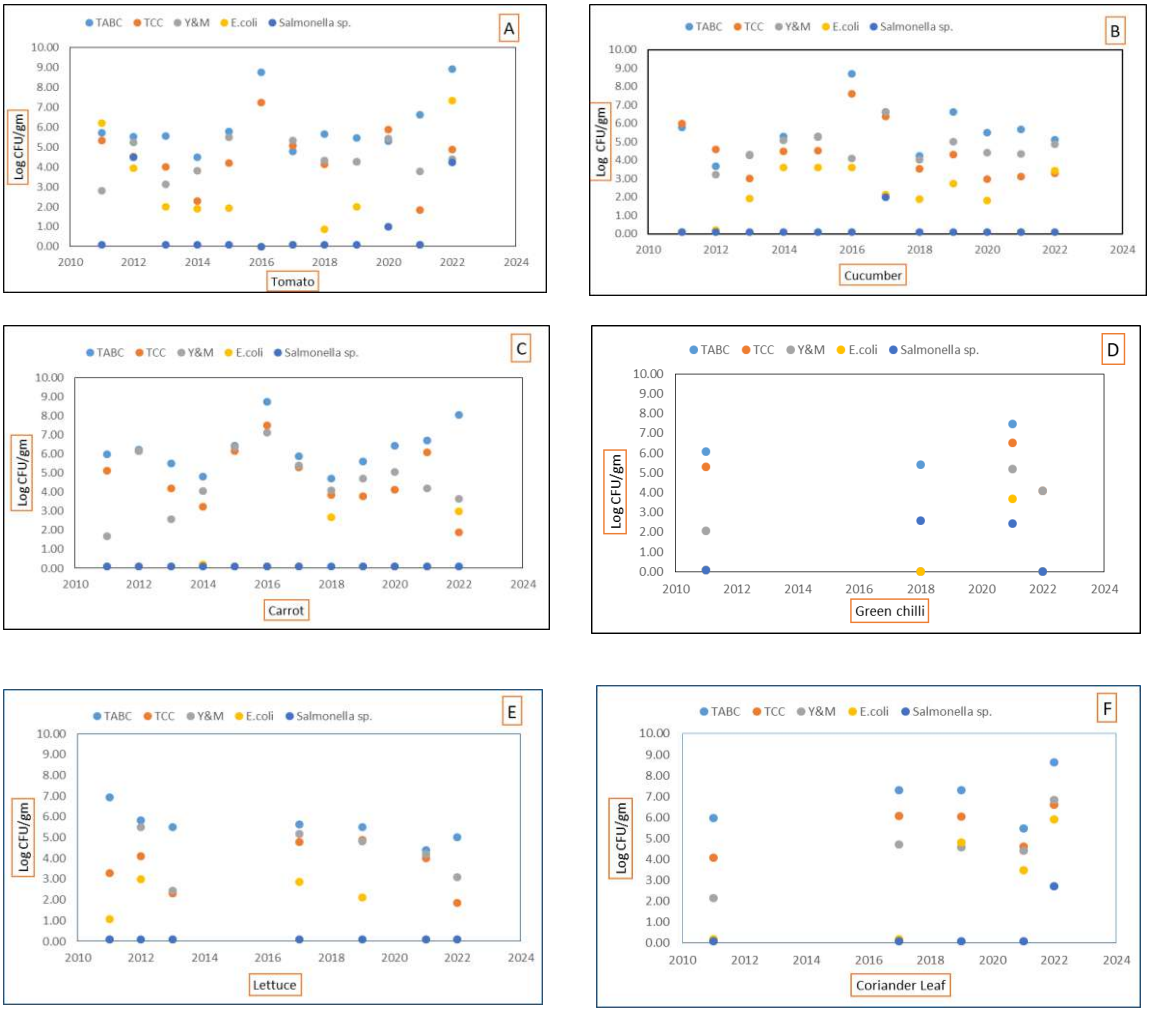

Figure 1. Indicators of microbiological quality (total aerobic bacteria, total coliform bacteria, total yeast, and mold) and safety (*E. coli* and *Salmonella*) were distributed on an annual basis in salad vegetables such as tomato cucumber, carrot, coriander leaf, green chili, and lettuce in Bangladesh between 2011 and 2022.

2. Use of Sanitizers

The most common reasons to use sanitizer are: 1. To eliminate any bacteria or pathogens and lower microbial load; 2. To control microbiological hazards from human sources; 3. To maintain a safe environment; 4. To control product cross-contamination; 5. To reduce fungi's ability to propagate; 6. To disinfect and/or clean the processing facility, machinery, and any surfaces that come into contact with the vegetable crop; 7. To make sterile water; 8. To sterilize water-holding vessels; 9. To maintain the product's cleanliness; and 10. To increase shelf life (Jing et al., 2020). Although various microbial agents are available for sanitizing fresh-cut produce, their efficacies differ, and none can eliminate pathogens without compromising sensory quality. Recent research has also demonstrated that chlorine is ineffective at reducing pathogens, and the creation of chlorine byproducts harms human health. As a consequence, there is considerable interest in developing a chlorine substitute that is both safer and better for the environment. Various fresh vegetables have been subjected to the application of calcined calcium, hydrogen peroxide, ozone, peroxyacetic acid, organic acid, natural antimicrobial agents, or combinations of these and other physical sanitization techniques. Generally, the ideal sanitizing agent should possess two key qualities: an adequate level of antimicrobial activity and little to no impact on the product's sensory quality. Consumer demands for safe and ecologically friendly products would increasingly influence sanitizer selection for both domestic and international use. There is a lack of information on a suitable alternative, more environmentally friendly sanitizers, their efficacy on various products, and their market acceptance. The US Environmental Protection Agency (EPA) has registered and authorized over 4000 sanitizers with 275 unique active ingredients as

antimicrobial sanitizers (Yeomans et al., 2021). Choosing the best sanitizer for specific vegetables (cucumber, tomato, carrot, capsicum, lettuce, green chili, and onion) can be challenging, given this lengthy list. The most commonly used sanitizers in the food business are chlorine-based, which include sodium or calcium hypochlorite, acidified chlorite, electrolyzed water, and others; they have been tested on a variety of fresh produce and are effective against a wide range of pathogens (Praeger et al., 2018). However, chlorine can interact with organic matter in the natural environment to produce halogenated byproducts like trihalomethanes or haloacetic acids (Praeger et al., 2018). Because these byproducts are carcinogenic and unfriendly to the environment, using chlorine to wash fresh vegetables has been prohibited in countries such as Belgium, Switzerland, and the Netherlands for safety reasons. (Aaliya et al., 2021). As a result, Table 3 includes a number of substitute sanitizers proven to improve the safety and quality of salad vegetables, such as calcinated calcium, aqueous chlorine dioxide (ClO_2), ozone, hydrogen peroxide, peroxyacetic acid, organic acid, and plant bioactive compounds or essential oils.

Table 3: List of various applicable non-chlorine sanitizers for washing fresh salad vegetables

Sanitizer	Allowable Levels	Advantages	Disadvantages	Rinse Step	Additional Comments	References
Calcinated Calcium (CCA)	Use 1g powder/10L water (giving a concentration of 0.01% and pH about 11) and 40 s wash	Calcinated calcium (CCa) is safe and eco-friendly, produced from marine waste (scallop shells)	If not dissolved properly may contribute residues on the vegetable surface. Can be affected by organic load in the wash water.	Yes	effective at lower doses, high pH, less contact time, available and cheap	Ahmed et al., 2019 Bari et al., 2002
Aqueous Chlorine dioxide (ClO_2)	ClO_2 5 mg/L, 60 s overhead spray, and brush roller system at 25 °C.	Easy to handle, inexpensive; It can be used in the form of spray, immerse, or washing; Concentration and contact can be maintained; Easy to adopt in industrial washing lines	Produce surface properties can affectClO_2 accessibility to microbes; Residual moisture after the water rinsing can promote microbial growth;	Yes	Not suitable for dried foods; Relatively less effect on microbial internalization	Praeger et al., 2018; Banach et al. (2017)
Hydrogen Peroxide (H_2O_2)	The typical concentration used: (0.04–2%); Environmentally friendly. Declared GRAS by FDA.	Breaks down eas ily, with no harmful by -products; Higher temperatures could produce a better reduction	Higher concentrations can cause browning or bleaching in certain products and can be corrosive and irritating. Unstable degrades fast	No	Commercially available at 31-70%, but 30-50% is most common. Dilute (3%) solutions are available to consumers.	McWatters 2002; Arslan-Alaton et al., 2009
Ozone (O_3)	No regulatory limit but typically used at 2-10 ppm for up to 5 min; Activity reduced in the presence of organic load	Declared GRAS by FDA. Environmentally friendly; Effective at low concentrations; No harmful end products.	Has to be generated on-site; unstable and highly reactive; Corrosive to equipment; OSHA requirements on employee exposure.	No	Solubility in water increases at lower temperatures and pH. Does not work as well at higher pH.	Ramos et al. 2013; Martin-Diana 2005; Alexandre 2011
Organic acids (acetic acid, citric acid, lactic acid, tartaric acid, oxalic acid, ascorbic acid, and phytic acid).	1% oxalic acid, 0.03% phytic acid, 0.5% CA 0.5% lactic 2 min or 2% acetic acid for 15 min	Organic acids have been used as sanitizers for fresh produce. The FDA recognizes organic acids as GRAS,	Their usefulness against microorganisms is generally low and requires high concentrations for long periods. Sensory quality might also be affected with 5-15 min treatment	No	Effective at higher conc, depends on water quality and costly; Antimicrobial efficacy is dependent on the microorganism strain and acid type.	Ramos et al. (2013);(Olmez 2009); Akbas (2007)

3. Calcinated calcium (Green Agrowash®):

Calcinated calcium is a baked and pyrolysis product of waste shell aggregate into a fine, odorless, natural, environmentally beneficial, and biodegradable powder. The Japan Standard of Food Additive (JSFA) has approved using this shell powder with microparticles in food. This powder is sparingly soluble in water and possesses antibacterial and antifungal activities, which is proven effective in killing bacteria and fungus and removing contaminants from the fruits and vegetables' surface (Zaman et al., 2021; Bari et al., 2002). One gram of powder in 10 L tap water or (0.01% solution) is recommended for 40-60s wash followed by clean water wash was able to eliminate pathogens and remove other contaminants from the surface of the vegetable. However, using more than the suggested concentration can leave a white stain on stainless steel or glass surfaces. Since calcinated calcium is made from natural ingredients, it is readily accessible, inexpensive doesn't harm the environment, and isn't toxic to humans or animals.

4. Hydrogen Peroxide

Hydrogen peroxide, also called hydrogen dioxide, can be used as a sanitizer of fruits and vegetables as a liquid or gas. It is considered "generally recognized as safe" or GRAS by FDA (U.S. Food and Drug

Administration) and EPA (U.S. Environmental Protection Agency) and is environmentally friendly because it breaks down to oxygen and water. It is affected by the organic load in the wash water but not by pH. The FDA has approved using hydrogen peroxide as a sanitizing solution on fresh produce at levels not exceeding 59 ppm. Recently, it has become more common to use hydrogen peroxide in combination with acetic acid (PAA) than to use it alone**.** Hydrogen peroxide causes cells to die by altering osmotic pressure, leading to the loss of cell wall integrity. This compound is cheap, easy to prepare, fast acting for bacteria, and can kill spores. Hydrogen peroxide must be used cautiously due to its instability in water, high allergenicity, and loss of effectiveness if not stored under the right conditions.

5. Ozone:

Ozone is also considered to have vigorous antimicrobial activity. It is highly reactive and has high penetrability (Ramos et al., 2013). Ozone generation/production has lower running costs, and it is GRAS. The effect of ozonated waters with different concentrations and contact times on fresh-cut produce's quality attributes and the microbial population was studied (Kim, 2012). Ozone does not produce any hazardous disinfection byproducts and decomposes into non-toxic products. Gaseous ozone is more effective against pathogenic and non-pathogenic microorganisms than aqueous ozone. However, gaseous ozone could be hazardous, toxic, and reactive in this form (Martin-Diana 2005; Añino, 2006; Alexandre 2011).

6. Organic acids:

Fresh fruit has been sanitized using organic acids like citric acid, acetic acid, and lactic acid as well as combinations with phosphoric acid and sulfuric acid. These substances can't stain or emit odors and aren't corrosive to stainless steel, making them more natural ingredients in food. On the negative side, yeasts, fungi, Gram-positive bacteria, and others are not destroyed by organic acids. Although organic acids are deemed GRAS by the FDA, their effectiveness against microorganisms is typically low and needs high concentrations for extended periods. Fresh and freshly cut vegetables have been sanitized using organic acids and acid compound sanitizers. To maintain the physical and chemical properties of many fresh-cut products and to stop microbial development, organic acids are crucial sanitizers. According to Ibrahim et al. (2009), decontaminating leaves of some particular veggies with 5% citric acid resulted in a noticeably lower microbial count than washing them with water. The ideal form of organic acid had no negative effects on flavor or taste, and it had no negative effects on the ecosystem. Fresh-cut fruit can have their shelf life extended by citric acid because it prevents food quality from deteriorating and the spread of disease. However, using these acids at greater concentrations may lead to a quality loss in some freshly cut leafy veggies due to off-odor and texture damage. It has also been investigated how citric acid and ethanol treatment in order affects the quality and microbial reduction of organic vegetables. As a result, an organic acid-based disinfectant has been developed using a combination of technologies in place of chlorine.

7. Chlorine dioxide

Fresh fruit can be effectively protected from bacterial, fungal, and viral contamination by using the oxidizing gas ClO_2 (Praeger et al., 2018; Sun et al., 2019). ClO2 is effective across a wide pH range and does not create any toxic byproducts or change the nutritive or olfactory qualities of food products. (pH 3–8). However, using ClO_2 to wash fresh products in gaseous and aqueous forms has benefits and drawbacks, which are listed in Table 4.

Table 4: Chlorine dioxide (ClO2) application in the aqueous and gaseous form: advantages and disadvantages

Aqueous ClO_2, (Praeger et al., 2018)	
Advantages	**Disadvantages**
Easy to handle, inexpensive	Produce surface properties can affect ClO_2 accessibility to microbes
It can be used in the form of a spray, immerse, or washing	Cross-contamination of wash water
Concentration and contact can be maintained	Water rinsing is required after the treatment
Easy to adopt in industrial washing lines	Residual moisture after the water rinsing can promote microbial growth
	Not suitable for dried foods
	Relatively less effect on microbial internalization
Gaseous ClO_2, (Sun et al., 2019)	
Advantages	**Disadvantages**
Higher antimicrobial activity	Needs onsite generation
It can be applied as batch treatment or continuous treatment	Needs technical knowledge
High accessibility to microbes, irrespective of surface barriers	laborious to perform, expensive
No water rinsing is required after the treatment	Explosive at higher concentration
It can impact microbial internalization	Challenging to maintain concentration and contact time
No issue of cross-contamination of wash water	Challenging to implement at the industry scale

8. Natural plant extracts:

Natural goods are increasingly being looked into as alternatives to conventional sanitizing agents in the washing processes for fresh produce. Essential oils (EOs) and hydrosols from aromatic plants are examples of natural plant extracts that are generally accepted as safe (GRAS) for use in the food industry and are also covered by EC Regulation No. 1334/2008 on flavorings and certain food ingredients with flavoring properties for use in and on foods (D'Amato, et al., 2018; European Commission, 2008). Numerous EOs and other natural extracts, such as sage, Greek oregano, eucalyptus, and rosemary, have been used to preserve fresh produce and barely processed vegetables. (Tzortzakis et al., 2010, 2016; Chrysargyris et al., 2021; Xylia et al., 2021). Furthermore, no variations in lowering *E. coli* O157: H7 and total coliforms in lettuce and spinach were discovered after washing with water and tannin solutions. (Engels et al. 2012). While washing spinach and lettuce samples in aqueous oregano extract for two minutes reduced E. coli O157:H7 counts by 2.1 log CFU g^{-1} and 3.7–4.0 log CFU g^{-1}, respectively, when coupled with Citrox® (a product containing citric acid and phenolic compounds) (Poimenidou et al. 2016). These findings suggested that plant extracts can successfully decrease the pathogenic load in fresh vegetables. Edible coatings with natural antimicrobial agents are becoming more popular as possible treatments to lessen the adverse effects of processing fresh vegetables. However, using natural edible coatings for freshly cut vegetables has not attracted attention, and no vegetable businesses have yet to wash or preserve fresh-cut vegetables using a natural antimicrobial agent due to fewer side effects than chemical sanitizers and non-economic efficiency. Plant extracts should also be kept in the dark because they are typically volatile and light-sensitive. Vegetables may become softer when plant extracts are in greater concentrations (0.5%). Washing fresh organic produce offered at a higher price can be done with natural detergents. The need for natural food preservation techniques, such as using natural antimicrobials and their combination with other obstacles, without adverse effects on the consumer or the environment, has been brought on by consumers' increasing demand for fresh and freshly cut produce (Tiwari et al., 2009). Essential oils are natural antimicrobial agents; however, it is practically difficult to use these oils because of their hydrophobic, volatile, and unstable nature (Deng et al., 2020).

9. Green tea extract:

Green tea extract (GTE; 60%) was shown by Randazzo et al. (2017) to exhibit a rise in antiviral activity with increasing pH. The cytoplasmic membrane damage, nucleic acid synthesis suppression, cell wall component inhibition, and cell membrane damage could contribute to the antibacterial activity. (Borges et al., 2013; Wu et al., 2013). Temperature, concentration, and contact time all impacted GTE's reaction. For lettuce and spinach, using 60% GTE also successfully lowered the bacterial count by 1.5 logs after 30 minutes of exposure (Table 5). A non-economic and occasionally greater dosage of GTE may result in an unpleasant odor and soften the vegetables because it requires longer times, less effectiveness, and a higher concentration than chemical sanitizers.

10. Conclusion

According to the majority of studies, the biggest threats to the food safety of vegetables come from irrigation or wash water, then from how the produce is managed during the postharvest process. Microbial contamination is a significant factor in postharvest losses for fresh vegetables, and washing them in water lowers the number of microbes on their surface by one or two logs (1 log reduction being a 10-fold reduction). The quantity of total dissolved solids (such as soil, dirt, and debris) in the water, water temperature, the quality of the incoming water (such as pH and mineral content), the contact time with the produce, and the texture of the produce are all variables that influence the effectiveness of the sanitizer in vegetable wash water (smooth or rough surface). Additionally, the majority of sanitizer effectiveness studies have only been conducted in laboratories; it is crucial to validate sanitizers in actual workplace settings. Furthermore, when determining the precise procedure (amount and mode of sanitizer delivery) to handle the vegetables, appropriate sanitation should consider the aforementioned factors.

Table 5: Efficacy of non-chlorine-based sanitizers in reducing bacterial pathogens from fresh salad vegetables' surface

Vegetables	Non-chlorine sanitizers (Conc and contact time)	Microorganisms	Maximum reduction (Log CFU/g)	Complete reduction/Number of samples	References
Lettuce	Peracetic Acid (PAA); (100 mg/L; 5 min	*Escherichia coli* O157:H7,	2.2	0/6	Singh et al., 2018
		Salmonella	6.8	6/6	

	at 65 rpm)	*typhimurium* DT104			
		Listeria monocytogenes,	2.4	0/6	
	Lactic acid (2%; 5 min at 65 rpm)	*E. coli* O157:H7,	1.7	0/6	
		L. monocytogenes,	1.7	0/6	
	Calcinated calcium: 0.01% for 40-60 sec	*E. coli*	2.1	3/3	Feroz et al., 2013
	Hydrogen peroxide (H_2O_2); (2% for 90 sec)	*E.coli* O157:H7	4.3	0/3	Lin et al., 2002
		S. enteritidis	4.3	0/3	
	Aqueous Ozone (O_3); (3 ppm for 5 min)	*E. coli* O157:H7,	5.9	0/5	Rodgers et al., 2004
		L.monocytogenes	5.9		
	ClO_2 3 ppm, 5 min	*E. coli* O157:H7,	5.8		
		L.monocytogenes	6.0		
	Plant extract (grape stem extract, 25 mg/ml)	*E.coli* O157:H7	0.7	0/5	Vázquez-Armenta, et al., 2017
		S. enterica	1.0	0/5	
		L. monocytogenes	0.8	0/5	
Tomato	PAA at 100 mg/L; 5 min@65 rpm (Laboratory scale)	*E. coli* O157:H7,	5.5	3/6	Singh et al., 2018
		S. typhimurium DT104	6.8	6/6	
		L. monocytogenes	2.4	0/6	
	Lactic acid (2%); 5 min@65 rpm	*E. coli* O157:H7,	2.4	0/6	
		S. typhimurium DT104	4.8	0/6	
		L. monocytogenes	2.3	0/6	
	ClO_2 5 mg/L, 60 s (Commercial scale)	*Salmonella* spp.	4.9	0/15	Chang et al., 2012
	PAA 80 mg/L, 60 s (Commercial scale)	*Salmonella* spp.	5.5	15/15	Chang et al., 2012
	Calcinated calcium for 1 min 0.01%	*E. coli O157:H7*	7.6	0/3	Bari et al., 2002
		Salmonella spp.	7.4		
		L. monocytogenes	7.5		
	H_2O_2; 5% for 2 min, 60 ºC;	*Salmonella* spp.	2.6	0/3	Sapers et al., 2006
		E.coli	1.4		
		L. monocytogenes	2.5		
	Aquous O_3; 0.45 ppm for 10 min	*Salmonella* spp.	4.5	0/6	Xu et al., 2004
	Green tea extract 60%; 5 min	*E.coli*	5.66±0.1	3/3	Nascimento, and São josé, 2022
		S. enteriditis	5.23±0.12	0/3	
Cucumber	Peracetic Acid (PAA) 0.5%	*S. typhimurium*	2.66 ± 0.20	0/12	Li et al., 2020
		L. monocytogenes	1.28 ± 0.35		
	Lactic acid (2%)	*S. typhimurium*	2.14 ± 0.26		

		L. monocytogenes	0.75 ± 0.43		
	Calcinated calcium (0.01 % for 1 min)	*E.coli*	3.62±0.1	3/3	Ahmed et al., 2019
	H_2O_2; 0.5% for 2 min	*S. typhimurium*	2.63 ± 0.19	0/12	Li et al., 2020
		L. monocytogenes	1.16 ± 0.40		
	Aqueous O_3; 2% for 5 min	-	-	-	-
	ClO_2; 100 ppm	*E.coli*	2.61±0.1	0/5	Chung et al., 2011
	Green tea extract 60%; 5 min	*S. enterica*	2.0±0.1	0/4	Xylia et al., 2022
		L. monocytogenes	2.07 ±0.1		
Carrot	Peracetic Acid (PAA) 40 ppm, 1 min;	*E.coli*	0.5	0/4	Ruiz-Cruz et al., 2007
		Salmonella spp.	1.5		
		L. monocytogenes	0.5		
	lactic acid (0.1%); 5 min	*E.coli* O157:H7	0.4	0/5	Gyawali et al., 2012
	Calcinated calcium (0.01% for 1 min)	*E. coli*	0.5	3/3	Amin et al., 2021
		Salmonella spp.	0.5	3/3	
	H_2O_2; 1.5% for 90 sec	*E.coli*	0.8	0/3	Augspole et al., 2013
	Aqueous O_3; 16.5 mg/L	*E.coli* O157:H7	1.85	0/3	Singh et al., 2002
	ClO_2: 20 mg/L	*E.coli* O157:H7	3.0	0/3	
	Plant Extract	-	-	-	-
Green chilli	Peracetic Acid (PAA)	-	-	-	-
	Lactic acid	-	-	-	-
	Calcinated calcium (0.01% for 1 min)	*E.coli*	0.5	0/3	Khadiza A. R. (2018)
		Salmonella spp.	0.5	0/3	
	H_2O_2; 0.5% for 2 min	*E.coli*	0.5	0/3	Khadiza A. R. (2018)
		Salmonella spp.	0.5	0/3	
	Aqueous O_3;	-	-	-	
	ClO_2;	-	-	-	-
	Plant extract	-	-	-	-
Coriander leaf	Peracetic Acid (PAA)	-	-	-	-
	Lactic acid	-	-	-	-
	Calcinated calcium (0.01% for 1 min)	*E.coli*	1.6±0.1	3/3	Khadiza A. R. (2018)
	H_2O_2	-	-	-	-
	Aqueous Ozone (O_3); 6% for 30 min	*E.coli*	2.5	0/3	Gibson et al., 2019
		S. typhimurium	2.7	0/3	

	Aqueous ClO_2	-	-	-	-
	Plant extract	-	-	-	-

-: data not found

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Vegetable Consumption in Vietnam: an Overview

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Abstract

Vietnamese cuisine is known for its abundant use of vegetables, herbs, and sauces. Vegetable cultivation occurs during spring, summer, autumn, and winter. Vietnam has experienced a consistent annual growth rate of 5% in vegetable production, reaching over 18 million tons in 2018. However, agricultural earnings remain low compared to average income levels. Vietnamese farmers heavily rely on imported pesticides, primarily from China. In urban areas like Hanoi and Ho Chi Minh City, households allocate around 8-9% of their monthly food budget to fruits and 12-13% to vegetables. Traditional markets dominate vegetable sales, with fruits accounting for 47-52% and vegetables for 58-67% of spending. Supermarket shopping is becoming more popular due to perceived cleanliness and preference for certified products. The Institute of Nutrition Vietnam recommends a daily consumption of 240-320g of ripe fruit and 480-560g of vegetables.

Keywords: Vietnam, agriculture, vegetables, fruits,

1. Introduction

Vegetables can refer to various vegetable parts that humans and animals consume (Nguyen Van Truong et al., 1991; Nguyen Thi Ngoc Hue and Vu Van Dung., 2016). In ancient times, when Vietnamese people used hieroglyphs letters, vegetables could be written by: 菱 or **蓌,** both pronounced as '*rau*'. The meaning can be referred to as the root, stem, leaf and leafstalk, flower, fruits, and seeds are the parts of plants eaten as vegetables (Dao Hung, 2013). Traditional Vietnamese meals always include a lot of veggies, herbs, and distinctive sauces. The main meal of Vietnamese people usually consists of a main dish (rice), a condiment (dipping sauce), and three basic dishes with sufficient quality and balance of *Yin* and *Yang*, making the meal medicine (Anonymous 2022; Huy Khoi H., 2000)

From the reported data, we found a vivid panorama of vegetable production and consumption in Vietnam, which shows clear development trends. In Vietnamese cuisine, vegetables and soups are very popular. Many vegetables, including water-spinach, amaranth, spinach, taro, and green papaya, are cooked in various ways, including boiled, stir-fried, soups, raw foods, and hot spots. In addition, flowers such as pumpkin flower, *Luffa Cylindrica*, water lily *Nymphaeaceae*, *Nesbania Sesban*, *Telosma Cordata*, *Sesbania grandiflora* Pers. can be used; and leaves of clove, mango, and young guava are also consumed in daily meals. The "cooling" plants, characteristic of *Yin*, are used to make a soft drink - juice. Vietnamese have used many fruits soaked in sugar (syrup) or salt and extracted water mixed with sugar to drink in fruit drinks. Drinks using pure or mixed fruit or modern style as slow-pressed fruit juice were learned from foreign cuisine (Dinh Hai H, 2010)

2. National Planning of Growing Vegetables

According to the report of Quang Ninh P., 2022, Vietnam has 4.1 million hectares of arable land to ensure food security for approximately 87 million people. The average arable land per capita is around 600m^2/person. According to Hoang Thi Van Anh (General Department of Land Management), from 2004 to 2010, nearly 750,000 hectares of land (more than 80% of which is agricultural land) were recovered to implement more than 29,000 industrial investment projects. Meanwhile, tens of thousands of hectares of agricultural land are lost yearly. Land acquisition and site clearance for housing and service works are always associated with urbanization (Quang Ninh P., 2022). After one decade, in 2022, these problems became more severe because the population reached more than 100 million people while Vietnam's economy increased more than 10 times to US$409 billion. As mentioned in recent Ministry of Agriculture and Rural Development statistics, the formation of industrial parks, export processing zones, golf courses, and so on has disrupted the irrigation system and caused heavy pollution in agricultural commodity production areas. Simultaneously, many areas of rice land near industrial parks and export processing zones are directly affected by wastewater, dust, and light, resulting in increased pests and diseases and a 15-30% decrease in productivity. According to calculations, each hectare of adjacent land that is unusable due to pollution for constructing an Industrial Park or a golf course usually entails about 1-2 hectares. According to the Ministry of Agriculture and Rural Development, in the period 2009 - 2030, about 500,000 hectares of rice land will likely be converted to other purposes, putting pressure on national food security and export demand in the future.

One of the major issues raised by many experts is that rice farmers' income is quite low compared to other occupations, at around 20 million VND per person per year by 2020. Dr. Do Kim Chung (Hanoi University of Agriculture) studied the last winter-spring rice crop and

discovered that the average cost of rice production was 17-19 million VND/ha, high-quality rice was 17-19 million VND/ha, while selling at 28-32 million VND/ha (for normal rice) and 31-35 million VND/ha (for high-quality rice) (for high-quality rice). This crop's profit rate is estimated to be 40-45%, significantly higher than the previous crop. However, even with this profit, most farmers cannot ensure a decent living compared to the general population. For example, if a family of four cultivates a hectare of land with two crops yearly, the maximum profit is only 20-22 million dong. This is a paltry sum in comparison to the expenses of the entire family. (Financial Ministry Report 2009 and 2022; General Statistics Office, 2022)

3. Growing Vegetables

Discover the intricacies of Vietnam's agricultural landscape as we delve into its regional variations and seasonal dynamics. With four distinct seasons and diverse climates, the availability of vegetables varies across different regions. We explore the cultivation practices and key crops each season, highlighting the rich culinary tapestry they contribute to. Additionally, we delve into the statistics of pesticide consumption, witnessing a shift towards natural alternatives derived from natural sources. The article also introduces modern approaches for soil improvement, emphasizing microbial products, agricultural lime, and neem powder. Lastly, we address the significance of postharvest procedures and explore alternative preservation methods. Join us on this journey of sustainable agriculture.

3.1 Overview by Locations and Seasons

Vietnam's diverse climate, with four distinct seasons: spring, summer, autumn, and winter brings about a corresponding variety of vegetables across different regions. However, vegetable cultivation in the country primarily occurs in two main seasons: spring - summer and autumn - winter. Summer vegetables in the north include spinach, jute, amaranth, water spinach, and pumpkin tops. Winter crops include kohlrabi, cabbage, tomatoes, chrysanthemums, sweet vegetables, etc. In the South, cruciferous vegetables, water spinach, herbs, bitter melon, tomatoes, cucurbits, cucumbers, and other vegetables are popular during the dry season. Also available during the rainy season are kohlrabi, zucchini, pumpkin, sweet potato, and other root vegetables. In the central region, popular summer vegetables include collard greens, glutinous spinach, spinach, rainbow spinach, and *Eryngium foetidum*. In the rainy season, usually, vegetables are cabbage, carrots, kohlrabi, cabbage, cove beans, and herbs (Hanoi Center for Investment, Trade and Tourism Promotion (HCITTP), 2020)

3.1.1. Vietnam's vegetables production and area

Over the years, Vietnam's vegetable production and area have steadily increased. Vietnam's vegetable production increased from 15 million tons in 2015 to approximately 18 million tons in 2018, to over 31 million tons in 2022, with an average annual growth rate of 5%, thanks to an increase in planting area converted from the forest or reclaimed wasteland. The cultivation area of major crops is approximately 2.600 million hectares, while the green vegetable growing area would be approximately 1 million hectares. Vietnam's growing fruit area constantly expands; fruit production is expected to reach 13.3 million tons by 2020. The area and output of Vietnam's main fruits have steadily increased over the years, with oranges and tangerines showing the greatest increase, nearly doubling in four years (Vietnam Credit, 2020). In 2022, the vegetable output will be around 31 million tons. The primary objective is that by 2025, the area of the agricultural land group for organic production will be about 1.5 - 2% of the total area of the agricultural land group; the area of organic arable land is about 1% of the total arable land, with crucial crops such as rice, vegetables of all kinds, fruit trees, tea, pepper, coffee, cashew, coconut, and so on. [Central Propaganda Committee (2022; VTPA & EPCR 2022; VFVMR 2017). Vietnamese Fresh vegetable production areas mainly concentrate in places with good land and climate conditions, such as the Red River Delta, Southeast region, and Da Lat. The Red River Delta is the most extensive fresh vegetable production area, and the Cuu Long River Delta is the second. They contribute more than half of the total national vegetable production. Vegetable production areas are divided into two main areas: The first is vegetable production around cities, towns, and major industrial zones that account for 35% of total national vegetable production and supply 37% of the total national vegetable output. The second is the vegetable rotational cultivation area which accounts for over 65% of the total national production area and 63% of the national vegetable output. Besides, vegetables are also planted in households with an average area of 36m2 per household. The average vegetable amount per capita is 65 kilograms per person. Many organic vegetable areas have been set up to bring high income to the farmers and supply organic vegetables for consumers. Many localities including Hanoi, Hai Phong (An Lao), Ho-Chi Minh City, Lam Dong (Da Lat), etc, have focused on setting up and expanding their organic vegetable areas. The Cuu Long River Delta accounts for nearly 40% of the national Fresh fruit production area. The next is the Northern mountainous region which accounts for about 23% (VTPA & EPCR 2022).

3.2 Statistics by pesticides consumption

By 2020-2030, Vietnam will use approximately 100,000 tons of pesticides per year in agricultural production, with a growth rate of 8-10% over the previous decade. In the last five years, Vietnam has spent from $500 to over USD 800 million per year on pesticides, primarily herbicides and pesticides, but also disease control, imported from more than 40 countries, with China accounting for 70% of the total.

Currently, the list of pesticides that can be used includes 424 active ingredients and 4,021 commercial products,

with biological drugs accounting for 18.26% of the total. Pesticides registered for use on vegetables are all low-toxicity drugs, with 50% being biological pesticides. Recently, the Ministry of Agriculture and Rural Development has removed 1,706 registered commercial products that are ineffective, harmful to human health, and hazardous to the environment, as well as 1,265 active ingredients from the pesticide list. Indeed, consumers' increasing demands for the quality of agricultural products drive producers to use biological pesticides, which are biological products researched and manufactured from materials derived from herbs or strains of microorganisms: bacteria, fungi, viruses, and nematodes cultured in various nutrient media by manual, semi-manual, or fermented methods (Phong, 2020; Hang, 2022).

3.3 Modern approaches for treatment and improvement of Soil quality

Recently, three modern methods for treating and improving soil quality have emerged. To begin, commercially packaged microbial products (biological products) containing the two main strains of microorganisms of *Trichoderma* spp. and *Bacillus subtilis* are available. This product has two main applications: composting and soil mixing for growing vegetables and plants. Second, the agricultural lime $CaCO_3$, and lime 98, CaO, will be more effective. Third, neem powder is made entirely from neem leaves, stems, and seeds that are pureed by cold pressing technology to keep the essence of azadirachtin - this substance is considered a "biological insecticide" (Xanh, 2022).

3.4 Postharvest procedure

Process of postharvest: preliminary, washing, drying at room temperature after rot prevention treatment. There are many methods to prevent fungi and diseases that cause postharvest rot of vegetables and tubers, such as $CaCl_2$ (salt solution 5%), about 5 min, or soak in hot water (47^{o}C) for 10-15 min, followed by sorting, packing, and transfer to logistic storage.

There are many harmful preservatives for fresh fruits and vegetables, such as antioxidants: BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), sodium nitrate and sodium nitrite, sodium benzoate, sulfur dioxide (SO_2), Substance 2,4 D, and Dioxin, have been restricted. However, depending on the type of vegetables, there may be ways to preserve them without using chemicals. As a result, other safe methods must be used to help eliminate microorganisms, and bacteria, prevent mold, inhibit ripening, and naturally store fresh fruit for a long time according to US FDA standards and Food Safety and Hygiene. Products that help reduce postharvest losses and ensure long-term commercial value for agricultural products for both domestic and export markets: Fruit Tray Made of Plastic, Sublimation, cold storage, and irradiation are all methods of preservation. Fruit and vegetable chemical preservation: antioxidants, air composition regulation (CO_2, O_2, Ethylene). However, around 30% of vegetables and fruits have insufficient processing capacity (Sanco et al., 2020; Mai et al., 2018). The output of fruits and vegetables in Vietnam in 2022 is 31 million tons, but the processing rate is only about 12-17%, implying that more than 70% of exported vegetables and fruits are still fresh or partially preserved. Vietnam's fruit and vegetable industry must continue to shift firmly in product structure, increasing processed products and decreasing fresh product exports. Exporting enterprises must increase their investment in processing technology, postharvest preservation, and logistics development to transport fruits and vegetables (Vin, 2022).

Meanwhile, according to a report from the Ministry of Agriculture and Rural Development, the cropping industry aims for a 2- 2.2% increase in production value by 2022; export turnover of primary agricultural products reached 20 billion USD; and the average output value per hectare of arable land is 110 million VND. The planting area for vegetables increased to 1.03 million hectares, with a total output of 19 million tons (Hong, 2018)

4. Domestic Consumption

There is a strong preference for consuming fresh vegetables and fruits in Vietnam. Green vegetables are integral to Vietnamese meals, while fresh fruits are enjoyed after meals in urban areas and throughout the day in rural regions. Water spinach, tomatoes, and bananas are the most commonly consumed vegetables. Vietnamese households prioritize food safety and allocate a significant portion of their budget to fresh food. Traditional markets are preferred for purchasing fruits and vegetables due to perceived freshness. However, supermarkets have introduced processed options. Challenges exist in distribution channels, leading to unreliable product quality and losses. Proposals include specialized regions for high-quality production and improved trade promotion. While supermarkets ensure safety, traditional markets remain important in fruit and vegetable distribution.

4.1 Consumption Habit

People in Vietnam practice eating a lot of veggies and fruits, especially fresh ones. Vietnamese people eat vegetables and fruits as part of their everyday diet. With a higher level of consumption, green vegetables have played an essential role in Vietnamese meals. While city dwellers frequently consume fresh fruits after meals and processed fruits on special occasions such as holidays or New Year's, rural dwellers, in contrast to city dwellers, frequently consume fresh fruits that are locally available due to demand and at varying times of day (VTPA & EPCR 2022).

Popular Vegetable Preparation Methods: According to a study conducted in Hanoi and Ho Chi Minh City (VFVMR 2017), distinct preferences in vegetable preparation methods were observed among the residents. Regarding potatoes, individuals in Hanoi exhibited a greater inclination towards stir-frying than soup-based

dishes, whereas those in Ho Chi Minh City displayed the opposite preference. Concerning tomatoes, most participants favored soup-based preparations; however, individuals in Ho Chi Minh City also consumed tomatoes raw. Notably, cabbage and water spinach were versatile in Ho Chi Minh City, with various cooking techniques, including stir-frying, boiling, and soup-making. Conversely, individuals in Hanoi predominantly favored boiling as the preferred method. It is worth mentioning that boiled water spinach emerged as a favored dish in Hanoi, albeit occasional stir-frying or soup preparations were noted.

Water spinach, tomato, and banana are the most commonly consumed vegetables in Vietnam, according to IFPRI1 (2002) and ICARD2 (2004) research. In detail, Water spinach is consumed by up to 95% of Vietnamese households, tomatoes by 88%, and bananas by 87%. In addition, Vietnamese households consume 71 kg of veggies per person per year, accounting for three-quarters of the total. Fruit and vegetable consumption in large cities: Ho Chi Minh City and Hanoi: Every year, Hanoi residents consume 86 kilograms of veggies and 68 kg of fruits. Annually, the population of Ho Chi Minh City consumes 84.6 kg of vegetables and 74.6 kg of fruits (VFVMR 2017). With more than 100 million people, the domestic market is extensive with rapid growth but may be under brutal competition due to importation. Indeed, Vietnamese consumers prefer imported fruits because of their original country, beautiful design, and longer shelf life (JICA, 2013; Chi, 2020).

4.2 Consumption power

Per Mr. Nguyen Huy Hoang, commercial director of Kantar World panel Vietnam Company, the top concern of Vietnamese consumers is food safety, followed by health and the environment/epidemic. Furthermore, families spend three times as much on fresh food as on fast-moving consumer goods. Mr. Nguyen Huy Hoang stated that in urban areas (i.e., Hanoi, Da Nang, Ho Chi Minh City, and Can Tho), each household spends approximately 18 million VND per month on expenditures, while in rural areas, the total expenditure is approximately 8 million VND per household (Hong, 2018).

In terms of value, consumption of vegetables and fruits accounts for about 4% of households' total average consumption volume. The total quantity of consumed vegetables and fruits per capita has doubled over the past ten years. The consumption trend of the Vietnamese people has changed much with a rapid increase in domestic demand. If the number of consumed vegetables per capita was 50 kg/person/year in 1993, 2002 it increased to 111kg/person/year. Similarly, the number of consumed fruits per capita was 18kg/person/year in 1993, and in 2002 it was 38 kg/person/year (VTPA & EPCR 2022)

The average urban family spends about 1.1 million VND per week on fresh food, three times the amount spent on Fast-moving consumer goods. The majority of this total expenditure is for fruit (19%), pork (14%), fish (12%), and seafood (12%). According to a 2017 study conducted by the Vietnam Academy of Agriculture, the average household in major cities such as Hanoi, Ho Chi Minh City, and others spends about 8-9% of their monthly food budget on fruits and 12-13% on vegetables (Tien Anh N., 2022).

Based on a survey of The Southern Center for Agricultural Policy and Rural Development Strategy, SCAP-IPSARD (VFVMR, 2017), The selection criteria of Vietnamese consumers for agricultural products: Freshness is an important consideration for consumers when choosing fruits and vegetables, with freshness being more crucial for vegetables than fruits. When purchasing fruits and vegetables, Ho Chi Minh City consumers pay more attention to the product's appearance, while consumers in Hanoi prioritize freshness.

4.3. Consumption trends

More than 90% of the money spent on fruits and vegetables is spent at traditional markets, with fruit accounting for 47-52% and vegetables accounting for 58-67%. The main reason consumers shop at traditional markets is for the freshness of the vegetables. Over the past decade, consumer sentiment regarding shopping places has remained consistent in Hanoi and Ho Chi Minh City. A significant majority, accounting for 58.6% of respondents, believe purchasing vegetables and fruits from supermarkets ensures better quality. However, a portion of 24.4% of individuals express concern about higher prices associated with supermarket shopping.

Additionally, a small percentage of shoppers (4.4%) prefer supermarkets due to their perceived convenience. These findings shed light on the prevailing attitudes of consumers towards shopping locations in these bustling cities. [VFVMR 2017]

However, "food safety" was the most important factor influencing consumers' decision to buy vegetables, followed by the "freshness" of the product. According to the Vietnam Association of High-Quality Products (2019) survey results, consumers are increasingly interested in quality, preferring safe organic goods and products. 80% of consumers care about safe products, and people with higher incomes are more concerned about safe products. All ages are concerned about product safety, from lower than 15 years old up to those older than 50 (VFVMR 2017). Specifically, 80% of those polled said they accept shopping at supermarkets because they believe the food is cleaner. Furthermore, up to 88% of consumers know and purchase products with high-quality Vietnamese logo brands, ISO, and VietGAP certifications. (JICA, (2013; Chi, 2020)

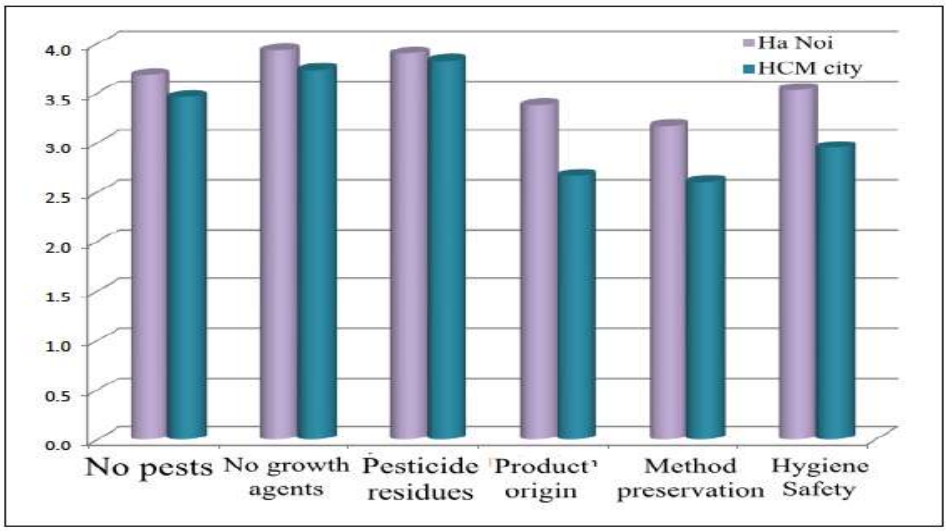


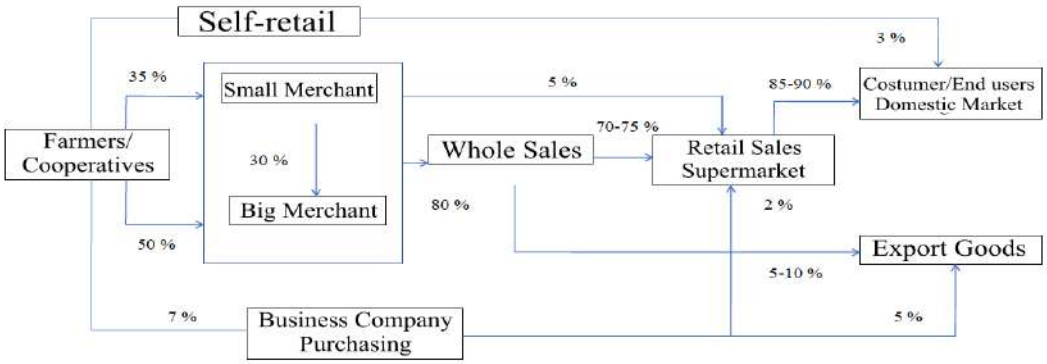
Diagram 1. What is product safety?. The Southern Center for Agricultural Policy and Rural Development Strategy, SCAP-IPSARD (VFVMR 2017)

This information confirms the consumption trends of Vietnamese people that they tend to consume fresh and organic vegetables and fruits more than processed ones. The demand for fruits and vegetables is increasing rapidly in quantity and quality for the domestic market. During the country's industrialization and modernization, nowadays processed vegetables and fruits are accepted by domestic consumers through an extensive system of developing supermarkets in urban areas (VTPA and EPCR 2022).

4.4 Distribution channels

The common distribution channel of Vietnamese vegetables and fruits is that the farmers harvest, then the traders collect, classify and transport to wholesale and retail markets or processing factories where vegetables and fruits will be again classified before packed up or processed. Some of above mentioned steps are manually done and distributed by farmers and small traders who are lack of knowledge about business, distribution and storage of vegetables and fruits. This makes the quality of fresh vegetables and fruits unreliable via each step during distribution. In fact, fresh vegetables and fruits can undergo some loss from 10% - 50% at the final step, making product expense increase higher, and so income of the farmers is not guaranteed. (Tran Cong Thang, 2017)

The Southern Center for Agricultural Policy and Rural Development Strategy, SCAP-IPSARD, proposes the establishment of a specialized region for high-quality fruit and vegetable production, following a large-scale and chain-oriented approach. Emphasis will be placed on solidly developing organic products and creating the brand identity for critical domestic and export products. Investments will be made in a fast transportation system, storage facilities, and a stringent quality control system, particularly at major wholesale markets.



Graphics 1. Model of Distribution channel for fruit and vegetable consumption in the Mekong Delta region. Data based on research on the Southern fruit and vegetable market, SCAP (VFVMR 2017)

A comprehensive trade promotion solution will be Producers to consumers to avoid oversupply and price fluctuations. While supermarkets provide safety assurances for products, they lack convenience, and traditional markets still play a significant role in the distribution of fruits and vegetables, where about 85% costumers prefer to shop fresh foods.

5. Diseases and healing related to lack of vegetables

In today's fast-paced world, the significance of incorporating vegetables into our daily meals cannot be overstated. However, the lack of vegetables in our diets poses grave health risks. Research has revealed that inadequate fruit and vegetable consumption contributes to a higher mortality rate in diseases like gastrointestinal cancer and stroke. This unbalanced dietary pattern is a prevalent issue globally, with many populations failing to meet recommended intake levels. Moreover, the repercussions of vegetable deficiency extend beyond nutritional gaps, leading to an increased susceptibility to severe ailments such as diabetes, high blood pressure, heart disease, and even depression. Conversely, increasing vegetable consumption has been instrumental in disease prevention and treatment, offering a wealth of essential nutrients and antioxidants. Promoting a diverse diet with ample vegetables and fruits is vital for overall health and well-being, supporting longevity and quality of life.

5.1 Diseases and mortality rates related to the Lack of Vegetables in the Diet

Insufficient consumption of vegetables and fruits poses health risks, including increased mortality rates from gastrointestinal cancer and stroke. In Vietnam, over 57% of adults consume fewer vegetables and fruits than the World Health Organization recommended. Non-communicable diseases, such as cardiovascular disease and hypertension, are significant causes of death in Vietnam, often linked to a lack of vegetables in diets. Including vegetables and fruits in daily meals is crucial in disease prevention. Recommendations from the WHO and the Ministry of Health emphasized the importance of vegetable and fruit consumption, yet overall intake still falls below the recommended levels. Promoting adequate vegetable and fruit consumption is vital for overall well-being and disease prevention.

5.1.1. Unscientific dietary imbalance

A daily diet deficient in vegetables and fruits poses several health risks. According to World Health Organization (WHO), data, inadequate fruits and vegetables consumption is linked to 14% of gastrointestinal cancer deaths and 9% of stroke deaths (Suc Khoe, 2018; Thuy, 2022). Eating fewer vegetables and fruits is linked to 19% of stomach and bowel cancer cases, 31% of ischemic heart disease cases, and 11% of stroke cases, according to research from around the world.

According to the 2015 survey on risk factors for non-communicable diseases in the 18-69 age group in Vietnam, more than 57% of adults consume fewer vegetables and fruits than recommended by WHO. Men have a higher

implemented, establishing direct sales channels from rate than women. Based on the WHO guidelines, eating about 400 grams of green vegetables and ripe fruit per day can help reduce the risk of cardiovascular disease and blood fats by two times and increase the ability to prevent other non-communicable diseases. Up to 80% of deaths in Vietnam are caused by non-communicable diseases, with the leading cause being unhealthy eating, specifically a lack of vegetables, excess protein, and excess alcohol, of which cardiovascular disease causes about 70,000 cases (Chiara Townley, 2018; Le, 2020; Huyen Infonet, 2020)

Vegetable deficiency has serious health consequences, including vitamin and mineral deficiency, manageable gastrointestinal diseases, increased cancer risk, increased likelihood of diabetes, high blood pressure, increased risk of heart disease, and depression. Meanwhile, Vietnam has approximately 12 million people with high blood pressure, but nearly 60% are undiagnosed, and more than 80% are not managed or treated. Nearly 70% of the more than 3.5 million diabetics are undiagnosed (Vinmec, 2022). A lack of vegetables causes nutritional deficiencies and is responsible for one in every twelve deaths from cardiovascular disease. People who ate more than five servings of fruits and vegetables daily had a 20% lower risk of cardiovascular disease than those who ate less than 3 servings.

Furthermore, inadequate fruit and vegetable consumption accounts for 14% of gastrointestinal cancer deaths and 9% of stroke deaths. Non-communicable diseases account for 75% of all deaths in Vietnam, with cardiovascular disease, hypertension, diabetes, and gout topping the list. Long-term vegetable deficiency in children has serious health consequences, including decreased resistance, increased appearance of harmful bacteria, obesity, growth retardation, and poor development (Van Tien, 2019)

5.2 Disease Prevention and Treatment

Vegetables play a crucial role in the daily diet, delivering essential vitamins and minerals that support overall well-being and aid in the absorption of nutrients. Dark green vegetables like spinach, collard greens, and water spinach boast abundant amounts of vitamin C, vitamin K, and folate. Similarly, citrus fruits such as oranges, tangerines, and grapefruits, along with vibrant vegetables like spinach, purple vegetables, tomatoes, broccoli, bell peppers, and papaya, are renowned sources of vitamin C, beta-carotene, and Flavonoids. These nutrients have been proven to combat oxidation effectively, enhance the body's resilience, and prevent diseases. Additionally, vegetables offer a substantial dietary fiber content that safeguards the digestive system, fortifies blood vessels, and helps lower cholesterol levels (Thao Trang, 2019)

5.2.1 Effect of disease prevention and control of food from vegetables and fruits

Consuming more fruits and vegetables reduces the risk of cardiovascular disease. They also protect customers from cancer, intestinal diseases, cataracts, and vision loss. Nutritionists recommend eating 5 to 13 servings of green vegetables and fruits daily, equating to 2,000 calories. If

you constantly feed your body enough nutrients from green vegetables and fruits, you can avoid the following dangerous diseases: Heart disease, high blood pressure, and cholesterol, certain types of cancer (WHO), and eye diseases (Thao Trang, 2019; Mai Phuong, 2021).

5.2.2 Recommandation for WHO and Ministry of Health

Based on the Vietnamese Adult Nutrition Tower, which has been approved by the Ministry of Health, the Institute of Nutrition recommends 480g - 560g of vegetables and fruits per day (equivalent to 6-7 units of vegetables, each unit being 80g of clean raw vegetables, excluding waste such as peels, seeds); vegetable consumption ranges from 240-320g/day, and ripe fruit consumption ranges from 240g/day. Compared to the WHO recommendation (MOHIN, 2020) of at least 400g of vegetables per day, the Institute of Nutrition recommendation is higher, consistent with the Vietnamese people's fruit and vegetable eating habits and the availability of vegetables and fruits. Despite this, according to the most recent national nutrition census 2019-2020 results, people's fruit and vegetable consumption has increased per capita from 190.5g of vegetables/person/day (2010) to 231g/person/day and 140.7g ripe fruit/person/day after 10 years (2020). However, the consumption of vegetables and fruits remained between 66.4% and 77.4% of the recommended demand. Therefore, the Vietnamese government has decided to launch the program 'Vision to 2030' to increase the use of vegetables in daily meals by 130% (MOHIN, 2012).

5.2.3 Diet for COVID-19 patients treated at home

According to the Ministry of Health's Guidelines on Nutrition for People Infected with COVID-19 at Home, the implementation and adherence to a nutritional regimen are essential, as nutrition helps to support and improve the "barrier" of health. Immune cells, antibodies, skin, respiratory mucosa, and gastric mucosa all contribute to increased resistance. Patients infected with COVID-19 have higher nutritional needs due to increased energy expenditure; if nutrition is not adequately supplemented, the patient will become severely malnourished. Malnutrition increases the risk of superinfection, disease exacerbation, mechanical ventilation duration, and treatment cost. Patients must ensure adequate and balanced nutrition by supplementing with various food groups such as starch, milk and dairy products, fat, vegetable, meat and fish, egg, and so on. To maintain normal physical condition, patients must eat normally with adequate and balanced nutrient groups and various foods (if possible). Add 1 to 2 extra meals, such as milk and dairy products, especially while suffering from a fever, cough, or fatigue. To prevent muscle atrophy and increase resistance, patients should consume more protein-rich foods (meat, lean fish..., beans, nuts), while to boost resistance, need more fresh fruits and juices, green vegetables, and spices (such as garlic and ginger) (Minh Anh, 2021).

6. Conclusion and Recommendation

In conclusion, this study highlights the critical importance of vegetable consumption for overall health, supported by statistical data from Vietnam. A deficiency in vegetable and fruit intake poses significant health risks, including gastrointestinal cancer, stroke, and heart disease. To address this issue, a multifaceted approach is necessary, involving raising awareness, promoting equitable access to fresh produce, and disseminating knowledge about the health benefits of a vegetable-rich diet.

Furthermore, sustainable farming practices and the support of local farmers are crucial in ensuring a consistent supply of high-quality vegetables, thereby enhancing the overall availability and accessibility of nutrient-dense options. In contrast to the average income level, overall, earnings from agricultural production is still lower than per capita income. Therefore, they must focus on building production areas and improving the efficiency of fruit and vegetable processing for matching the requirements from supermarket that could improve living standards and can export to increase income. The dietary fiber content in vegetables plays a significant role in maintaining digestive health, contributes to disease prevention and treatment. Prioritizing vegetable consumption is crucial for improving health outcomes, reducing non-communicable disease burdens, and promoting overall well-being.

Adhering to recommendations from authoritative bodies such as the World Health Organization and the Ministry of Health is crucial. Despite progress, per capita vegetable and fruit consumption in Vietnam remains suboptimal. Therefore, Gorvement's efforts should be directed towards bridging this consumption gap and encouraging individuals to incorporate an adequate amount of vegetables and fruits into their daily diets. Implementing comprehensive strategies, raising awareness, and supporting sustainable agricultural practices are key steps towards achieving these goals.

Acknowledgment

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Assessment of Fecal Contamination in Multiple Water Samples Associated With Diarrhea in Urban Communities of Dhaka City

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Abstract

Rapid urbanization and poor hygiene knowledge among city dwellers have led to a growing sanitation crisis in urban areas of Bangladesh and potential exposure to fecal contamination in the urban water systems due to inadequate sanitation and poor fecal sludge management. This study was carried out from April to June 2022 to assess the fecal indicator bacteria and *Vibrio* spp in point-of-use water, including glass water, bath water, kitchen water, and holding tank water in the households of Dhaka city dwellers. In addition to collecting and analyzing drinking water, field notes, and a semi-structured questionnaire were used to survey randomly selected households. The analysis revealed that 30% of glass, 25% of the kitchen, 75% of the storage tank, and 45% of bath water were contaminated with E. coli and fecal coliform bacteria, i.e., *Enterobacter faecalis*. Although no Vibrio species were detected in the glass water, *Vibrio chlorea* was found in bath and storage tank water. Bangladesh standard did not allow any coliform (0 CFU/100ml) or fecal coliform (0 CFU/100ml) in drinking water. However, s 40% of the glass water sample was contaminated with E coli, and 100% was contaminated with fecal coliform. *Escherichia coli* in drinking water is a public health concern and may contribute to diarrheal diseases. The findings suggest that recontamination and post-treatment contamination at the point of drinking play a significant role in household water contamination. The water holding tank should be cleaned periodically, and intermittent investigation needs to ensure a safe water supply.

Keywords: Potable water, *E. coli*, *Vibrio cholera*, water reservoir, fecal contamination, indicator bacteria, low-income urban area, and Dhaka city.

Introduction

Safe drinking water is critical for people's health, productivity, and livelihoods. Safely managed drinking water is defined as using an improved water source on the premises, available when needed and free from fecal contamination. Dhaka is the capital city of Bangladesh, with a population of 20.20 million (Zinia et al., 2018). Since it is one of the world's most densely populated cities, water demand is exceptionally high, and access to safe drinking water is one of the essential public health priorities. Contaminated water and poor sanitation are linked to the transmission of diseases such as cholera, diarrhea, dysentery, hepatitis A, typhoid, and polio. The presence of bacteria does not mean the water is unsafe to drink. Only disease-causing bacteria, known as pathogens, lead to disease. If these pathogenic bacteria are resistant to multiple antibiotics, that can cause treatment failure, resulting in an increased mortality risk and an unnecessary burden on healthcare infrastructure. Antimicrobial resistance (AMR) of the waterborne pathogen is a global public health threat, especially for low and middle-income countries (LMIC) where the threat has not been fully identified. In Bangladesh, drinking water quality is also threatened, particularly in urban areas, due to increased rural migration and growth (Juthi et al., 2009). The presence of the index microorganisms as microbial contaminants, such as thermo-tolerant coliforms and *E. coli* in drinking water, has been commonly reported in Bangladesh (Moniruzzaman et al., 2011; Saha et al., 2019). It has recently been shown that antibiotic-resistant genes have adapted to the environment through pathogenic bacteria. Antibiotic-resistant bacteria in drinking water have become an emerging problem worldwide (Nishan et al., 2020). Our previous study on the street tea-stall drinking water quality results revealed that all 246 samples from two city corporation areas were contaminated with MDR fecal coliform bacteria or *Escherichia coli* (data not shown, NFSL Survey, 2021).

On the other hand, household potable water is often contaminated with sewage lines, and various human activities might pollute groundwater. Residential, metropolitan, and business activities would all affect groundwater quality. Household withdrawal of underground water might be contaminated due to the nearness of a faulty septic tank or channels to the pipe and the depth of the pipe. Because of spillage, distribution pipes might pollute supplied water (Parvez,

2016). The total water pollution status of city supplies and the sources of this severe condition is crucial to evaluating public health risks. A recent report stated that controlling microbial contamination of in-house drinking water might be an essential interim strategy until a safe, reliable water connection is provided to the household (Clasen et al., 2015). Researchers have repeatedly observed that the microbiological quality of water can change throughout the collection, transport, home storage, and consumption. However, there have been very few attempts to assess water samples of all household activities and water sources to understand the complete picture of the fecal contamination levels at the household level. These investigations would be helpful for the SDG database and the policymakers in taking preventive water contamination interventions for low-income urban residents. Therefore, this study aimed to assess the water quality of household potable water in a low-income urban area of Dhaka City. This research used a One Health approach to evaluate the water quality of household potable water in a Dhaka City neighborhood with a low income. Warm-blooded animals' intestinal flora contains bacteria like fecal coliform and enterococcus, which are indicators of the possible existence of fecal material and associated fecal pathogens. (EPA, 2017).

2. Methods and Materials

2.1. Sample collection

This cross-sectional study was carried out from April to June 2022. One hundred and fifty (150) low-income households from two areas (Mirpur and Jatrabari) were selected based on the higher diarrheal disease episodes this year. In addition to collecting and analyzing drinking water, field notes, and a semi-structured questionnaire were used to survey randomly selected households. Water samples of all household activities and groundwater, water reservoirs, supply water, household storage, etc. were collected and analyzed for fecal indicator bacteria and *Vibrio* spp. The bacteriological analysis includes total aerobic bacteria, *Enterococcus* sp., and *Vibrio* sp., carried out according to the USFDA Bacteriological Analytical Methods (2015).

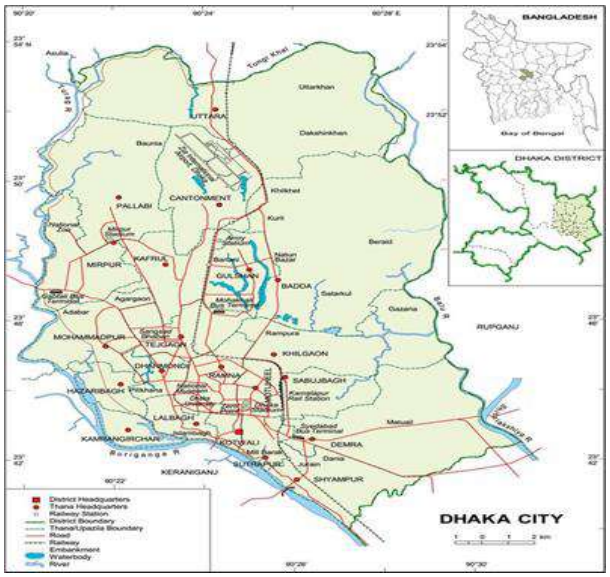

Fig 1: Map **of** sample collection areas

3. Results

The study was carried out to investigate the water quality of household potable water in a low-income urban area. Water samples of all household activities and groundwater, water reservoirs, supply water, household storage, etc. were collected and analyzed for fecal indicator bacteria and *Vibrio* spp.

Water quality of Jatrabari area: Glass water and stored water after boiling or filtering for drinking were found free from *Vibrio* but contaminated with *Enterobacter* and *E. coli* accordingly. Water from the kitchen, bath, and reservoir tank/drum was contaminated with *E. coli*, *Enterobacter* spp., and *Vibrio* spp. Maximum number of *Enterobacter* in reservoir tank/drum water (45/100ml), 34 in bath water, and 24 in kitchen water, respectively. Samples of bath water

collected from the Jatrabari area showed that 60 % of bath water was contaminated with *Vibrio* spp.

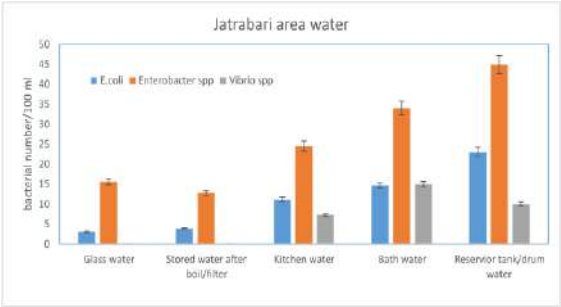

Fig 2: Water quality of Jatrabari area

Water quality of Mirpur area: Glass water and stored water after boiling or filtering for drinking were found free from *Vibrio* but contaminated with *Enterobacter* and *E. coli* accordingly. Water from the kitchen, bath, and reservoir tank/drum was contaminated with *E. coli*, *Enterobacter* spp., and *Vibrio* spp. Number of *Enterobacter* in reservoir tank/drum water (54/100ml), 33 in bath water, and 25 in kitchen water, respectively. *Escherichia coli* in drinking water is a public health concern and may contribute to diarrheal diseases. Bangladesh standard did not allow any coliform (0 CFU/100ml) or fecal coliform (0 CFU/100ml) in drinking water. However, 40% out of 100% of the glass water sample showed it was contaminated with *E. coli* and fecal coliform. During sample collection, questions were asked to the respondents of both the Mirpur and Jatrabari area: what is the source of water supply, house type, what about the last time of cleaning the water tank etc. The answers from the Mirpur area were: water supply from WASA and ground level water tank to overhead tank water was pulled out through submersible motor, all the respondents staying in their own house, could not remember when the reserve tanks were cleaned. The respondent from Jatrabari answered that about 53% of householders stayed in tin sheds instead of buildings. All respondents stated that they have tube wells. However, 53% of householders have both WASA and submersible tube well for water supply. Nevertheless, 80 % of the respondents use shared bathrooms.

4. **Discussion:**

In the study, glass water and stored water after boiling or filtering for drinking were found free from *Vibrio* but contaminated with *Enterobacter* and *E. coli* accordingly. *E. coli* in drinking water is a public health concern and may contribute to diarrheal diseases. Water from the kitchen, bath, and reservoir tank/drum was contaminated with *E. coli*, *Enterobacter* spp., and *Vibrio* spp. Based on the analysis, most of the urban households surveyed in the Jatrabari area withdraw underground water with a submersible pump twice a day to fill the tank, and from the tank, water was supplied to each tenant household of 5 storied buildings. On the other hand, all households surveyed in the Mirpur area use WASA supply water stored in the underground reserve tank, and from the tank, water was supplied to each tenant household of 5 storied buildings. The results draw parallel findings to the water quality thematic report by UNICEF & BBS September 2021 as water collected directly from a source on premises; it was less likely to be contaminated with *E. coli* compared to water collected from covered or uncovered storage containers. The study also indicates that; water quality deterioration at household level is not just a reflection of the cleanliness of vessels used to serve drinking water, but the processes of transport and storage within the home are likely to increase *E. coli* risk level. Results of the present study were similar to a study done by Farhana et.al. (2007) titled Study on Assessment of Drinking Water Quality Consumed by Floating People in Dhaka City, Bangladesh, reveals that about 90% of people consume water without adopting any purification measures such as boiling, filtering etc. The presence of alarming levels of fecal and total coliform indicates the existence of pathogenic organisms, which may induce potential health threats to the people. Hossain *et al*. 2021 found in a study that 75% of tap water and 50% of the water distribution line were contaminated with coliform. About 56% of the respondents agreed that the faulty water distribution line is the main reason for water contamination. The result obtained from this survey is also comparable to the findings of the Ministry of Local Government and Rural Development, in which reports detect the presence of coliform and heterotrophic bacteria in the water samples of the Jatrabari area (Daily Star, 2022).

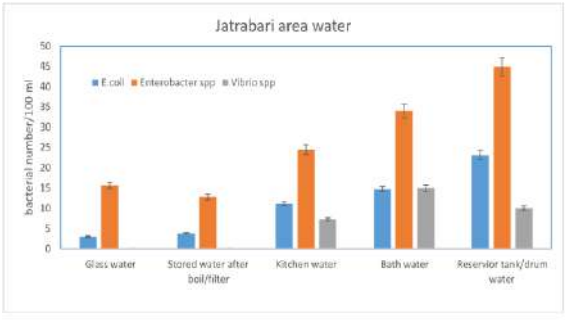

Fig 2: Water quality of Jatrabari area

5. Conclusions:

The current significant obstacles to human health in developing countries are well understood, and a significant component relates to unsafe water, poor sanitation, and inappropriate hygiene (WHO, 2003). From the results and findings obtained in the present

study, it can be concluded that water from the source and point-of-drinking water showed 30% of glass water, 25% kitchen water, 75% of storage tank water, 45% of bath water was contaminated with *E. coli* and fecal coliform bacteria i.e., *Enterobacter faecalis*. Although no V*ibrio* species were detected in the glass water, *Vibrio chlorea* was found in bath and storage tank water. In conclusion, despite various efforts, recontamination and post-treatment contamination at the point of drinking play a significant role in water contamination in households. Thus, strong awareness of personal hygiene practices among consumers may contribute to reducing the risk of waterborne diseases.

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Section E: Miscellaneous

Role of Flow Experience in the Digital Age on Consumer Purchase Intention: A Case Study of Fresh Food in Vietnam

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Abstract.
Since the Covid 19 epidemic, shopping for fresh food online are an upward trend. This study determined and measured the factors influencing the flow experience on the intention to buy fresh food from Vietnamese consumers. The Partial Least Squares- Structural Equation Modeling (PLS-SEM) approach was applied in this study, and information was gathered from Vietnamese customers purchasing fresh food online. The research results show that the factors: Concentration, Advertising Value, Website/App Quality, Informativeness, Interest, and Perception of Risk have a direct impact on Flow Experience. At the same time, the flow experience substantially impacts purchase intention. In particular, the most decisive factor in the consumer's flow experience is the online advertising value. From the research results, the team proposes many solutions to enhance the flow experience to stimulate consumers' purchase intention.

Keywords. Fresh food, Vietnamese consumers, purchase intention, flow experience.

Introduction

E-commerce has helped people remove all barriers of space and time in business activities. Online shopping is gradually becoming a daily routine among Vietnamese people's activities. Vietnam is considered a developing country with the most growing market in Southeast Asia, with a total value of e-commerce goods estimated at $56 billion by 2026. In 2021, the number of product categories Vietnamese consumers shopped online increased by 50%. In addition, the 40% increase in online stores has led to a 1.5 times increase in total online retail spending nationwide compared to 2020 (Anh, 2021).

Notably, in 2021, the Covid-19 pandemic devastated many traditional business activities in the distribution of goods. Consumers cannot buy directly at stores, supermarkets, and markets because they need to ensure epidemic prevention factors. Therefore, online shopping has become even more of a "must," indispensable to satisfy essential needs in the context of people minimizing outside movement. According to the E-commerce White Paper statistics, the percentage of consumers shopping for food online is about 53%, about 43% of consumers buy shoes and cosmetics, and about 33% need household appliances (Duy, 2021). Food and medical products are essential items that are always of concern and play an important role in protecting the health and maintaining life during the raging epidemic. In the products purchased in addition to personal items and the fact that traditional markets or supermarkets are temporarily suspended from direct sales is difficult for families with a habit of buying fresh food there. Therefore, shopping for fresh food online is even more essential. However, transitioning from shopping in-person to online with fresh food takes much work. Because consumers easily find it difficult to consider and evaluate product quality through images. Moreover, many food businesses still need to fully grasp the factors influencing consumers' intention to buy food through flow experience. Most previous studies have focused on groups of clothing, cosmetics, or digital devices but have yet to pay attention to fresh food items. Therefore, this study is necessary and suitable for the current situation through the topic "Research on the factors affecting the flow experience to the intention to buy fresh food of Vietnamese consumers". After giving an overview of the research, part 2 presents the theoretical basis for this research. Research models, hypotheses, and methods are presented briefly in parts 3 and 4. Section 5 presents research results, including the testing hypothesis. The discussion of the research results is presented in the last section.canned foods, fresh food is the new priority to be searched during the epidemic season. The need to use fresh food always exists in every Vietnamese family because of the priority of health. Consumers cannot buy directly at stores, supermarkets, and markets because they need to ensure epidemic prevention factors.

Flow Experience

According to flow theory, *flow* is a psychological state in which an individual feels cognitively

productive, motivated, and happy (Mihaly, 1990). Flow is a complex concept that many researchers operate and measure across multiple dimensions. Models of two, three, four, or five constructs, including enjoyment, attention, concentration, curiosity, and control, can be used to measure (Patanasiriand Krairit, 2019). Research (Morales-Solanaet al., 2019) also shows that concentration and personalization are two antecedent factors, which are also factors that directly affect the flow experience. Flow experience consists of 5 impact variables: informativeness, reliability, entertainment, irritation, and incentives (Martins et al., 2019). Study (Mustafi, 2020) on the impact of advertising value and perception of the brand to purchase intention through flow. Research results show that 68% of respondents answered that the degree of flow is a psychological life. Personal experiences are closely associated with efficiency, motivation, and pleasure. Specifically, each performance, happiness, and motivation category is all in the 70%-75% range, indicating that the actual conditions are sufficient for acceptance. According to (Patanasiriand Krairit, 2019), the flow experience can be used to examine the Website's impact on the flow experience or use the flow experience as an intermediate variable for website quality and purchase intention.

According to many previous studies, the flow experience has a favorable impact on consumers' purchase intention. One of the studies showed that the degree of flow factor involvement is different with different electronic devices (Barta et al., 2021). According to the author, feedback, focus, time distortion, and enjoyment are important factors affecting the flow experience. Research paper (Morales-Solanaet al., 2019) on "Purchase from Online Supermarkets: Key Factors Influencing Experiences of Flow, Purchase Intent and Loyalty" has shown that concentration also affects the flow experience. Simultaneously, personalization, risk perception, and perception of product assortment diversity also directly influence flow and indirectly purchase intention. Another study analyzed the factors affecting consumers' purchase intention after seeing a smartphone advertisement (Martins et al., 2019). The author applied structured equation modeling (SEM) to examine the causal relationships and estimate the conceptual model. It is shown that advertising value is positively influenced by informativeness, reliability, entertainment, and incentive.

Reliability was the most substantial positive factor, followed by entertainment and information. Second, the flow experience is positively influenced by prestige, entertainment, incentives, and promotional value. Information and stimulation have a negative effect. Incentives are the strongest influencers, followed by prestige and entertainment. Third, adding emotional value and website design quality directly impacted brand perception. Finally, the results indicate that web design quality, advertising value, flow experience, and brand awareness are the main factors to explain purchase intention in the context of smartphone advertising. Another study (Mustafi, 2020) shows that the following factors: Information, stimulation, entertainment, irritation, and prestige all affect the consumer's flow experience. According to (Wibowo et al., 2020), when further research on the relationship between two variables of advertising value and brand awareness directly affects purchase intention indirectly. Through the author's research, the advertising value and flow experience significantly influence purchase intention. Also, the study "Enhancing consumer flow experience in China through interpersonal interactions in social commerce." (Liu et al., 2016) showed that expert knowledge of perceived similarity and familiarity directly affect the flow experience and indirectly affects purchase intention through the flow experience.

Online shopping intention

Online shopping is defined as consumer behavior in purchasing goods through Internet stores or websites using online shopping transactions (Monsuwé et al., 2004). In addition, the study (Chiu et al., 2009) looked at it from a different angle and said that online shopping is the exchange of time, effort, and money to receive products and services in return. Most online shopping customers are consumers with little time; they do not like shopping directly at the stores to avoid noise, seller's push, or waiting in line and wait for a long time. In this procurement process, every transaction is done through the Website, and buyers and sellers are not in contact with each other (Kolesar and Galbraith, 2000). Consumers can buy the goods and services they need on the Internet quickly. The Website can show customers many different models and specific prices to make it easier for buyers to choose. In addition, customers can also refer to reviews, product reviews, or comments from customers who have shopped online before to make their own choices. This information is helpful to customers when giving them a multi-dimensional view of the product they want to buy (Chatterjee, 2001). Sellers can quickly sell online and reach more potential customers. Thus, the definitions of online shopping behavior all describe the relationship between purchasing and selling products and services over the Internet.Procurement is a process where people must make many decisions and are influenced by many factors. Purchase intention refers to the customer's ability to plan or prepare to purchase a product or service (Wu et al., 2011). It is a popular metric to measure the online shopping experience of customers. Online shopping intention is based on consumer behavior through a process by which an individual or an organization can select, delete, use, and accumulate products/services to satisfy their needs and customers' wishes. Consumers can face and make many purchasing decisions to satisfy their own needs. However, researching and finding the real

reason is complex. Moreover, the answers inside the mind and thoughts are always tied into each individual's "black box" (Kotler et al., 2012).

According to (Lin, 2007), purchase intention is assessed through the ability to retain and prolong customers' time when visiting the Website. This factor has an important impact on e-commerce websites to help customers evaluate and experience shopping on the website/app (Zott et al., 2000). Customers' purchase intention also depends on many other objective factors, such as income, price, trust, and expectation of the product (Kotler et al., 2012). So quickly pushing customers to the buying decision stage takes work. According to research (Mustafi, 2020), information, stimulation, entertainment, encouragement, and prestige directly and indirectly positively influence the intention to purchase online through intermediaries called flow. Moreover, advertising value on website and application platforms is considered one of the important factors affecting the intermediate flow experience and through that affects online shopping intention. With the research goal of determining the role of online advertising on customers' intention to buy a phone online, the author has pointed out three factors: flow experience, advertising value, and another factor, including control variables, gender, age, experience, and education all impact purchase intention. The research model (Wibowo et al., 2020) also explains that flow experience directly affects purchase intention, which the author explains as concentration, enjoyment, desire, and action. The research "Purchase from Online Supermarkets: Key Factors Influencing Experiences of Flow, Purchase Intent and Loyalty" (Morales-Solana et al., 2019) with 651 valid responses from the survey online. The author has proposed a model where the mediating variable is flow positively impacts purchase intention. The study (Hsu et al., 2011) shows that website quality indirectly affects the flow experience and purchase intention. In addition, research also shows that service quality is more important than information and website quality. In the study "Online Consumer Behavior and Its Relationship with Website Airflow: Insights on Online Travel Agencies in China," flow experience is a cognitive state that leads to behavioral outcomes, including purchase intention and satisfaction. In other words, the research analyzed by testing the scales' similarity and the confirmatory factor analysis method (CFA) shows that the flow experience directly impacts the purchase intention (Gao and Bai et al., 2014) to buy quickly and easily.

Research Model and Hypotheses

Based on studies (Barta et al., 2021; Morales-Solanaet al., 2019; Martins et al., 2019; Mustafi, 2020; Wibowo et al., 2020; Liuet al., 2016), the proposed model includes six factors that directly affect the flow experience of customers when customers shop online: informativeness, concentration, website/app quality, enjoyment, risk perception, and online advertising value. At the same time, according to (Mustafi, 2020; Wibowo et al., 2020; Morales-Solanaet al., 2019; Hsu et al., 2011; Gao and Baiet al., 2014), purchase intention is affected by flow experience.

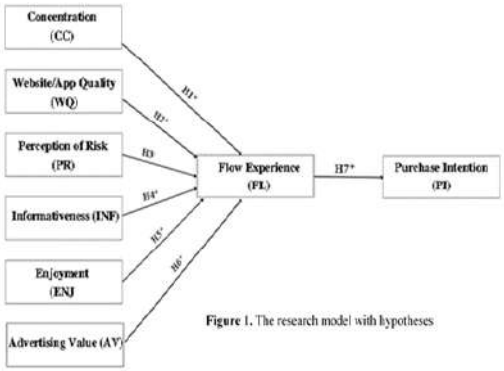

Figure 1. The research model with hypotheses

Concentration

Concentration *is* defined as attention devoted to a limited stimulus field. The element has the most substantial influence on the flow experience, and the individual must be focused on experiencing it (Barta et al., 2021). Expressions of concentration, curiosity, and interest in an activity describe the flow experience. According to (Morales-Solanaet al., 2019), centralization and personalization are the direct precursors of flow in online activity. Concentration is one of the two direct and prerequisite factors for online consumers' musical experience in the study (Patanasiriand Krairit, 2019) on purchase intention on e-commerce platforms. The author has relied on the Website and social networking platform to show that the perception of interest, concentration, and curiosity are all part of the flow factor. Research (Ghani & Deshpande, 1994)has given four scales to evaluate the impact of concentration: I am strongly attracted by my activities when shopping on the website/app; I am fully focused and active when shopping on the website/app; I am very focused on my activity when shopping on the website/app; I only focus on this web/app when shopping experience.

Hypothesis 1: Concentration has a positive effect on the flow experience

Website/App Quality

According to (Martins et al., 2019), research on motivating factors stimulates consumer purchase intention through interaction with advertising. The author uses Ducoffe's web advertising model and the flow experience theory. Research shows that advertising value, website quality, and flow experience explain consumer purchase intention. Research (Hsu et al., 2011) on the influence of website quality on customer satisfaction and purchase intention proposed a model and examined perceived enjoyment and perceived flow mediating the relationship between website quality. At the same

time, the study also evaluates the correlation between feelings of enjoyment and the flow of feelings. Research shows that website quality affects customers' perceived enjoyment, satisfaction, and purchase intention. Website quality is expressed by three scales information, system quality, and service quality. According to (Gao and Bai et al., 2014), the information, effectiveness, and entertainment constitute the website atmosphere and directly affect the customer's flow experience.

Hypothesis 2: Website quality has a positive effect on the flow experience

Perception of risk

Risk perception (Bauer, 1960) has been studied as psychological uncertainty. Another definition of *perceived risk* is a consumer's subjectively expected loss (Peter and Michael, 1976). Customers encountering unexpected problems in their shopping process will give rise to "perceived risk," making the customer unpredictable and influencing their decisions (Bauer, 1960). Research model (Chen et al., 2018) has shown that perceived sacrifice and perceived risk affect customers' perceived value. According to (Lim et al., 2013), perceived risk in online shopping is determined by four causes: Supplier, consumer, technology, and product. The risk is perceived when the consumer is uncertain and afraid of getting undesired results. These issues make the buyer feel hurt or lost, thus hindering the flow of experience and purchase intention. In addition, the lower the perceived risk, the greater the flow experience will be; in other words, the perceived risk negatively impacts the flow experience (Morales-Solanaet al., 2019).

Hypothesis 3: Perceived risk harms flow experience

Informativeness

According to (Proffitt et al., 20145), the informativeness of advertising impacts consumers by a significant 78%. Consumers have a habit of finding out information about products before making a purchase, especially when making an online purchase. Therefore, providing complete and accurate information to consumers and regularly updating the website/application is necessary. Research (Mustafi, 2020) on the role of online advertising on smartphone purchase intention has shown that informativeness directly impacts flow experience and indirectly on purchase intention. Informative, enjoyment, and enjoyment are the determinants of advertising value (Ducoffe, 1995) and positively impact customer flow experience (Wibowo et al., 2020). in studying how smartphone advertising affects consumer purchase intention (Martins et al., 2019), the author shows that informativeness, directly and indirectly, impacts the flow experience. The study of (Gao and Baiet al., 2014) also determined the informativeness that constitutes the website atmosphere and directly influences the flow experience. Along with the point of previous studies, (Patanasiriand Krairit, 2019) also said that the more information provided, the more likely it is to make a shopping decision on the Website.

Hypothesis 4: Informativeness has a positive effect on the flow experience

Enjoyment

The interaction between people and technology creates a feeling of comfort and ignores all risks. This feeling is defined as enjoyment (Davis et al., 1992).The interaction between technology and consumers makes them feel satisfied and excited when they experience and shop on the Website (Webster et al., 1993). Consumers not only go to the Website to find out information and shop, but the Website can give them satisfaction and satisfaction when experiencing the Website. Make them immersed in the space that the Website brings. According to (Webster et al., 1993), *enjoyment* is the degree to use the system satisfied. They are ignoring any other consequences of its use. According to (Barta et al., 2021), flow experience is associated with enjoyment and leads to buying behavior of online consumers. Research (Moneta and Csikszentmihalyi, 1996)also shows that for optimal experience, individuals need to feel complete concentration and enjoyment.

Hypothesis 5: Enjoyment affects the flow of experience

Advertising value

Advertising value is a measure of advertising effectiveness, defined as a subjective assessment of the relative value or utility of advertising to consumers (Ducoffe, 1995). In (Hoffman and Novak, 1996), it is shown that the perceived advertising value contributes to the development of the flow experience. The reason is that consumers entirely focus on the messages and information they receive while eliminating extraneous speculations and thoughts. Consumers will rate newly received messages as worthy if they are relevant to their needs. Consumers show confidence in the value of advertising, and customers' purchase intentions will be increased (Ko et al., 2005). Especially in the food sector, this is one area requiring the need to convey the "green-clean" message. When customers trust the delivery of that message, the customer experience flow and purchase intention will increase positively.

Hypothesis 6: Online advertising value positively affects the flow experience

Flow Experience to Purchase Intention

In today's online shopping problem, the problem of studying the relationship between flow experience and purchase intention has always been a problem studied by many authors. In 1975, psychologist

Csikszentmihalyi proposed the first flow experience model (flow experience). The flow experience increases the user's time using the site (Hsu et al., 2011). Many studies suggest that the flow experience affects the attitudes and intentions of consumers. Research (Mathwick and Rigdon, 2004) has revealed that flow experience affects consumers' attitudes. Thereby increasing purchase intent and website experience time. In the sports stream research paper, (Carlson and O'Cass, 2010) showed that stream experiences are perceived as enjoyable for individuals who want to re-experience the experience. The author has proven that individuals will engage and expand as they experience the flow of online shopping. Research (Koufaris, 2002) has shown how the customer experience flow can affect the likelihood of a purchase that the customer could not have foreseen. The flow experience positively affects the customer's intention to play the game (Chin-LungHsu, 2004). Research (Morales-Solanaet al., 2019) also shows that flow positively impacts purchase intention and online customer loyalty. In the same opinion, according to (Martins et al., 2019), the greater the flow experience, the higher the customer's purchase intention.

Hypothesis 7: Flow experience positively affects consumer purchase intention

Research Methods

Qualitative research was conducted to determine the factors affecting consumers' intention to buy fresh food online through flow experience. The research team conducted a filter to interview eleven students who intend to buy fresh food online. The scale was adjusted to suit the ongoing group study based on the information obtained from the group interviews. Accordingly, the team provides a model with six independent factors affecting the consumer's flow experience. An *intermediate variable* is the flow experience, which directly impacts purchase intention The official study uses a quantitative method with a non-probability sampling technique. It conducts filtering to select appropriate survey subjects who are consumers that intend to shop for fresh food online. The questions in the survey are based on the scales after being adjusted in qualitative research. Then quantitative research uses the method of partial least squares Partial Least Squares (PLS) with Smart PLS software to test the research model and hypotheses from the scales adjusted from the preliminary study. This test is done through the measurement model test and structural model test (Hair et al., 2014). Research

Results

The results of the study are described below.

Respondent Characteristics

After being collected, the questionnaires were cleaned and analyzed. Preliminary statistics about the research sample are shown in Table 2 through essential information. Characteristics of users such as gender, occupation, and income. In addition, the parameters related to online shopping activities on e-commerce platforms were also reported, such as the frequency of purchases on e-commerce sites in the month and the usual time frame of 355 respondents.

Table 2. Respondent characteristics

		Research Sample	Percentage
Frequently shopping	Frequently shopping	279	79
	Not frequently shopping	76	21
	Total	355	100
Gender	Male	149	42
	Female	206	58
	Total	355	100
Age	Under 18	32	9
	18 to 22 years old	185	52
	23 to 30 years old	104	29
	30 years old and up	34	10
	Total	355	100
Accommodation	Ha Noi	45	12,6
	TP.HCM	278	77,9
	Other	32	9,5
	Total	355	100
Income	Under 5 million	138	38,9
	5 to 10 million VND	99	28
	10 to 15 million VND	75	21
	Over 15 million VND	43	12,1
	Total	355	100
Occupation	Student	183	51,5
	Office Staff	123	34,6
	Worker	25	7
	Other	24	6,8
	Total	355	100
Frequency of buying	Less than 3 times/week	192	54
	3-5 times/week	103	29
	More than 5 times/week	60	17
	Total	355	100

Measurement model

When evaluating the reliability of the scale, the study used the composite reliability (CR) coefficient R, the average Extracted variance index (AVE), and the single load factor (Outer loading). For the measurement model to be accepted, the CR coefficient must be greater than 0.7; the outer loading coefficient must be> 0.4. The scale must reach the reliability value (F. Hair Jr et al., 2014). Moreover, AVE > 0.5 reaffirms the reliability and convergence level of the scale (Fornell and David, 1981). The results of the parameters all show that the combined reliability, the average extracted variance, and the load factor are satisfactory in terms of reliability and aggregate value.

Table 3. Convergence validity and construct reliability results

	Cronbach's Alpha	Composite Reliability (CR)	Average Variance Extracted (AVE)
Advertising Value Online (AV)	0.882	0.919	0.739
Concentration (CC)	0.847	0.897	0.686
Enjoyment (ENJ)	0.906	0.935	0.782
Flow Experience (FL)	0.876	0.911	0.675
Informativeness (INF)	0.819	0.881	0.649
Perception of Risk (PR)	0.834	0.889	0.667
Purchase Intention (PI)	0.831	0.888	0.664
Website/App quality (WQ)	0.890	0.924	0.752

In addition, Table 3 also has Cronbach's Alpha coefficient values with a significant value of 0.8, showing that the scales have high reliability when analyzing the linear structural model, the discriminant value is evaluated through the Fornel-Lacker criteria presented in Table 4. To further test the measurement

model, the authors conducted a discriminant test to test the model further when comparing the relationship between variables with the average AVE. There are shows that the square root value of AVE of the component scales is larger than the correlation between concept pairs. Therefore, the research sample ensures the discriminant of the measurement factors. In addition, the Heterotrait-Monotrait Ratio (HTMT) coefficients are all satisfactory when the values of these coefficients are all < 0.9, ensuring the discriminant value. Specifically, the coefficients of HTMT in this study shown in Table 4 are all lower than 0.7.

Structural model

The results of testing the linear structural model are presented in Fig 2. The path analysis results show the factors' impact level based on β Standardized Coefficients. The explanatory level of the independent variables to the dependent variable is FL and PI, respectively, with R Square values of 0.587 and 0.412, respectively. A detailed evaluation of the f square will be analyzed when discussing the research hypothesis. According to Table 5, the effects of factors are reflected through the f value, which shows the most significant effect of AV on FL with f square= 0.075, followed by CC, WQ, ENJ, INF, and PR. In addition, all Q square values are more important than 0, showing that all six factors that affect FL and FL impact PI.

Table 1. Design scale.

Item	Scale	Source
	1. Concentration (CC)	
CC1	I am strongly attracted to my activities when shopping on the website/app	
CC2	I focus entirely on my activity when shopping on the website/app	(Barta et al., 2021)
CC3	I am very focused on my activity when shopping on the website/app	
CC4	I only focus on this web/app when the shopping experience	Qualitative research
	2. Website/App Quality (WQ)	
WQ1	The website/app looks attractive	
WQ2	Websites/apps use fonts properly	(Ha and Im, 2012)
WQ3	Websites/apps that use colors sensibly	
WQ4	Websites/apps that use multimedia features properly	
	3. Perception of Risk (PR)	
PR1	I think buying food online increases payment risk	
PR2	When I buy food online, I think it is risky if I do not get fresh food as expected	(Chen et al., 2018)
PR3	I am worried that buying food online may leak my data	
PR4	I find it difficult to compare food quality when shopping online	(Tráng, 2019)
	4. Informativeness (INF)	
INF1	Does the web/application provide information related to the fresh food that I want to buy?	(Mustafi, 2020)
INF2	The information provided by the website/app is a reliable source	
INF3	Food information on the web/app is well updated	
INF4	Web/app that provides product-related promotions	Qualitative research
	5. Enjoyment (ENJ)	
ENJ1	The experience of buying food online is desirable	
ENJ2	The experience of buying food online is gratifying	(Barta et al., 2021)
ENJ3	The experience of buying food online brings to happiness feeling	
ENJ4	The experience of buying food online is fun	
	6. Advertising Value Online (AV)	
AV1	I feel that advertising online food shopping in online media is helpful	(Martins et al, 2019)
AV2	I feel that online advertising provides the perceived value of online food	
AV3	I feel that online advertising for the new way of purchasing is important	
AV4	I feel that online ads give me better food choices	
	7. Flow Experience (FL)	
FL1	I have experienced the process of shopping for food online	(Barta et al., 2021)
FL2	When I buy food online, I often feel that time passes quickly	
FL3	When I buy food online, I often ignore what is going on around me	(Chen et al., 2018)
FL4	When buying food online, I often focus a lot and forget to do other things	
FL5	I am not distracted by other online shopping activities other than this shopping web/app	Qualitative research
	8. Purchase Intention (PI)	
PI1	When I need to buy food, I like to shop on websites/apps	
PI2	I would recommend people buy food on websites/apps	(Chen et al., 2018)
PI3	I will continue to buy food on websites/apps in the future when the need arises	
PI4	Overall, I am happy to buy food on websites/apps	

Hypotheses testing

The research results are evaluated through Bootstrap analysis, once again reaffirming the reliability of the research model. In this study, the authors used Bootstrapping technique with a repeated sample size of 5000 observations to test the research hypotheses, and the results are shown in Table 6

Table 4. Fornel-Lacker criterion and Heterotrait-Monotrait ratio (HTMT)

		AV	CC	ENJ	FL	INF	PR	PI	WQ
Fornell-Larcker Criterion	Advertising Value (AV) Online	0.859							
	Concentration (CC)	0.531	0.828						
	Enjoyment (ENJ)	0.618	0.554	0.884					
	Flow experience (FL)	0.648	0.586	0.627	0.821				
	Informativeness (INF)	0.535	0.507	0.571	0.588	0.806			
	Perception of risk (PR)	-0.483	-0.405	-0.450	-0.473	-0.546	0.817		
	Purchase intention (PI)	0.641	0.529	0.570	0.641	0.589	-0.478	0.815	
	Website/App quality (WQ)	0.555	0.431	0.547	0.589	0.565	-0.485	0.521	0.867
HTMT		AV	CC	ENJ	FL	INF	PI	PR	WQ
	Advertising value (AV) Online								
	Concentration (CC)	0.610							
	Enjoyment (ENJ)	0.691	0.628						
	Flow Experience (FL)	0.737	0.675	0.704					
	Informativeness (INF)	0.629	0.605	0.662	0.695				
	Purchase Intention (PI)	0.747	0.625	0.654	0.752	0.712			
	Perception of Risk (PR)	0.562	0.474	0.515	0.551	0.661	0.572		
	Website/App quality (WQ)	0.625	0.491	0.607	0.664	0.662	0.604	0.566	

Table 5. Results of f Square

	Matrix f Square						
	Advertising Value Online (AV)	Concentration (CC)	Enjoyment (ENJ)	Informativeness (INF)	Perception of Risk (PR)	Website/App quality (WQ)	FL
Flow Experience (FL)	0.075	0.057	0.035	0.023	0.002	0.042	
Purchase Intention (PI)							0.700

The study model's hypotheses include seven relationships, as illustrated in Fig 1. The results of estimating the relationship between research concepts show that there are six accepted hypotheses. Hypothesis H3 demonstrating the impact of PR on FL is rejected because there is no statistical significance at 5%. The results of the parameter evaluation of the respective hypotheses are shown in Table 6. Specifically, the research hypotheses are discussed in detail as follows:

Table 6. Results of hypotheses testing

Hypothesis	β Standardized Coefficients	Standard Deviation (STDEV)	T Statistics	P Values	Result
H1	0.198	0.060	3.312	0.001	Supported
H2	0.176	0.063	2.791	0.005	Supported
H3	-0.035	0.047	0.750	0.453	Not Supported
H4	0.137	0.062	2.220	0.026	Supported
H5	0.172	0.078	2.217	0.027	Supported
H6	0.249	0.066	3.757	0.000	Supported
H7	0.642	0.042	15.434	0.000	Supported

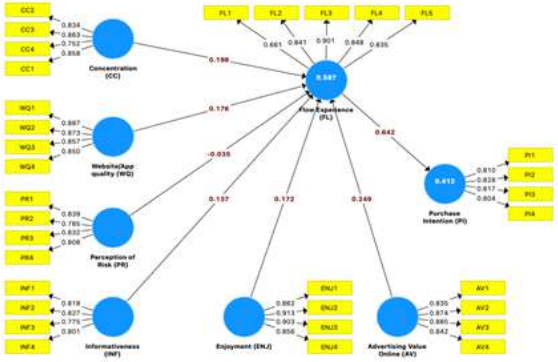

Figure 2. Path analysis and R Square

Verifying hypotheses H1 and H2, the study's results confirm that CC and WQ positively affect FL with β Standardized Coefficients of 0.198 and 0.176, respectively, with T-Statistics = 3.321 and 2.791 with significance. Statistics at the 1% level. Thus, it is appropriate to accept hypotheses H1 and H2, in which

the impact of CC and WQ on FL is also relatively significant, as shown in the f square value presented in Table 5. This result. Also similar to the conclusions in the study of (Barta et al., 2021; Morales-Solanaet al., 2019; Patanasiriand Krairit, 2019; Martins et al, 2019).The positive relationship of PR to FL is tested in hypothesis H3. The results in Table 6 support the acceptance of this hypothesis (T Statistics = 0.750; P-value = 0.453). This result is not consistent with the study of (Morales-Solanaet al., 2019; Patanasiriand Krairit, 2019; Liu et al., 2016; Chen et al., 2018). Specifically, this will be further explained in the section on governance implications. Meanwhile, with β equal to 0.137 statistically significant at a 5% level, hypothesis H4 about the impact of INF on FL is accepted based on Bootstrapping results. However, this effect is relatively low, even the lowest among the factors that have a real impact on FL, as shown by the value of f square = 0.023. This conclusion is consistent with the research of (Mustafi, 2020; Wibowo et al., 2020; Martins et al, 2019).The positive correlation between ENJ and FL is tested, corresponding to hypothesis H5. The results in Table 6 support the acceptance of this hypothesis when they all reach statistical significance at the 5% level in T-Statistics. With the coefficient β reaching 0.172, the hypothesis that ENJ has a positive effect on FL is accepted with a medium level of impact shown at the value of f square = 0.035. This result shows a higher level of impact than in the study of (Barta et al., 2021), although the results are similar in accepting the hypothesis. This is also a new variable included in some recent studies. In addition, with hypothesis H6 about the positive effect of AV on FL. The study's results support the acceptance of this hypothesis, with β reaching 0.249 and showing the most substantial impact on the factors affecting FL with statistical significance at 1%. This is also shown in the f-square matrix when this value in Table 5 reaches 0.075. This effect is also similar to the study of (Mustafi, 2020; Wibowo et al., 2020).Finally, the author tested hypothesis H7 about the positive effect of FL on PI. Research results show that accepting this hypothesis with β = 0.642 is statistically significant at a 1% level. In addition, FL substantially affects PI with f square = 0.700.

Discussions and Further Research

Managerial implications

Research results show six accepted hypotheses out of seven proposed hypotheses, except for the risk perception factor. The perceived risk factor has the lowest impact on the flow experience. The remaining factors include: Concentration, website/app quality, informativeness, enjoyment, and online advertising value, all have a significant positive impact on the flow experience. In particular, the online advertising value factor significantly influences the flow experience. In addition, the research results also show a strong impact of flow experience on purchase intention.

Advertising will influence customers' shopping intent and drive quick online purchase decisions. When attracted by advertising values, consumers tend to be more interested in the product or business mentioned in the advertisement. Therefore, building quality online advertising value will help increase customer shopping experience. Especially with fresh food products, the reputation of product origin is always a concern. Investing in the right advertising message about the product is essential to promote consumers' purchase intention.

With the concentration factor, retailers need to study more deeply the factors that make up the concentration of each individual, especially the concentration when participating in the product shopping experience. Improve the quality of sound and images, and diversify products to prolong the focus time of the customer experience. Along with the focus, the sales website/app quality significantly impacts the customer's shopping experience. Factors such as interfaces, fonts, and images, need to be researched to suit consumers' tastes. In particular, websites/apps with many customer support features, such as 24/7 support chat, also increase customers' sympathy for the website/app and simultaneously improve the customer flow experience.

The flow experience is also influenced by liking and being informative. It makes customers feel excited and attracted when shopping will indirectly stimulate purchase intention. The attractiveness of images, content, and service quality will make customers more comfortable when shopping, reinforcing purchasing decisions. Information is the factor that has a weaker effect on the flow experience than the rest. This may be due to a preference for visuals rather than spending too much time reading information. Therefore, the information retailers give should be more intuitive and vivid, easier to read and understand. The communication to the customer should be the necessary information presented logically and reasonably. Customers who want to save more time reading information will feel satisfied when the information is condensed and concise. The perceived risk factor is the null hypothesis. This factor has the lowest impact on the flow experience compared to the rest. It can be seen that most customers believe that risk perception has no significant influence on flow experience. At the same time, it does not seriously affect purchase intention. It can be explained that when shopping for fresh food online, the perception of risk does not have too significant a direct influence on the customer's flow shopping experience and indirectly on the purchase intention.

Finally, the results are similar to many previous studies showing that consumer flow experience strongly influences purchase intention. Enhancing the flow experience can stimulate purchase intention, prompting action to decide to purchase a product. Therefore, retailers should enhance the factors that positively impact the flow experience by boosting customer purchase intention.

Limitations

This study focuses on the factors that affect the flow experience and consumers' intention to buy products online. Many factors influence customer purchases due to consumer intention and behavior. There are also many other factors affecting the online shopping intention of consumers mentioned as behavioral characteristics of the surveyed customers, such as gender, place of residence, and income, ….and there are many factors that the team has not yet explored. However, this study only focuses on the online shopping intention of individual consumers and their flow experience when buying fresh food on the website/app.

The application of Partial Least Squares- Structural Equation Modeling (PLS-SEM) analysis is optimal for performing an exploratory study and is appropriate for developing and testing new theories. PLS is relatively optimal in research data that cannot meet the strict requirements of covariance-based SEM (CB-SEM) or when the sample size needs to be increased. Future developments might circumvent these constraints when the access data is large enough and normally distributed to test the research hypothesis again according to the wrong structural equation model based on the covariance. These solutions help assess the model's fit in a more holistic perspective, thereby better evaluating the theoretical model.

Online shopping is a topic that attracts the attention of many researchers, especially in today's modern science and technology era. Everything follows the trend of technologization from consumers' habits and usage methods. When consumers have different needs, their buying intentions are also different. The online advertising value is the most important thing driving customers to buy intent. Online advertising messages must bring consumer enjoyment. This study shows the factors affecting the customer flow experience, such as website/app quality, informativeness, interest, online advertising value, and focus. From there, researchers can develop more factors that affect customer flow experience. Therefore, this research is vital to help retailers understand the needs and factors to enhance the flow experience to stimulate consumers' purchase intention.

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Factors Influencing the Purchase Intention of Vegetarian Foods: The Case Study of Vietnamese Consumers

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Abstract
This study aimed to analyze the factors influencing the purchase intention of vegetarian food. The research method included Cronbach's Alpha reliability analysis and Exploratory Factor Analysis (EFA) using the SPSS 20.0 program. The Theory of Reasoned Action (TRA) and the Theory of Planned Behaviour (TPB) were used in conjunction with adjustment. The sample size included 300 vegetarian consumers in Viet Nam. From the results, the authors suggested a series of repercussions to help vegan restaurant owners develop an understanding of consumer demand for vegetarian food in Viet Nam to plan a sustainability strategy, keep existing customers and promote consumers' purchase intentions for vegetarian food in Viet Nam.

Keywords: Vegetarian Food, Vegetarianism, Purchase Intention, Purchase Intention, Viet Nam.

Introduction

Vegetarianism is well known and is becoming a global trend (VTV Newsletter, 2020). According to VTV e-Newsletter (2020), the trend of consumers choosing environmentally friendly and healthy food has been guided by consumers in the past, but it is expected to explode further in the next few years. In 2022, many vegan chain shops were established in China. According to a study by Market Research Firm W&S (2012), a vegetarian diet helps protect the environment and reduces greenhouse gas emissions, which cause floods and droughts. On the other hand, waste and residues emitted by livestock are a source of pollution to the water and air. Because the advantage of vegetarianism is reducing the demand for animal food, reducing livestock and poultry breeding is beneficial to protect the surrounding environment, human health, and life (Nezlek and Forestell, 2020). According to The Economist (2022), the proportion of vegetarians and vegans worldwide is growing faster than expected. The Vegan Society (2022), researched by Lotte, shows that 87% of Vietnamese consumers have tried plant-based milk, 46% have tried other milk alternatives, 49% have tried plant-based meat, and 32% have tried vegan egg substitutes. The main motivations were health (61%), interest in animals (40%), and wanting to try new food trends. According to Industry and Trade Daily (2022), Ms. Kim Hanh said that the current vegetarian trend among consumers is an opportunity for Vietnamese vegetarians. Due to Vietnam's tropical agriculture and diverse vegetarian food processing industry, the country has many opportunities to meet the growing consumer demand for vegetarian food. This trend is an opportunity for the market, manufacturers, and experts of vegetarian products. Vegetarian food has now found its way and brings many benefits to customers, realizing the growth potential of the Vietnamese vegetarian market, especially in Viet Nam. Hence, this paper aims to determine why Vietnamese consumers purchase vegetarian food. The findings are to develop solutions to build the willingness of Vietnamese people to consume vegetarian food in a sustainable direction.

Vegetarian concept and the intention to buy vegetarian food

According to the Vietnamese Food Safety Act (2010), food is a product that people eat and drink in a new form or preliminarily processed. Vegetarianism is a way of life that does not use food produced from animal foods and animal additives such as eggs, milk, and honey. Various forms of vegetarianism depend on the extent to which products of animal origin are excluded from consumption (Franco and Rego, 2005; Craig and Mangels, 2009). Vegetarians can be divided into the following categories: milk, dairy, and eggs; vegetarians with eggs; vegetarians with milk and dairy but no eggs and egg products, etc. In a vegetarian diet, honey may be included, depending on the individual's interest (Franco and Rego, 2005). There is also a vegan diet called vegan or vegetarian. Vegans do not use animal products, so they do not eat animal-based foods like meat, eggs, dairy, or honey. In addition, they do not use clothes made from animals, such as feathers, silk, and leather, or products tested on animals (Craig and Mangels, 2009; Franco and Rego, 2005). A vegetarian diet includes all fruits, vegetables, nuts, grains, seeds, beans, and legumes - all of which can be prepared in unlimited ingredient combinations.

According to the Vegetarian Society UK (2018), dishes may be suitable for vegetarians. Many concepts present different arguments, but vegetarianism used in this study is a diet consisting of only foods of plant origin, or no dairy, eggs, or honey, and does not eat animal food. In the discussion of eating habits, in addition to the functional aspects of food, research also focuses on health and sustainability aspects such as environmental, social, and economic (Miguel et al., 2010), primarily vegetarian food (Souza et al., 2013). Vegetarianism has many motivations (mainly related to ethical, religious, social, economic, health, and environmental), resulting in many different thoughts and attitudes, so efforts are made to understand better the vegetarian consumer group (Janssen et al., 2016). The growing popularity of vegetarianism makes it even more critical for marketers. They understand this convexity and the importance of vegan motivation on consumers' willingness to purchase vegan products (Janssen et al., 2018). According to Ajzen (1991), purchase intention includes consumers' motivation, willingness to try, and effort to complete the behavior. Furthermore, purchase intention is the willingness to purchase and continue to use the product (Tirtiroglu and Elbeck, 2008). Dodds et al.(1991) argued that purchase intention represents the ability to purchase a particular product and what an individual would like to purchase in the future (Long and Ching, 2010). Behavioral intention is an essential concept, especially in business and other fields in general (Giao and Tra, 2020). Purchase intention can be measured by consumers' shopping expectations and consideration of the good/service (Laroche, 1996). In addition, according to Blackwell (2005) and Win and Do (2016), intention is a factor used to assess future executive behavior ability. Purchase intention is buying something people think they would buy (Huang et al., 2014). According to Ajzen (1991), intentions are directly influenced by Subjective Norms, Attitudes, and Perceived Behavioural Control. Kotler et al.(2009) proposed six stages in purchasing a product: cognition, information search, product interest, preference, persuasion, and purchase decision. (Schiffman et al., 2005) insist that consumer behavior is the interplay of factors that influence behavior, perception, and the environment, and it is human changes that change their lives. According to Bennet (2007), purchase intention is the probability that a customer will purchase a specific product or service.

To measure purchase intention, marketers use predictive models to help determine the likelihood of future outcomes. Purchase intention is a traditional, long-standing, and widely used metric in consumer market research. It uses various research processes such as product and new product testing, packaging testing, advertising content testing, innovation and brand positioning, and loyalty.

Theoretical framework

Purchase Intention

According to Kotler (2001), studying customer spending behavior is a top priority for managers because it significantly impacts a firm's strategic marketing and sales intention—research consumer behavior to understand what customers want, what they want, and what services they expect. Businesses will sell products and services that customers need, not what the business owns. It is a trend for all businesses worldwide to study consumer behavior to determine goals and develop appropriate production planning strategies. Satisfy customer needs through shopping behavior. Therefore, marketers must be sensitive and flexible to understand customer needs and influence shopping behavior. According to Kotler (2001), the buyer behavior that leads to purchase intention comes from the initial method, such as the initial method where the product appears in high consumer demand, reasonable price, market conditions, and location. Attractive advertising strategies and promotions will arouse consumers' conscious thinking about products and services. From the initial factors affecting buyers, matching the characteristics of consumers will lead to purchase intention. The purchase intent process is the series of stages consumers go through when purchasing a product or service.

Theory of Reasoned Action (TRA)

The theory of Reasoned Action (TRA) (Ajzen and Fishbein, 1980) show that behavioral intent is the most important predictor of human behavior, consumption, and user choice. The theory of reasoned action (TRA) consists of three elements: Behavioral Intention (BI), Attitude (AT), and Subjective Norm (SN). Behavioral intention is influenced by attitude and subjective norms, and more attention should be paid to the factors that contribute to the tendency to use, and people will consider these two factors. Attitudes are personal expressions of a user's positive or negative beliefs about a product or service. Subjective norms represent the impact of social relationships (family, friends, and colleagues) on individual users. In the TRA model, beliefs about a product attribute profoundly affect consumer attitudes, as measured by product attributes. Consumers will focus on attributes that are essential to various degrees. When evaluating options for product attributes, consumers intend to select beneficial attributes. Besides, attitudes are measured by perceptions of product attributes and characteristics, and if the weights of these attributes are known, the outcome of consumer choice can be predicted. Subjective norm is measured by what stakeholders like or dislike about what they buy and use. The influence of subjective normative factors on consumers' purchase intention depends on the degree of support/opposition of consumers to purchase and use and the motivation and effect of influencing consumers to act according to consumers' wishes. The influence degree of related persons on consumer

behavior trends and the motivation of consumers to pay attention to related persons are two essential factors for evaluating subjective indicators. The closer a stakeholder is to a consumer, the greater the influence on their purchase and usage intentions. The more trust consumers have in the relevant group, the greater the influence on purchasing and usage trends. Consumers' willingness to buy will be affected to varying degrees by their power.

Theory of planned behavior (TPB)

The theory of Planned Behaviour (TPB) consists of three elements: attitudes toward behavior, subjective norms, and control. Perceived behaviors are three functions of an individual's behavioral intentions.TPB was developed from the theory of reasoned action (Ajzen and Fishbein, 1975), arguing that behavior can be predicted or explained by behavior and tend to perform that behavior. It is assumed that behavioral dispositions include motivational factors that influence behaviors and are defined as the level of effort people make to try the behaviors (Ajzen, 1991). Behavioral propensity is a function of three factors. First, attitudes are conceptualized as positive or negative evaluations of performance behaviors. The second factor is social influence, which refers to perceived social pressure to perform or not perform an action—the TPB theory of expected behavior by adding cognitive behavioral control to the TRA model. The perceived behavioral control component reflects the ease of performing the behavior, which depends on the availability of resources and the opportunity to perform the behavior. However, TPB models are limited in predicting behavior (Werner, 2004). The first limitation is the intention factor, which is not limited to attitudes, subjective norms, and perceived behavioral control (Ajzen, 1991). The TPB model predicts an individual's behavior based on specific criteria. However, individuals do not always behave as these criteria predict (Werner, 2004).

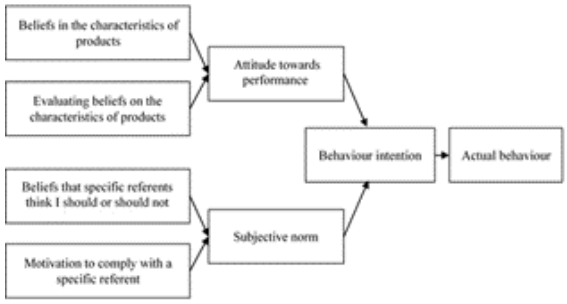

Figure 1. Theory of Reasoned Action (TRA) model (Ajzen and Fishbein, 1975)

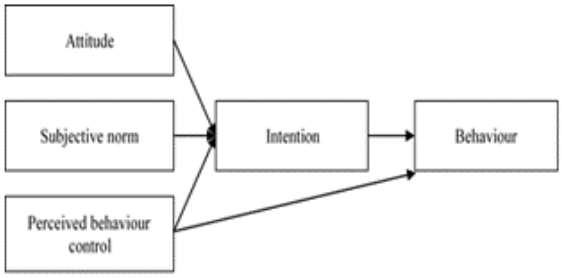

Figure 2. Theory of Planned Behaviour (TPB) (Ajzen, 1991)

Hypothesis Development

On this theoretical basis, the research results and 2 theoretical models, TRA-TPB and 6 models, come from previous research results and the author's judgment. The authors suggest the following research hypotheses on the influencing factors of vegetarian willingness in Viet Nam.

Environmental concern

According to (Kollmuss and Agyeman, 2002), personal environmental concern is an individual's understanding of the impact of human actions on the environment. The more people understand and become aware of environmental issues, the more clearly, they will be aware of the impact of future consumption behavior on the environment and thus better understand and understand changes in their willingness to consume. Environmental causes are essential to changing consumer and food behavior today (Pino et al., 2012). Reducing or stopping meat consumption is increasingly seen as a possible way of reducing pollution (Kersche-Risch, 2015; Ploll and Stern, 2020; Nguyen et al., 2020). Environmental concerns are a decisive driver in reducing meat consumption (Lai et al., 2020). Likewise, animal welfare is an essential moral driver for individuals to switch to more plant-based diets, and Empathic sensitivity to animals is one of the main drivers of positive attitudes toward plant-based foods (Cliceri et al., 2018). It is crucial for vegetarianism (Zamir, 2004; Dyett et al., 2013; Radnitz et al., 2015; Hargreaves et al., 2021).

Hypothesis 1: Concerns about environmental issues positively affect the intention to purchase vegetarian

FOOD IN VIETNAM.

Cognitive value

The theory of planned behavior (Ajzen, 2005) describes how consumers make choices. In consumers' evaluation of product characteristics, attitude affects product cognition and choice; likewise, it directly impacts consumers' preferences and purchasing decisions. Perceived value is essential in consumers' purchasing decisions, reinforcing willingness to spend and showing the connection between products and consumers (Hur et al., 2013; Wang and Yu, 2016). The conceptual framework for this study is based on the critical dynamics of Ajzen's Theory of Planned Behaviour (2001) and (Zanoli and Naspetti, 2002). It has been suggested that attitudes

toward vegetarians directly impact the consumption of vegetarian diets.

*Hypothesis 2: Cognitive value positively affects the purchase intention of vegetarian food in Vietnam***.**

Animal Welfare

Looking at the intent-attitude path suggested by the broader framework of rational action theory (Fishbein and Ajzen, 1975), this work asks buyers with spare time to purchase a brand that separates it from vegans, i.e., Regular and occasional buyers. Lacoeuilhe et al. (2017) found that different perception factors drive the choice of regular and occasional buyers. The role of ethical motivations (i.e., environmental, animal welfare, health, and ethical concerns) and perceived factors (i.e., perceived quality, brand trust, ownership of a vegan brand, and perceived value) were the driving forces behind the intention to purchase private vegan labels. The two main ethical motivations behind consumers switching to vegetarian and vegan diets are animal welfare and environmental certification. Environmental concerns are the responsibility of consumers to be aware of the environmental impact of their consumption choices, while animal welfare concerns include respect for consumers' rights to animal rights and cruelty-free practices. Some researchers have studied the two together without distinguishing those (Radnitz et al., 2015). In contrast, it would be more relevant to view them as two different aspects of emerging research (Martinelli et al., 2021).

*Hypothesis 3: Concerns about animal welfare positively affect the purchase intention of vegetarian food in Vietnam***.**

Product features

While there is still debate about whether whole foods are more nutritious than conventional foods (Hasselbach and Roosen, 2015), many reports point to happiness as a high-value driver for the consumption of Organic/Natural Foods (Kriwy and Mecking, 2012; Nguyen et al., 2020). Following a vegan lifestyle includes avoiding animal products or products that have been tested on animals. Understanding consumer attitudes towards vegan diets can explain in consumption of vegan diets. In-depth literature shows that attitudes can predict consumer behavior and influence consumption. For example, (Gumpo et al., 2020) found that consumers' attitudes influence their decision-making. However, understanding consumer attitudes and behaviors are critical to effectively connecting with the market and consumer trends.

Hypothesis 4 (H4): Product features positively affect the purchase intention of vegetarian food in Vietnam.

Social Influence

Social influence occurs when lifestyles, behaviors, thoughts, and preferences are combined with the behavior of others, and their behavior changes in the company of others (Markowskiet al., 2019). Individuals can be influenced by their social environment in several ways (Wescombe et al., 2019; Ngo et al., 2021). Social influences, directly and indirectly, affect mood, attitudes, and ultimately destructive behavior. Traditional social influence is also associated with credibility. Today, however, influence can come from unknown individuals on social media. Affected people do not need direct contact. This influence disrupts principles, attitudes, and standards and affects purchases. The importance of understanding social influence stems from the fact that it helps to understand social behavior, which in turn aids decision-making, steers public opinion, and facilitates change (Peng et al. 2017). Consumers receive information through multiple channels, and social influence is essential when analyzing scammers' attitudes and purchase intentions (Stibe et al., 2016). Social influence is a broad topic that strongly and steadily influences consumer behavior and attitudes. There are two types of social influence: information society and normative social influence and social impact on information related to trust. The social influence strategies studied include social learning, social identity, and social comparison. Social learning occurs when behavior is changed through direct observation and imitation of others (Rao et al., 2001). Social evidence arises when some people do not know how to behave and behave and therefore behave the same way as the group, assuming that the group knows the correct social norms. Social comparison occurs when a person compares himself to others who are similar. Normative social influence involves meeting group expectations. In some studies, informational social influence replaces overt normative social influence. It was concluded that healthy behaviors are transmitted through social networks and that social influence in severe health conditions can help to change food choices, influence consumption to eliminate negative habits, and steer individuals in positive directions (Kim and Choo, 2022).

Hypothesis 5: Social influence positively impacts the purchase intention of vegetarian food in Vietnam.

Attitudes

According to Davis et al. (1989), attitude is the individual's positive or negative feelings about the performance of the target behavior. Engagement occurs when interest, connection, commitment, or focus on a specific issue, product, or situation leads to action (Richin et al., 1986). It refers to the understanding and relationship between a person and a target or subject. Engagement is influenced by topic

and interest (Ghali-Zinoubi et al., 2019). This trend happens when an individual participates, and thus, participation determines the purchase/choice of a product. Engagement occurs when individuals search for information about a product and connect with something they intend to buy. It is the perceived relevance of an object to its values, interests, and needs. Attitude is a crucial factor in the consumer engagement process, affecting decisions at the purchase time. Individuals are more likely to buy a particular product when there is engagement – engagement is influenced by human personality traits, knowledge, and experience (Baldini et al., 2018). The several levels of engagement, from high to low, are rare and elated to acquisition purposes (Park et al., 2019). High engagement requires much research, effort, consideration, comparison, and evaluation (Kim et al., 2019; Nguyen et al., 2020; Rokonuzzaman et al., 2020). Low levels of effectiveness do not require research, and brands play a significant role (Jeseviciute Ufartiene, 2019), but when the issue is related to health, values , and needs (Dodds et al., 1991) or product purchase quality (Rokonuzzaman et al., 2020), participation is always higher. The resulting engagement combines situations and long-term events in which various cognitive and behavioral complexities influence buying. In a nutshell, engagement can be understood as the importance of a product according to the consumer's standards and attitudes, preferences, and desires, as people try not to buy goods or services that harm their life. Engagement relates to consumer interests, preferences, beliefs, lifestyles, and attitudes. Therefore, it can be expected that attitudes toward the consumption of vegan products may impact the use of vegan products.

Hypothesis 6: Attitude positively affects the purchase intention of vegetarian food in Vietnam.

Purchase intention

Purchase intention can be understood as the willingness to purchase products and services. Purchase intent is observed when attempting to purchase a product or service and occurs when a supplier exhibits characteristics that satisfy a consumer's needs (Dodds et al., 1991). Purchase intention can also be seen when there is a possibility or plan to buy (Luo et al., 2011; Wu et al., 2011); the more substantial the intention, the more likely it is to purchase these products and services. Consumers' propensity to satisfy their needs and active buying interests suggests that purchase intent is triggered by emotions, which can associate with a brand, product, or service (Luo et al., 2011). Motivations, needs, or new information related to purchase intent. Media influences the purchase intention process, making the process more complex and influenced by many factors (Luo et al., 2011). In addition to product knowledge and information, product imagery also plays an important role (Imelia et al., 2017), but product familiarity also generates purchase intent, and product familiarity is more likely to generate purchase intent (Liu et al., 2016). Regarding consumer purchase intention, consumer purchase intention shows the relationship between products and consumers (Dodds et al., 1991). Attitudes toward a brand, product, or service strongly impact purchase intention and propensity to purchase (Hsu et al., 2017).

Conceptual frame work

Accordingly, empirical studies and theories, this study proposes 6 factors that affect consumers' willingness to purchase intention of vegetarian food in Viet Nam. The relationship between the variables is described as follows:

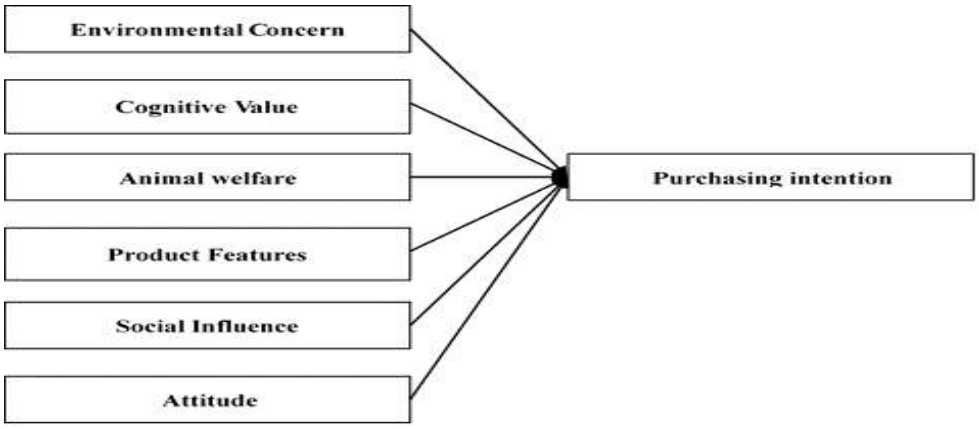

Figure 3. Conceptual framework

Research Method

Research design

Phase 1: Preliminary Research

Step 1: (Preliminary Evaluation) directly communicate with experienced sellers through the phone function, evaluate and verify the variables in the questionnaire and their experience, to understand the willingness of Vietnamese consumers to buy vegetarian food.

Step 2: (Preliminary Quantification) Edit and reconstruct the appropriate questionnaire based on the supplier's assessment. A limited predictive questionnaire with 50 samples was submitted to assess the reliability and correlation of variables.

Phase 2: Formal Research

Formal quantification: After the initial quantification of the results, the questionnaire was edited and reconstructed. Design a questionnaire and proceed with the official survey. The data were analyzed by SPSS 20.0 software, descriptive statistical analysis, Cronbach's Alpha scale, exploratory factor analysis (EFA), and linear regression model. Finally, conclusions and implications for the work are built.

4.2 Research procedure

This study uses a quantitative approach based on the objectives, questions, and research subjects. Quantitative studies were used to assess reliability, scale values, and test model hypotheses; primary data were collected through questionnaires sent directly to Vietnamese consumers. The collected data were processed using SPSS software.

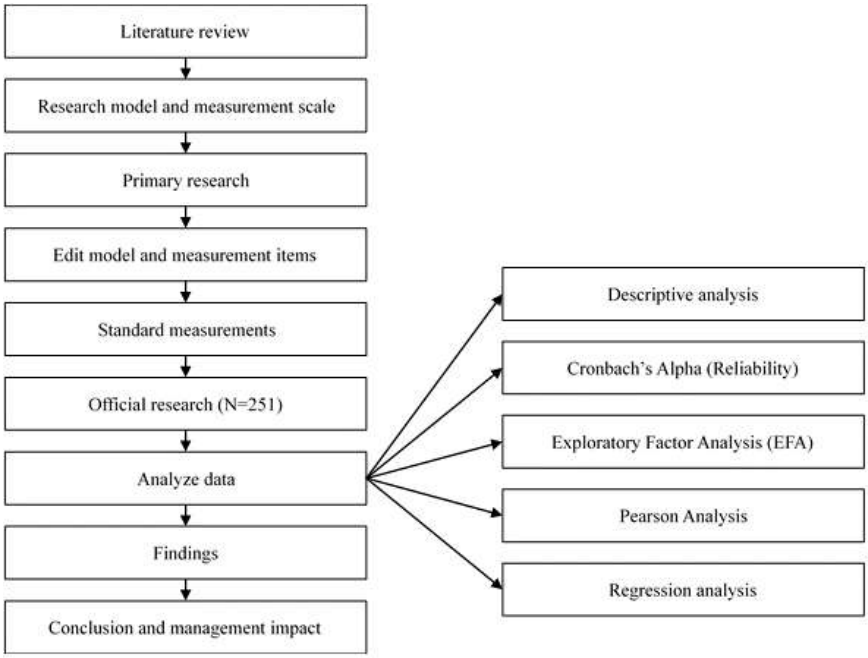

Figure 4. Research stages

Research Sample and Research Scale

The minimum sample size must be 5 times the number of variables observed in the study model (Tho, 2014). The number of observed variables of factors in the research model is 25 observed variables. Therefore, the minimum sample size should be 5 x 25 = 125. The sample size, which includes 300 samples collected for the analysis, is appropriate.

Data collection

A survey was conducted to collect data and analyze the factors influencing consumers' willingness to purchase vegetarian food in Viet Nam. A total of 307 questionnaires were distributed, and the collected data will be cleaned before analysis. After the survey, 307 answers were obtained, of which 251 were valid and 56 were invalid (1 answer was not in Viet Nam, and 55 wrong answers were included in the question set).

Reliability Test (Cronbach's Alpha):

The scale's reliability is assessed by the Cronbach Alpha coefficient, which only measures the scale's reliability (including 3 or more observed variables) and does not calculate the reliability of each observed variable of the scale. The above coefficients have variable values in the range [0, 1]. According to Nunnally (1978) and Hair et al. (2009), Cronbach's Alpha coefficient values from 0.7 or higher are considered reliable and reliable scales. In theory, the higher this coefficient, the more reliable the scale. However, when Cronbach's Alpha coefficient is too large (greater than 0.95), it indicates that many variables in the scale are not different, a phenomenon known as scale overlap (Tho, 2014). Another vital

measure, according to Cristobal et al. (2007), is the correction of total correlation, which is a good scale when the observed variable has an index of 0.3 or higher.

Table 1. The measurement of the research constructs

No.	Measurement items	Code	Source
Environmental Concern (MT)			
1	I take responsibility for the impact of my food choices on the world	MT1	(Martinelli, 2020)
2	I care about the impact of my food choices on the world	MT2	
3	My food choices are the vital impact I can have on the world	MT3	
4	The way I eat expresses concern for the world	MT4	
5	My food choices matter to the world	MT5	
Cognitive Value (NT)			
6	Vegetarian foods have higher nutritional value	NT1	(Tobias, 2021)
7	Eating a vegetarian diet will improve my long-term health benefits	NT2	
8	Vegetarianism is a symbol of status and a wealthy lifestyle	NT3	
9	Vegetarian food makes a positive contribution to an environmentally friendly world and protects animal rights.	NT4	
Animal Welfare (DV)			
10	It is important to treat animals naturally	DV1	(Miguel et al., 2020)
11	I care about animal welfare	DV2	
12	Animals cannot suffer	DV3	
13	The idea of a "natural" env ironment applies to both domestic and wild animals.	DV4	
14	Companies must consider their profits and animals welfare	DV5	
15	Companies must consider animals, their market value, and cost	DV6	
Product Features (SP)			
16	Vegetarian food is better than non-vegetarian food	SP1	(Tobias, 2021)
17	Vegetarian food is suitable for consumption because it does not contain animal products and biological products derived from animals.	SP2	
18	Vegetarian food has better quality	SP3	
19	Vegetarian food tastes better than traditional food	SP4	
Social Influence (XH)			
20	My friends often recommend vegetarian foods to me	XH1	(Miguel et al., 2020)
21	My friends often go shopping for vegetarian food with me	XH2	
22	My friends often share their experiences and knowledge of vegetarian products with me.	XH3	
23	Most of the people whom I consulted intend to keep buying vegetarian food	XH4	(Thu and Tuyen, 2017)
24	Their opinions and suggestions influence vegetarian purchases	XH5	
Attitudes (TD)			
25	I like to consider vegetarian products as one of my shopping options	TD1	(Miguel, 2020)
26	I love buying vegetarian products	TD2	
27	I feel great when using vegetarian products	TD3	
Purchase Intention (YD)			
28	I am happy to buy vegetarian products	YD1	(Miguel, 2020)
29	I wish to eat vegetarian products	YD2	
30	I plan to eat vegetarian products	YD3	
31	I intend to buy vegetarian products in the following days	YD4	

Exploratory Factor Analysis (EFA):

After testing reliability, the convergent and discriminant validity of the concepts in the research model needs to be tested by exploratory factor analysis. This reduction is based on the linear relationship of the factor to the observed variable. The applicability of the EFA analysis method was assessed by KMO and Bartlett tests.

Bartlett's test: to see whether the correlation matrix is a unit matrix (a unit matrix is a matrix with a correlation coefficient between variables of 0 and a correlation coefficient of 1 with itself). If the p-value of the test is < 0.05 (at the 5% significance level), then the observed variables are correlated with each other in the factor. Therefore, use the appropriate EFA.

4.4.3 KMO coefficient (Kaiser-Meyer-Olkin):

It is an index to evaluate whether factor analysis is applicable. The higher the KMO factor, the higher the rating. (Kaiser, 1974) suggested: KMO ≥ 0.9: very good; 0.9 > KMO ≥ 0.8: good; 0.8 > KMO ≥ 0.7: normal; 0.7 > KMO ≥ 0.6: temporary; 0.6 > KMO ≥ 0.5: poor; KMO < 0.5: no acceptance. Coefficients in

[0.5; 1] are the basis for appropriate factor analysis. The scale's one-way, convergent, and discriminant validity were assessed using EFA.

Table 2. Determining the Importance of Independent Variables by Percentage

Demographic factors		Frequency	%
Gender	Male	77	30.7
	Female	174	69.3
Age	18-25	166	66.1
	26-35	64	25.5
	36-54	20	8.0
	>54	1	0.4
Education	High school	25	10.0
	Intermediate/ College	24	9.6
	University	182	72.5
	Post Graduate	20	8.0
Occupation	Student	121	48.2
	Office worker	96	38.2
	Businessman	8	3.2
	Housewife	26	10.4
Income level	< 5,000,000 VAN	104	41.4
	5,0000,000 VND - 10,0000,000 VND	80	31.9
	10,0000,000 VND - 20,0000,000 vnd	41	16.3
	> 20,0000,000 VND	26	10.4
Total		251	100

*VND: Vietnamese Dong

Multiple Regression Analysis:

Multiple regression analysis aims to assess the degree and direction of the influence of independent variables on the dependent variable. The dependent variable is usually denoted Yi, and the independent variables are denoted Xi, where i ~ (1, n), where n is the number of observations and k is the number of independent variables in the model. For example:

Yi = 1 + 2X2i + 3X3i +…+ kXki + Ui (3.1)

Regression analysis is designed to test whether the effect of the independent variable (Xi) on the dependent variable (Yi) is statistically significant through the corresponding regression parameter (), where Ui is the remainder corresponding to Ui ~ N(0, 2). The following basic steps to this analysis are completed:

To check the overall fit of the model and assumptions:

$$H_0 : \beta_2 = \beta_3 = \ldots = \beta_k = 0$$

H_1: is there at least one other regression parameter

This hypothesis is tested with the parameter F. As the formula is following:

$$F = \frac{ESS/(k-1)}{RSS/(n-k)}$$

Where: ESS is the portion of variance explained by the model, and RSS is the portion of variance not explained by the model. If F > Fα (k-1, n-k), reject H0; otherwise, H0, where Fα (k-1, n-k) is F at significance level α and numerator (k-1) and denominator (n-k) degrees of freedom critical value. Alternatively, if the p-value obtained from the calculation of F is sufficiently small, the regression model fits the survey data at the chosen significance level. The multiple coefficients of determination (R^2) were used to determine how well the independent variables explained (%) the dependent variables in the model. The F-test is a reciprocal, similar to the R^2.

Test for multicollinearity by VIF coefficients. The magnitude of this coefficient is also inconsistent, and generally, VIF < 10 is considered a model that does not violate the multicollinearity assumption.

4.4.5 Autocorrelation Test:

Use the Durbin-Watson index. As a rule of thumb, if 1 < Durbin-Watson < 3, you can conclude that the model is autocorrelation-free.

Tests for statistical significance of individual regression parameters. For example, from equation (3.1) to test whether parameter 2 is statistically significant at the 5% level:

Hypothesis:

$H_0 : \beta_2 = 0$

$H_1 : \beta_2 \neq 0$

Calculate the parameter t with n-k degrees of freedom as $t = \frac{\hat{\beta}_2 - \beta_2}{Se(\hat{\beta}_2)}$. Among them: $\hat{\beta}_2$ is the sample regression parameter; β_2 is the regression parameter to be tested, and $Se(\hat{\beta}_2)$ is the corresponding sample regression parameter. If the calculated t-value exceeds the critical value t at the chosen significance level (α = 5%), the null hypothesis H0 can be rejected, indicating that the independent variable corresponding to this parameter is significant for the dependent variable. Alternatively, if the p-value obtained from the calculation of t is sufficiently small, the regression parameters are statistically significant. When analyzed using SPSS 22.0 software, p-values are indicated with symbols (Sig.)

Results

Descriptive statistics

Among the 251 people, 77 were male, accounting for 30.7%, and 174 were female, accounting for 69.3%; 25 were high school graduates, accounting for 10%;

24 were intermediate/college education, accounting for 9.6%, and 182 were college graduates, accounting for 10%. 72.5%; 20 graduate students, accounting for 8%, 121 students/students, accounting for 48.2%, 96 office workers, accounting for 38.2%, 8 business people, accounting for 3.2%, 26 homemakers, accounting for 10.4%; There are 104 people with income below 5 million, accounting for 41.4%, 80 people with income between 5 million and 10 million, accounting for 31.9%; 41 people with income between 100 million and 20 million, accounting for 16.3%; 26 people with income exceeding 20 million dongs, accounting for 10.4%.

According to the results, environmental concern (MT), cognitive value (NT), animal welfare (DV), Product Features (SP), social influence (XH), attitude (TD), and purchase intention (YD). The Cronbach's Alpha coefficients of 0.826; 0.766; 0.805; 0.792; 0.817; 0.887; 0.904 were all greater than 0.6, and the total correlation coefficients of each scale's observed variables were greater than 0.3, so the scales were reliable.

5.3 Factors Influencing the Willingness to buy vegetarian food. The adjusted coefficient of determination *R*2 and the F test, while the VIF coefficient predicted multicollinearity result obtained is as follows: n

Table 4.Result of multivariate regression analysis

Model	R	R^2	Adjusted R^2	Std. Error of The Estimate	Durbin-Watson
1	0.745[a]	0.555	0.544	0.549	1.906

5.2 Reliability

As a result, we have a coefficient *R*2 adjusted by 0.544, which means that the established multiple linear regression model is suitable for 54.4% of the dataset, i.e. the independent variable explains 54.4% of the variation in the dependent variable. In addition, the autocorrelation phenomenon in the model was tested by the Durbin-Watson coefficient. The model is said to have no autocorrelation if the Durbin-Watson coefficient is greater than 1 and less than 3. The model has autocorrelation if Durbin-Watson is less than 1 or greater than 3. The results in Table 4 show that the Durbin-Watson coefficient is 1.906, so the model has no autocorrelation.

Based on Table 5, the Sig. = 0.000 < 0.01 and F = 50.793 indicates that the model is consistent with the data. In other words, the independent variable is linearly related to the dependent variable at the 99% confidence level. The analysis of the variance table of the regression model shows that the model has F=50.793, Sig=0.000<0.05, indicating the overall fitting of the regression model; the combination of independent variables in the model can explain the variation of the dependent variable. According to the findings, the regression coefficients of all variables MT, NT, DV, SP, XH, and TD have Sig levels of less than 0.05. It indicates that these variables are significant at the 95% confidence level. So at the 95% confidence level, the variables MT, NT, DV, SP, XH, and TD all have statistical significance, or the MT, NT, DV, SP, XH, andTD variables affect the dependent variable. Also, all variables have positive coefficients, indicating that the variable has the same effect as YD. It can also be seen from the above result table that the variance inflating factor VIF is in the range of (1.106-1.343) < 2, so it can be concluded that the model does not have multicollinearity. It indicates that the relationship between the independent variables has no significant effect on the interpretation results of the regression model.

Table 5. ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	91.812	6	15.302	50.793	0.000[b]
Residual	73.508	244	0.301		
Total	165.320	250			

By analyzing the results, the regression equation extracted according to the unstandardized coefficients is as follows:

YD = -0.443 + 0.130MT + 0.131NT + 0.172DV + 0.158SP + 0.115XH + 0.429 TD

Table 6. Regression analysis

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	-.443	0.257		-1.726	0.086		
	MT	0.130	0.059	0.117	2.194	0.029	0.642	1.558
	NT	0.131	0.063	0.114	2.062	0.040	0.599	1.671
	DV	0.172	0.060	0.154	2.850	0.005	0.621	1.612
	SP	0.158	0.061	0.139	2.573	0.011	0.622	1.609
	XH	0.115	0.052	0.116	2.195	0.029	0.654	1.528
	TD	0.429	0.041	0.465	10.512	0.000	0.931	1.074

Based on the above equation, if the environmental concern factor increases by 1 unit, the purchase intention increases by 0.130 units on average. Suppose the cognitive value factor increased by 1 unit, and purchase intention increased by an average of 0.131 units. Every 1 unit increase in the animal welfare coefficient, the purchase intention increases by 0.172 units on average. If the product Features factor increases by 1 unit, the purchase intention increases by 0.158 units on average. For each additional unit of social influence factor, the purchase intention increases by 0.115 units on average. Finally, for every 1 unit increase in the attitude, the purchase intention increases by an average of 0.429 units. The regression equation extracted according to standardized coefficients is as follows:

YD=0.117MT+0.114NT+0.154DV+0.139SP+ 0.116XH + 0.465 TD

Table 3. Cronbach's Alpha				
	Scale means if items deleted	Scale variance if item deleted	Corrected item-total correlation	Cronbach's Alpha if the item deleted
Cronbach's Alpha = 0.826				
MT2	11.27	5.270	0.670	0.774
MT3	11.27	5.230	0.660	0.778
MT4	11.29	5.125	0.648	0.782
MT5	11.45	4.816	0.637	0.791
Cronbach's Alpha = 0.766				
NT1	7.73	2.188	0.632	0.648
NT2	7.73	2.176	0.630	0.651
NT4	7.67	2.341	0.537	0.754
Cronbach's Alpha = 0.805				
DV1	11.43	5.150	0.614	0.758
DV3	11.49	5.091	0.601	0.765
DV5	11.47	5.026	0.632	0.750
DV6	11.41	5.123	0.631	0.750
Cronbach's Alpha = 0.792				
SP1	7.52	2.107	0.683	0.664
SP2	7.50	2.371	0.610	0.744
SP3	7.51	2.355	0.613	0.740
Cronbach's Alpha = 0.817				
XH2	7.39	3.095	0.660	0.759
XH3	7.36	2.703	0.723	0.692
XH4	7.23	3.032	0.629	0.789
Cronbach's Alpha = 0.887				
TD1	7.46	3.321	0.783	0.837
TD2	7.51	3.163	0.805	0.817
TD3	7.38	3.388	0.752	0.864
Cronbach's Alpha = 0.904				
YD1	11.47	6.458	0.775	0.881
YD2	11.38	6.029	0.798	0.872
YD3	11.41	6.108	0.776	0.880
YD4	11.53	5.970	0.795	0.873

Standardized regression coefficients show that the effect of independent variables on purchase intention, ranked in the following order: attitude (β = 0.465), animal welfare (β = 0.154), Product Features (β = 0.139), environmental concerns (β = 0.117), social influences (β = 0.116) and cognitive value (β = 0.114).

4. Conclusion

This study examines the impact of environmental concern, cognitive value, product features, animal welfare, attitudes, and social influences on the willingness to consume vegetarian food in Vietnam. The study examines the influence of factors promoting vegetarian consumption in Vietnam with 95% confidence and helps to provide detailed information on factors promoting vegetarian consumption in Vietnam. Therefore, the results and directions will help in future decisions on general food purchases. Research on motivation and barriers, especially vegetarianism, on the other hand, this study helps strengthen the theoretical basis of previous studies and provides a new model for the research field suitable for the actual situation. In Vietnam, especially in Vietnam, this study assessed consumers' willingness and willingness to purchase vegetarian food and the gap between perceptions, including motivators and disincentives. The research suggests managerial implications to help businesses and companies understand incentives and disincentives to implement appropriate strategies to improve company performance while helping balance the ecosystem and ensure consumers make healthy choices when purchasing vegetarian food. Managerial Implications Based on the findings of our analysis, the authors propose some management implications for vegetarian suppliers. First of all, for environmental concern factors, vegetarian companies need to understand the relationship between humans and nature and implement production strategies that protect the living environment and ensure ecological balance because they are a bridge to encourage users to choose to buy and to use. Additionally, businesses need to raise awareness and awareness of vegan consumption and nature conservation through training programs at all levels, emphasizing that their brands are environmentally friendly, such as using recycled materials for packaging. When attitudes towards the living environment improve, consumers will have more altruistic behaviors for social interests rather than personal interests, which helps to form the willingness to buy vegetarian food in Viet Nam, especially in Vietnam. Enterprises need to have a sense of responsibility and professional ethics, strictly follow the production process, from seed selection and breeding to harvesting, processing, supply, and linkage, cater to the market, control the market, and thoroughly inspect the products. Follow pre-commercial standards. Expired products need to be thrown away, and ensuring food safety cannot reuse. The brand reputation of a business can create trust and purchase intentions. Publicity campaigns by authorities and business media regarding the vegan product/vegetarian consumption should focus on the positive environmental impact of vegan product/vegetarian consumption. More scientific

research is needed to demonstrate the impact of vegetarianism on overall environmental protection.

Secondly, for the cognitive value factor, since the perceived value is so significant to buyers, entrepreneurs need to highlight the perceived quality of vegetarian food and its associated health benefits. Health knowledge can be imparted through different platforms, leading customers to demand vegan products while enhancing consumer confidence in their branded products, as well as environmental concerns and the animal consumers may recommend in-store, thereby increasing the benefits—the overall perceived value of what differentiates its product from its competitors. Trade promotion activities should focus on vegetarian food's health benefits and value.

Thirdly, regarding the animal welfare factor, since consumer concerns about animal welfare influence consumer eating behavior, vegan manufacturers and retailers must adjust their marketing. Regulators can influence the psychology of consumers who love animals, hoping to ensure fairness and that animals are treated naturally, reducing the need to eat meat and increasing the need for vegetarian food.

Fourth, manufacturers provide more and more healthy products for Product Features factors, and the core value brought is not only to provide good products but also to bring valuable life to customers. Businesses should implement a nutrition counseling program that provides information on healthy eating to improve and maintain health. Additionally, marketing campaigns to promote the benefits of a vegetarian diet highlight the dangers of consuming contaminated food, which can be harmful to health and life-threatening.

Fifth, it is for the social influence factor. The survey shows that family members and friends influence consumers in Viet Nam to organize activities by establishing promotional activities. Training activities related to vegetarian cooking, using vegetarian ingredients to contribute to the brand, bring participants with relatives and friends.

Sixth, as for the attitude factor, attitude also influences the willingness to buy vegetarian food. This result is consistent with the background theory of consumer buying behavior. "I am happy to buy a vegetarian product." Attitude factors must excite consumers when choosing a vegan option, engaging consumers with unique and creative marketing strategies. In addition, it is necessary to ensure the quality factor of the product, increase the unique creativity of the product, and meet the taste needs of consumers, thereby increasing the authenticity of the influence of fun on customers and consumers' willingness to consume vegetarian food.

Strengthen the relationship between institutions and enterprises. A coordinated advocacy effort to increase the campaign's reach to publicize the names of companies dedicated to environmental protection and the creation of environmentally friendly goods. Businesses need to demonstrate that the product brings development to the community, and this perspective has led vegan food businesses to ethically and socially market to the community. Activities that could be solutions include: organizing charity rallies; developing funds to support social activities from the profits of vegan businesses. Another suggestion is that companies must identify each product's potential consumer groups and develop appropriate strategies. Initially, food manufacturers can focus on products catering to vegetarian and vegan customers. After vegetarian consumption became a trend that affected other customer groups, merchants began to expand their products to serve lower market segments.

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Customer's Behavior of Using Shopee Pay E-Wallet after the Covid-19 Pandemic: Food Industry

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Abstract:
The COVID-19 pandemic raged and significantly affected human social life. Food became scarce and challenging to buy during social distancing. In addition to being able to buy directly, some regions are limited in the amount of food they buy. Moreover, the implementation of the social distancing directive also has a significant impact on purchasing behavior. Especially in the field of e-commerce, namely Shopee, many people use this application more and more. Therefore, the payment method through ShopeePay e-wallet is also gradually used by Shopee users to pay when buying goods in general and food in particular. In this study, the team analyzed 314 surveys, then analyzed the influence of factors through SmartPLS software. User behavior is measured and evaluated through the following factors: trust, perceived usefulness, awareness of security, Perceived Ease of Use, price-saving orientation, and social impact on ShopeePay users after the COVID-19 pandemic. This research paper's results will provide a more theoretical basis for further studies to refer to and supplement to improve.

Keywords: Perceived Usefulness; Perceived Security; Perceived Ease of Use; Price saving orientation; Usage behavior; Food industry; Shopee Pay e-wallet

Introduction

Information technology is developing increasingly, leading to improving people's social life and following the trend of modernization. In response to today's pace of life, many technology platforms and products are born to serve people. Almost every field has been innovated and applied many technological measures. In particular, in the financial sector, there is the appearance of e-wallets. An E-wallet is an application used to store credit card information and for users to perform financial transactions on an e-commerce platform. Payment via e-wallet is one of the most used transaction methods thanks to its convenience, usefulness, and security (Uddin et al., 2014). According to research by Merchant Machine, the COVID-19 pandemic has indirectly promoted the growth of digital payments in general and digital e-wallets in particular. This study predicts that digital e-wallet users will increase from 44.5% in 2020 to 51.
7% in 2024. Also, according to statistics from payment platforms for mobile payments of Merchant Machine, the top e-wallets on the list of most used are Airpay with 650 million users, followed by WeChat with 550 million users. The e-wallets included in this list include ApplePay, with 507 million users, GooglePay, with 421 million users, and Paypal, with 377 million users.Talking about the strong growth of non-cash payment methods of e-wallets in Vietnam, it is impossible not to mention Shopee Pay (formerly AirPay wallet). This is a product built on the cooperation of AirPay and Shopee. Mr. Tran Tuan Anh, Managing Director of Shopee in Vietnam, commented that: 2020 will bring many significant changes in the way of living, working, and shopping online, especially the acceleration of digital payments and towards a cashless social future. Why is it said that "after the severe devastation caused by the COVID-19 pandemic, the e-wallet industry in Vietnam has become extremely vibrant"? It can be seen that during the violent outbreak of the COVID-19 pandemic, the whole society had to implement the policy of distancing, limiting contact, and gathering. Therefore, traditional trading activities were affected a lot, with some places closing for a long time. Meanwhile, with the solid foundation available before, shopping on e-commerce platforms is increasingly used thanks to its relevance to the social situation. Accompanied by a non-cash payment method that does not need to be in direct contact is also more widely used. According to the study "Analysis of Intent to Use E-Wallets During the Covid-19 Pandemic" in Indonesia by (Kusuma, G. B. I., and Dirgantara, I. M. B., 2021) the outbreak of the Covid-19 pandemic in some countries has brought significant change in consumer behavior. Due to the social distancing policy, people's outside activities are reduced. Small habits began to change, leading to big habits gradually changing, from which digital payment accounts appeared (e-wallets) replacing cash payment methods. This study's results also indicate that each individual's innovation greatly influences the intention to use e-wallets in this context. These can be mentioned as "Intent to use e-wallet through the perception of ease of use" experience has a significant influence on intention to use e-wallet through the perception of ease of use" affects society has a significant influence on intention to use e-wallets through perceived usefulness" easiness of use has a significant influence on intention to use e-wallets through perceived usefulness" and perceived

usefulness have a significant influence on intention to use e-wallets." So, after the COVID-19 pandemic, what changes have been made in customer service usage behavior in Vietnam to create a boom in the e-wallet market? What factors have influenced the behavior of customers using e-wallets, and to what extent? To learn more about that issue, we chose the topic: "Research and analysis of customers' behavior using ShopeePay e-wallet after the COVID-19 pandemic".

This research project aims to determine the factors affecting the user's behavior in using Shopee Pay e-wallet. From there, a research model was built, and data collection through the survey for data analysis. The results of the research paper can be used as a premise for subsequent studies or help organizations and individuals learn more about customer behavior in the e-wallet market, specifically Shopee Pay. The main objective of this study is: to analyze user behavior by Shopee Pay after the COVID-19 pandemic by identifying factors affecting usage behavior, locating the influence of factors on usage behavior, and drawing conclusions as a basis for future studiesThe research object is to determine the factors affecting the behavior of using Shopee Pay e-wallet after the COVID-19 pandemic. Based on the theory of customer usage behavior, the topic focuses on the relationship and influence level from independent to dependent factors. From there, we will give results based on this relationship and the level of influence. The scope of the study is limited to the e-commerce platform, digital payment, namely Shopee Pay e-wallet—a survey of customers who use social networks and Shopee Pay e-wallets. The survey conditions are users who use Shopee Pay e-wallet to make financial payments at 18 years or older. This study was approximately 3 months, from September 2022 to December 2022. This study is conducted by a combination of two methods: qualitative research and quantitative research

Research Methods

Qualitative research methods

Qualitative research aims to understand, edit, supplement, and complete the questionnaire. The study was carried out in the following order.

- The first step is to study the theoretical bases in previous studies to build a model of factors affecting the behavior of using e-wallets.
- The next step uses a qualitative and group discussion method to focus on the factors affecting the behavior of using e-wallets after the COVID-19 pandemic. Explore the theoretical underpinnings of perceived usefulness, perceived security, and perceived ease of use, social influence, price-saving orientation, and trust. Then complete the construction of the questionnaire for the survey.

Quantitative research method

- First, conduct a preliminary survey by sending a survey form to 10 people to sample test results. Then check for mistakes and unclear questions to adjust accordingly.
- The next step is to conduct an official survey with a survey form on the behavior of using Shopee Pay e-wallets after the COVID-19 pandemic through a questionnaire that has been added and completed after the preliminary survey.

The result of the questionnaire is that the answers of customers who have been using the Shopee Pay e-wallet will be collected, checked, and analyzed. Using SPSS software, the data will be analyzed, and descriptive statistics, tested by scale, factor analysis, and linear regression analysis.

Technology Acceptance Model – TAM

Davis (1989) states that the Technology Acceptance Model (TAM) has two factors influencing perceived usefulness and ease of use. Perceived usefulness is the belief that users using a system can improve the performance of their activities" (Davis et al., 1989). Although perceived usefulness did not affect usability (Davis, 1993), it significantly impacted the intention to use technology (Davis, 1989). (Cheong et al., 2004) found that perceived usefulness positively affects consumer attitudes.

Unified Technology Acceptance and Use Model (UTAUT)

The UTAUT model was developed by Venkatesh et al. (2003). They were based on the most common view of studying user acceptance of a new information system to create a combined model of the previous 8 models.The authors expected that three factors had a direct impact on behavioral intent (expected effect, expected effort, social influence) and 2 factors that directly impacted actual use behavior (convenient conditions and behavioral intent). Besides, intermediate factors: Gender, age, experience, and voluntary use indirectly affect behavioral intentions and usage behavior through the main factors.Facilitation is the degree to which one believes an organization's technical infrastructure is qualified to make the system work efficiently (Venkatesh et. al., 2003). In addition, the expected effort is defined as the ease of use of the system and expected performance is the degree to which an individual believes that when they use new systems, to be productive at work. Social influence is also the degree to which an individual perceives the importance of being influenced by those around them to use the new system.

User behavior

The term "user behavior" consists of two separate parts: "user" and "behavior." The user can be understood as the person using the product in terms of language. Whereas behavior has a more complex meaning, Haider's (1958) analysis of behavior states that the essential components of behavior are: a person

is trying to do something, intends to do something, and can do something. Bergner (2011) also agrees with this definition and relates it to his 8-parameter behavioral model. Bergner pointed out in this post that the "want" parameter is another way of expressing the above concept of doing and intending to do something.

Trust

According to the research of Doney and Cannon (1997), "trust" is a set of beliefs related to the honesty, goodwill, and competence of the partner. Trusted consumers will direct their perceptions and attitudes toward technology. Users agree that the technology is reliable, secure, and keeps its promise, which positively affects the intent of use (Maulita et al., 2022). When trust is strengthened, user behavior and intent will also be affected (Oliveira et al., 2014). Therefore, this study proposes the following hypothesis:

4. Hypotheses (H1- H7)

H1: Trust has a positive impact on ShopeePay usage behavior

Perceived security

According to Ooi and Tan (2016), "Perceived Security" is the perception of protection from the worrisome risks associated with mobile payments, such as the risk of data loss, and loss of personal data that can lead to financial losses. Security-related concerns are critical in the case of mobile payments and e-wallets related to financial services, which subsequently influence usage decisions (Liébana-Cabanillas et al., 2018). ShopeePay wallet helps users make safe digital payments with absolute security of personal transaction information according to PCI DSS standards. From there, influence the decision to use ShopeePay. Therefore, this study proposes the following hypothesis:

H2:Perceived security has a positive impact on ShopeePay usage behavior.

Perceived Ease of Use

Perceived ease of use is the degree to which users expect less effort to use a system (Davis, 1989). Since e-wallets have distinct characteristics and require consumers to have a certain level of knowledge and awareness, perceived ease of use may influence consumers' attitudes to accept technology plays a vital role in that (Makanyeza, 2017). Perceived ease of use helps users to have a positive attitude toward service, thereby increasing the intention to use that service (Pavlou and Fygenson, 2006). Therefore, this study put forward the following hypotheses:

H3:Perceived Ease of Use positively impacts ShopeePay usage behavior.

After the COVID-19 pandemic, people will tend to find e-Wallets useful when they feel a variety of gadgets are offered. According to research by KusumaandDirgantara (2021), "Perceived ease" of use has an influence on perceived usefulness in using e-wallets." According to (Singh et al., 2020), no matter how great the benefits received from technology are, it is useless if the technology is struggling to use." Therefore, this study proposes the following hypothesis:

H4: Perceived ease of use has a positive effect on the perception of usefulness in using ShopeePay.

Perceived Usefulness

According to Davis (1989), "Perceived usefulness" is defined as the degree to which a person believes that the use of a particular idea, technology, or innovation will improve their tasks and performance. Individuals seem attracted to embrace new technologies when they consider them more valuable and relevant to their daily lives (Rehmanand Shaikh, 2020). The electronic payment brings flexible access to time and place convenience, especially in the COVID-19 pandemic limiting face-to-face contact (De' et al., 2020). In particular, it is impossible not to mention the ShopeePay e-wallet. When users feel the higher the usefulness of using ShopeePay e-wallet, the more users want to use ShopeePay. Therefore, this study proposes the following hypothesis:

H5.Perceived usefulness positively affects the behavior of using ShopeePay.

Social influence

The study of Singh et al. (2020) shows that "social influence" can positively impact people using ShopeePay as a means of payment. This argument means that "the more social influence ShopeePay uses, the higher the likelihood of users using ShopeePay to complete their transactions continuously. Social influence is the degree of influence of family, friends, and colleagues on consumers' opinions about using a product or service and system (Riquelme, 2010). According to (Venkatesh et al., 2003), "Social influence" refers to the extent to which users are influenced to react to technology by their significant acquaintances and relatives." Social influence influences the Intentions of a person using a particular technology (Verkijika, 2018). Based on the stated studies, the authors hypothesize the following:

*H6:Social influence positively affects consumer behavior using ShopeePay as a means to pay***.**

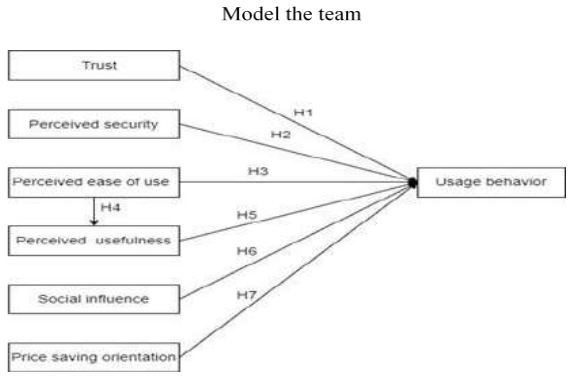
Price saving orientation

In the study (Ly, H. T. N.et al., 2022), the author mentioned, "Promotions" are key to shedding light on reducing the amount of money in each transaction. With a price-saving orientation,

promotions are also incorporated to clarify how much customers feel they can save while completing transactions. Having attractive discounts when using ShopeePay services also increases their desire to use ShopeePay. Therefore, the proposed price-saving orientation is included in the model of the study, hence the proposed assumption:

H7: Price-saving orientation positively impacts user behavior when paying via ShopeePay.

Based on previous research and proposed hypotheses on factors influencing ShopeePay usage behavior after the COVID-19 pandemic. The study shows that the proposed model includes the variables shown in the proposed model that is synthesized by the authors, as shown in Figure A.1



Source: Synthetic Research

Research Methods

This research paper is carried out using a combination of two methods:

Qualitativeresearch method:

After understanding the theoretical basis of factors such as perception of usefulness, perception of security, perception of ease of use, social influence, price saving orientation, and trust. The team will complete the development of the questionnaire for the survey.

Quantitative research method:

It was conducted an official survey on the behavior of using the ShopeePay e-wallet after the COVID-19 pandemic through questionnaires that have been supplemented and completed after the preliminary survey. The number of surveys obtained was 358, but only 314 were valid, specifically invalid questionnaires due to: choosing multiple options in one question, hitting the screening question (unused), and typing only one type of answer on the Likert scale.

In terms of age: with the ages of 18-30 years old, 207 survey votes accounted for the high estate with 65.9%, under18 years old accounted for nearly 19.1%, ages from 31 to 40 years old accounted for about13.4%,agesfrom41to50 years old accounted for nearly 1.3% and 50yearsand older accountedfornearly0.3%.

Regarding career: The rate of behavior using ShopeePay e-wallet of customers with the proportion of students accounting for the highest proportion in the survey sample, accounting for 60.5%. In second place were office workers, accounting for 24.8%. Freelancers accounted for a low 14.1%. The lowest was the proportion of sales and homemakers, accounting for only 0.3%.

On gender: The survey sample showed that the percentage of men participating was 30.3% less than that of women.

Regarding income: through the distribution table, we see that the income level of less than 5 million VND is higher than the rest, accounting for more than 51.3%. Accounting for the lowest proportion is the income level from 20 million – less than 25 million, accounting for 4.8%.

About the time to start using: We found that the proportion of people using ShopeePay wallet before the Covid-19 pandemic accounted for the most, with a rate of 57.6%. And the number of people using ShopeePay wallet to pay accounts for the lowest rate after the Covid-19 pandemic.

The results are the answers of customers who have been using the ShopeePay e-wallet will be collected, checked, and analyzed usingSmartPLS software; the data will be analyzed and statistically described and verified by the scale and factor analysis.

Result

Cronbach's Alpha test

Cronbach'salpha is a statistical test of the rigor (explainability of research concepts) of a set of observed variables with Cronbach's Alpha reliability coefficient. In theory, the higher Cronbach's alpha, the better (the more reliable the scale). However, multicollinearity occurs if Cronbach's alpha is too large ($\alpha > 0.95$). This means that the scale of many variables does not change (Nguyen DinhTho, 2011, pp.350 - 351).After running the analysis, all initial scales are reliable (Cronbach's alpha coefficient is more significant than 0.6). All seven scales have reliable values and vary in the range (0.7 - 0.9) shown in Table 1:

Table 1. Composite of Cronbach's Alpha coefficient for 7 scales(Source: Results of SEM model analysis in SmartPLS 4

Ordinal numbers	Scale	Number of observed variables	Cronbach's Alpha
1	Social influence	3	0.752
2	Perceived Security	3	0.814
3	Perceived Ease of Use	3	0.771
4	Usage behavior	3	0.725
5	Trust	3	0.754
6	Perceived Usefulness	3	0.770
7	Price saving orientation	3	0.761

Structural Equation Model Analysis (SEM)
Measurement model definition

Scale Reliability

The composite reliability coefficient CR, the total variance of AVE, and the single-factor loading coefficient (Outer loading) are used by us to evaluate the reliability of the scale. In particular, if the composite confidence coefficient is greater than 0.7 and the external load factor is more significant than 0.4 (Hair et al., 2014), then they are significant in terms of reliability. In addition, according to (Fornell and Larcker, 1981): "The total variance is greater than 0.5, confirming the reliability and convergence of the scale. The calculation results of the combined reliability, factor loading factor, and method error of the component scales show that the concept scales all meet the reliability and convergent value requirements." (Table 2).

Table 2. Measurement model testing (Source: Data processing results using SmartPLS 4)

	Cronbach's Alpha	Composite reliability(rho_a)	Composite reliability(rho_c)	Total Variance (AVE)
AHXH	0.752	0.845	0.849	0.656
BM	0.814	0.814	0.89	0.729
DSD	0.771	0.779	0.867	0.685
HVSD	0.725	0.727	0.845	0.645
LT	0.754	0.802	0.852	0.659
THI	0.77	0.77	0.867	0.685
TKG	0.761	0.769	0.862	0.676

Discriminant Validity

The study used the Heterotraite-mono trait ratio to evaluate the difference in the scale. The scale difference table clearly shows that all HTMT values are not higher than 0.85. Therefore, the elements all meet the requirements of value discrimination. (Table B.3)

Table 3. Correlation matrix between conceptual structures according to the Fornell–Larcker standard table (Source: Data processing results using SmartPLS 4)

	AHXH	BM	DSD	HVSD	LT	THI	TKG
AHXH	0.81						
BM	0.229	0.854					
DSD	0.26	0.641	0.828				
HVSD	0.243	0.676	0.678	0.803			
LT	0.122	0.282	0.207	0.259	0.812		
THI	0.322	0.558	0.662	0.693	0.241	0.828	
TKG	0.208	0.624	0.623	0.619	0.234	0.543	0.822

Structural model verification

The team used VIF, R Square, and f Square along with the path coefficient to evaluate the structural model. Collinearity Statistics (VIF) The regression of each variable to estimate the path coefficient depends on the predictor variable (Hair et al., 2014). The path coefficient will not be guaranteed if a polylinear multiplication occurs between the independent variables. The VIF results have shown that "the association between the predictors does not violate the hypothesis of multilinear multiplication because all the coefficients are in the acceptable range (VIF = 1.347 - 2,106 <5), so the model does not violate this phenomenon."(Table B.4)

Table 4. VIF coefficient table (Source: Results of SEM model analysis in SmartPLS 4)

	VIF
AHXH1	1.539
AHXH2	2.106
AHXH3	1.535
BM1	1.743
BM2	1.814
BM3	1.828
DSD1	1.574
DSD2	1.622
DSD3	1.538
HVSD1	1.496
HVSD2	1.467
HVSD3	1.347
LT1	1.426
LT2	1.588
LT3	1.572
THI1	1.481
THI2	1.699
THI3	1.597
TKG1	1.449
TKG2	1.56
TKG3	1.672

R Square

The R2 value of 0.25 represents a weak endogenous structure, a level of 0.5 is relative and 0.75 is high (Hair et al., 2014). The results of the analysis showed that the adjusted R2 value of the Intent model was 0.620, and the adjusted R2 value of the trust model was 0.514. Through analysis of the impact of online reviews affecting purchase intent, it was shown that factors of timeliness, usefulness, reviewer expertise, trust, quality, and participation value explained 62% of the variance of the intention to buy online products at a statistically significant 5% and the remaining 38% The explanation is due to other factors that have not yet been included in the model. (Table B.5)

Table 5. Table R Square and R Square Adjusted (Source: Results of SEM model analysis in SmartPLS 4)

	R-square	R-square adjusted
HVSD	0.638	0.631
THI	0.439	0.437

f Square

Cohen (1988) proposed the "f Square index" table to assess the importance of independent variables as follows:

- The impact level is extremely small or has no impact: f Square < 0.02:
- Small impact: 0.02 ≤ f Square < 0.15
- Average impact: 0.15 ≤ f Square < 0.35
- Large impact level: f Square ≥ 0.35

According to f Square figures in the SEM model (Table B. 6):

- AHXH and LT variables have extremely small or no impact on HVSD variables
- BM, DSD, and TKG variables have a small impact on HVSD variables
- THI variable average impact on HVSD variable

- DSD variable has a significant impact on the THI variable

Table 6.Table f Square (Source: Results of SEM model analysis in SmartPLS 4)						
	AHXH	BM	DSD	HVSD	LT	THI	TKG
AHXH				0			
BM				0.098			
DSD				0.041		0.782	
HVSD							
LT				0.002			
THI				0.152			
TKG				0.03			

Model results diagram

PLS-SEM results for the measurement model as shown in Figure B.1.

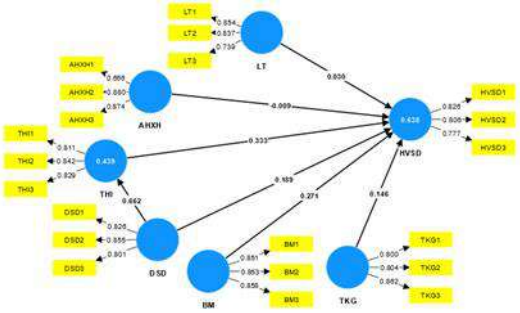


Figure B.1.PLS-SEM results for the measurement model (Source: Results of SEM model analysis in SmartPLS 4)

Figure B.1.PLS-SEM results for the measurement model.

Boots trapping inspection

Because the data analyzed by PLS is assumed to be non-normally distributed, the regression analysis's parametric significance test cannot be used to test the significance of coefficients such as path coefficients. Instead, PLS relies on non-parametric bootstrap analysis to test the significance of the coefficients (Hair et al., 2014). Bootstrapping is used to calculate t-values to see if the path coefficient deviates. significantly from zero. In this study, 314 observations were tested with 5000 repetitions by the non-parametric Bootstrapping technique to ensure linear structural model validation requirements.(Table B.7 and Table B.8).

Table 7. Table results in Bootstrapping model structure (Source: Data processing results using SmartPLS 4)					
	Original sample (O)	Sample mean (M)	Bias	2.5%	97.5%
LT -> HVSD	0.028	0.029	0.001	-0.049	0.105
BM -> HVSD	0.271	0.272	0.001	0.139	0.398
DSD -> HVSD	0.409	0.408	-0.001	0.275	0.544
DSD -> THI	0.662	0.661	-0.001	0.553	0.748
THI -> HVSD	0.333	0.330	-0.003	0.186	0.477
AHXH -> HVSD	-0.009	-0.006	0.003	-0.086	0.060
TKG -> HVSD	0.146	0.144	-0.002	0.023	0.275

Compare the impact of these variables on usage behavior variables, trusts, and usefulness perceptions in descending order as follows: we found that the perceived ease of use variable has the most substantial impact on perceived usefulness (β = 0.662), followed by perceived ease of use variable impacting usage behavior (β = 0.409); perceived usefulness impacts usage behavior (β = 0.333);perceived security variable impacts usage behavior (β 0.271); price-saving orientation variable impacting usage behavior (β = 0.146); trust variables that impact usage behavior (β = 0.030); and finally, the social influence variable that impacts usage behavior (β = -0.009).

Table 8. Direct impact outcomes of relationships (Source: Data processing results using SmartPLS 4)					
	Original sample (O)	Sample mean (M)	Standard deviation (STDEV)	T statistics (|O/STDEV|)	P values
LT -> HVSD	0.030	0.033	0.038	0.792	0.429
BM -> HVSD	0.271	0.272	0.066	4.129	0.000
DSD -> HVSD	0.409	0.408	0.069	5.908	0.000
DSD -> THI	0.662	0.661	0.050	13.349	0.000
THI -> HVSD	0.333	0.330	0.075	4.434	0.000
AHXH -> HVSD	-0.009	-0.006	0.037	0.256	0.798
TKG -> HVSD	0.146	0.144	0.063	2.306	0.021

Thus, hypotheses H2, H3, H5, and H7 are supported because their hypotheses act in the same direction, and the p-value is less than 0.05. H1 and H6 were not statistically significant, with p-values of 0.471 and 0.801, respectively.We also examined the relationship between perceived usefulness (intermediate variables) and usage behavior through the impact of perceived ease of use. We showed that the relationship is strongly correlated and has a specific relationship: THI -> HVSD through the impact of perceived ease of use with impact factor (β = 0.333).Perceived Security, Perceived Ease of Use, Perceived usefulness, and price-saving orientation directly affect user behavior. Furthermore, perceived ease of use indirectly affects perceived usefulness to influence user behavior, and the results of linear structure analysis and tests show that all assumptions H2, H3, H5, and H7 are accepted, and the H1 and H6 hypotheses are not accepted. That is, trust and social influence do not affect user behavior.

Discussion

All the results of this study are in agreement with previous studies. According to the research of Budyastuti, 2019, trust does not significantly affect the behavior of using ShopeePay but also depends on many other factors. According to Ngoc, L. B. et al., during the COVID-19 pandemic, people are reluctant to engage in money-related activities due to numerous scams and scams. Therefore, users realize that ShopeePay has good security features for online transactions, and their personal information wants to use more enthusiastically. Therefore, security is fundamental in e-wallet payments in financial services, which influences the decision to use it. The research findings of (Widjaja and Setyawan, 2021; Candy et al., 2022; Alwi, 2021) state that a person would use an e-wallet more often if it were straightforward and did not require much effort to use. This may be due to the post-COVID –19 pandemic with lifestyle changes and the new normal, and ShopeePay must help ease the

payment process. According to the studies of (Widjaja and Setyawan, 2021; Kusuma and Dirgantara. 2021), greater the confidence in the benefits that users will receive when using ShopeePay. Research by (Widjaja and Setyawan, 2021; Candy et al., 2022; Kusuma and Dirgantara, 2021) confirms that consumers will be willing to consider and use e-wallets that provide greater usefulness, such as efficiency and time savings. Ease of use has a significant effect on behavioral intent. Especially when trading during the COVID–19 pandemic limited all forms of outside activities and physical distancing, leading to future use. Previous research by Ly, H. T. N. et al., 2022, social influence from family, friends, or social networks does not affect the behavior of using e-wallets during the COVID-19 pandemic. Users will prefer to use e-wallets with many discount programs that reduce costs or spend both tangible and intangible value while meeting their needs.

Conclusion

Perceived Ease of Use is considered a factor that greatly influences customers' usage behavior on ShopeePay when buying food items. These factors are evaluated through Online customer reviews of the user manual, customer reviews about the ShopeePay wallet interface, Evaluate the level of simplicity and accuracy of the application. The popularity of online payment types, in general, and e-wallets, is increasing, so users must always have appropriate knowledge and skills to use them more effectively. However, ShopeePay's customers are of many ages and industries, and complex operations are inappropriate. According to the survey results via the Arkrt scale, in the evaluation questions about ShopeePay's operation manual, interface, and operation, most customers participating in the survey chose "yes" and "complete consent" of understandability and simplicity. Therefore, it can be seen that ShopeePay's operations and interface always need to be simplified as much as possible so that anyone can use it easily.Perceived usefulness also influences usage behavior, second only to perceived ease of use. Furthermore, perceived ease of use also affects perceived usefulness. Because when simple, uncomplicated utility operations ensure users successfully perform transactions and functions of ShopeePay, its functions are fully implemented and used more. According to the survey results, most participants chose "yes" and "complete consent" to questions about the usefulness of ShopeePay. This shows that the usefulness of ShopeePay in the post-pandemic period is also shown by helping users not need to have direct contact, saving time when buying food, ensuring safety, and being faster and easier than traditional payment methods.Choosing to use a platform is related to the security of that platform. Perceived security is an essential and indispensable factor in the 4.0 era when people gradually use online platforms full of risks of information leakage and cyberattacks. And according to the survey results, the higher the user's confidence in the ease of use, the most participants have excellent feedback on information security and safety when transacting on ShopeePay. Thereby, it can be seen that the trust of users in the security of ShopeePay is substantial.Price savings significantly influence the customer's decision to buy food and pay, especially during the post-pandemic economic recovery period. Promotion campaigns and issuing promo codes are always a magnet to attract customers from major e-commerce platforms such as Shopee, Lazada, and Tiki. While exchanges have discounts on food products, e-wallets will have discounts and cashback (Shopee) when paying through the application. This also attracts users to use the wallet to pay for transactions because of the "discount." As a result, most participants agreed that preferential policies ultimately attracted them. Thereby, it can be seen that one thing that attracts customers to use ShopeePay is the preferential policies and promotions. A large part of customers always consider spending, and they always prioritize the most economical options for buying food and drinks.This paper has completed quantitative and qualitative research steps by descriptive statistics, Cronbach's Alpha testing, and PLS-SEM linear structure model validation through SmartPLS software. The results of this research paper have met the initial goal of identifying factors affecting the usage behavior of ShopeePay users after the COVID-19 pandemic in the food industry. In the research paper, 6 factors were identified: perceived usefulness, perceived ease of use, trust, security, price-saving orientation, and social influence. These factors are determined based on relevant research and an overview of the testing theory and techniques from the SEM model. Through this, the team identified the level of influence of the factors that the group gave and sorted by the level of influence from high to low: Perceived Ease of Use, Perceived usefulness, perception of security, and orientation to save prices. Based on the measurement results, two factors have very little and almost no influence: trust and social influence.Because the scope of the study is only in the area of customers who have been using the ShopeePay e-wallet, the general level of e-wallet behavior is not high. Therefore, in the following study, the group is oriented to expand the research to identify the factors affecting customers' behavior using e-wallets after the COVID-19 pandemic. Thereby not only measuring the influencing factors but also comparing the popularity and trust of consumers for online payment platforms.Perceived ease of use is assessed as a factor that greatly influences customers' usage behavior on ShopeePay when buying food items. These factors are assessed through online customer reviews of the user manual, customer evaluation of the ShopeePay wallet interface, and rate of the simplicity and accuracy of the application. The popularity of online payments, in general, and e-wallets is increasing, so users must

have appropriate knowledge and skills to use them more effectively. However, ShopeePay's customers are of different ages and industries, and complicated manipulation is inappropriate. According to the survey results through the Likert scale, in the evaluation questions about the instructions for use, interface, and operation of ShopeePay, the majority of customers participating in the survey chose "agree" and "strongly agree" on ease of use and simplicity. Therefore, the operations and ShopeePay interface must be simplified as much as possible so anyone can use it easily.Perceived usefulness also affects usage behavior, the second largest after perceived ease of use. In addition, perceived ease of use also affects perceived usefulness. Because when the simple and uncomplicated utility operations ensure that users successfully perform transactions and functions of ShopeePay, its functions are fully implemented and used more. According to the survey results, most participants chose "agree" and "totally agree" on questions about the usefulness of ShopeePay. This shows that the usefulness of ShopeePay in the post-pandemic period is also reflected in helping users not need to be in direct contact, saving time when buying food, and ensuring safety and faster easier than traditional payment methods.Security awareness is an essential and indispensable factor in the 4.0 era when people gradually use online platforms fraught with risks of information leakage, cyber-attacks, etc. And according to the survey results, most participants have excellent feedback about information security and safety when transacting on ShopeePay. Thereby, it can be seen that users' trust in the security of ShopeePay is substantial. Choosing to use a platform is related to the security of that platform.Customers' decision to buy food and pay is significantly influenced by the intention to enjoy price savings, especially during the post-pandemic economic recovery period. Promotion campaigns and codes are always a magnet to attract customers from major e-commerce platforms such as Shopee, Lazada, Tiki, etc. At the same time, the floors have discounts on actual products. Products and e-wallets will have discounts and refunds (Shopee) when paying through the application. This also entices users to use the wallet to pay for transactions because it"gets a discount."Thereby, it can be seen that one thing that attracts customers to use ShopeePay is preferential policies and promotions. As a result, most participants agreed that the preferential policies attracted them. Many customers always consider spending; they prioritize the most economical way to buy food and drink. This study has completed quantitative and qualitative research steps by using descriptive statistics, Cronbach's Alpha test, and PLS-SEM linear structural model testing through SmartPLS software. The results of this research paper have met the initial main goal of determining the factors affecting the usage behavior of ShopeePay users after the COVID-19 pandemic in the food industry. In the research, there are 6 factors identified, namely: perceived usefulness, perceived ease of use, trust, security, price-saving orientation, and social influence. These factors are determined based on relevant studies, theoretical overview, and testing techniques from the SEM model. Through that, the research team has determined the influence of the factors the team has given and arranged in order of influence from high to low: Perceived ease of use, perceived usefulness, security awareness, and price-saving orientation. Besides, two factors have little influence and almost no influence based on the measurement results: trust factor and social influence.Because the scope of the study is only in the area of customers who have been using ShopeePay e-wallets, the general level of behavior of using e-wallets is not high. Therefore, in the subsequent study, the group is oriented to expand the research problem to determine the factors affecting customers' behavior using e-wallets after the COVID-19 pandemic. Thereby, the influencing factors can be measured, and the popularity and trustworthiness of consumers for online payment platforms can be compared.

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Research on the Impact of Marketing-Mix Factors on Brand Awareness and Brand Image – The Case of TH TrueMilk in Ho Chi Minh City, Vietnam

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Abstract.
The purpose of the study was to investigate the variables influencing consumer brand awareness and brand image of fresh milk products made by TRUEMILK in Ho Chi Minh City. The research used expert-conducted preliminary qualitative research and a formal linear structural model for quantitative analysis [Partial Least Squares - Structural Equation Modeling(PLS-SEM)]. Distribution intensity and promotion are the two aspects that have been found to have an impact on the awareness of the TH milk brand. The distribution intensity and brand image both have an impact on the perception of the TH milk brand. Brand image is unaffected by price. In which the "Intensity of distribution" element has the greatest influence on customers' brand awareness and perception of the TH milk brand. Nearly 40.7% and 57.6% of the variation in brand awareness and brand image are explained by independent factors. The study then makes some managerial recommendations to improve the brand image and awareness of TH Truemilk.

Keywords. Brand Image, Brand Awareness, Marketing impact, Promotion, and Price Deals.

Introduction

Consumers tend to make wise purchases and maintain healthy lifestyles when their income is increasing. It has been demonstrated, as stated in a 2019 article by Investment Bridge Magazine that "the food and beverage industry has always been one of the significant economic sectors with considerable potential for growth in Vietnam. This industry is the largest in Vietnam's economic sectors in terms of Gross Output and ranks second in terms of total income. Thanks to increased consumer spending power and the trend toward higher-value foods and beverages that will rule the market, the industry is predicted to keep growing strongly through 2020 at an average growth rate of 10.9 %/year. In the future, the demand for healthy foods and beverages will positively affect all market categories and lead to a move toward high-value food goods, providing clean food and healthy drinks companies with significant development potential. (Trang, 2019).According to Securities Investment Magazine (2020) "In 2020, the Food and Beverage Industry is greatly affected by two "shocks" in a row, namely Decree 100/2019 and the Covid-19 pandemic. The article also included: "In a brief survey of firms in the sector conducted by Vietnam Report in August 2020, nearly 50% of companies reported that their operations had been negatively impacted, with alcohol consumption being particularly hard hit by Decree 100. The Covid-19 pandemic has come to light with several flaws in the food and beverage sector, most notably issues with distribution, logistics, and human resource administration. 2020 (Hieu Minh). Thus, it is evident that the food and beverage industry in the Vietnamese market has gone through a great deal of change in the years 2020–2021. However, as a result of these significant fluctuations, many companies have identified numerous flaws in their operational framework, as stated in the Securities Investment 2020 article: "In a quick survey of enterprises in the industry conducted by Vietnam Report in August 2020, 94.7% of companies have identified weaknesses related to distribution and logistics when the demand for essential food and packaging increases but the inventory is insufficient and is adjusting its proportion structure and distribution network to be able to adapt to the crisis" (Minh,2020).Despite facing many difficulties in the first quarter of 2020, the Food and Beverage industry businesses are gradually stabilizing in the most recent quarter allowing them to continue to grow and achieve new positions in 2021. To have a long-term existence and sustainable development, businesses must come up with plans and solutions to enhance and develop their products differently and address consumers' psychology by 2021.Currently, in the Vietnamese market, there are many businesses with strong competitiveness in the beverage market such as Vinamilk, Suntory Pepsico, Tan Hiep Phat, Coca-Cola... Customers tend to demand purity, safety, quality when used, and familiarity with the product, especially with the appearance of such a wide variety of beverages. TH True Milk is a representative to meet

the demands of a safe, high-quality product and give consumer's familiarity. This milk brand of pasteurized fresh milk uses organic technology to preserve nutrients and is ideal for young people, students, and students since it lifts their spirits and gives them the energy for a long active day.Until this point, TH True Milk has always delivered product improvement technology and offered attractive marketing strategies for its fresh milk product line. However, are people aware of TH true Milk pasteurized fresh milk? How do users feel after using the product? How well-known is the TH true Milk brand among consumers? To establish a successful brand, it is crucial to develop recognized symbols and increase consumer brand recognition since these factors have a big impact on consumers' purchasing decisions. Based on the aforementioned rationale, the author's study in the "Research on Marketing-Mix Factors Affecting Brand Awareness and Brand Image - The Case of TH True Milk in Ho Chi Minh City" is essential. The study's findings will aid in TH true Milk brand development, offering comprehensive solutions, boosting value, and reaffirming the company's position in the Vietnamese beverage market**.**

Relevant background theory

Marketing-mix

According to economist McCarthy (1960), marketing mix is classified according to the 4P model including Product, Price, Place, and Promotion used in the marketing activities. Marketing-Mix is the combination and arrangement of marketing tools that a business uses to influence the demand for its product in a selected target market. Those tools include Product strategy, Price strategy, Place strategy, and Promotion strategy.

The relation between Marketing-mix and brand image

The efforts made to raise consumer awareness and influence their decision to purchase a company's goods or services can be viewed as part of the marketing mix. If the goal of marketing is to introduce customers to experience a company's goods and services for the first time, a strong brand image will keep them coming back to the business again and again.Businesses in the modern marketplace cannot dispute that brand image will affect and determine consumer behavior. Furthermore, it's also a competitive advantage that enhances the prestige and credibility of the brand. Building a brand image through effective marketing methods will showcase the brand's identity, put it ahead of rivals, bring it closer to customers, and encourage brand sentiment. Therefore, a brand's long-term potential to increase sales is greatly influenced by the relationship between the marketing mix and brand image.

The relation between Marketing-mix and Brand awareness

According to Anker's (1996) model, brand awareness is the first criterion that encourages consumer demand for product ownership and serves as a crucial metric to assess the power of the brand and the size of potential customers. The more the brand has coverage and is known by many people, the more attractive their products are and the public trusts them. Brand awareness is crucial in determining if a brand will survive when there is more competition in the market. Additionally, a process of "communicating" to present the product to more people is required for the brand to become more well-known; in other words, the consumer is a combination of marketing-mix components. Utilizing its six dimensions, the Brand Identity Prism enables brand managers to evaluate a brand's advantages and disadvantages. Based on 06 aspects: (1) Physique, (2) Personality, (3) Culture, (4) Relationship, (5) Reflection, (6) Self-image. Once customers instill the brand's message, they have a better chance to engrave the brand image in their minds.

The purpose of this model is to aid brand specialists and business owners in comprehending the various components of the brand, thereby generating a distinction in identification. The model is divided into three parts corresponding to three phases, respectively: (1) Brand strategy analysis; (2) Brand identity system; (3) Brand identity enforcement system. These stages operated sequentially, continuously, and interconnected.

Relevant research model

Research of Yoo et al (2000): "Examination of selected marketing mix factors and brand equity". The study's major goal is to investigate the relationship between brand equity and the elements of the marketing mix. The company and customer's value is only included in the conceptual framework to suggest a worthwhile avenue for further study of the structure of brand equity. Pricing, store image, distribution channel, advertising, and price transaction all play important roles in determining a brand's relevance in terms of perceived quality, brand loyalty, and brand identity. The most suitable strategic alternatives for each brand within the firm are chosen, and the least competitive businesses are chosen for each brand based on the findings.Thao's research (2011) "The influence of some marketing mix factors on brand awareness and brand image". The goal of marketing is to strengthen the brand, which entails raising consumer awareness of the brand name. The structural model of how the marketing mix components affect brand equity was developed in accordance with the results of existing theoretical investigations. The study's findings suggest that brand managers should use the marketing mix of price agreements very sparingly. Even though certain short-term financial gains from short-term sales can result from price

trading.Research by Huang (2012) “How brand awareness is related to market outcomes, brand equity, and marketing mix”. This study addresses the connection between brand awareness and brand value as well as the connection between brand awareness and brand outcome. The findings support the positive relationship between brand awareness and brand equity by demonstrating how consumer brand experience affects brand awareness, which in some situations comes before perception. The study also shows the connection between brand awareness and brand equity market performance metrics, such as sales premium, equity premium, and selling price, as well as the relationship between marketing mix factors and brand awareness. Finally, it shows the significance of price distribution and promotion in raising brand awareness among consumers. Businesses choose a recognized brand identification model based on the result. Research of Lin et al. (2013) “A study on factors affecting brand loyalty of Taiwanese adolescents towards mobile phone purchase. The case of Taichung City”. This study's primary goal is to improve understanding of the connections between adolescent consumption habits, brand image, and brand loyalty by examining the variables that affect teenagers' brand loyalty toward mobile phone purchases and analyzing the relationships among these variables. In which brand image is influenced by product image, business image, and competitor image. Based on these factors, the brand image is determined, and prominent in customers.Research of Rajhet al (2015) - "Effect of marketing mix factors on service brand value". To establish brand recognition and brand image in the model, the author claims that price, advertising, price offers, delivery service method, physical proximity, and staff are necessary components to the identity of the author's brand. Research results indicate that brand awareness is highly influenced by advertising intensity rather than brand image because increasing brand awareness is a task, easier than developing a positive brand image.

Research models and methods

Proposed model

In the study of Yoo et al. (2000), there are two factors related to marketing mix strategies that have the strongest impact on brand awareness, namely distribution and advertising intensity. This is repeated in Thao's study (2011). Based on the set models, (Rajh et al., 2015 and Huang, 2012) in their recent Based on the research (Rajhet al., 2015 and Thao, 2011), this research proposed a study model with 4 components connected to marketing mix tactics that have an impact on brand image: proposed a study model with 5 components connected to marketing mix tactics that have an impact on brand image: promotion, distribution intensity, advertising, store image, and price transaction. The detailed research model is shown in Figure 1.

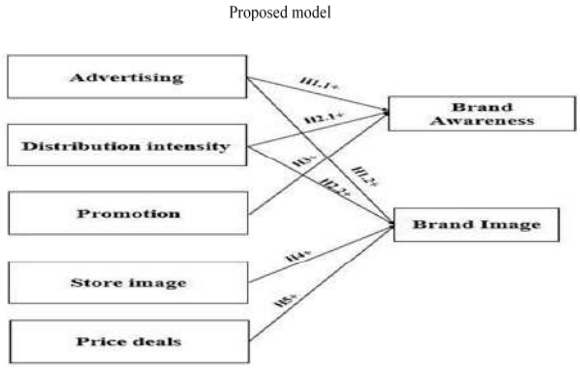

Figure 1. Proposed research model

Research Theory

Advertising.

According to the authors' research, advertising is connected to brand identity and image, which makes it more likely that employees would identify with firms that are popular with outsiders. According to (Bhattacharya et al., 1995), an organization's reputation often acts as a predictor of organizational performance because well-regarded organizations are regarded as successful. If the brand image is perceived by consumers as reputable, successful, and popular, it will also enhance their pride in identifying brand reputation (Ahearne et al, 2005). Research articles by (Jemal, 2014; Lien, 2016; Altoveros, 2016; Khan, 2018; and Haque, 2018) have demonstrated that this factor has an impact on the level of brand recognition. Therefore, hypothesis H1.1, H1.2 are proposed:

H1.1: Advertising has a positive effect on consumers' brand awareness
H1.2: Advertising Has an Impact on Consumers' brand image

Distribution intensity – The most important factor of a brand. Because when it comes to a brand, customers often think of the brand's coverage through its distribution channels, showing the level of brand awareness of the product, combined with the dense distribution intensity, the brand image will be widely disseminated to consumers. Researchers (Yooet al., 2000; Kohli et al., 2003) demonstrate that there is an impact on the level of brand recognition.Therefore, hypothesis H 2.1, H 2.2 are proposed:

H2.1: Distribution intensity has an impact on consumers' brand awareness and brand image.

Item	Scale	Source
1. Advertising		
AD1	The company's advertising campaign is invested more cost than the competitors	
AD2	The company's advertisement appears frequently	Yoo et al. (2000) Lang et al. (2014
AD3	I remember the company's advertising campaigns recently	
AD4	The company's advertisements made a good impression on me	
AD5	Overall, I enjoy the company's advertising campaign	Thao (2011)
2. Distribution intensity		
DI1	The company's products are sold in more stores than competitors	
DI2	The number of stores sells more company's dairy products than competitors' products	(Yoo et al.,2000) (Rajh et al., 2015)
DI3	The company's brand is distributed in many stores	
DI4	I can purchase company products anywhere.	
3. Promotion		
PR1	The company's sales promotion is attractive	
PR2	I enjoy participating in the company's sales promotions.	(Lang et al., 2014)
PR3	I like the company's sales promotions	
4. Store the image		
SI1	I can buy high-quality company milk at the company's retail stores.	
SI2	The company's retail stores will be prestigious places	(Yoo et al., 2000), (Thao, 2011) and (Lang et al., 2014)
SI3	The store sells the company's products as well as those of famous companies	
SI4	The company's retail stores do not have a comfortable environment.	
SI5	Each store where I buy the company's milk has its distinct characteristics.	
5. Price deals		
PD1	The company regularly offers price incentives	
PD2	The company's selling price is more affordable than other brands	
PD3	The company has repeatedly offered price incentives	(Yoo et al., (2000), and (Rajh et al., 2015)
PD4	Brand X fulfilled my expectations	
PD5	The company often offers many promotions in the form of product discounts	
6. Brand image		
BI1	I can immediately identify some features of the company's brand	
BI2	I can recall company logos and symbols quickly	(Yoo et al., 2000)
BI3	The company's brand value is the reason I use this product	
7. Brand awareness		
BA1	I can easily recognize this brand among competitive rivals.	
BA2	This brand is familiar to me.	(Yoo et al., 2000)
BA3	I am knowledgeable about the company's brand.	

H2.2: Distribution intensity has an impact on consumers' brand image

Promotion. According to economists, brand awareness is impacted by promotion (Huang, 2012). Promotions are actively being used in the context of the current market with a stimulus program and appear to have evolved into a sales culture. Many companies view promotions as a marketing and branding activity in addition to a tool to boost consumption and sales. As a result, H3 is suggested:

H3: Promotion has an impact on consumers' brand awareness.

Store image. Pinar et al based on the model of Aker and Keller (2012) to show that store image have a decisive influence on brand image. Customers who purchase goods from conspicuous, well-known retailers are more likely to develop a brand identity. Therefore, it is suggested that:

H4: Consumer brand perception is influenced by store image.

Price deals. Price and brand awareness are strongly correlated, according to (Kleineet al., 1993). A media connection can be a communication message sent from a supplier to a customer. Bhattacharya and Sen (2003) went on to say that customers are more likely to recognize the brand because of price agreements and discounts. Therefore, it is suggested that:

H5: Consumer brand recognition is enhanced by firm price.

Research methods

By adjusting the scale of previous research to fit the object and scope of the study, which are consumers who are university students in the Go Vap district of Ho Chi Minh City, this study aims to explore additional factors affecting the relationship between Marketing-mix and brand awareness and brand image of consumers in Ho Chi Minh City - case

of TH True Milk brand. The study has found 5 independent factors influencing 2 dependent variables, namely "brand image" and "awareness" of the TH True Milk brand, following prior research and theoretical underpinnings. A total of 27 observed variables corresponding to 5 components are presented in Table 1. The research uses quantitative methods with non-probability sampling techniques and conducts filtering to select suitable survey subjects who have used freight forwarding services. In the context of the Covid-19 epidemic, information and data were gathered through a thorough online survey. The research model and research hypotheses are next tested quantitatively using the partial least squares PLS method with Smart PLS software utilizing scales modified from the preliminary study. This test is carried out using a structural model test and a measurement model test (Hair et al., 2014).

Research results

Preliminary assessment of the research sample

The questionnaire responses are filtered and analyzed. Table 2 shows preliminary information on the research sample based on the gender, age, and income of survey participants. There are 300 questionnaires sent to consumers through an online survey; the total number of responses collected is 310, with 10 responses invalid due to choosing many options in a question or leaving required questions blank. People under the age of 35 are the majority of survey participants, accounting for around 84.67% of total respondents. Approximately 46.3% of survey respondents had a monthly income of less than 10 million VND, which is close to the average salary. Besides, the career fields of respondents are diverse such as students, housewives, freelancers, and office workers.Table 2.

Table 2. Preliminary statistics on research

Criteria		Research Sample	Percent (%)
Age	Under 25	109	36.3
	25 to 35	145	48.4
	Above 35	46	15.3
Income	Under 5 million VND	70	23.3
	5 to 10 million VND	69	23.0
	10 to 15 million VND	91	30.3
	Above 15 million VND	70	23.3
Sales locations	TH True Mart	45	15.0
	Supermarket	119	39.7
	Convenience store	107	35.7
	Others	29	9.7

Structural equation model analysis (SEM)

In this study, the quantitative research design is used through structural equation modeling (SEM) using SmartPLS software. The model tests the level of impact with the participation of 7 factors, including (1) Advertising, (2) Distribution intensity, (3) Promotion, (4) Store image, (5) Price deals, (6) Brand image, and (7) Brand awareness. These 7 factors are specifically measured by 28 scales that have been preliminarily evaluated through qualitative research.

Testing of the measurement model

This study uses the Composite Reliability (CR), the Average Variance Extracted (AVE), and Outer loading to assess the reliability of the scale. For the measurement model to be accepted, the CR coefficient must be greater than 0.7; the outer loading coefficient greater than 0.4, the scale reaches the value of reliability (Hair et al., 2014), and the AVE greater than 0.5 to reaffirm the reliability and convergence of the scale (Fornell & Larcker, 1981). The results of the parameters indicate that the Composite Reliability, the Average Variance Extracted (AVE), and the outer loading coefficient are satisfactory. In addition, Table 3 shows the value of Cronbach's Alpha coefficient. Table 3.

Table 3. Results of reliability and converging value of the scale

	Cronbach's Alpha	Composite Reliability (CR)	The average variance extract (AVE)
Advertising (AD)	0.919	0.937	0.711
Distribution Intensity (DI)	0.867	0.904	0.654
Promotion (PR)	0.902	0.931	0.772
Store Image (SI)	0.785	0.860	0.609
Price Deals (PD)	0.893	0.919	0.695
Brand Awareness (BA)	0.876	0.915	0.730
Brand Image (BI)	0.871	0.912	0.722

For further assessment of the measurement model, the study conducted a discriminant test, when comparing the relationship between variables with the Average Variance Extracted (AVE). The discriminant validity assessed through the Fornell-Larcker criterion presented in Table 4 shows that the square root of the Average Variance Extracted (AVE) is larger than the correlation coefficient between variables. Therefore, the research sample ensures the discriminant of the measurement factors. In addition, the coefficient of HTMT is satisfactory when the value of this coefficient is less than 0.9, ensuring the discriminant validity (Garson, 2016; Henseler et al., 2016).Table 4.

Table 4. Results of value analysis differentiated by Fornel-Lacker and HTMT

		AD	BA	BI	DI	PD	PR	SI
Fornell-Larcker Criterion	Advertising (AD)	**0.843**						
	Brand Awareness (BA)	0.195	**0.854**					
	Brand Image (BI)	0.217	0.758	**0.850**				
	Distribution Intensity (DI)	0.245	0.622	0.667	**0.809**			
	Price Deals (PD)	0.520	0.273	0.289	0.320	**0.834**		
	Promotion (PR)	0.623	0.235	0.262	0.284	0.651	**0.878**	
	Store Image (SI)	0.199	0.665	0.657	0.481	0.173	0.198	**0.780**
Heterotrait-Monotrait Ratio (HTMT)		AD	BA	BI	DI	PD	PR	SI
	Advertising (AD)							
	Brand Awareness (BA)	0.216						
	Brand Image (BI)	0.235	0.852					
	Distribution Intensity (DI)	0.269	0.706	0.758				
	Price Deals (PD)	0.577	0.298	0.256	0.380			
	Promotion (PR)	0.692	0.315	0.287	0.276	0.737		
	Store Image (SI)	0.225	0.797	0.757	0.552	0.170	0.240	

Structural model inspection

The results of testing the linear structural model are shown in Figure 2. The influencing factors are 40.7% and 57.6%, respectively, representing the variance of the variables BA and BI. In addition, the test of

multicollinearity gives VIF less than 5, it is concluded that the model does not violate this phenomenon (Hair et al., 2014). Figure 2 also shows detailed values of the paths in the model through β standardized coefficients.Table 5 shows the f2 value to evaluate the impact factor, with DI having the greatest influence on BA (f2= 0,549). This coefficient of the remaining factors affecting BA is not significant. Meanwhile, DI and SI are the two most influential factors out of the four factors when considered concerning BI, with f2 values of 0.338 and 0.304, respectively. The f2 value will be analyzed further in the discussion of the research hypothesis. On the other hand, the Q2 values are all greater than 0 indicating that all factors have a significant impact on the dependent variables BA and BI.

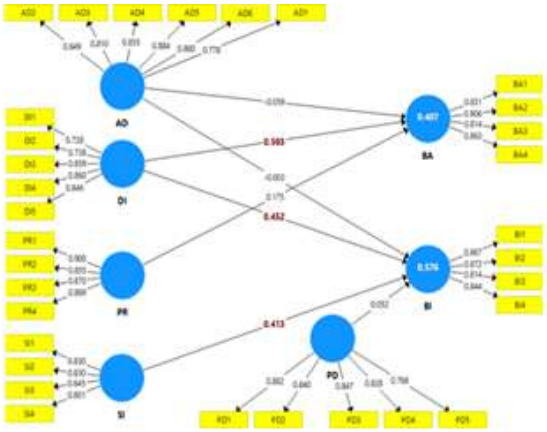

Figure 2. The results of the linear structural model inspection

Hypothesis testing

The research results are evaluated using the Bootstrap method, which confirms the reliability of the research model. To evaluate the research hypotheses, the authors employed Bootstrapping with a repeated sample size of 5000 observations in this study; the findings are shown in Table 6.

Table 5. Index result of f Square

	Advertising (AD)	Distribution Intensity (DI)	Price (PD)	Deals	Promotion (PR)	Store Image (S
Brand Awareness (BA)	0.004	0.549			0.031	
Brand Image (BI)	0.000	0.338	0.004			0.304

As presented, Figure 1 shows the hypotheses including 7 relationships in the research model, and 5 hypotheses showing the impact of the control variable on the level of brand awareness and brand image of customers. The results of estimating the relationship between research concepts show that there are 5 accepted hypotheses, in which hypotheses H1.1 and H1.2 show the impact of AD on BA and BI is rejected because its statistical significance is greater than 5%.

Table 6. Hypothetical test results

Relationship	Hypothesis	Standardized Coefficients	Standard Deviation (STDEV)	T Statistics	P Values	Result
AD→BA	H1.1	-0.059	0.041	0.974	0.33 0	Not Support
AD→BI	H1.2	-0.003	0.042	0.073	0.93 8	Not Support
DI→BA	H2.1	0.593	0.046	12.838	0.00 0	Support
DI→BI	H2.2	0.432	0.054	8.236	0.00 0	Support
PR→BA	H3	0.175	0.063	2.824	0.00 4	Support
SI→BI	H4	0.413	0.054	7.654	0.00 0	Support
PD→BI	H5	0.052	0.050	1.038	0.29 9	Not Support

AD= Advertising , BA= Brand Awareness , BI= Brand Image , DI= Distribution Intensity , PD= Price Deals , SI= Store Image , PR= Promotion

The results of the evaluation of the parameters of the respective hypotheses are shown in Table 6. Specifically, the research hypotheses are discussed in detail as follows: Testing hypothesis H1.1 and H1.2, the research results confirm that advertising has the opposite direction to brand awareness and brand image with the β-coefficients of -0.059 and -0.003 and is not statistically significant at the 5%. Thus, hypotheses H1.1 and H1.2 are rejected. This conclusion is consistent with the study of Bhattacharya et al. (1995), Ahearne et al., 2005, Research articles of Jemal (2014), Lien (2016), Altoveros (2016), Khan (2018), and Haque (2018).Hypothesis H2.1 and H2.2 to determine the impact of brand awareness and brand image. Which, hypothesis H2.1 and H2.2 show a positive impact on two factors of brand awareness with a standardized β coefficient of 0.593. Besides, the statistical significance level of this factor is 0.000 less than 0.05, thereby it is accepted. The conclusion of this hypothesis is quite similar to the study (Yoo et al., 2000; Kohli et al (2003).The test of the relationship between Promotions in the same direction is shown in hypothesis H3 in Table 6. With the value of β reaching 0.175, the promotion factor has a positive impact on the brand awareness level of consumers. And the index value of this factor is 0.004 less than 0.05, so this factor is accepted. The level of impact of promotion on both dependent factors is also quite high, as shown by the value of f2 in Table 4. This result is similar to the conclusions in the study (Huang, 2012).Hypothesis H4 shows a positive impact on the brand image with β reaching 0.413 and statistically significant at 0.000 and 5%, so the store image factor is accepted. This conclusion is also consistent with most of the studies (Aker, 1996 Keller, 2012).In the Hypothetical test results table, the standardized β coefficient of the price is 0.052 with a statistical significance level of 0.299 > 0.05, so the price factor is not accepted. This conclusion is not consistent with the studies (Kleine et al., 1993; Bhattacharya, 1995 Sen, 2003).

Discussions

The regression results show that: both factors have a considerable impact on the quality of consumer's relationship with brand awareness of TH's product: "Intensity of distribution", and "Promotion". At the same time, "advertising" also affects "brand

awareness” to build loyal customers. Both elements have a significant impact on how well consumers relate to the brand image of TH’s products "Intensity of distribution" and "store image". Additionally, "price trading" and "promotion" have a favorable effect on "brand image" to develop devoted customers.Promotion, as already mentioned, is a component of consumer relationships. Lots of customers would like the store to feature member-only specials and incentives nearby when they are shopping for products. The statistical chart of measurement variables' frequency for the "promotion" scale, on the other hand, reveals that the quality of promotion has not yet adequately matched customers' needs. “I don't find TH's promotions appealing because they are exclusive to me”. Promotions and incentives for customers enable stores to attract more customers who are interested and willing to spend time shopping there more frequently.Advertising is an element whose importance depends largely on how the product marketing program reaches its target audience. To better service customers, organizations must understand the psychology and needs of customers. TH spends more money but is not appreciated by consumers. To meet customer needs, organizations must develop a stronger advertising strategy.Intensity of distribution: this is a factor that affects the quality of relationships with customers. TH’s product is distributed in numerous stores, making it easier for customers to purchase products; customers can easily purchase products on e-commerce sites to meet their convenience needs; and TH has made changes to the distribution process so that customers can purchase products wherever they are. Customers will have more priority to choose from in today's retail sector due to the severe rivalry there, where the supply of goods meets the demand more completely.Store image is one of the factors for consumers to be impressed with the products they will purchase. TH's retail locations will be prestigious establishments that consumers value and care about. However, in addition to offering goods from well-known companies that customers tend to overlook, the store also sells goods made by TH.Price transaction. One of the factors that buyers take into consideration when making a purchase; hence, it may be regarded as a crucial one. The vast majority of customers are pleased with the cost of TH. Additionally; TH frequently runs several sales on top-rated products. Consumers, however, believe that TH provides no incentive regarding selling price.The regression results show that the intensity of distribution, advertising, promotion, store image, and price transaction all affect the brand awareness and brand image of TH.

Propose solution

To improve brand awareness and brand image, more distributions to res should be opened in Ho Chi Minh City's offline and online channels:

All variables have average coefficients or more, according to the results of the descriptive statistical analysis of the "Intensity of Distribution" components. The amount of "agree" among consumers who claim that distribution intensity has an impact on brand awareness and image is likewise very high. In comparison to the other variables, the observed variable "I locate TH's items in more stores than competitors" had the lowest mean value.

TH True Milk is available on supermarket shelves or the shelves of convenience stores, but the frequency and selection are not as "dense" as those of competitors' products. Customers find it easy to disregard the product and find it difficult to decide whether to buy it because of this. The distribution of goods is increased through the establishment of more retail locations, aiding firms in gaining new clients, expanding their market reach, and entering untapped market niches. Region development in comparison to rival VinaMilk, which has 104 stores, TH True Milk now has 52 retail stores in Ho Chi Minh City, according to websosanh.vn. Enterprises have to deal with the challenge of raising brand awareness among consumers in comparison to rival businesses. To efficiently reach customers with possible goods, businesses must research the potential market, as well as the competitors' distribution networks, and consider variables like location, demographic makeup, spending power, etc.

Implement retail marketing strategy through online marketing cooperation to increase brand awareness and brand image:

Customers believe that "I can buy things on e-commerce sites simply”. Many enterprises choose the highest average score for this measurement variable compared to other variables.

Sales through direct distribution channels are currently being hampered by the difficult Covid pandemic situation and social distance restrictions. Businesses should make investments to grow their internet sales channels and their business markets. As a result of information technology, firms will be able to optimize labor, reduce operating expenses, and free up management time.

The main remedy is for companies to offer coupons to customers via partners and inversely. Diversify existing sales channels and consider multichannel sales. In the era of quickly evolving technology, there are delivery applications in addition to online sales channels like websites and social networks to help firms keep up with trends and reach more prospective clients. Using websites like Shopee, Lazada, Baemin, Now, Gojek, etc., consumers may effortlessly and conveniently purchase goods anywhere. When it comes to retail marketing strategy, the form of marketing cooperation between businesses cannot be ignored. This will help expose your brand to a wide range of potential customers. This marketing cooperation helps businesses both promote each other and increase the possibility of getting more customers to use the company's services/products.

Retail businesses, supermarkets, and convenience stores are examples of linked businesses that can complement one another. TH True Milk's target clients can cooperate with some possible partners such as Shopee, Lazada, Baemin, Now, Gojek, and Grab.

Strengthen marketing activities to directly support sales taking place at the point of sale to increase brand image:

The majority of consumers are extremely interested in the significance of the shop image, according to the findings of a statistical survey detailing the elements of the "Store Image" scale. Consumers perceive how crucial the brand image of TH True Milk products is. Customers believe that "TH retail stores will be reputable sites," specifically. Compared to the other variables, this measurement variable's average score is the greatest. Additionally, "The Company's retail store does not have a pleasant space" has a pretty high average review score. When new clients approach a product at the point of sale, first impressions are advantageous. The counter, which includes the main fixed shelf, the top of the shelf, the cash register, and the display point, is typically where the purchase decision is made. The success or failure of marketing efforts is significantly influenced by the buying area. Businesses must utilize display and installation media at the point of sale to advertise the product or brand. One of the essential components that will influence that emotion in many ways is image. More than half of customers' purchase decisions are based on ability, thus even if the firm's purpose is not to sell, the corporation still wants to manipulate consumers' sentiments about the goal, mission, and brand awareness. One of the most important factors in influencing how the customer feels will be images.

In short, the company's retail stores will be reputable places, TH True Milk should focus on the art of product presentation along with a pleasant space with its characteristics. This will help reinforce the buyer's decision in favor of the retailer and plays a huge role in the overall success of TH True Milk's brand marketing program and retailers.

Creating attractive promotions to raise brand awareness among customers:

According to the survey results, descriptive statistics on the scale of the components of "Promotion" reveal that consumers like the promotion of TH True Milk. Consumers say, "I enjoy participating in TH's promotions". Hence, "Promotion" has a significant impact on brand image and brand awareness; the average score for this variable is the highest compared to the remaining variables. With the 4.0 technology era, people are becoming increasingly interested in company marketing for convenience and benefits. The observed variable "I feel TH's promotions are exclusive to me" had the lowest mean score. All measuring factors have a significant influence on brand awareness and brand image. Promotion programs not only assist businesses in solving difficulties such as increasing sales and pushing inventory sales, but they also provide businesses with an opportunity to improve brand awareness and the shopping demand among customers.

Offering a discount on the cart's total price or number of items.

Offering a discount on the total price of the cart or the number of items is an efficient strategy to encourage customers to spend more, hence increasing the company's average order value. Implement this strategy by assessing the customer's average order amount over the past several months, then give a bonus discount or free delivery on all purchases that exceed 10-20% of the previous mean. Instead of giving cash, company owners frequently give discount coupons to encourage customers to continue purchasing their items. It might be a purchase coupon for 100.000, 500.000, or several million VND, or it can be a voucher used to purchase items at a percent discount...For example, if a consumer purchases 5 packs of TH 180 ml fresh milk, they will receive a voucher for 2 bags of TH fresh milk. This voucher will be applied to the customer's next purchase. Using vouchers and discount coupons is an excellent approach to keep clients using your products for a long time, which is an important type of company marketing.

Auxiliary messages of the brand:

Shape and style: Maintain and improve your body shape. Give others a sensation of lightness, health, and young attractiveness.

P**hysical**: The drops of milk extracted from nature through closed processing bring essential nutrients to the body.

TH's target customers are people who prefer natural products and care about health issues, especially ladies and mothers with young children.The majority of customers are married women. Many factors have impacted their purchase decisions. In, "Delicious, nutritious, cheap" is the most important criterion for women's shopping decisions. In addition, sale promotions always increase customer purchasing desire. Most businesses take advantage of this mentality by offering several promotions and discounts on special occasions or days.The majority of customers are married women. Many factors have impacted their purchase decisions. Which, "Delicious, nutritious, cheap" is the most important criterion for women's shopping decisions. In addition, sale promotions always increase customer purchasing desire. Most businesses take advantage of this mentality by offering several promotions and discounts on special occasions or days.For example,

when a customer buys a carton of TH true MILK UHT Pure Fresh Milk HILO 180 ml box (48 boxes), they will get 1 free yoga mat and a 30-day yoga voucher at X center. This program focuses on brand value and its communication message, addressing customers' basic demands for clean milk and a beautiful body shape.

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