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FOOD SAFETY AND FOOD SECURITY

**held at Dong Nai University of Technology,
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on August 15 – 18, 2014**

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PREFACE

The Asian Food Safety and Security Association (AFSSA) is a voluntary, non-profit, charitable organization established in 2010. The 5th annual general meeting of Bangladesh-Japan Association for Science and Technology (BJAST) at Dhaka in 2010 decided to establish the Asian Food Safety and Security Association (AFSSA). The first annual meeting of AFSSA was held at Osaka Prefecture University, in September 15-17, 2012. This meeting decided to hold 2nd annual meeting of AFSSA to be held in Bien Hoa City, Dong Nai province, Vietnam in August 15-18, 2014.

The main objective of AFSSA is as follows:

- To forge effective cooperation among the member states in areas including climate change, food safety and food security.
- To disseminate research findings and future research in food safety and security.
- To help in building Asian Food Safety and Security Network.
- To help educational institutions by supplying equipment, books, materials, ideas, etc.
- To develop strong relationship between the Asian countries
- To exchange views, ideas by organizing seminars, symposia, conferences and meetings.
- To help secure funds for students to attend seminar, conferences, training programs.
- To lunch a journal on Food Safety and Food Security with a focus on current trends and needs in Asia.

This conference discusses the key food safety, and food security issues. It brings together renowned experts, entrepreneurs and government officials from different countries to discuss current and emerging food safety and food security issues, find out common problems among the countries, future direction and collaborative research plans on food safety, food security and related topics.

The plenary sessions, and concurrent symposia, will also address the challenges facing Asia to ensure food safety in the development of new food products and processing technologies, internationalization of food trade, safety of foods derived from biotechnology, microbiological risks, emergence of new and antibiotic-resistant pathogens, particularly from emerging pathogens, directing research to areas of high risk, focus intervention and establishment of target risk levels and target diseases or pathogens.

The two-days scientific and poster presentation sessions will provide multiple opportunities for experts and participants to discuss key food safety, and Food security issues. Experts from governmental organizations, international organizations, entrepreneurs and universities from different countries will be able to share their ideas, address challenges, and discuss strategies and collaborative programs to enhance the continuing global efforts to deliver safe foods for a better health and environment of the people of the developing countries.

There are 32 articles have been selected to publish in the Journal of Food and Nutrition Science (JFNS) (http://www.sciencepublishinggroup.com/j/jfns). The last papers were selected and published in this proceeding.

The proceeding contains papers submitted by speakers from different countries based on presentation made in the conference. The views expressed are those of the authors, and the editorial committee takes no responsibility for the contents or comments, or the views of the Asian Food Safety and Security Association. However, it is believed that book will act as a good source for relevant information in relation to food safety and security.

The editorial committee is thankful to Asian Food Safety & Security Association for giving the permission and financial support to publish the proceedings.

Ryohei Kada
Jeyam Subbiah
Chowdhury Rafiqul Ahsan
Md. Latiful Bari
Dam, Sao Mai
Ngo, Ke Suong

Message from the President of AFSSA

Takashi UEMURA, DVM, Ph.D.
Professor Shijonawate Gakuen University,
Osaka, Japan

It is my great pleasure and honor to extend the gratitude on the grand opening of the "2nd AFSA Conference on Food safety and Food Security in the presence of many distinguished guests.

The passions of some scientists in ASIA who believe that bio-science and biotechnology contribute to improvement in the quality of life and welfare of human beings of the world and know the importance of co-laboratory works and direct exchange of idea and information made this series of Conference start. The first Conference was held in Osaka on September, 2012 hosted by Osaka Prefecture University, JAPAN.

Sustaining of our passion, by holding the conference bi-annually, has been supported by private and public institutes/organizations of Lincoln University, Osaka Prefecture University, International Union of Microbiological Societies (IUMS), University of Dhaka, People Committee of Dong Nai Province, and Dong Nai University at Vietnam as the host, as well as by students, volunteers and financial supporters, and many distinguished guests have strongly encouraged us. I express my sincere thank to all those hosts and supporters.

This International Conference is qualitatively improving as an international meeting; researchers from 20 different countries join in this 2nd conference, where higher level of research works will be presented and hot discussions will be expected.

Hopefully, I want to give the opportunity as many as possible for young researchers to attend the international meeting. Our endless voyage for obtaining tomorrow's higher QOL and welfare of human beings by bio-science and biotechnology necessitates continuous supply of fresh and innovative wisdom.

Thank you all very much

Takashi UEMURA, DVM, Ph.D

Message from People Committee of Dong Nai Province, Vietnam

TRAN VAN VINH
Vice President of People Committee of Dong Nai Province, Vietnam

It is my great pleasure and honor to extend the gratitude on the grand opening of the "2nd AFSA Conference on Food safety and Food Security" in the presence of many distinguished guests.

Dong Nai province is one of the industrial province in Vietnam. There many manufactures are located in this area, including the food producing and food related companies, Vietnamese and foreign plans, such as: Vedan, Nestle, Vina coffe, Dona food,… and so on.

Food safety and Food Security Associate is the organization, which connects the people in Asian and other place in the word that are interesting in the food safety and food security. That's why, the conference, which is organizing by Food safety and Food Security Associate is very helpful for Vietnam and other countries.

The "2nd AFSA Conference on Food safety and Food Security" has been supported by private and public institutes/organizations of Lincoln University, Osaka Prefecture University, International Union of Microbiological Societies (IUMS), University of Dhaka, People Committee of Dong Nai Province and Dong Nai University at Vietnam as the host, as well as by students, volunteers and financial supporters, and many distinguished guests have strongly encouraged us. I express my sincere thank to all those hosts and supporters.

There are many researchers from 20 different countries join in this conference, where higher level of research works will be presented and hot discussions will be expected.

Hopefully, I want to give the opportunity as many as possible for every people who are joining to the conference to meet each other, share the researches and find opportunities of the development

Thank you all very much

Mr. Tran Van Vinh

Dong Nai University of Technology

PHAN NGOC SON

Principle of Dong Nai University of Technology

Dong Nai University of Technology (DNTU) was established in 2005.

Dong Nai University of Technology is a major educational institution in higher education and vocational training system of Southern Viet Nam as well as a top leading private university in terms of teaching and research excellence, providing education services with disciplinary and professional diversity, and multi-level learning for thousands of students from all parts of the country. DNTU provides a creative and stimulating environment for students within its distinctive and traditional disciplines.

Students may be gaining a formal qualification to a wealth of industry experience, or they may be fulfilling a life-long dream to undertake further study for the sheer pleasure of learning.

The university is located at the central area of Bien Hoa City, 30km north of Ho Chi Minh City. Bien Hoa City lies in the center of the most dynamic quadrangle economic zone including Southern Dong Nai, Ho Chi Minh City, Vung Tau, Binh Duong, which attract the most foreign investment. Dong Nai has many large industrial zones. Each year, numerous skilled labors are needed for expansion of industrial activities in production facilities and of business services. The demand for qualified and trained manpower is great; however, the available labor force has not met the recruitment needs for human resources of the industrial zones. This is a great opportunity for education and training sector. In light of it, DNTU equips students with good knowledge and qualification so that they can be able to find a job easier after graduation.

In recognition of local and national concerns, we continually update our range of programs and research directions, leading, in turn, to graduates who are well-qualified, well-respected and in-demand locally and nationally. Graduates will forever have a worldwide network of support from DNTU and its alumni as they embark on their careers.

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CONTENT

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Research on antibacterial activity of various cellulose derivatives and application to food safe field

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Abstract:

Cellulose derivatives $(C_6H_{10}O_5)_n$ are mainly used in the construction and the engineering field, the ceramics and the paper coating. And they are also used in the fields of demanding the safety, such as medical supplies, cosmetics and toiletries. However, there are a few reports about the antibacterial activities of a cellulose derivative, and it will be considered to be a significant matter to examine the detailed antibacterial activities.

In this research, 7 kinds of cellulose derivatives $(C_6H_{10}O_5)_n$ was used in the antibacterial test. The antibacterial activities test against *Staphylococcus aureus* and *Escherichia coli* was examined by the shake flask method and Minimum Inhibitory Concentration (MIC) measurement method.

Among the cellulose derivatives used for the examination, MIC test was carried out with the samples which soluble in water. On the other hand, the samples that soluble few in water was tested by the shake flask method, respectively.

As a result, although the test result had a little variation in the structure of the cellulose derivatives, some cellulose derivatives tested had possessed the antibacterial effect against test bacteria. MIC values of water-soluble samples (two samples) were 25 and 100 μg/mL against *S. aureus* and 100 and 200 μg/mL against *E. coli*, respectively. In the water insoluble samples (5 samples), 3 samples were effective and 5-log reduction was succeeding within 2 hours.

By preparing the film sheet etc. that are added the cellulose derivative of antibacterial agents, it will be also applicable to the preservation field of several kinds of foods.

Keywords: Cellulose derivatives, Antibacterial activity, Shake flask method, MIC

1. Introduction

Cellulose derivatives $(C_6H_{10}O_5)_n$ are used in many fields such as the medicines, cosmetics and toiletries that will be requested the safety, construction and engineering works, ceramics, and the paper coating [1]. Cellulose is the recyclable polymer that is synthesized by water, carbon dioxide, and solar energy during all parts of the world (the dry base: about 15.5 billion tons/year). Cellulose derivatives are cheaper than the cheap synthetic polymer such as polyethylene (180-200 Japanese yen/kg), which could be developed as the functionality cellulose [2]. The cellulose derivatives are also valuable material, because it is possible to obtain at low price. Moreover, a lot of preparation methods can be made with the antibiotics such as oxytetracycline and ampicillin etc. [3]. It is also used as a coating agent for dry syrup. Moreover, a part of cellulose derivative can be made with diamines, sustained delivery medicines and the immobilized enzymes [4]. The cellulose derivative is easily processed material. The cellulose derivatives have different character according to the cellulose material such as shape (for instance, the fiber form, the fiber length, the fiber distribution, the

specific surface area, the surface figure, and the shape of cross section, etc) [5]. The cellulose derivatives are widely used in various fields.

In this research, the presence of the antibacterial effect of these materials was investigated for additional functional value.

2. Materials and Methods

2.1.Test agents

The basic structure and the structural formula of various cellulose derivatives used for experiment were shown in Table 1 and Fig. 1.

Table 1. *Functional group of various cellulose derivatives tested in this experiment.*

Sample No.	R (substituent)	X	DS (substitution degree)
K-012	Et	Br	0.45
K-013	Pr	Br	0.41
K-014	Bu	Br	0.27
K-015	Bu	Br	0.56
K-016	Bu	Br	1.16
K-017	Hex	Br	0.55
K-019	Oct	Br	0.47

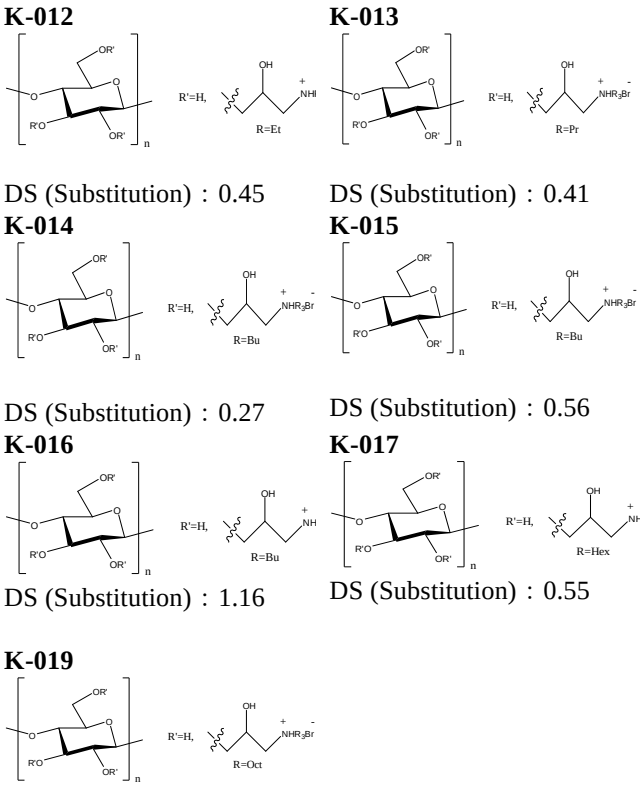

Fig.1*. The structural formula of various cellulose derivatives used to experiment*

2.2. Test bacteria

Escherichia coli (*Escherichia coli* NBRC 3972; *E. coli*) and *Staphylococcus aureus* (*Staphylococcus aureus* NBRC 13276; *S. aureus*) were used as tested bacteria.

2.3. Shake-flask method

2.3.1. Test agents

The cellulose derivatives of test agents, K-013, K-014, K-015, K-017 and K-019, were shown in Table 1.

2.3.2. Test medium

Soybean-Casein Digest Agar（Nihon Pharm. Co., Ltd, Japan : SCD Agar）and Soybean-Casein Digest Broth（Nihon Pharm. Co., Ltd, Japan : SCD broth）was prepared, and was used for the test after the sterilization at 121℃ for 15 min.

2.3.3. Preparation of test bacteria numbers

Pre-incubation test bacteria was added into 2 mL of SCD broth, and incubated at 37℃ for 16～18 h under shaking condition (rotation speed : 100～120 rpm/min). The incubation bacterial suspension was diluted by SCD broth, and the absorbance at 630 nm (OD_{630}) was adjusted to 0.12 ± 0.02 (Bacterial numbers: about 1.0×10^8 CFU/mL). The bacterial suspension was 100 hold dilutions by the sterilized physiological saline.

2.3.4. Shake-flask method

K-013, K-014, K-015, K-017 or K-019 was added to each of flask, respectively (no sample was added to blank flask). Test bacterial suspension (each of 20 mL) was added to each of six flasks, and incubated at 25℃, 100 rpm/min under shaking condition.

2.4. Measurement of bacterial numbers

Each of test suspension (0.5 mL) was sampled at 0, 20 min, 40 min, 1 h and 2 h incubation. 10, 100 and 1000 hold dilution was performed by the sterilized physiological saline, and each of diluted sample (0.1 ml) was smeared to each of two SCD Agar by using the bacteria spreader, respectively. After 2 days incubation, the viable bacterial numbers were measured.

2.5. MIC（Minimum Inhibitory Concentration）test method

2.5.1. Test agents

The cellulose derivatives, K-012 and K-016 were used as test agents (refer Table 1).

2.5.2. Test medium

SCD Agar, SCD broth and Mueller Hinton Broth (Becton Dickinson Microbiology System, USA : MH broth) was used after the sterilization at 121℃ for 15 min.

2.5.3. Preparation of test bacterial suspension

SCD broth was poured into the sterilized test tube, and added one colony (before incubation at 37℃ for 16～18 h). The bacterial suspension was diluted with SCD broth (OD_{630}), which was adjusted as 0.12 ± 0.02 (bacterial numbers: about 1.0×10^8 CFU/mL), and used as the test bacterial suspension.

2.5.4. Preparation of test solution

K-012 and K-016 were prepared to 800 μg/mL by using MH broth. Two hold dilution was performed, and prepared 800～6.25 μg/mL solution and used as MIC measurement.

2.5.5. Measurement of MIC values

The prepared solutions (each of 100 μL) were poured into 96 well micro plate and added each of 5 μL of test bacterial suspension, and mixture well. Incubation at 37℃ for 16～20 h. When OD_{630} on micro plate reader was below 0.3, its concentration was judged as effectively, and also judged as MIC. At lease, three tests were reaped and calculated each of MIC values.

3. Results and Discussion

3.1. Shake-flask method

The results by using *S. aureus* and *E. coli* were shown in Fig.2 and Fig.3, respectively.

There are some different results by cellulose derivatives and bacteria tested. K-013 was the most effective activity against *S. aureus* and more than 5 Log reductions were found after 60 minutes reaction. K-015 and K-019 showed the antibacterial effect, and more 5 Log reductions were succeeded at 120 minutes later. Against *E. coli*, K-013 also showed the most effective activity, and 5 Log and/or more reduction was found after 20 minutes later. K-014 showed the effect, and 5 Log and/or more reductions were found after 120 minutes later.

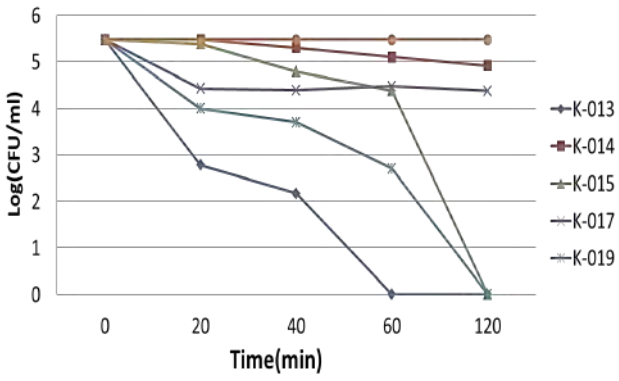

Fig.2 *The antibacterial activity against S. aureus tested.*

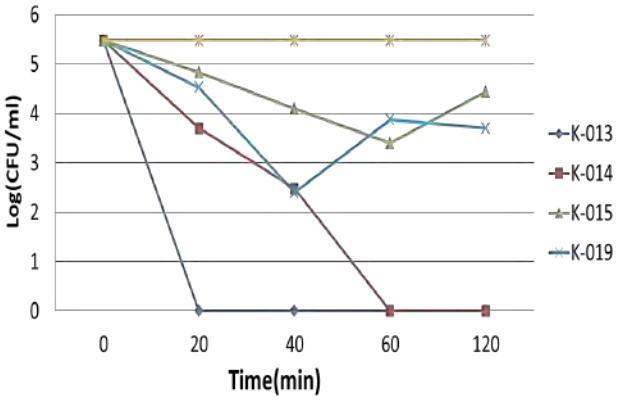

Fig. 3 *The antibacterial activity against E. coli tested.*

3.2. MIC Measurement

MIC values of K-012 and K-016 against *S. aureus* were 25 and 100μg/mL, respectively. MIC values of K-012 and K-016 against *E. coli* were 400 and 100μg/mL, respectively.

4. Conclusion

The antibacterial effect of seven kinds cellulose derivatives against two kinds of bacteria such as *E. coli* and *S. aureus* were evaluated by using MIC measurement or Shake-flask method.

From Shake-flask method, all cellulose derivatives tested possessed the antibacterial activity (Fig. 2 and 3). K-013, 15, and 19 killed the test bacteria finally. Especially, K-013 possessed stronger antibacterial activity, and killed the test bacteria at 60 minutes. On the other hand, K-014 and K-17 possessed the weak antibacterial activity. The result was greatly different depending on the kinds of the test cellulose derivatives.

There was occasionally only the temporary control effect in K-015 and K-019 for *E. coli* (Fig. 3). In K-013 and K-014, the tested bacteria were killed finally, and on K-013, the tested bacteria were killed within 20 minutes. Therefore, the antibacterial activity against *E. coli* was recognized. These results suggest that K-013 was the strongest and possessed the speedy bactericidal rate.

In the result of MIC measurement, the antimicrobial effect by K-012 against *S. aureus* at 25 μg/mL and 400 μg/mL in *E. coli*. On the case of K-016, 100 μg/mL was effective against *E. coli* and *S. aureus*.

In these results, all cellulose derivatives tested possessed the antibacterial activity; its activity level would depend on the difference of structural formula. Further research will be necessary to examine the influence of the functional group.

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Trial of construction of HACCP for food safety and security in Nara Prefecture of Japan

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Abstract:

HACCP has spread globally as an international standard system of food hygiene management. On securing the safety and security of the food, which prevent the food poisoning, it is the indispensable subject in the food business of our country to practice the process control by HACCP.

While "Japanese revival strategy named to JAPAN is BACK" focused the introduction of TPP was carried out in June, 2013 by Cabinet decision, the spread and promotion of HACCP to the safety standards of the agriculture-and-forestry marine product and foods will be necessary in the planning of export promotion in food field.

Nara is one of the sightseeing prefectures (Nara prefecture is consisted of many sightseeing places). Many tourists of inside and/or outside of the country will be coming to Nara. Therefore, Guarantee of the safety in the agriculture-and-forestry marine product, the general food, and the food restaurants (food of the store which offers cooking) supplying in Nara prefecture is an important problem. The construction of good environment, which the consumer used comfortably, will be important duty for many persons engaging in the food field of Nara Prefecture.

In the present situation, a non-profit organization (NPO) Nara HACCP study group under the progress of the introduction and the spread activities of HACCP in Nara Prefecture will be explained. NPO is the cooperation organization between Nara food hygiene association and Nara Co-op. We would like to introduce the details as the promotion model of original HACCP in Nara.

Based on the above, in this time the motivation of HACCP activities, the beginning and the details of this activity, the independent activities, and the future plan of NPO Nara HACCP study group, are introduced.

Keywords: Construction of HACCP, Food safety, Food security, Nara Prefecture of Japan

1. The motivation of recognition institutions of the hazard analysis critical control point (HACCP)

There is only one company (two factories), using HACCP system in Nara Prefecture. The authorization company possessing high advanced plan about HACCP support method is very few comparing with other prefectures. In addition, few opening special study session concerning about HACCP is held within Nara prefecture, many food business have gone out the other prefectures for the receiving seminar or workshop by paying the transportation fee and the attendance fee.

In this situation, on the Nara food hygiene association (public corporations), the necessity of the specialized education about HACCP by the committee members (20 persons) was necessary for performance of the support work based on the food hygiene inspection system instruction plan based on HACCP of Nara Prefecture.

Moreover, in a Nara Co-op, the contribution to local community is a mission, which is made as a basic policy. In addition, the necessity for local supply and consumption was also advocated. HACCP was the basic policy of the co-op to improve the health, the quality control level of food safety, security of local agriculture-and-forestry marine product and the processed foods and food business within Nara prefecture.

In order to reach agreement with the measure of HACCP, and both organization may collaborate and attain purposes and ideas in this way, the establishment of HACCP organization within Nara prefecture was advanced on the support of cornering sections of Nara Prefecture.

2. Beginning and Progress of the Measure

Nara Prefecture has organized "the Nara food safety and security conference" (social meeting) which "reflecting the opinion of the people of the prefecture in decision of the measure about reservation of the food safety from each representation". Nara food hygiene association (public corporations) participated as the representative of the food production and Nara Co-op also attended as the representative of committee of the production trading position.

The social meeting was held twice per year. Several questions and answers are given about the measure (the food hygiene inspection system instruction plan at a given fiscal year), result about food safety and security of Nara Prefecture, explanation of the subject at the time, and the proposal matter from the committee members.

The measure of HACCP was discussed friendly in the 10th meeting held in September, 2008. The booklet about HACCP entitled "The food safety and security which cooperated with us" was published by the expert committee members.

In the 12th meeting held in November, 2009, the committee members proposed the necessity of establishment of the food hygiene management system and the HACCP standard plan of Nara Prefecture was presented.

As shown in Table 1, a proposal and a report of this subject were positively performed in "the report of holding progress of spontaneous HACCP study session" and each time shown in Table 1 "an establishment request of a Nara version mini HACCP system."

After the first proposal, through the committee meeting in four years (9 times) the friendly discussion was performed, and it determined that the direction of establishment of tentative system and a plan about the original HACCP authorization system on Nara Prefecture will be made in 20th meeting held in September, 2009.

Table 1. *Programs of Subject concerning about Main HACCP in Nara Food Safety and Security Meeting*

Meeting day	Main selected theme concerning HACCP	Remarks
10th (March, 2008)	Publication of book entitled " "(Situation of introduction in our prefecture, theme and future plan)	Introduction of activities of food safety and security from farm to distribution field in Nara prefecture
11th (March, 2009)	Preparation of document to the consumer about food safety and security; and opening proposal of seminar	The thing of the contents like an explanatory of the booklet of the 10th publication
12th (November, 2009)	Opening proposal of executive workshop of HACCP The establishment proposal of a Nara mini HACCP recognition system The establishment proposal of the ordinance about the food safety of Nara Prefecture	Subject institution was carried out as a proposal and exchange of opinions also of any was carried out about the possibility and the problem of realization.

13th (March, 2010)	HACCP executive workshop re-opening proposal The establishment proposal of the ordinance about the food safety of Nara Prefecture The subject of the present condition of GAP (Good Agricultural Practice) in Nara Prefecture	About the executive workshop, the holding proposal by the Nara food hygiene association was made.
14th (November, 2010)	Re-establishment proposal of the ordinance about the food safety of Nara Prefecture Present condition explanation of the safe and safe measure of the agricultural products of Nara Prefecture	・A tie-up proposal etc. with the Ministry of Agriculture and Forestry office ・The proposal of risk communication such as a seminar
15th (March, 2011)	The opinion proposal to the Nara food sanitation inspection system instruction plan (proposal) in the Heisei 23 fiscal year The proposal of risk communication of the security of the production site of agricultural and livestock products	・Including the relation with HACCP ・Proposal of concrete plan such as symposium
16th (September, 2011)	Explanation of 2nd HACCP study session, a request of support of a gathering for friendly discussion A report and evaluation of 1st HACCP study session of a holding result	・Support of 2nd HACC workshop ・Including HACCP study group concept proposal
17th (March, 2012)	A report and evaluation of 2nd HACCP study session of a holding result The starting proposal and exchange of opinions of the Nara HACCP study group	Construction plan of HACCP research group on the extended line of HACCP workshop
18th (September, 2012)	A report and evaluation of 3nd HACCP study session of a holding result Exchange of opinions about the subject of a Nara mini HACCP system	HACCP system of Nara Prefecture and debate of a problem which will be desired in the future
19th (March, 2013)	A report and evaluation of 4th HACCP study session of a holding result Presentation of the Nara HACCP study group fundamental plan proposal, explanation The construction proposal and exchange of opinions of a right prefectural version HACCP attestation system	Comprehension by which a study group is entitled Nara Prefecture A study group considers institutionalization towards examination and attestation, and Nara Prefecture recognizing.
20th September, 2013)	A report and evaluation of the HACCP study session in the H 25 fiscal year of the conclusion of a result Explanation of the HACCP study session holding point by the Ministry of Agriculture, Forestry, and Fishery subsidy The request of establishment of a Nara version mini HACCP recognition system etc.	Based on the orientation at the time of the 19th gatherings for friendly discussion, an institutionalization design will be tackled in a Nara version HACCP recognition system.

3. Independent Trial Activity

In parallel to the proposal and/or the report activities, between Nara food hygiene association (public corporations) and Nara Co-op, the basic education study session about HACCP was held the twice per year (4 times) from July, 2011.

The appeal to participants for study session was performed with the receiver's address (public corporations) of "technical newsletter" of the Nara industrial development synthesis center, the members of Nara food hygiene association and the customer of food production companies of Nara Co-op.

In holding of study session, we have started with Nara Prefecture HACCP society (a private organization). The HACCP introduction training meeting (Open lecture, a day) and the leader training meeting (3 times, every continuousness three weeks) were held at July and August 2013 by the subsidy of the Ministry of Agriculture, Forestry and Fisheries (refer Table 2). There were many participants in the above-mentioned training meeting.

From the participant, there were many opinions that importance of HACCP has well understood and the participant members would try to their company understands about the performance of HACCP. We think that the satisfactory results were obtained. This is valuable to continue to this HACCP promotion activity.

Table 2. *Opening points of HACCP Workshop from 2011 to 2013 year*

Times	Opening day	Name of workshop	Theme and Lecturer	Partici-pants	Remarks
First	26th June, 2011	Challenge from the basic program of food hygiene to the practice of HACCP (Basic edition)	5 theme 5 person	41 person	
Second	15th November, 2011	Challenge from the basic program of food hygiene to the practice of HACCP (Practice edition)	6 theme 6 person	42 person	
Third	20th June, 2012	How to introduction of HACCP ? (Trial and introduction case in Nara Prefecture	7 theme 7 person	43 person	
Forth	30th November, 2012	How to introduction of HACCP ? (Base and practice of precondition program)	6 theme 6 person	45 person	
Fifth	21st August, 2013	Nara Prefecture HACCP Introduction Workshop	6 theme 6 person	55 person	Carried out by the support of Ministry of Agriculture, Forestry, and Fisheries in 2013.
Sixth	28th August, 4 and 11 September, 2013	Nara Prefecture HACCP Reader Training Workshop	13theme 7 person	38 person	Carried out by the support of Ministry of Agriculture, Forestry, and Fisheries in 2013.

Opening place: Nara Prefecture Institute of Industrial Development (Former Nara Prefecture Institute of Industrial Technology

4. Nonprofit Organization (NPO) Nara Prefecture HACCP Study Group

The Nara HACCP study group which will establish the improvement in the food safety in inauguration of an NPO Nara HACCP study group, activation of industry and local symbiosis decided that higher reliability, continuity, and continued existence are considered as necessity, and establish the application of a non-profit organization (NPO).

Chairman of the board of director is Yoshikazu Sakagami, vice president of Society for Antibacterial and Antifungal Agents, Japan (also a professor of the Kinki University agricultural department). Vice president of Hideo Okayama is the president of Nara food hygiene association (public corporations). The main establishment purposes is "bottom up of the health and the quality control level of the Nara food business planned", "The private complement of spread and education of the HACCP technique of publication is aimed at in the Nara food hygiene inspection system instruction plan", and various required related papers were drawn up, registered completely and established to Nara Prefecture on presentation at 3 February, 2014, and finished the registration.

We think that it can be completed on the private position over the HACCP related system in Nara Prefecture due to institutionalize in the future.

The specific activity purpose and main enterprise in our NPO activities are shown in Table 3 and Table 4, respectively.

Table 3*. The purpose of activity on NPO Nara HACCP study group*

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(1) Based on the plan of HACCP, which our country promotes this organization try to bottom-up the health and the quality control level of the food business in Nara by opening of holding the study session. In production sites such as an agriculture-and-forestry marine product in Nara prefecture, the keeping of food safety and security by HACCP would be tried. This organization contributes to keep the international level concerning about the safety by HACCPs (GAP is included) such as an agriculture-and-forestry marine product and food in Nara prefecture.

Table 4*. Project of NPO Nara HACCP Workshop*

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(1) Presentation of latest (new) information about the food safety and security
(2) Opening of the study session about HACCP and the issue enterprise of an attendance vote

The following enterprises are scheduled to perform two above-mentioned enterprises.

① The presentation of latest information concerning about the food safety and security, the food hygiene management, and food safety management system

② Opening of the study session about HACCP and the registration of Nara food hygiene management system concerning holding business
③ Creation instruction of HACCP related data, and the opening enterprise of the creation exercise and the presentation meeting
④ Enforcement enterprise of the training in factory concerning HACCP introduction
⑤ Opening enterprise of the inspection meeting of HACCP introduced factory
⑥ Performance of the enterprise of above-mentioned ③～⑤ about the Nara food hygiene management system concerning HACCP
⑦ The interchange program of the members and the related companies

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5. Future plan (Future ambition)

The future trial of HACCP in our country has a high promotion of improving introduction and the food business companies were shown the registration of the announcement meaning in December 2013 [1].

Moreover, we think that the local adhesion type measure, which has firmly a sense of purpose and promotion through the manager of food business HACCP - tackles. It is tackled on the basis of the detailed cooperation with a local governing body and is required the measure especially in food business recognized the situation in and outside the country.

The persons concerned engaged in food of Nara Prefecture must keep the heavy responsibility of the food safety and security to people living in Nara prefecture and Nara visiting tourist from the outside of the country as sightseeing prefecture. Based on 5 times study session, we would like to aim to contribute the measure of HACCP which country and Nara Prefecture will advance in the future NPO activity. Moreover, we hope that our trial would become to a model to contribute construct a model measure, this is our pleasure if the future district food hygiene association and local purchase co-op in other prefectures become one model which carried out the collaboration aiming the introduction and spreading HACCPs. And we also hope that our trial will contribute the local community promotion model.

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Prevalence and antimicrobial susceptibility of *Listeria* spp. in dairy food products and water samples in Dhaka, Bangladesh

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Abstract:

A total of 57 samples, of which 17 were surface water samples and 40 were dairy food samples (raw milk, pasteurized milk, icecream, sweet, milk based drink like matha and borhani) were tested for the isolation of *Listeria* spp. Putative *Listeria* isolates were identified by conventional microbiological tests and Analytical Profile Index. Overall prevalence of *Listeria spp.* in both food and water samples were 8.77%, of which one was (1.75%) *Listeria monocytogenes*, 2 (3.5%) were *Listeria innocua* and 2 were (3.5%) *Listeria welshimeri*. When compared between two types of samples, water samples contained two *Listeria* spp. (11.76%) of which one was pathogenic *Listeria monocytogenes* and the other was *Listeria innocua*. In case of food samples, three *Listeria* spp. (7.5%) were isolated of which one was *Listeria innocua* (icecream sample) and two were *Listeria welshimeri* (icecream sample and raw milk). No *Listeria* was found in pasteurized milk, sweet, matha and borhani. Antibiotic resistance profile of the *Listeria* isolates showed that 60% isolates were resistant to Ampicillin and Erythromycin, 20% isolates were Sulphamethoxazole and Ciprofloxacin resistant. No resistance was observed to Chloramphenicol for any *Listeria* isolates.

Keywords: Prevalence, Antimicrobial susceptibility of *Listeria* spp., Dairy food products, Water.

1. Introduction

Listeria monocytogenes is associated with listeriosis, a foodborne infection which can lead to severe conditions like meningitis, endocarditis and has high mortality rate. It is widespread in the environment and has been isolated from water, soil, dust, plants, animal feed, feces and sewage and has been associated with mammals, birds and possibly fish (Sauders *et al*., 2012). This pathogen has also been isolated from food products like unpasteurized (raw) milk or foods made from unpasteurized milk, red meats, poultry, seafood, vegetables, fruits and ready-to-eat food products (Kasalica *et al*., 2011, Karakolev, 2009, Malek *et al*., 2010) . Of these, milk and other dairy products have been implicated in about half of all the listeriosis outbreaks and in several sporadic cases worldwide (Molla *et al*., 2004; Mahmoodi, 2010). The ability to grow or survive at low temperatures, low pH and low water activities makes *L. monocytogenes* an important hazard in foods (Mahmoodi, 2010). Multi-drug resistance among *Listeria monocytogenes* isolated from food or the environment have also been described, which imposes an additional risk to public health (Coner *et al*., 2009; Jamali *et al*., 2013; Gomez *et al*., 2014). However, in Bangladesh information on the occurrence and distribution of pathogenic *Listeria monocytogenes* as well as other *Listeria* spp. is very limited. This study is aimed to study the prevalence of *Listeria spp.* in dairy food products and water samples.

2. Materials and Methods

2.1. Collection of samples

A total of 40 dairy milk products (such as ice-cream, Sweet, Sondash, Matha, Borhani), raw and pasteurized milk from shops and restaurants and 17 water samples from various ponds around Dhaka city have been collected in sterile container. These samples were collected during the period of April, 2013 to June, 2014. The samples were transferred under aseptic conditions in an ice box to the laboratory at the Department of Microbiology, University of Dhaka.

2.2. Sample Processing and isolation of *Listeria* sp.

Samples were processed according to combined method of United States Department of Agriculture (USDA) and U.S. Food and Drug Administration (FDA). 25 gm or 25 ml food/water sample was mixed with Oxfoid Listeria selective enrichment broth and was incubated at 30°C for 48 hours. In case of solid sample, it was homogenized in 0.1% Peptone Water before inoculating into broth. After 48 hours, from each broth loopful inoculum was taken and was streaked on Oxoid Listeria selective agar followed by incubation at 37°C for 48 hours. Each plate was examined for typical colonies of *Listeria* and colonies with characteristics typical of *Listeria* spp was subcultured on Tryptone soya agar supplemented with 0.6% yeast extract at 37 °C for 24 hours.

2.3. Identification of *Listeria* sp.

To identify *Listeria* species, all isolates were screened for their Gram reaction by Gram staining method. Isolates which appeared as Gram positive, rod shaped organisms were further screened for their biochemical tests (Catalase test, Oxidase test, xylose and mannitol fermentation test, motility test and hemolysis on blood agar) as suggested in the Bergey's Manual of Systematic Bacteriology Volume 2 (2005). Isolates showing typical properties of *Listeria* were identified by Listeria API.

2.4.Determination of Antimicrobial Susceptibility

Susceptibility of the *Listeria* isolates to different antimicrobial agents was measured *in vitro* by employing the modified Kirby-Bauer method (Barry and Thornsberry, 1985). Commercially available antimicrobial discs (Oxoid limited, England) were used for the test. An inoculating needle was used to pick single, isolated colony, and inoculated into 3 ml of Muller-Hinton broth. The broth cultures were then allowed to incubate at 37°C for 4 h to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a McFarland 0.5 standard (3×10^8 CFU/ml). To streak on the surface of agar medium, a sterile, nontoxic cotton swab was dipped into the tube containing young culture and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked evenly in three directions over the entire surface of the agar plate to obtain a uniform inoculum. A final sweep was made around the agar rim with the cotton swab. This plate was then allowed to dry for 3 to 5 minutes, before the discs were applied. Antibiotic impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface. Within 15 min after the discs were applied, the plates were inverted and placed in an incubator at 37°C. After 16 to 18 h of incubation, the plates were examined and zone size was measured.

3. Results and Discussion

3.1. Isolation of *Listeria spp.* from food and water samples

Among 57 collected samples, bacterial isolates showing black halo colonies typical of *Listeria* sp. were obtained from 15 samples: in particular 12 (30%) of 40 food samples and 3 (17.65%) of 17 water samples. No growth was obtained from pasteurized milk and borhani. Further investigation showed that all were Gram positive rod and showed similar morphology as *Listeria spp.* For further identification biochemical tests were performed which included Catalase test, Oxidase test, sugar (xylose and mannitol) fermentation test and motility test. As a positive control, *Listeria monocytogenes* ATCC 43256 strain was included in the test. Results are summarized in Table 1.

Table 1. *Biochemical properties of putative Listeria isolates*

Isolate no	Catalase	Oxidase	Sugar fermentation		Motility	
			Xylose	Mannitol	At 25^0C	At 37^0C
1	+	-	-	-	+	-
2	+	+	+	+	+	+
3	+	-	+	-	+	-
4	+	-	+	-	+	-
5	+	-	+	+	+	+
6	+	-	+	+	+	+

7	+	-	+	+	+	+
8	+	-	+	-	+	-
9	+	-	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+	-	-	-	+	-
13	+	-	-	-	+	-
14	+	+	-	+	+	+
15	+	-	+	+	+	+
Listeria monocytogenes ATCC43256	+	-	-	-	+	-

All *Listeria* sp. are catalase positive, oxidase negative, motile at 30^0C and non-motile at 37^0C. 6 out of 15 isolates showed these properties (isolate no. 1, 3, 4, 8, 12, 13, Table 1). All of these isolates failed to ferment mannitol, while only two could ferment xylose, indicating to differentiation at species level.

3.2. Identification of the *Listeria* isolates by Analytic Profile Index (API)

The six isolates that showed biochemical characteristics typical of *Listeria* sp. were further tested for identification using Listeria Analytical profile Index (API). As a positive control, *Listeria monocytogenes* ATCC 43256 was included in the test. Results from API showed that among the six isolated tested, two showed characters that matched those of *Listeria weilshmeri*, two with *Listeria innocua* and one with *Listeria monocytogenes* (Table 2). Isolate no. 13 did not match with those of *Listeria* sp.

Table 2. *Identification of the Listeria isolates by Analytic Profile Index*

Isolate no	Species
1	*Listeria innocua*
3	*Listeria welshimeri*
4	*Listeria innocua*
8	*Listeria welshimeri*
12	*Listeria monocytogenes*

Following the results of *Listeria* API, it can be concluded that this study isolated 5 (8.77%) *Listeria* sp from 57 samples tested, of which one was (1.75%) *Listeria monocytogenes*, 2 (3.5%) were *Listeria innocua* and 2 (3.5%) were *Listeria welshimeri* (Figure 1).

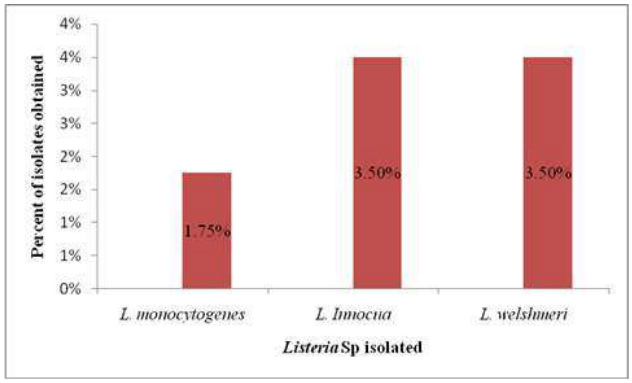

Fig 1*. Prevalence of Listeria sp in total number of samples*

When the prevalence of different species of *Listeria* were compared between the different types of samples it was observed that, among 17 water samples tested, two *Listeria* spp. (11.76%) were found of which one was pathogenic *Listeria monocytogenes* and the other was *Listeria innocua* (Figure 2). In case of 40 food samples tested, three *Listeria* spp. (7.5%) were isolated of which one (2.5%) was *Listeria innocua* (icecream) and two (5%) were *Listeria welshimeri* (raw milk and icecream) (Figure 2).

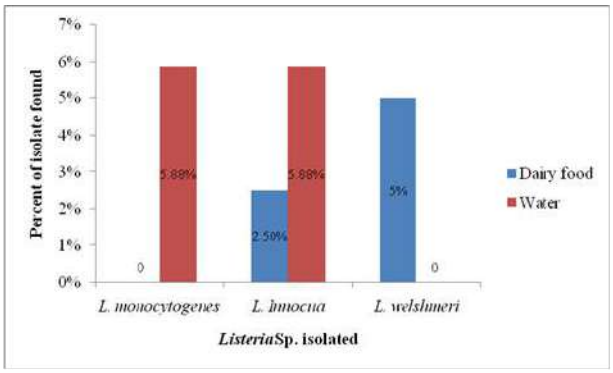

Fig. 2. *Prevalence of Listeria spp. in dairy food and water samples*

3.3. Antibiotic Susceptibility Pattern of the *Listeria* isolates

Identified *Listeria spp.* were tested for their antibiotic susceptibility against the five (5) commonly used antibiotics, which were Ampicillin (AMP), Chloramphenicol (C), Erythromycin(E), Ciprofloxacin (CIP) and Sulphamethoxazole (SXT) by disc diffusion assay (ref). The pathogenic *Listeria monocytogenes* isolate was resistant to Ampicillin and Erythromycin, while intermediate resistance of this isolate was observed against Chloramphenicol, Ciprofloxacin and Sulphamethoxazole (Table 3). Both *Listeria welshmeri* isolates were Ampicillin and Erythromycin resistant. There was difference between the sensitivity patterns of these isolates to other antibiotics tested. The *Listeria innocua* isolate did not show resistant to any of the antibiotics tested and was sensitive to Ciprofloxacin and, while intermediately resistant to other four antibiotics. When the sensitivity patterns of these isolates to different antibiotics were combined, it was observed that 60% isolates were resistant to Ampicillin and Erythromycin, 20% isolates were Sulphamethoxazole and Ciprofloxacin resistant (Fig. 3). No resistance was observed to Chloramphenicol for any *Listeria* isolates

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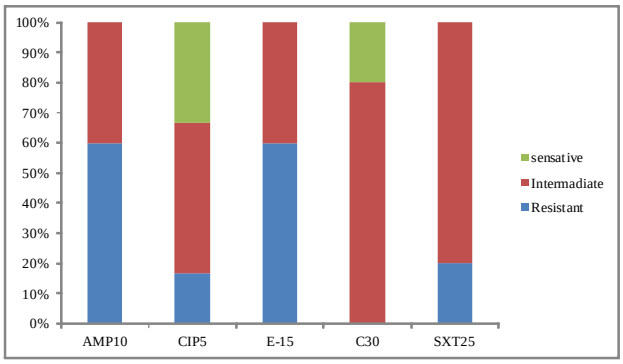
Table 3. *Antibiotic sensitivity pattern of the Listeria isolates*

Isolate	AMP-15	CIP5	E 15	C30	SXT25
Listeria innocua isolate 1	I	S	I	I	I
Listeria welshemeri isolate 1	R	R	R	S	I
Listeria innocua isolate 2	I	S	R	S	S
Listeria welshemeri isolate 2	R	I	R	I	R
Listeria monocytogenes	R	I	R	S	S



S=Sensitive, R=Resistant, I= Intermediate

Fig. 3. *Antibiotic sensitivity pattern of Listeria isolates*

AMP- Ampicillin, C-Chloramphenicol, E-Erythromycin, CIP-Ciprofloxacin and SXT-Sulphamethoxazole.

Among 40 dairy food samples, three samples (7.5%) were positive for *Listeria* spp., two from icecream and one from raw milk. Of these two were *Listeria welshimeri* (5%), one from raw milk and the other from one icecream sample. Another species isolated was *Listeria innocua* (2.5%), which was isolated from another icecream sample. Similar prevalence (6.397%) of *Listeria* sp. in dairy food products and environmental samples has been reported in a study conducted in Istanbul, Turkey (Atil. *et al*, 2011). In another study, the overall prevalence of *Listeria* in dairy food was 7.2%, of which *L. innocua* was the most commonly recovered species (66.6%) (Rahimi *et al.*, 2012).

Among 17 water samples, 2 (11.764%) samples were positive for *Listeria* spp, of which one was *Listeria innocua* and one was *Listeria monocytogenes.* Isolation of *Listeria* in water in low prevalence has been reported by Atil, *et.al*, (2011), who found 1 (0.8%) *Listeria monocytogenes* and 2 (1.5%) *Listeria innocua* from 132 water samples tested. The presence of *Listeria monocytogenes* in water in the current study indicates to contamination of water by fecal materials of animals, birds or human. In Bangladesh, water-borne infection is a major health issue and presence of *Listeria monocytogenes* imposes additional threat to public health. It also suggests that further study of water bodies in Bangladesh is necessary for investigating the prevalence of pathogenic *Listeria monocytogenes.*

Resistance of pathogenic as well as non-pathogenic bacteria to antibiotics has become a concern for last few decades and this problem is intensifying worldwide. Several studies report the isolation of multi-drug resistant pathogenic and non-pathogenic *Listeria* sp. (Conter *et al.*, 2009; Jamali *et al.*, 2013; Gomez *et al.*, 2014). Antibiotic resistance not only interferes with effective treatment measure, the antibiotic resistance gene pool in bacteria facilitates horizontal transfer of these genes among different bacterial strain that poses a huge threat to humankind. It was observed that the pathogenic *Listeria monocytogenes* was resistant against ampicillin and erythromycin and had intermediate susceptibility to Ciprofloxacin. Arslan and O¨ zdemir (2008) isolated *L. monocytogenes* that was resistant to 2 antimicrobials among 11 tested. In another study, 89% of *L. monocytogenes* isolates were sensitive to 14 different antibiotics. Resistance to ampicillin is significant, as it is a first choice of antibiotic in

listeriosis treatment for humans (Conter *et al*., 2009). 100% susceptibility of *Listeria* sp. to chloramphenicol has also been reported by Marian *et al*., 2012.

Listeria welshimeri isolate obtained in this study was most resistant against antibiotics, while *Listeria innocua* was not resistant against any tested antibiotics. McDowell *et al*., (2000) observed resistance to one or two antibiotic tested among 0.6% *Listeria monocytogenes*, while no resistance was observed among *Listeria seeligeri or Listeria welshimeri.* Therefore it seems like antibiotic resistance has not become such big threat in *Listeria* sp, though much more investigation is needed to draw any conclusion.

4. Conclusion

Outbreaks of Listeriosis in some countries, caused by consumption of milk and dairy products contaminated with *Listeria monocytogenes*, indicates the risk and danger to consumer health. The purpose of the present study was to find out the prevalence of *Listeria spp.*, in particular the pathogenic *Listeria monocytogenes* from dairy food products and water samples. Detection of *Listeria* sp in food samples indicate the overall standard of sanitation and hygiene in food processing in Bangladesh is very poor. Presence of *Listeria monocytogenes* in water sample is of particular concern for public health and suggests for further detailed investigation of food products as well as environmental samples in Bangladesh.

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Survey the Polyphenol Extraction from *Artocarpus Altilis* of Vietnam by Enzymatic Method

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Abstract:

Polyphenols has potential applications asantioxidant component that obtained good application in the food processing, medicine and cosmetic. *Artocarpus altilis* is a very common plant in Asian country and in Vietnam. The leaves of this plant contain high polyphenols. The polyphenols extraction was surveyed, with pectinase and cellulase using. The experiment was conducted according to a central composite design, with the following variables: with pectinase, solvent pH (pH-P, 3.0–5.0), the concentration of pectinse (C-P, 0.1-0.3%), solvent temperature (ST-P, 30–50 °C) and extraction time (ET-P, 20–40 min); with cellulase, solvent pH (pH-C, 4.0–6.0), the concentration of cellulase (C-C, 0.1-0.3%), solvent temperature (ST-C, 40–60 °C) and extraction time (ET-C, 20–40 min).

*Artocarpus altilis*were ground with the solvent, vacuum filtered, centrifuge in 20 min with 6000 rpm and evaluated for polyphenol contents. A highly significant model was obtained for polyphenol extraction efficiency (PEE), which was positively affected by ST-P (ST-C) and C-P (C-C), and inversely affected by pH. The most extraction conditions with pectinase were pH, 4.0; C-P, 0.1%; ST-P, 40 °C and ET-P, 25 min; at such conditon, 68.607 mg/g raw material was extracted. The most extraction conditions with cellulase were pH, 5.0; C-C, 0.2%; ST-C, 50 °C and ET-C, 25 min; at such condition, 131.927 mg/g raw material was extracted.

Keywords: *Artocarpus altilis*, polyphenols, pectinase, cellulase, antioxidant

1. Introduction

Sake (*Artocarpus altilis*) is under the Moraceae, grown in the tropical area of Southeast Asia and the Pacific Islands. Sake is the big trees with the high about 15 - 20m [1], [3], [7].

Pradhan et al. (2013) [5] have published about the presence of phenolic compounds and terpenoids in sake via using ethyl acetate and mathanol to extract.

Polyphenols are known as an antioxidant, found in onions, cocoa, tea, apples,… According to the American Cancer Society, polyphenols help prevent the risk of cancer, high blood pressure, heart disease and diabetes. By chromatography and spectroscopic methods, scientists have isolated and identified the compounds in sake, such as flavonoids, triterpene, chalcone, stilbene ... [2]. Flavonoids are an important group of polyphenol compounds and biological activity. Therefore, the study of extraction of phenolic compounds with biological activity in food applications will be highly beneficial.

Some recent studies have also published polyphenol extraction by using pectinase and cellulase. [4].

In this study, we extracted polyphenols from the leaf of sake by using pectinase and cellulase.

2. Materials and Methods

Materials: Sake leaves that were used in this study were collected from Vinh Loc Industrial Park, Binh Chanh, Vietnam

Basic extraction: Material was extracted in water with enzyme pectinase/cellulase, solution was vacuum filtered, centrifugal in 20 min with 6000rpm/min.

Survey the effect of pectinase enzyme on the extraction: four surveys were done: extraction temperature (30, 35, 40, 45, 50^{0}C); extraction time (20, 25, 30, 35, 40 minutes); pH (3, 3.5, 4, 4.5, 5); enzyme concentrations (0.1, 0.15, 0.2, 0.25, 0.3%).

Survey the effect of cellulase enzyme on the extraction: four surveys were done: extraction temperature (40, 45, 50, 55, 60^{0}C); extraction time (20, 25, 30, 35, 40 minutes); pH (4, 4.5, 5, 5.5, 6); enzyme concentrations (0.1, 0.15, 0.2, 0.25, 0.3%)

Methods of analysis: the quantification of total polyphenols by the Folin - Denis method [6]

Data analysis: All experiments were repeated 3 times. Data is processed by ANOVA - one way using statistical software Statgraphics Centurion XV to test differences between pairs of samples with a significance level of 5%.

3. Results and Discussion

3.1. Effect of temperature on the extraction

Fixed time of 20 min, pH 4.5 (pectinase), pH 5 (cellulase), 0.15% enzyme concentration, the ratio of raw material/water solvent: 1:8. Pectinase enzyme changes in temperature: 30^{0}C, 35^{0}C, 40^{0}C, 45^{0}C, 50^{0}C (enzyme cellulase: 40^{0}C, 45^{0}C, 50^{0}C, 55^{0}C, 60^{0}C). The results was shown in Figure 1.

The velocity of the enzyme reaction will increase with temperature, but it increases only to a certain limit.

The highest polyphenol content at 40^{0}C when using the enzyme pectinase and 50^{0}C with cellulase enzyme.

3.2. Effect of the extracted time on the extraction:

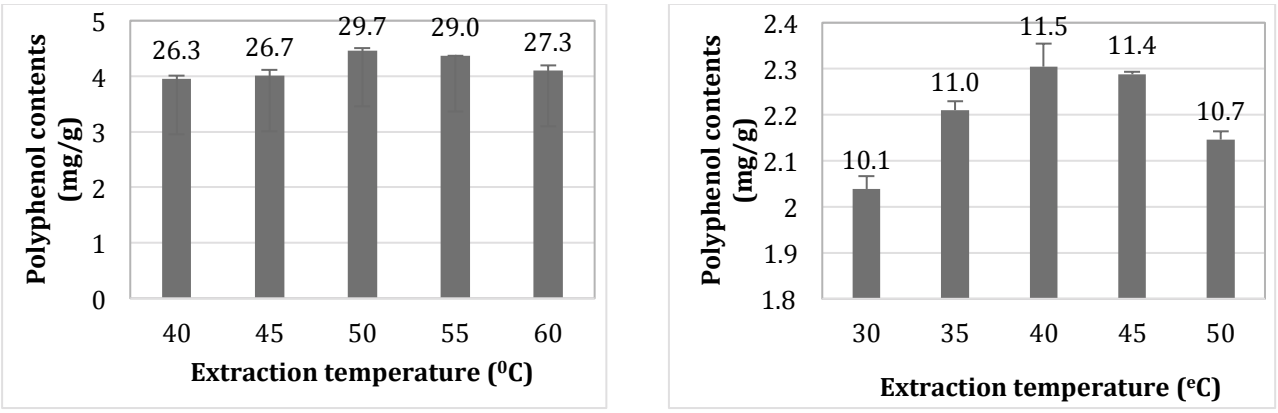
Fixed pH 4.5 (pectinase), pH 5 (cellulase), 0:15% enzyme concentration, the ratio of raw material/water solvent: 1:8, temperature extract of pectinase enzyme 40^{0}C (50^{0}C cellulase enzyme). Changing time of 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes. The results were shown in Figure 2.

Increasing extraction time, the reaction rate increases. Polyphenol content increased slightly from 25 to 30 minutes. Polyphenols are only stable in acid environment, and unstable in base environment. So there should be appropriate extraction time for enzyme reaction.

3.3. Effect of the extracted pH on the extraction

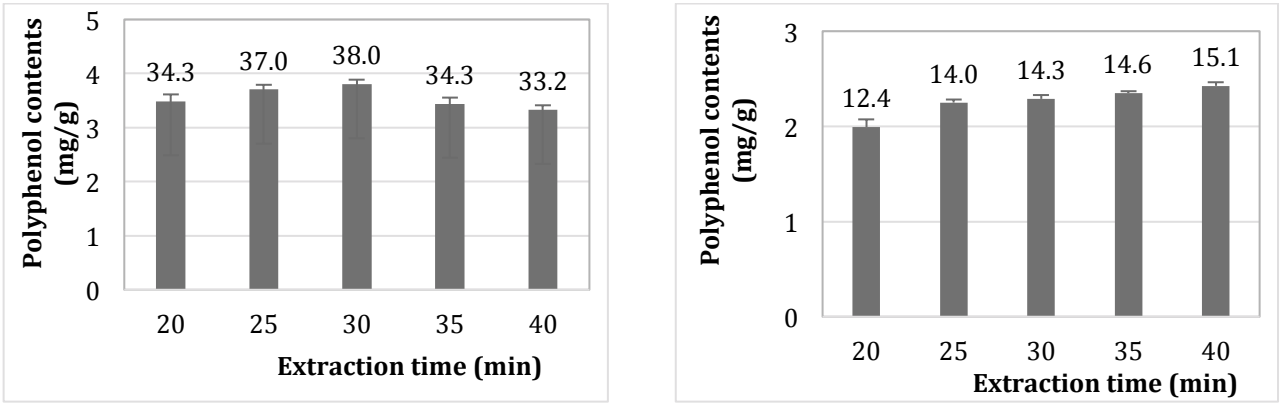
Fixed time of 25 minutes, 0:15% concentration of enzyme, the ratio of raw material/water solvent: 1:8, temperature extract of pectinase enzyme 40^{0}C (50^{0}C cellulase enzyme), pH changes: 3; 3.5; 4; 4.5; 5 (cellulase: 4, 4.5, 5, 5.5, 6). The results were shown in Figure 3.

pH affects to reaction of the enzyme and durability. Polyphenol content between different pH values is not much difference. The highest polyphenol content at pH 4 (enzyme pectinase), pH 5 (enzyme cellulase).



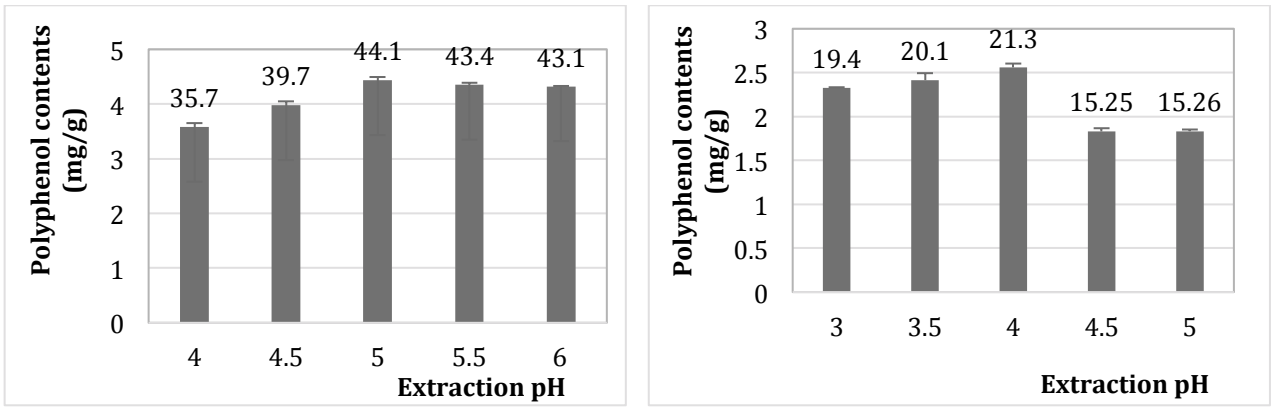
(a) (b)

Fig 1. *Effect of temperature to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)*



(a) (b)

Fig 2. *Effect of extraction time to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)*



(a) (b)

Fig 3. *Effect of pH to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)*

3.5. Effect of the concentration of enzyme on the extraction

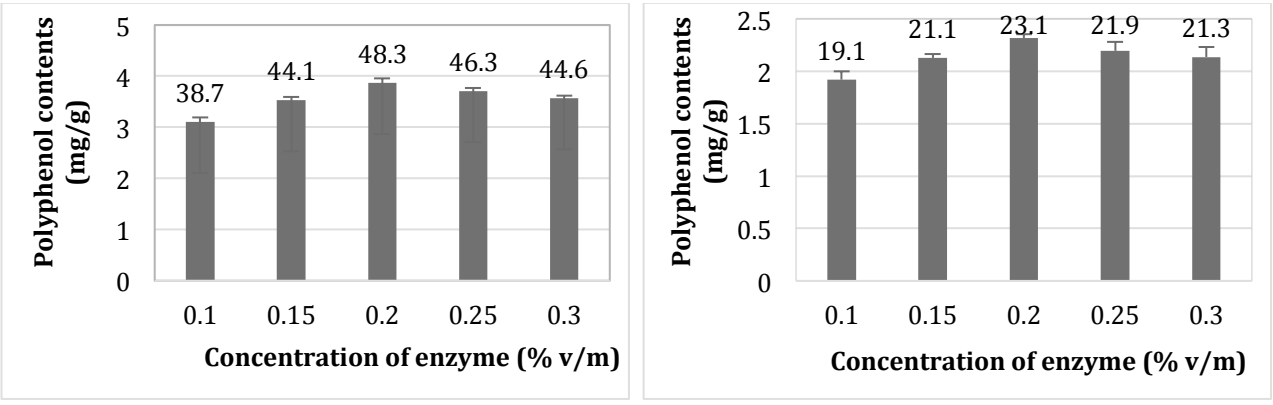
Fixed time of 25 minutes, pH 4 (pectinase), pH 5 (cellulase), the ratio of raw material/water solvent: 1:8, temperature extract of enzyme pectinase 40^0C (50^0C enzyme cellulase), Changing enzyme concentration of 0.1%, 0.15%, 0.2%, 0.25%, 0.3%. The results was shown in Figure 4.

Extractable polyphenol content corresponding to the enzyme concentration is not much difference. Increasing concentrations of 0.1 - 0.2%, the concentration of polyphenols increased and decreased slightly at the next level.

When excess substrate, the reaction rate increased with increasing concentrations of enzyme but when the concentration of enzyme saturation with substrate concentration, reaction rate did not change or not increased with increasing enzyme concentration.

3.6. Effect of the extracted time on the extraction

Total 4 times extraction using pectinase enzyme, polyphenol content obtained is 68.607mg/g, and when using enzyme cellulase with 4 times extraction, polyphenol content obtained is 131.927mg/g.



(a) (b)

Fig 4. *Effect of concentration of enzyme to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)*

4. Conclusion

In this study we found that temperature, time, pH, enzyme concentration are the factors that is affecting the extraction process.

The extraction method using enzyme pectinase after 4 times extraction, total obtained polyphenol content is 68.607mg/g at 40^0C temperature, time of 25 minutes, the ratio of raw material/water solvent 1:8, pH 4, concentration of enzyme 0.2%. And when using enzyme cellulase after 4 times extraction, the total obtained polyphenol content is 131.927mg/g at 50^0C temperature, time of 25 minutes, the ratio of raw material/water solvent 1:8, pH 5, concentration of enzyme 0.2%.

So using cellulase in this case will give more affection on the polyphenol extraction from sake leaves. The researched method is easy to apply in the industry. The product can be used in food industry

Acknowledgements

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Aquaponic production of lettuce (*Lactuca sativa* L.) with foliar fertilizer supplementation

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Abstract:

Lettuce is popular high value leafy vegetable grown worldwide which contains vitamins and minerals necessary for body metabolism. This study aimed to determine the growth, yield performance and postharvest qualities of lettuce (*Lactuca sativa* L.), investigate the effect of foliar fertilizers on lettuce, and evaluate the cost and return of lettuce production in aggregate aquaponic system. The experiment was conducted in a Randomized Complete Block Design using lettuce as test crop with the following treatments: T1 – no supplementation, T2 – supplemented with effective microorganism (EM), T3 – supplemented with algafer foliar fertilizer, T4 – supplemented with mega yield foliar fertilizer. Lettuce was harvested after 23 days transplanting with 100 percent survival rate and all the harvested plants were marketable. The growth parameters of lettuce were not significantly affected by the experimental treatments except on lettuce supplemented with algafer that produced more number of leaves but has the shortest roots. The free radical scavenging activity and pigment contents of lettuce showed no significant difference in all treatments. Plant tissue analysis of lettuce also indicated no significant difference in terms of percent nitrogen, phosphorous and potassium. The cost and return analysis has indicated lower profit of vegetable production in treatment without foliar fertilizer supplementation but higher net income in algafer supplementation. This revealed that sustainable production of lettuce using an aggregate aquaponic system is inevitable and profitable.

Keywords: aggregate, aquaponics, lettuce, foliar fertilizer, free radical scavenging activity, pigment

1. Introducion

Several technologies in producing quality and safer vegetables have been identified and promoted. With these, aquaponic production of vegetables has been anticipated as a system promoting of low cost production of leafy vegetables with fishes accompaniment. Aquaponic is the combination culture of fish and plants in recirculating systems [8,2] in which nutrients are excreted from the fish and use by the microorganism for breaking down of organic wastes into available form of nutrients and absorbed by plants cultured hydroponically [9]. Since this waste product is toxic to aquatic life when it accumulated in higher concentration, however this harmful effect can be minimized through integration of crops diversification to aquatic life [10]. In this system, the effluent circulates and sustains nutrients, which are needed to the growing crop. Usually, tilapia fish was adapted in aquaporin system because it is tolerant of fluctuating water conditions such as pH, temperature, dissolved oxygen, and dissolved solids [2,3]. In aquaponics system, it is possible to produce a constantly rotating supply of fresh, organic vegetables with minimal effort and expenditure. In addition, better yield performance and postharvest qualities can be attained as well with lettuce and pakchoi production in aggregate hydroponic system using novel nutrient solutions [1]. Most of the crops grown in aquaponic system are leafy and fruit vegetables [4]. Lettuce [7,10,15], basil [8,9] and herbs [3] are grown best in aquaponic system. In addition fruiting plants such as cucumber [12,14,15], tomato [15] and pepper will grow, though aquaponic production of fruit vegetables is not sufficient in supplying all the nutrient requirements needed by the plants for higher production [3]. One of the nutritious leafy

vegetables is lettuce, which provides minerals and nutrient that are essential for body metabolism. It contains calories, sodium, carbohydrates, dietary fiber, sugar, protein, vitamin A, vitamin C, and calcium [13] which help body metabolism and cultivated in different ways.

Different technologies have evolved for the production of lettuce. It is an important challenge nowadays to produce safe and quality round year vegetables to sustain the food requirement of a rapidly growing populace. Vegetable industries face difficulty in attaining the optimum requirement for production amidst urbanization. It is very difficult to attain the necessary food requirement with conventional field production due to environmental problems such as heavy rainfall, pest infestation, and climate change. These may lead to the fluctuation of supply and prices of the commodity in the market.

Recently, demand for safe and high quality foods with low cost cultivation and production systems is increasing [6]. This is highly preferred for the sustainability of vegetable production. This study was conducted in order to assess the growth, yield performance, and postharvest qualities of lettuce in aquaponic system supplemented with foliar fertilizers. The study was done at the Vegetable Research Area of the Department of Horticulture, College of Agriculture and Food Science, Visayas State University, Visca, Baybay City, Leyte, Philippines. It aims to determine the growth, yield performance, effect of foliar fertilizers' supplementation, postharvest qualities and the cost and return of lettuce under aggregate aquaponic system.

2. Materials and Methods

Preparation of fish tank

The fish tank was made of plastic barrel with a volume capacity of 150 liters. Three barrels were used and each barrel served as a replication of the study. A rectangular cut was done at the side of the barrel to serve as entrance and discharge of water. The tanks were cleaned properly to get rid of the odor and undesirable particulate matter. Each tank was filled up with 120 L of water prior to the introduction of tilapia (*Oreochromis sp*) fish. Each tank was provided with an aerator to provide oxygen supply for the fish in the aquaculture.

Rearing of fish

Sixty (60) pieces of two-month-old tilapia fish with average weight of 50 grams and length of 6 cm were introduced into each tank. The fishes were feed two times daily with commercial feed grower pellet at a rate of 3-4 grams per tank. The commercial pellet has a guaranteed analysis of crude protein (31%), crude fat (6%), crude fiber (6%), ash (12%) and moisture content (12%).

Storage tank

The storage tank was made of plastic container. The effluent from the fish-rearing tank was manually transferred to the storage tank at 1 week interval. This solution served as fertilizer to the growing plants in beds and discharges into the sump and manually applied back into the vegetable growing beds.

Bed and medium preparation

The bedding material was made of polyvinyl chloride (PVC) pipe material. It is about 1.40 m long and was cut lengthwise along the PVC for about ¼ of the size of the pipes. There were 24 pieces of PVC pipes used in the study. These served as the container of the aggregates composed of fine river sand and coco coir for the plant to grow. The hauled river sand was strained to acquire more or less uniform sizes. The strained sand particles were washed thoroughly with flowing water to eliminate the undesirable salts. On the other hand, coco coir was soaked in water for 1 day and then dried by air. The dried coco coir was sterilized for at least an hour to eliminate harmful microorganism. The mixture was composed of 3 parts fine river sand and 1 part coco coir by volume.

Lettuce seedlings preparation

Lettuce (Romaine variety) vegetable was used in the study. The seeds were secured from the local market distributor of East-West Seed Company. The medium used for the seedling production consisted of garden soil, carbonized rice hull and vermicompost at 1:1:1 ratio. Sterilization of garden soil was done in order to eliminate harmful microorganisms that can cause diseases to the seedlings. The medium was placed in a seedling tray ready for the sowing of seeds. Seeds of the vegetables were sown individually into seedling tray. A starter solution was made by dissolving 1 tablespoon of urea (46-0-0) in a 1 gallon of water. Application was done after the leaves were fully developed. Ten days after sowing, seedlings were hardened for some times to reduce transplanting shock. Watering of the seedlings was done whenever it is necessary.

Transplanting

The hardened seedlings were transplanted into the aggregate in PVC pipes beddings for the aggregate aquaponic system. The beds were 1.40m long and 20 cm in diameter with 7 experimental plants in each bed. Each seedling was planted at a distance of 17 cm between plants and 30 cm between beds. Transplanting was done during afternoon to reduce the stress of the experimental

plants.

Experimental design and treatments

The study was arranged in Randomize Complete Block Design with four (4) treatments replicated 3 times. Data gathered were analyzed by using the analysis of variance (ANOVA). Treatment means having significant differences were subjected to Duncan's Multiple Range Test (DMRT). Statistical analysis was carried out using Sirichai Statistics version 6.07. T_1 – Lettuce grown in aggregate aquaponics system. T_2 - Lettuce grown in aggregate aquaponics system enhance with effective microorganism foliar fertilizer. T_3 - Lettuce grown in aggregate aquaponics system supplemented with "Algafer" foliar fertilizer. T_4 - Lettuce grown in aggregate aquaponics system supplemented with inorganic (Mega Yield) foliar fertilizer.

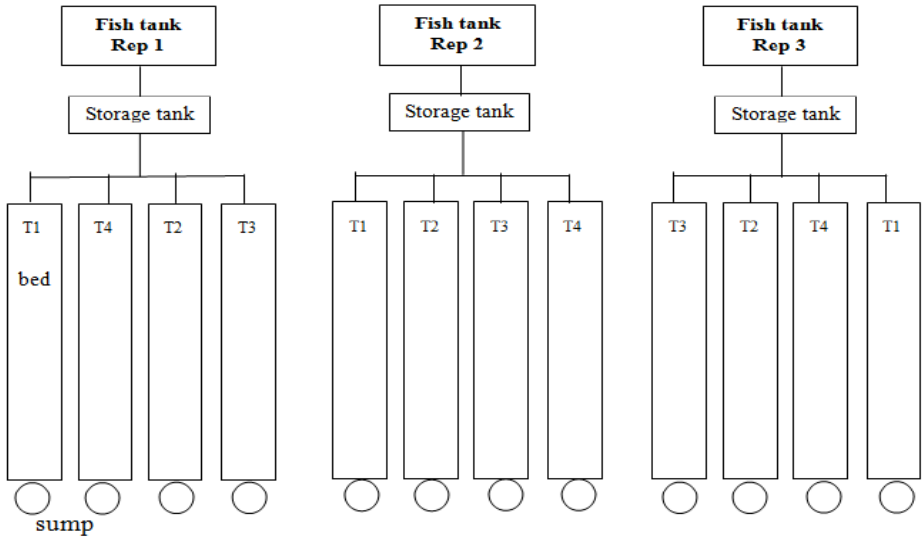

Fig. 1. *Schematic set up of the study*

Protective Structure

The existing protective structure of the Department of Horticulture in the Vegetables Area was used in the study. These were made up of improvised structure using bamboo as the skeleton within its structure with UV plastic roofing materials.

Foliar fertilizer application

"Algafer" is an organic liquid foliar fertilizer derived from marine algae which can enhance plant growth and increase crop yield while "Mega Yield" is an inorganic liquid foliar fertilizer containing essential nutrients needed for plant growth [5]. In addition, "Effective Microorganism (EM)" is a foliar fertilizer formulation containing microbial inoculant. These three foliar fertilizers were used in the study following the recommended concentrations of 1 tablespoon per liter of water for "Algafer", 8 tablespoon per 16 liters water for "Mega Yield", and 2 milliliters per liter of water for "EM". The preparation was based on the manufacturer's instruction as indicated on its label. The application of the foliar fertilizers on lettuce was done every after five days in all experimental set up three (3) days from transplanting.

Foliar Fertilizer Analysis

The analyse of the foliar fertilizers were given by its manufactory. Mega Yield foliar fertilizer has N (4%), P2O5 (7%), K2O (10%), B (2%), Cu (7%), Fe (15%), Mn (5%), Mo (5%), Zn (0.06%) and Cl (0.03). Algafer foliar fertilizer has N (11%), P2O5 (3%) and K2O (4%), trace elements, growth hormones and organic chelates.

Water effluent analysis

The water effluents from the fish tank were collected, submitted and analyzed about total nitrogen, phosphorous, potassium and magnesium at the Central Analytical Services Laboratory of PhilRootcrops, Visayas State University, Baybay City, Leyte.

Water effluent application

The stored water effluent was manually fertigate to the growing beds through a sprinkler. Before the application of nutrient water to growing beds, volume measurement was done in order to determine the total volume of water used for the whole duration of the study. Furthermore, the discharge water in the sump was applied back to the growing beds. The application of this effluent to the plants was done at least a week after storage.

Growth and yield parameters

Days from Transplanting to Harvesting. This was determined by counting the days from transplanting to harvesting.

Weight per Plant (g). This was determined by weighing the individual sample in every replication of the treatment.

Yield per Plot (g). This was determined by weighing all the plants harvested in each plot.

Leaf-Size (cm). This was determined by measuring the length and width of leaves at the time of harvest in individual sample in every replication of the treatment.

Number of Leaves. This was determined by counting the total number of leaves in every sample plant.

Plant Height (cm). This was determined by measuring the height of the plant from above surface of the medium up to the highest part of the plant.

Length of Roots (cm). This was determined by measuring the three longest roots of the sample plants at the time of harvest.

Number of Marketable and Non-marketable Plants. This was determined by counting the marketable plants and non-marketable plants harvested that were free from any damage either caused by insect or mechanical damage.

Weight of Marketable and Non-marketable Plants (g). This was determined by weighing the marketable and non- marketable harvested plants.

Percentage of Survival (%). This was determined by counting the number of dead plants per plot divided by the total number of plants in every plot times 100 as indicated in the formula.

$$\% \text{ survival} = \frac{number\ of\ dead\ plants}{total\ number\ of\ plants}\ x\ 100$$

Physico-chemical Properties of Feed Waste Solutions

Feed waste solutions from the fish tanks were collected and sent to the Research Laboratory of the Department of Pure and Applied Chemistry for pH, total dissolved solids (TDS expressed in ppm), oxidation-reduction potential (ORP expressed in mV), and electrical conductivity (EC expressed in µSiemens) tests [11].

Plant Tissue Analysis

Preparation for plant tissue analysis was done at harvest. Plant leaf was prepared, chopped, oven-dried, and submitted for N, P, and K analysis at the Central Analytical Services Laboratory of PhilRootcrops, Visayas State University, Baybay City, Leyte. Total N, P, and K in plant tissue samples were analyzed by using the Kjeldahl, Ultraviolet-visible, and atomic absorption spectrometric methods, respectively.

Determination of Free Radical Scavenging Activity

Samples in every treatment of lettuce was prepared and submitted for free radical scavenging assay at the Department of Pure and Applied Chemistry, College of Arts and Sciences, Visayas State University, Visca, Baybay City, Leyte.

Pigment Analysis of Lettuce

Samples in every treatment of lettuce was prepared and submitted for chlorophyll *a* and *b* and total carotenoids analyses at the Department of Pure and Applied Chemistry of the Visayas State University. The pigments were extracted with 95 % acetone and quantified by UV-vis spectrophotometer.

Cost and Return Analysis

The cost and return of production was determined by recording all the expenses throughout the conduct of the study and income from the crops. Gross incomes were calculated by multiplying the total weight of marketable plants with the prevailing market price of lettuce per kilogram, and do similarly with the tilapia fish. The difference between the gross income and the expenses represented the net income as shown in the formula:

$$GI = (WMP\ x\ P) + (YF\ x\ P)$$

GI – Gross income
WMP – Weight of marketable plants (Kg)
P – Price (PhP)
YF – Yield of fish (Kg)

$$NI = (GI - E)$$

NI – Net income (PhP)
E – Expenses (PhP)

3. Results and Discussion

3.1. Growth and yield parameters

Lettuce was harvested 23 days after transplanting as shown in Table 1. Harvesting time of lettuce was comparable to all treatments. This means that the treatments being employed did not affect the days to harvest of lettuce. From the result of the study, lettuce has 100 percent survival rate, which implies that treatments have no detrimental effect to the growth of lettuce and thus all were found marketable. This means that the foliar supplementation is unnecessary considering the better or the best management of the crop.

Table 2 shows the plant height, root length and number of leaves of lettuce grown in aggregate aquaponic system with foliar fertilizer supplementation. There was no significant difference of plant height of lettuce among treatments. This means that the use of fish feed waste without foliar fertilizer supplementation is sufficient enough to produce lettuce plants with desirable height. This also illustrates the availability of nutrient in fish feed waste available for plant uptake.

The length of roots in lettuce was highly significantly different among treatments. The longest root was observed on plants supplemented with "Mega Yield" foliar fertilizer, but comparable with those lettuce plants that received no supplementation. Lettuce plants supplemented with algafer had the shortest roots. The lettuce plants that received foliar fertilizer supplementation showed greater number of leaves than the lettuce plants without supplementation, which is statistically different. This can perhaps be explained by the contribution of foliar fertilizer application, which contain nutrient elements on lettuce plants. Mega yield foliar fertilizer has N (4%), P2O5 (7%), K2O (10%), B (2%), Cu (7%), Fe (15%), Mn (5%), Mo (5%), Zn (0.06%) and Cl (0.03) and Algafer foliar fertilizer has N (11%), P2O5 (3%) and K2O (4%) necessary for plant growth.

Table 3 shows the leaf size, weight per plant and yield per plot of lettuce grown in aggregate aquaponic system with foliar fertilizer supplementation. No significant difference was found on lettuce plants in terms of leaf size, weight per plant, and yield per plot among treatments. This only shows that growing lettuce in aggregate aquaponic system is sustainable in terms of leaf sizes and yield. In addition, this system is more profitable by removing other expenses such as foliar fertilizer supplementation.

Table 4 shows the marketable weight and free radical scavenging activity of lettuce grown in aggregate aquaponic system with foliar fertilizer supplementation. As it was revealed, no significant difference was observed in lettuce plants in terms of marketable weight and FRSA. This contradicted with the findings of others that free radical scavenging activity is influenced by an enhanced plant biomass from organic fertilization (Personal communication with Dr. Felix M. Salas).

Table 1. *Days from transplanting to harvesting, percent survival and number of marketable plants of lettuce as affected by foliar supplementation in aggregate aquaponic system*

Treatment	Days from Transplanting to Harvesting	% Survival	Number of Marketable Plants
No supplementation	23	100	5
Enhanced with effective microorganism	23	100	5
Supplemented with "Algafer"	23	100	5
Supplemented with "Mega yield"	23	100	5

Table 2. *Plant height, root length and number of leaves of lettuce as affected by foliar supplementation in aggregate aquaponic system*

Treatment	Plant Height (cm)	Root Length (cm)	Number of Leaves
No supplementation	27.30	16.71 ab	18.67 b
Enhanced with effective microorganism	27.83	15.05 bc	19.40 a
Supplemented with "Algafer"	28.61	13.55 c	20.53 a
Supplemented with "Mega yield"	27.25	17.90 a	19.00 a
CV (%)	5.27	6.26	2.79

Means in a column with no letter designation are not significantly different from each other based on 5% level of significance in DMRT

Table 3. *Leaf size, weight per plant and yield per plot of lettuce as affected by foliar supplementation in aggregate aquaponic system*

Treatment	Leaf size (cm)		Weight per plant (g)	Yield per plot (g)
	Length	Width		
No supplementation	20.30	10.78	86.07	430.33
Enhanced with effective microorganism	21.23	11.21	91.53	457.67
Supplemented with "Algafer"	21.06	11.14	97.87	489.33
Supplemented with "Mega yield"	20.47	10.84	89.73	448.67
CV (%)	4.33	4.7	10.72	10.72

Means in a column with no letter designation are not significantly different from each other based on 5% level of significance in DMRT

Table 4. *Marketable weight and free radical scavenging activity of lettuce as affected by foliar supplementation in aggregate aquaponic system*

Treatment	Marketable weight (g)	Free radical scavenging activity (µmol/100g)
No supplementation	430.33	318.50
Enhanced with effective microorganism	457.67	329.04
Supplemented with "Algafer"	489.33	325.31
Supplemented with "Mega yield"	448.67	298.90

CV (%)	10.72	5.49

Means in a column with no letter designation are not significantly different from each other based on 5% level of significance in DMRT

Table 5. *Pigment contents of lettuce as affected by foliar supplementation in aggregate aquaponic system*
Means in a column with no letter designation are not significantly different from each other based on 5% level of significance in DMRT

Treatment	Chlorophyll		Carotenoids (ppm)
	a **(ppm)**	***b*** **(ppm)**	
No supplementation	22.84	29.90	2123.33
Enhanced with effective microorganism	25.58	25.55	2430.00
Supplemented with “Algafer”	25.43	30.87	2293.33
Supplemented with “Mega yield”	24.72	23.76	2403.33
CV (%)	9.24	22.42	12.89

Table 6. *Tissue analysis of lettuce as affected by foliar fertilizer supplementation in aggregate aquaponic system*

Treatment	%Nitrogen	% Phosphorous	% Potassium
No supplementation	4.88	0.05	7.93
Enhanced with effective microorganism	4.68	0.05	8.85
Supplemented with “Algafer”	5.08	0.05	9.00
Supplemented with “Mega yield”	5.11	0.05	10.45
CV (%)	7.02	2.30	6.44

Means in a column with no letter designation are not significantly different from each other based on 5% level of significance in DMRT

Table 7. *Overall expenses and net income (PhP) of growing lettuce as affected by foliar supplementation in aggregate aquaponic system*

Treatment	Yield/sample (g)	Expenses (PhP)	Net income (PhP)
No supplementation	86.07	868.99	1,476.93
Enhanced with effective microorganism	91.53	971.57	1,466.19
Supplemented with “Algafer”	97.87	982.77	1,561.39
Supplemented with “Mega yield”	89.73	973.97	1433.55

Note: 168 plants
Price: lettuce – 100/kg

Table 5 shows the pigment contents of lettuce grown in aggregate aquaponic system with foliar fertilizer supplementation. There was no significant difference between treatments. This means that the pigment development in lettuce plants is not significantly influenced by foliar fertilizer supplementation and the visual quality of lettuce derived from pigmentation is well afforded by aggregate aquaponic system of production. Moreover, this no significant difference of lettuce plants in terms of pigment contents can also be used to explain the result on FRSA of lettuce plants grown in aggregate aquaponic system with foliar fertilizer supplementation.

3.2. Plant Tissue Analysis

Table 6 shows the tissue analysis of lettuce as affected by foliar fertilizer supplementation in aggregate aquaponic system. There was no significant difference between treatments. This means that there was no important contribution of foliar fertilizer with respect to nitrogen, phosphorus, and potassium contents in the tissue of lettuce.

3.3. Cost and Return Analysis

Table 7 shows the overall expenses and net incomes of growing lettuce as affected by foliar fertilizer supplementation in aggregate aquaponic system. Lettuce grown supplemented with foliar fertilizer has higher expenses compared to other treatments. This was due to additional inputs such as effective microorganism and foliar fertilizer, which is considered, added cost in growing lettuce. Lettuce grown without supplementation has lower net income compared to treatment supplemented with algafer. This may be due to the lower weight per plant and lesser number of leaves. With foliar supplementation higher net income was obtained due to production of heavier weight per plant of lettuce. Thus, algafer supplementation has the highest net income. In as much as lesser expenses can be incurred in growing lettuce without supplementation but would still produce comparable yield, then it safe to infer that growing these vegetables in aggregate aquaponic system is potentially adaptable.

4. Conclusion

From the result of the study, the following conclusions were drawn:

1. The growth, yield performance, and postharvest qualities of lettuce grown under aggregate aquaponic system without supplementation were the same as that with foliar fertilizer supplementation.

2. The production of lettuce under aggregate aquaponic system was not affected by organic foliar fertilizer supplementation; and
3. Aggregate aquaponic system is economically viable and has a potential in the production of lettuce in the country because it is cheap, easy to handle, friendly to environment and the net income was just comparable with foliar fertilizer supplementations.

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Minor millets cultivation for agricultural sustainability and nutritional security

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Abstract:

Many people's livelihood security depends on the sustained management of various biological resources that are important for food and agriculture. Selective utilization of crops and varieties in recent times have threatened agro biodiversity leading to rapid erosion of natural resources and consequently affecting the nutritional security of people. One of the possible pathways for conservation of such neglected agro biodiversity resources is to bring them into use thereby making them viable crops within the contemporary social and economic context. Agro biodiversity is the result of the interaction between the environment, genetic resources and management systems and practices used by culturally diverse peoples. The management of agro biodiversity by the indigenous communities is for a variety of reasons. The land use types, land use stages, aspect, soil fertility, availability; of water and quality seed of crops etc. are essential variables which an indigenous farmer considers while cultivating a diversity of traditional landraces in the farm for food security. India is the largest producer of many kinds of millets, which are often referred as coarse cereals. However, realizing the nutrient richness of these grains they are now considered as nutria-cereals. Small millets, as a group includes several grain crops namely finger millet (ragi), proso millet, barnyard millet, Italian millet, kodo millet and little millet. In the four decades since 1961, the area under millets declined by nearly 50% from about 18 million hectares to about 9 million hectares. During this time, production of millets declined from about 8.8 million tons to about 7.2 million tons; a decline of 18%. Tremendous decrease in area and production is directly or indirectly resulted in the loss of agro biodiversity of these crops as these crops are grown on marginal or rained areas without any fertilizers. Therefore, more emphasis should be given for maintaining bio diversity of these highly rich crops by increasing area under these minor millets and developing proper agro techniques for increasing its productivity.

On the other side, India ranks second in the incidences of malnutrition among children and more than one third of the world's malnourished children live in India. Therefore, there is an urgent need to increase production of these crops as these are good source of calcium, iron, potassium, magnesium and other micronutrients. Among small millets, ragi has about 10 times more calcium than wheat or rice. These crops also have the potential for tackling the hidden hunger caused by micronutrient deficiencies but, they are grown in marginal land with improper management cultural practices. Therefore need of hour is to conserve these underutilized crops for food security and also for sustaining the agro-diversity. Traditional farming systems need to be strengthened to achieve the goals of sustainable development and biodiversity conservation.

Keywords: Agro biodiversity, conservation, sustainability, food security and minor millets

1. Introducion

Agro-biodiversity can be understood as the diversity within and among species found in an agro-ecosystem that contribute to food and agriculture, including domesticated biodiversity i.e., the diversity of crops and livestock genetic resources as well as all other plant and animal genetic resources i.e. crop wild relatives [10, 12, 14]. On the other hand, sustainability refers to, the properties and assets of a system that sustain the ability of agents to adapt and meet their needs in

new way [10]. In present context, in the period of drastic change and increasing uncertainty, the adaptability of a system plays a major role. Selective utilization of crops and varieties in recent times have threatened agro biodiversity leading to rapid erosion of natural resources and consequently affecting the nutritional security of people. One of the possible pathways for conservation of such neglected agro biodiversity resources is to bring them into use thereby making them viable crops within the contemporary social and economic context. Farmers may have to change their farming systems by switching to new crop species/varieties or livestock breeds that are better adapted to the new conditions. Agro biodiversity can thus be seen as a crucial asset to keep multiple options open, sustaining the ability to rapidly adapt and transform farming systems under unpredictable future conditions [10].

The small farmers, particularly in regions of rich agro-biodiversity immensely contribute to the on-farm conservation and enrichment of this diversity, often at personal cost. The agricultural progress in the past and present would not be possible if these genetic resources and associating knowledge were not conserved by the farmers. In the four decades since 1961, the area under millets declined by nearly 50% from about 18 million hectares to about 9 million hectares. During this time, production of millets declined from about 8.8 million tons to about 7.2 million tons; a decline of 18% [15]. Tremendous decrease in area and production is directly or indirectly due to negligence of scientists and farmers for these crops and this all resulted in the loss of agro biodiversity.

In the present scenario, lack of attention from researchers, policy makers, donors, farmers and consumers are increasingly threatening the genetic diversity of minor millets. This is an irreversible loss to those farmers, particularly who depends on these crops for their food and nutritional security and meager income generation. Therefore, there is urgent need to conserve these crops for the improving genetic diversity and sustainability of the system. Conservation of minor millets plays crucial role to the future global food and nutritional security. In addition to this, agro biodiversity conservation needs to be demonstrated and captured for the future agricultural development for sustainability of resources.

With hunger still widespread in India and distribution of rice and wheat having little impact, growing nutritionally rich crops at a household level can improve diets and curb malnutrition. And since these same households struggle with rising food prices, environmental impacts caused by climate change, and few options for new jobs, combining efforts to curb malnutrition, increase farmers' yields and expand job opportunities together with a locally grown grain proven to withstand harsh environments, means a large impact on improving their lives. Therefore, there is urgent need to conserve these crops for the improving genetic diversity and sustainability of the system. Conservation of minor millets plays crucial role to the future global food and nutritional security. In addition to this, agro biodiversity conservation needs to be demonstrated and captured for the future agricultural development for sustainability of resources.

India ranks second in the incidences of malnutrition among children and more than one third of the world's malnourished children live in India [2]. Global food security has been increasingly narrowing down to only few crops. Over 50% of the global requirement proteins and calories are met mainly by three grains, such as: maize, wheat and rice. The narrowing base of global food security is limiting livelihood options for the rural poor, particularly in marginal areas. Minor millets, which are high in nutrients such as calcium and iron, are grown primarily in hilly, arid areas of India where, because of their high tolerance to drought, they are often more productive than other grains [1]. While locally important, in the past they were rarely traded outside the farming communities because of the grain's reputation as food for the poor. More recently, farmers have turned to cash crops such as cassava, resulting in a decline in millet cultivation.

So, the farmers, scientists and the policy makers should fit in the minor millets in their cropping systems as these crops have a comparative advantage in marginal lands and can withstand stress conditions and contribute to sustainable production with low inputs at low cost of production. Thus, they can also contribute to the diversity-richness as well as to the stability of agro-ecosystems. There are hardly any alternatives to these species for their strategic role in fragile ecosystems, such as found in arid and semi-arid lands, in mountains, steppes and tropical forests.

Minor millets are drought resistant crop and can be stored for a long time without insect damage [1]; hence, it can be important during famine and therefore these are taken as "famine reserves". This aspect is very important as Indian agriculture in the context of climate change, which is the burning problem of our country at the local level. Therefore, farmers, scientists and policy makers should develop certain new eco-friendly strategies for obtaining higher production and for maintaining the agro-biodiversity of these crops.

2. Change in Scenario of Small Millets

India has the third largest area under small millets cultivation in the world, which is mainly confined to semi-arid, hilly and mountainous regions. These crops are hardy and quite resilient to

a variety of agro-climatic adversities. They are grown mostly in marginal areas under agricultural conditions in which major cereals fail to give substantial yields [1]. Presence of these minor millets has been declining in the Indian food basket over the years. From 1955-56 to 2008-09, there was drastic reduction in the area and production of small millets i.e. 83.0% decrease in area and 78.26% decrease in its production but about 26.5 % increase in productivity was observed during this period (Table 1).

The main reason of this decline is the inception of green revolution which tremendously increased the area, production and productivity of wheat crop due to release of HYV's which are high input responsive. In addition to this, during last five decades, there is an increase in the availability of other cereals like rice, maize etc (Fig.1 and Fig.2). Another reason is the negligence of farmers and scientists in its development and planning of its production techniques. The lack of modern technologies for their effective processing and utilization may also be the reasons for their declining acreage.

Table 1. *Change in area (lakh ha), production (lakh tons) and productivity of (kg/ha) of ragi and small millets from 1955-56 to 2008-09*

Particulars	1955-56	1965-66	1975-76	1985-86	1995-96	2008-09	% decrease /increase
Ragi							
Area (lakh ha)	23.7	26.9	26.3	24.01	17.74	13.81	41.7 % decrease
Production(lakh tons)	18.5	13.3	28.0	25.2	25.0	20.4	10.3 % increase
Productivity (kg/ha)	800	492	1064	1049	1410	1477	84.6% increase
Small millets							
Area (lakh ha)	53.4	45.6	46.7	31.6	16.6	9.1	83.0% decrease
Production(lakh tons)	20.7	15.6	19.2	12.2	7.8	4.5	78.3% decrease
Productivity (kg/ha)	388	341	412	386	469	491	26.5 % increase

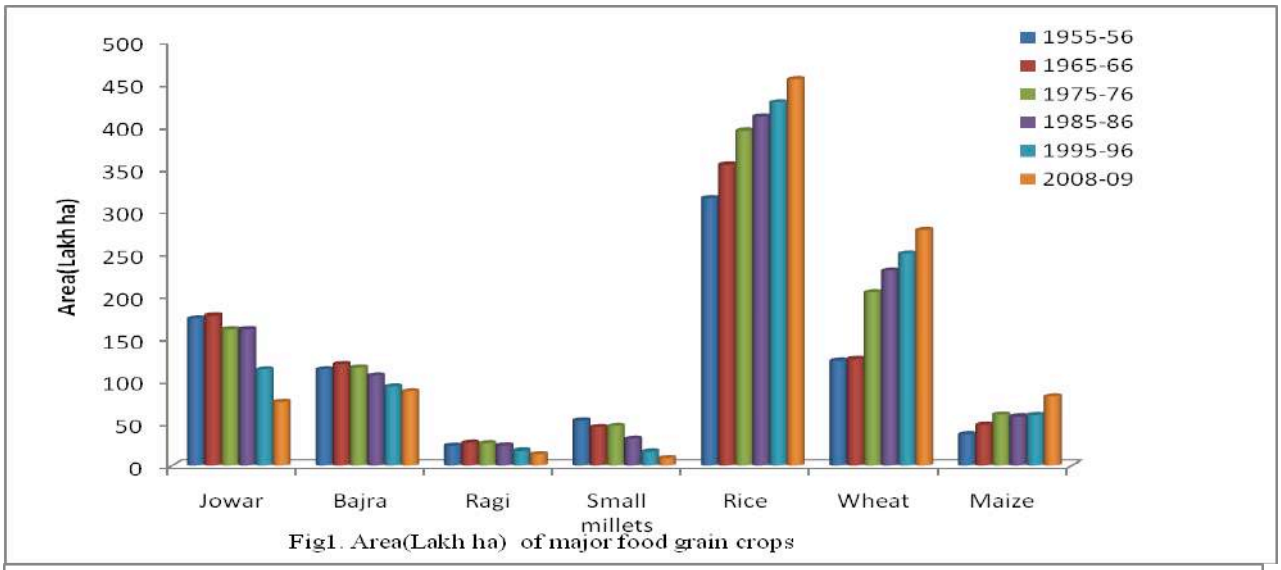

Fig1. Area(Lakh ha) of major food grain crops

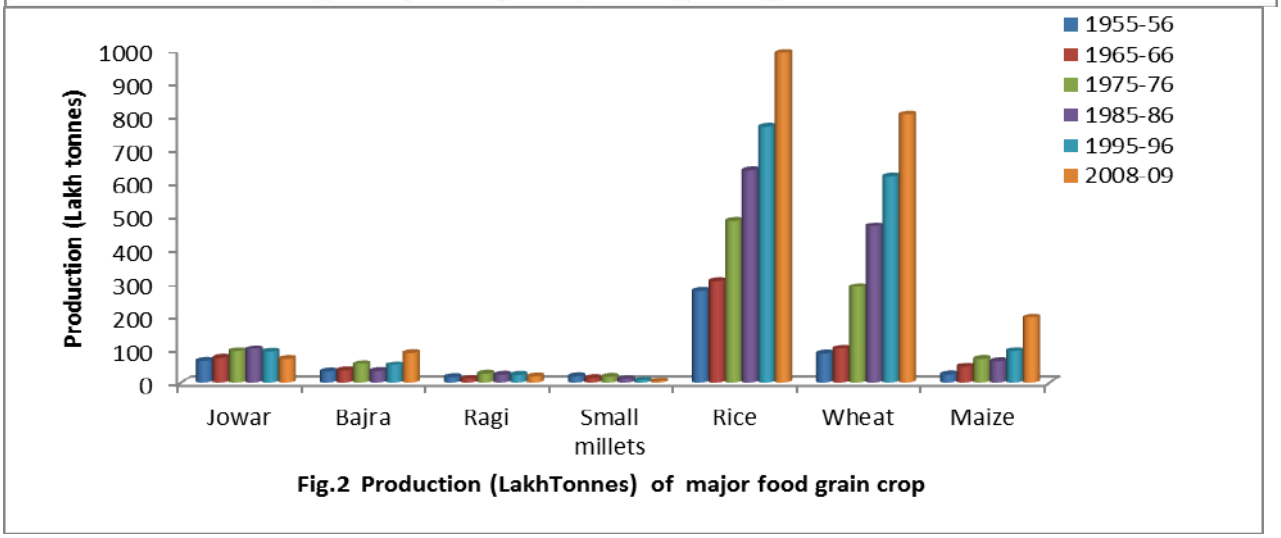

Fig.2 Production (LakhTonnes) of major food grain crop

3. Minor millets: Overview

Minor millet may be defined as millets cultivated for their small grains, which are borne on short, slender grassy plants. In other words they refer to a group of small seeded cereal crops. The most important minor millets cultivated in India are finger millet (ragi), foxtail millet, proso millet, barnyard millet, kodo millet and little millet. The detail description is as given in Table 2.

Table 2: *Common name, scientific name, area and major growing states of small millets in India*

Crop	Scientific name	Chromosome No.	Area of Domestication	Major States
Ragi (Finger millet)	Eleusine coracana	2n=36 (4x)	East Africa	M.P.,Maharashtra, Orissa, Uttarakhand, U.P. and Tamil Nadu
Foxtail /Italian millet/Kauni	Setaria italica	2n=36 (2x)	Central Asia-India	Karnataka, Rajasthan, M.P. and Chattisgarh
Proso or Common millet (cheena)	Panicum miliaceum	2n=36 (4x)	Central Asia-India	Bihar, North East and Maharashtra.
Indian Barnyard millet (Sawan)	Echinochloa frumentacea	2n=54 (6x)	India	Karnataka, M.P., Uttarakhand, U.P. and North East
Kodo millet	Paspalum scrobiculatum	2n=40 (4x)	India	Maharashtra, Tamil Nadu and Chattisgarh
Little millet/ Kutki	Panicum miliare	2n=36 (4x)	India	Karnataka, Tamil Nadu, Andhra Pradesh, M.P., Jharkhand, Orissa Maharashtra & Chattisgarh

3.1. Nutritive value of small millets

Minor millets are highly nutritive in nature, therefore these are also termed as Nutrea cereals (Table 3). Minor millets are high energy, nutritious foods comparable to other cereals and some of them are even better with regard to protein and mineral content. They are particularly low in phytic acid and rich in dietary fibre, iron, calcium and B vitamins. As the millets are consumed by the poor, they guard them against food and nutritional insecurity imposed by various agronomic, socio economic and political factors. Minor millets can thus act as a shield against nutritional deficiency disorders and provide nutritional security. Therefore minor millets could be a better option for overcoming this problem of malnutrition in India

Table 3 *Nutrient composition of small millets and other cereals (per 100 g edible portion; 12 % moisture)*

Crops	Food Protein (g)	Fat (g)	Ash (g)	Crude fibre (g)	Carbo-hydrate (g)	Energy (kcal)	Ca (mg)	Fe (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)s
Rice (brown)	7.9	2.7	1.3	1.0	76.0	362	33	1.8	0.41	0.04	4.3
Wheat	11.6	2.0	1.6	2.0	71.0	348	30	3.5	0.41	0.1	5.1
Maize	9.2	4.6	1.2	2.8	73.0	358	26	2.7	0.38	0.2	3.6
Sorghum	10.4	3.1	1.6	2.0	70.7	329	25	5.4	0.38	0.15	4.3
Pearl millet	11.8	4.8	2.2	2.3	67.0	363	42	11.0	0.38	0.21	2.8
Finger millet	7.7	1.5	2.6	3.6	72.6	336	350	3.9	0.42	0.19	1.1
Foxtail millet	11.2	4.0	3.3	6.7	63.2	351	31	2.8	0.59	0.11	3.2
Proso millet	12.5	3.5	3.1	5.2	63.8	354	8	2.9	0.41	0.28	4.5
Little millet	9.7	5.2	5.4	7.6	60.9	329	17	9.3	0.3	0.09	3.2
Barnyard Millet	11.0	3.9	4.5	13.6	55.0	300	22	18.6	0.33	0.10	4.2
Kodo millet	9.8	3.6	3.3	5.2	66.6	353	35	1.7	0.15	0.09	2.0

**N × 6.25 (Source: Hulse, et al., 1980) [7]*

These crops are micro nutrient supplement crops, especially with regard to calcium and iron, and high dietary fibre [5]. Finger millets has about 8-10 times more calcium than other food crops while barnyard millet has 2-10 times more Iron as compared to other crops (**Table 3)**. The grain protein is rich in sulphur-containing amino acid and other essential amino acids than all other major cereals. For these reasons, many people depend for their nutritional, food and livelihood security on these crops. The grain is also rich in phytochemicals, including phytic acid, which is believed to lower cholesterol, and phytate, which is associated with reduced cancer risk [4]. These health benefits have been partly attributed to the wide variety of potential chemo preventive substances, called phytochemicals, including antioxidants present in high amounts in foods such as millets [8]. Health benefits of eating millets Lignans, an essential phytonutrient present in millet, are very beneficial to the human body, which act against different types of hormone-dependent cancers, like breast cancer and also help reduce the risk of heart disease. Regular consumption of millet is very beneficial for postmenopausal women suffering from signs of cardiovascular disease, like high blood pressure and high cholesterol levels. Recent research has indicated that the regular consumption of millet is associated with reduced risk of type 2 diabetes mellitus.. This is mainly due to the fact that whole grains like millet are a rich source of magnesium, which acts as a co-factor in a number of enzymatic reactions in the body, regulating the secretion of glucose and insulin. Magnesium is also beneficial in reducing the frequency of migraine attacks. It is even very useful for people who are suffering from atherosclerosis and diabetic heart disease

Millet is gluten-free, therefore an excellent option for people suffering from celiac diseases often irritated by the gluten content of wheat and other more common cereal grains. It is also useful for people who are suffering from atherosclerosis and diabetic heart disease [6]. Choi *et al.,* (2005) and Park *et al.,* (2008) concluded that proso millet protein could be a potential therapeutic intervention in type 2 diabetes [3, 11]. Devi *et al.,* (2011) review the nature of polyphenols and dietary fiber of finger millet and their role with respect to the health benefits associated with millet [5]. According to research and recent studies, consumption of millet can help women combat the occurrence of gallstones, as they are a very high source of insoluble fiber. Millets extract from the seed coat where reported to have shown high antibacterial and antifungal activity compared to whole flour extract due to high polyphenols content in seed coat [16]. Therefore, there is an urgent need to increase production of these crops, as these are good source of calcium, iron, potassium, magnesium and other micronutrients. Health benefits of eating millets

Supplementation of cereal-based products with millets has become increasingly popular due to nutritional and economic advantages. With proper preparation, 30 per cent of minor millets can be gainfully substituted in value added foods belonging to the categories of traditional foods, bakery products, extruded foods and allied mixes for the convenient preparation by rural and town folk at low cost. Poroso millet flour is used as a substitute for rice flour in various snack foods.

3.2. Limitations for growing millets

- Mostly millets are grown on marginal lands under rainfed conditions. Some of these are still grown in the hilly areas under shifting cultivation
- Lack of proper package and practices for small millets is being followed by the farmers. Seeds are mostly sown through broadcasting and no fertilizers and manures are used..
- Quality seeds of improved varieties are not available to the farmers.
- Agriculture operations like tillage, sowing, inter culturing are not done timely.
- Lack of availability of latest post harvest operations techniques.
- Marketing facilities for the disposal of surplus produce at a remunerative price is not available.

3.3. Strategies for enhancing productivity and maintaining Agro biodiversity

- Strengthening of basic and strategic research in crop improvement.
- Development of varieties tolerant to biotic and abiotic stresses.
- Strengthening the delivery system of proper technology for growing small millets.
- Strengthening of research on post harvest technologies like processing, value-added foods, medicines, nutraceuticals, bio-fuel and other products.
- Development of integrated strategy for the conservation and sustainable use of agricultural biodiversity.
- Changing the mindset of farmers and imparting information about the nutraceutical properties of the minor millets.

5. Conclusion

Since genetic diversity tends to reduce when local varieties are replaced by improved cultivars. Increasing efforts should be done to store local crop varieties and wild relatives in seed collections. Such gene banks can function as a low-cost conservation instruments in order to safeguard genetic resources for many years, thereby avoiding loss through changing environmental or economic conditions.

These crops also have the potential for tackling the

hidden hunger caused by micronutrient deficiencies but, they are grown in marginal land with improper management cultural practices. Therefore need of hour is to conserve these underutilized crops for food security and also for sustaining the agro-diversity. Traditional farming systems need to be strengthened to achieve the goals of sustainable development and biodiversity conservation.

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Optimization of parameters for extraction and conversion from color contain in achiote (*Bixa orellana* L.) into norbixin by response surface methodology

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Abstract:

Response surface method was used in this research to optimize the technical parameters of the extraction and transformation color compound in annatto seeds into norbixin. Parameters affected the extraction efficiency and norbixin recovery yield were solvent concentration, rate of solvent/seed, incubation time, and incubation temperature. The optimization results including: concentration of ethanol is 51.82^0; concentration of NaOH is 0.52M; ratio of solvent/seed is 7.1/1; incubation time is 33.12 minutes, incubation temperature is 58.6 ^{0}C, the rate of 36% HCl/color solution is 0.5/5 (v/v).

Keywords: Cellulose derivatives, Antibacterial activity, Shake flask method, MIC.

1. Introduction

Bixa orellana L. grows mostly in the tropics, is native to Central and South America, now widely grown in many tropical countries. Colonial period, *Bixa orellana* L. were imported to Vietnam, this plant grow wild or is cultivated in scattered in the South Delta, Central Highlands and central to harvest seeds, ornamental or medicine (Vo Van Chi, 1998). The color of Annatto compound that extracted by cashews'nut (*Bixa orellana* L.). Annatto is a natural color that has been widely used for a long time in the traditional cuisine of many nations in the world. In the food industry, Annatto natural color is the second most important behind the caramel and is recognized by CODEX as food coloring and color safe pharmaceuticals. Bixin and norbixin are two major compounds building to Annatto. Bixin is insoluble in vegetable oil. Norbixin soluble in water at high pH become yellow to orange colour. The chemical structure of bixin ester group should be able to convert bixin and norbixin in cashew colored sodium or potassium salt of norbixin to increase solubility in water (Le Thi Anh Dao et al, 2000; Nachtigall et al, 2009; Silva et al, 2009), thereby increasing the usability of color annatto preparations.

2. Materials and Methods

2.1. Materials

Ripped annatto seeds were purchased in Vinh Cuu district, Dong Nai province. After shelling, removing the impurities, floaters and small grains, seeds were dried in an oven at temperature of 40^0C until the moisture content reached about 10% as recommended by Mantovani group (2013).

- Acetone 99.7%; VN-Chemsol Co., Ltd.; Vietnam.
- Ammonia 25 ~ 28%; Guangdong Guanghua Chemical Factory; China.
- Chloroform 99%; Guangdong Guanghua Chemical Factory; China.
- CH_3COOH 99.5%; Guangdong Guanghua Sci-Tech Co, Ltd.; China.
- KOH 86%; Guangdong Guanghua Chemical Factory; China.
- 96% NaOH; Guangdong Guanghua Sci-Tech Co., Ltd.; China.
- Ethanol 99.5%; VN-Chemsol Co., Ltd.; Vietnam.

2.2. Research Methodology

2.2.1. Analysis of Norbixin

Sample was weighed as 0.25 g (± 0.02 g) (W) then dissolved by distilled water or appropriate solvent after that transferred into volumetric flask with a capacity of one liter. Next step, diluted by water or suitable solvent to the desired concentration, the concentration is depending on the concentration of the solution, and the last step, measuring the absorbance of the diluted solvent (A). (Distilled water or solvent were used as blanks)

Pigment content (%) = 100 x (A/A1% 1cm) x (F/W)

Among them:

- A: absorbance of the diluted solution
- $A^{1\%}_{1cm}$: absorbance of standard solutions 1% (the thickness of cuvette is 1 cm)
- F: Factor of dilution (F = volume of diluted solvent / volume of standard solvent);
- W: volume of sample was diluted.

2.2.2. Evaluation method norbixin free recovery yeild from extractive solvent

Add 5 g acid on extraction solvent, waiting deposited about 1 hour (Hyman et al, 1990) then centrifuging to collect the precipitate of norbixin after that washing the precipitate dissolved, dissolved by KOH solvent 0.5% to volume extraction initial translation (5 g). Dissolving with 0.5% KOH to measure the absorbance by wavelength at 482 nm (FAO JECFA, 2006). Extraction yield and norbixin recovery yield were calculated by the formula as below:

Extraction yield (%) = $(m_1 \times 100)/m_0$

Recovery yield (%) = $(m_2 \times 100)/m$

Among them:

- m: volume of Annatto contain in raw materials;
- m_0: mass of dry matter contain in material;
- m_1: mass norbixin be extracted;
- m_2: mass of norbixin be extracted.

2.2.3. Assessment of solubility method

Following to Santos'method (Barbosa and coworker, 2005). Powder was stired lightly in water as 0.4 revs per minute until become to a homogeneous solution then take note the time to dissolve. The time required less than 5 minutes was considerd as good powder.

2.3. Conduct

2.3.1. Optimizing concentration of solvent by response surface method 2 factors (Central Composite Design - CCD).

After weigh 5g cashews, the sample was stired in solvent about 30 revs per minute then incubating at 600°C for 30 minutes. After filling, take 1g solvent to 1000 mL of 0.5% KOH. Determine norbixin extraction yield (%) by measured at a wavelength of 482 nm. Experiments were illustrated by table number 1.

Table 1. *Survey with 2 factors*

Elements	Indicator variables				
	–α	–1	0	+1	+α
Ethanol (^{0})	21.72	30	50	70	78.28
NaOH (M)	0.36	0.4	0.5	0.6	0.64

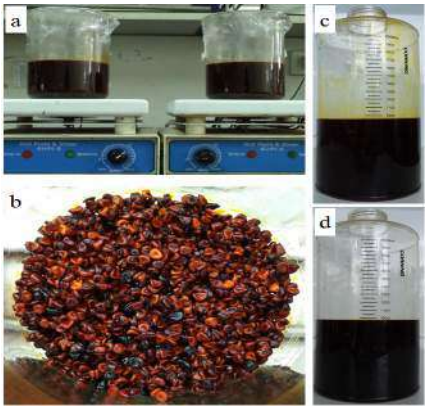

Fig 1. *Extracting and converting process into norbixin*
(a: stirring process, b: waste matter, c: extractive solvent was filted, d: extractive solvent was incubated)

2.3.2. Optimization process ratio of solvent / material and conditions for saponification reaction by response surface method 3 elements (central composite design (CCD).

The study was carried out as same as 2.3.1 item. The ratio of solvent/material, temperature (°C), incubation time (minutes) were surveyed. Determine norbixin extraction yield (%) was measured by wavelength at 482 nm. Experiment processed by table number 2.

Table 2*. Survey with 3 factors*

Elements	Survey				
	–α	–1	0	+1	+α
Ratio of solvent /material	2.64	4	6	8	9.36
Incubation temperature (^{0}C)	43,2	50	60	70	76.8
Incubation time (min)	13.18	20	30	40	46.82

2.2.3. Survey volume of concentrated chlorhydric acid to precipitate free norbixin

Weigh 5 g of the solution was extracted then drip Solid Acid 36% in (Solid Acid was driped slowly into a centrifuge tube (Haque, 2011) volume of centrifuge are 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6 and 0.65 mL, waiting for 1 hour and then centrifuged precipitate collected. Wash precipitate several times with distilled water. Dissolve the precipitate by KOH 0.5% into 5 g. Taking 1 g dilute to 1000 mL of 0.5% KOH. Determine the norbixin free recovery yield (%) by wavelength at 482 nm.

2.2.4. Design and Data Processing

The experimental were designed by software JMP 9.0.2 program (SAS Institute Inc., 2011, USA), analysis of variance ANOVA, comparing average by Tukey's HSD method.

3. Results and Discussion

3.1. Optimization concentration of solvent

Result of extraction efficiency and color conversion in center point are higher than others. This is a sign that the optimum point can be located near the center survey.

Table 3. The result of experience

Variable	Encryption	X_1 – concentration of ethanol (^{0})	X_2 – concentration of NaOH (M)	Extraction of norbixin yield (%)
1	−−	30	0,4	1,14
2	−+	30	0,6	1,28
3	+−	70	0,4	1,3
4	++	70	0,6	1,42
5	a0	21,72	0,5	0,98
6	A0	78,28	0,5	1,11
7	0a	50	0,36	1,18
8	0A	50	0,64	1,46
9	00	50	0,5	1,78
10	00	50	0,5	1,74
11	00	50	0,5	1,72
12	00	50	0,5	1,7
13	00	50	0,5	1,8

Influence level of these factors and interactions each other on the response Y is illustrated and arranged on a Pareto chart (Figure 2). Accordingly, influence level extraction efficiency yield and color conversion into norbixin descending as X12 interactions have the greatest impact, followed by interactive X22, the third is the concentration of NaOH (X2), after the concentration of ethanol(X1) and finally the interaction X1X2 not significantly affected. Thus, if you compare between ethanol and NAOH, the effect of ethanol is important than NaOH

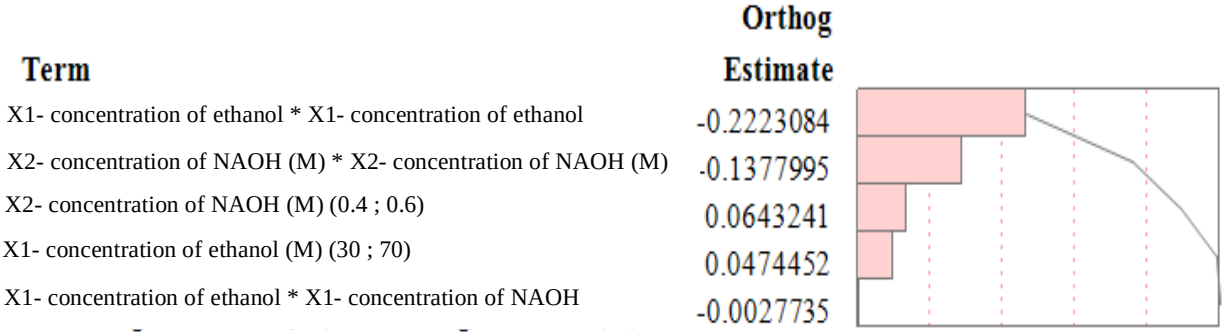

Fig 2. *Pareto chart showing the effects of these factors*

The relationship of the response Y - extraction yield X1 - ethanol concentration X2 - concentration of NaOH was demonstrated by a equation as: Y = 1.748 + 0 , 06X1 X2 + 0.082 - 0.326 X12 - X22 .188. Response surface showing the influence of the concentration of two-component solvent extraction performance and norbixin convert color to look like in Figure 3. Surface above the peak point, is extraction efficiency and norbixin color transformation into the highest ranges survey.

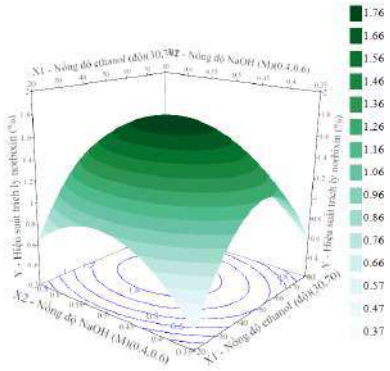

Fig 3. *Response surface showing the relationship between yield and concentration of two ingredients solvent*

Optimal results are obtained optimal optimum concentration of ethanol (51,820) and NaOH (0.52 M) will be used to conduct subsequent experiments as a basis for the construction and manufacturing processes.

3.2. Optimized ratio of solvent / material and reaction conditions saponification

After conducting experiments with random order, the results are summarized in Table 4.

Table 4. *Experimental results 3 CCD elements*

Variable	Encryption	X_1 – Ratio of solvent / material	X_2 – Incubation temperature (^{0}C)	X_3 – Incubation time (min)	Y – Extraction of norbixin yield (%)
1	−−−	4	50	20	1,03
2	−−+	4	50	40	1,17
3	−+−	4	70	20	0,83
4	−++	4	70	40	0,98
5	+−−	8	50	20	1,46
6	+−+	8	50	40	1,57
7	++−	8	70	20	1,34
8	+++	8	70	40	1,46
9	a00	2,64	60	30	0,85
10	A00	9,36	60	30	1,59
11	0a0	6	43,2	30	1,38
12	0A0	6	76,8	30	1,28
13	00a	6	60	13,18	0,98
14	00A	6	60	46,82	1,6
15	000	6	60	30	1,8
16	000	6	60	30	1,69
17	000	6	60	30	1,78
18	000	6	60	30	1,82
19	000	6	60	30	1,75
20	000	6	60	30	1,82

Overall, the results in Table number 4 show that extraction efficiency and color conversion into the norbixin (from 15 to 20 treatments) at the solution in central is the best. This is a sign that the optimum point can be located near the center survey. Influence level of these factors and their interactions on the response Y is illustrated and arranged on a Pareto chart (Figure 4). Accordingly, extraction efficiency and color conversion into norbixin as follows: X1 - the ratio of solvent / Most affected grain, X32 interaction affects less than second place, followed by interactive X12 and X22, then the annealing time and temperature of incubation X2 X3, 3 pairs of interacting pairs of 3 factors significantly influence the performance extracted and converted into norbixin color. Thus, if you compare the effects of three factors, the impact of the rate of extraction efficiency and color conversion into the more important norbixin incubation time, incubation temperature was at number 3 most influential factors in survey

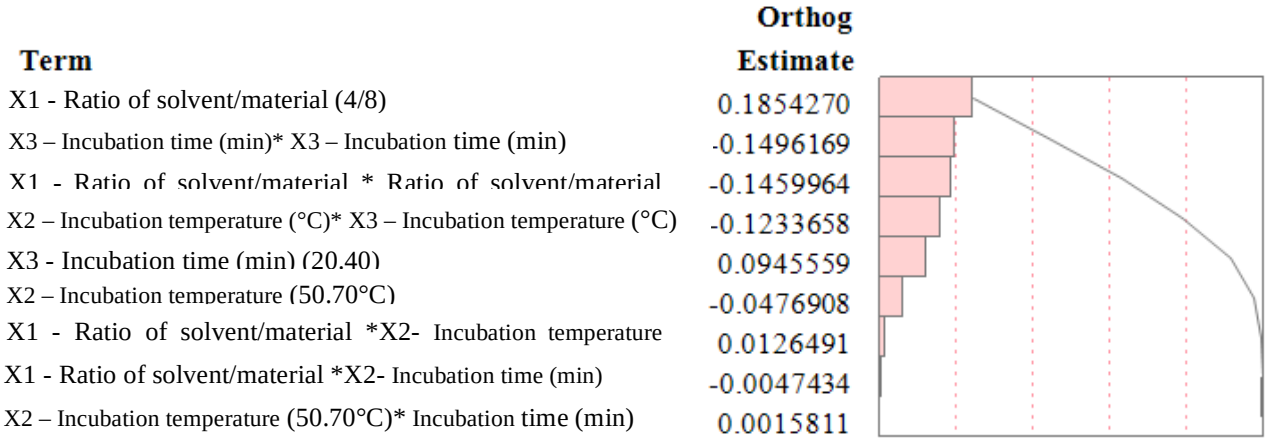

Fig 4*. Pareto chart showing the effects of these factors*

The results obtained can represent the relationship between the response Y - Extraction

yield and convert color into 3 elements norbixin with X1 - ratio solvent / particle, X2 - X3 and annealing temperature - incubation time by the following regression equation: Y = 1.777 + 0.224 X1 - 0.058 X2 + X3 0.114 - 0.201 0.162 X12-X22 - X32 0.176. Response surface corresponding to each pair of elements is shown in Figure 5, the response surface on the top side, ie the highest performance ranges.

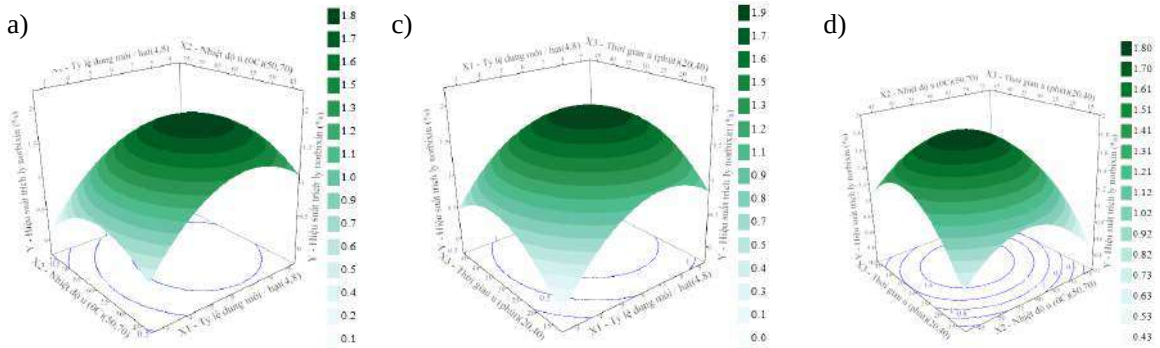

***Fig 5**. Response surface showing the correlation between the extraction efficiency between: a) annealing temperature and the ratio of solvent / seed; b) incubation time and the ratio of solvent / seed; c) annealing temperature and time*

The result is optimum solvent ratio / seed is 7.1 / 1, 58.58 ^{0}C annealing temperature and annealing time is 33.12 minutes, while the extraction efficiency and color conversion into norbixin estimated 1,86%; equivalent to 86.92% recovery efficiency. Optimal parameters are consistent with the recommendations of Nachtigall et al. (2009) that the implementation of the saponification reaction of bixin norbixin should not raise the temperature too high for a long time, the temperature should be below 70 0C and shorter time to 1 hour.

3.3. Effect of hydrochloric acid concentration to precipitate free norbixin

The survey of the impact of volume of 36% HCl acid on norbixin retrieval performance is shown in Figure 6

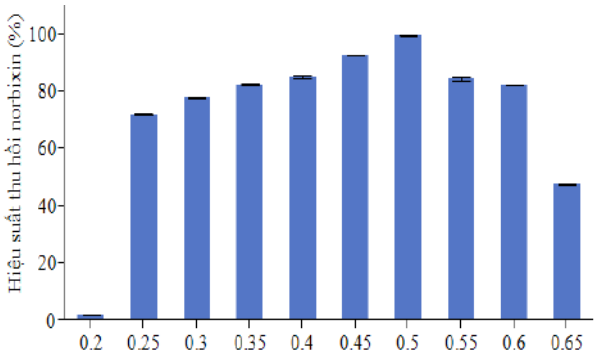

***Fig 6.** Influence of volume of 36% chlohydric acid recovery performance norbixin*

From the average results compare with Tukey's HSD method showed that the volume of concentrated hydrochloric acid precipitates the best norbixin 0.5 mL and mean differences with retrieval performance when using different volumes of HCl in p = 0.05 level. With the amount of fluid extracted in each treatment is 5 g (5.43 mL), then 0.5 mL of 36% HCl when dissolved in translation extract equivalent to a concentration of 1 M. This result is consistent with the method of Dinda et al. (2008) when the authors use dilute hydrochloric acid to precipitate norbixin obtained after solvent extraction with 5% KOH.

3.4. Solubility of the powder precipitated at pH norbixin in different countries

Using 0.1 g norbixin precipitated dough for each treatment to the survey conducted in water solubility of precipitated dough norbixin at different pH value, the results shown in Figure 7.

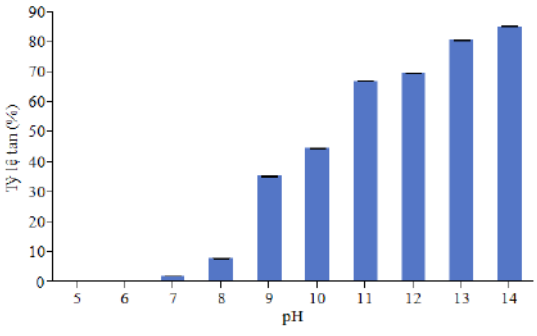

***Fig 7.** Norbixin ratio dissolved in water at different pH*

After comparing the average rate of melting of norbixin at different pH by means of Tukey's HSD showed that only at pH 5 and 6 percentage tan difference not significant, the remaining percentage tan differences significant pairs. Thus norbixin precipitated powder soluble in water at a pH below 7, while at pH above 7, the melting rate proportional

to the pH value. Experimental results with the correct reference information about the solubility of the precipitate norbixin powder in water (Dinda et al., 2008), the reason is freedom in powder precipitated norbixin have long hydrophobic carbon vessels, namely HLB value is 2.65 lower than 7

4. Conclusion

Annatto Colour in Vietnam's cashew color can be extracted and converted into ethanol solvent norbixin alkaline by NaOH. The recovery of 87% at best in the conditions as follows: ethanol concentration of 51,820; concentration of 0.52 M NaOH; ratio of solvent / material is 7.1 / 1; annealing temperature and time respectively 58.6 0C and 33.12 minutes. After extraction, norbixin freedom can be separated from the service by means of acid extraction chemistry. Concentration of 1 M hydrochloric acid (HCl or 9.2% volume concentration versus fluid volume extracted) is best suited to precipitate withdrawal norbixin freedom. After drying at 50 0C precipitate obtained powder precipitated with 44.25% norbixin.

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Food technology learning scheme and students' satisfaction: The case of Đong Nai University of technology

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Abstract:

The primary objective of this study is to identify the current satisfaction level of customer at Dong Nai University of Technology. Exploring previous studies enables the context of customer satisfaction shall be impacted by antecedents, including (1) Non – academic factor, (2) academic factor, (3) reputation factor, (4) access factor, (5) program issues factor and (6) understanding factor. Measuring the impacts of each antecedent to customer satisfaction requires applying quantitative research method with linear regression technique. Achieved results show that antecedents above are modified after exploratory factor analysis and new components consists of (1) academic aspect, (2) reputation aspect excepting well-known level of the university, (3) program issues aspect in excepting flexibility in structure and syllabus of Food Technology learning, (4) access aspect, (5) understanding aspect, (6) attitude, stipulated time, and connection of non-academic staffs, (7) availability and communication skills of non-academic staffs, (8) well-known level of the university, and (9) flexibility in structure and syllabus of Food Technology learning. All new components can explain for 59.8 percent of total respondents and it is showed through R-Square value in model summary table. Statistically, all new components can explain for 59.8 percent of changes in student satisfaction as well. Among those components, reputation aspect excepting well-known level of the university as it has estimated beta value of 0.174 compared than estimated beta values of other components.

Keywords: learning scheme, food technology, student's satisfaction, learning and teaching

1. Introduction

Vietnam has been benefited by growth in its economy of which GDP of Vietnam in 2012 stood at US$141.7 billion for the population of more than 88 million of people (World Bank, 2013), leading to the opportunities for the development of many industry sector and the country is also determined as good location for taking tourism. Wisconsin International Trade Team (2012) asserts that the dollar for food imports in Vietnam has increased more than 25 times in last decade, it was only US$20 million in 2000 and now reaching to US$525 million in 2010. Along with the economic development in general, Vietnam is also well known as the country with good competitive advantage in natural food production as well as its imported foods are also consumed high proportion in importing structure of the country (State Government Victoria, 2009).

With the fact that food technology is considered as major sector of the economy, Ministry of Agriculture and Rural Development and Ministry of Education and Training have cooperated with each

other in opening new education programs and trainings. This encourages people participate in learning scheme and then they will utilize the knowledge into the real life context. Dong Nai University of Technology (DNTU) has also been integrated into this program.

Considering the fact that DNTU is now located in the biggest province in Southern Vietnam with the main kinds of land are bazan and alluvial soil. Dong Nai province is determined as the best place for growing industrial plants such as rubber, coffee and orchard. Dong Nai is also determined as famous industrial zones as it is surrounded by major economic areas such as Lam Dong in the north, Binh Thuan in the east, Ba Ria - Vung Tau in the south, Binh Duong, Binh Phuoc and Ho Chi Minh City in the west. Additionally, the climate in the province is always between 25.4 and 27.2 Celsius degree and such climate supports to growing many kind of vegetables and feeding animals. In this context, the study is strongly emphasized that DNTU has to enhance its higher education scheme on food technology in order to take the competitive advantage of geographic area and gain the benefit from food industry. In more detail, the quality of education and training of food technology subject is the most important one whether student satisfaction is crucial factor for assessing the effectiveness of education. The problem should be addressed by this study about the quality of higher education and training on food technology at DNTU has not been reviewed and accessed before. And until now, there is no official research on the quality of education and training of food technology faculty through assessing the level of students' satisfaction. The author would like to choose the topic named: "**Food technology learning scheme and students' satisfaction at Dong Nai University of Technology**".

The conceptual model of the research is based on the HEDPERF model of Firdaus, which will be presented in the next section, as below:

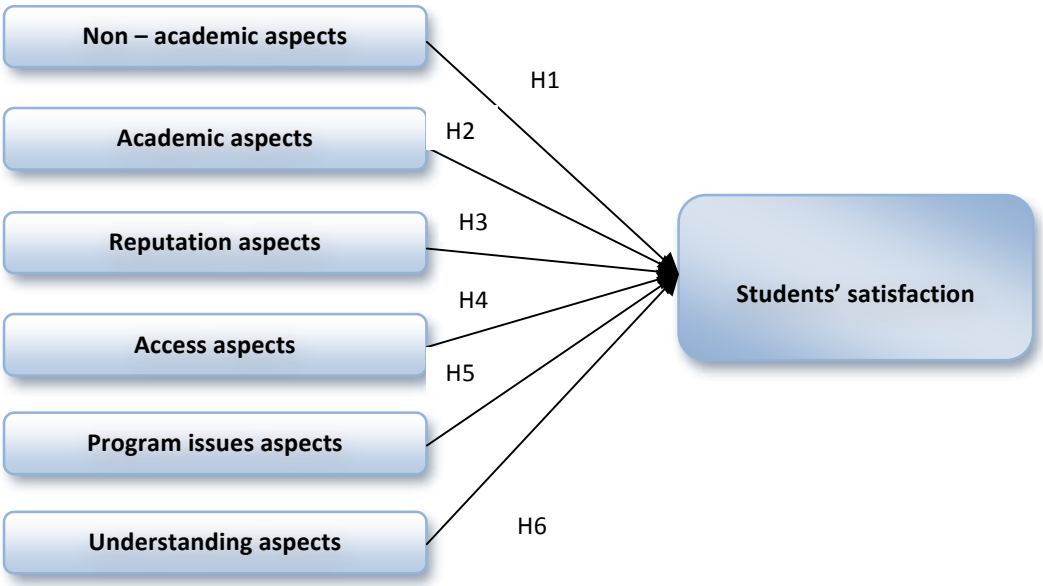

Fig 1: *The conceptual model of the research. Source: adapted from Firdaus (2006)*

According to the conceptual model of the research as well as the analysis in the above part about the relationship between six factors and student's satisfaction, the hypotheses of the research is again illustrated as below:

$H1_0$: There is no relationship between non-academic aspect and student satisfaction with learning program on food technology at DNTU

$H1_a$: There is a relationship between non-academic aspect and student satisfaction with learning program on food technology at DNTU

$H2_0$: There is no relationship between academic aspect and student satisfaction with learning program on food technology at DNTU

$H2_a$: There is a relationship between academic aspect and student satisfaction with learning program on food technology at DNTU

$H3_0$: There is no relationship between reputation aspect and student satisfaction with learning program on food technology at DNTU

$H3_a$: There is a relationship between reputation aspect and student satisfaction with learning program on food technology at DNTU

$H4_0$: There is no relationship between understanding aspect and student satisfaction with learning program on food technology at DNTU

$H4_1$: There is a relationship between understanding aspect and student satisfaction with learning program on food technology at DNTU

$H5_0$: There is no relationship between access aspect and student satisfaction with learning program on food technology at DNTU

$H5_a$: There is a relationship between access aspect and student satisfaction with learning program on food technology at DNTU

$H6_0$: There is no relationship between program issues aspect and student satisfaction with learning program on food technology at DNTU

$H6_1$: There is a relationship between program issues aspect and student satisfaction with learning program on food technology at DNTU

According to figure above, the dependent variable of the model is students' satisfaction and the independent variables of the models are: non – academic aspects, academic aspects, reputation aspects, access aspects, program issues aspects and understanding aspects.

2. Experimental methodology

2.1. Research method

In this research, the author would like to adopt both of qualitative and quantitative research method, however, quantitative method will be the main research method as the one of the main objective of this research measuring the relationship between student's satisfaction and its antecedents in order to predict the trend of student's satisfaction based on the factors impacting their satisfactions. Moreover, the deductive research approach, which is considered as the approach commonly used for quantitative research method according to Bryman & Bell (2007) has been chosen to apply in this study as mentioned in the earlier part of the research. In more detail, the qualitative method was conducted by the application of survey of questionnaire with a sample of students studying in food technology faculty of DNTU.

2.2. Sample size

As mentioned above, the author would like to collect the primary data from survey of questionnaire on a sample of people who are studying in food technology faculty of DNTU. The author self – recognized that it was necessary to conduct the sample technique to generate adequate sample size for later data analysis.

According to Tabachnick & Fidell (1996), the sample size should be calculated from the number of predictor factors used in the research, the calculation for the sample size is illustrated as below:

Sample size = 50 + 8 * Number of predictor factors

As mentioned from Chapter II, the conceptual model of the research contains 6 factors including: non – academic aspects, academic aspects, reputation aspects, access aspects, program issue aspects and understanding aspects. The author applied the calculation for sample size of Tabachnick & Fidell (1996) for sample size as: Sample size = 50 + 8 * 6 = 98. However, 98 respondents as sample size is quite small whether the student's satisfaction is considered as the new phenomenon which was studied in DNTU.

In another opinion, Roger (2006) stated that the sample size for social phenomenon should be range from 150 – 200 observations. The author decides to choose 200 people as the size of the sample because this number is suitable with the number of students in food technology faculty of DNTU (278 students). However, 278 survey of questionnaire were sent out by the author to make sure that the target number of respondents will be met due to the occurring of unqualified answers.

3. Data analysis and results

3.1. Overview of respondent information

There are four demographic information are collected in survey with 278 students who are learning Food Technology in DNTU. It is worth to denote that there are 78 questionnaires that have not been passed the quality of answers due to these respondents have not answered many questions. Hence, the response rate is about 72 percent (200/278 = 0.719). The table below is prepared to summary about demographic information of 200 respondents:

Table 1: *Demographic information*

Variable	Attributes	Number	Percent
Marital status	Single	181	90.5%
	Divorce	0	0.0%
	Marriage	19	9.5%
	Others	0	0.0%
Age	Less than 20 years old	68	34.0%
	20-24 years old	132	66.0%
	24-30 years old	0	0.0%
	>30 years old	0	0.0%
Sex	Male	102	51.0%
	Female	98	49.0%
Natio-nality	Vietnamese	200	100.0%
	Others	0	0.0%

As shown in the table above, marital status has four attributes, including single, divorce, marriage, and other (i.e. living alone or living with family). Result shows that more than 90 percent of students are single and they live in Dong Nai City for learning Food Technology in the university. High number of single student is true as the students in DNTU are still young and they are learning higher education on Food Technology and they do not want to get marriage.

The second demographic information is age, there are four age group categories, including less than 20 years old, 20-24 years old, 24-30 years old, and more than 30 years old. Among age categories, there are 34 percent and 66 percent of respondents lying in the first and second groups. Indeed, the students are young due to Vietnamese culture encourage people to go to school very early. It is different from students in Western countries where students often go to work in trials before they go to university for achieving higher education. The third demographic information is sex or gender of respondents; there are 51 percent of male and 49 percent of female students involved in the survey. The number of male students is higher than female one accordingly. The last demographic information is nationality of students. The result shows that all students are from Vietnam. Or on the other hand, there are not students come from other countries.

3.2. Reliability test analysis

The table below is prepared to provide results of reliability test analysis for six aspects in conceptual research model:

Table 2: *Result of reliability test analysis*

No	Factors	Cronbach's alpha	Notes
1	Non-academic aspect	0.768	All attributes have Item-Total Correlation higher than 0.3
2	Academic aspect	0.911	A3 is removed as it has Item-Total Correlation lower than 0.3
3	Reputation aspect	0.896	All attributes have Item-Total Correlation higher than 0.3
4	Access aspect	0.877	All attributes have Item-Total Correlation higher than 0.3
5	Program issues aspect	0.899	All attributes have Item-Total Correlation higher than 0.3
6	Understanding aspect	0.830	All attributes have Item-Total Correlation higher than 0.3

As shown in the table, non-academic, academic, reputation, access, program issues, and understanding aspects have mean values of 0.768, 0.911, 0.896, 0.877, 0.899, and 0.8310 that are higher than the minimum value of 0.6, according to Hair et al. (2011). Among these factors, only non-academic aspect has one attribute (A3) with Item-Total Correlation of 0.287 that is lower than 0.3. Thus, A3 attribute has to remove from data analysis and Cronbach's alpha value will be 0.786 after this attribute is removed (Appendix II). It is worth to indicate that non-academic aspect is good reliability of survey scale while other factors have Cronbach's alpha values higher than 0.8 or they are very good reliability of survey scale.

Additionally, academic aspect is recorded as highest Cronbach's alpha value (0.911) and this result is true since the students take into account what they can learn when joining in the university. In this context, their satisfaction and interests will be aligned with academic programs in food faculty at DNTU. Program issues aspect has second highest Cronbach's alpha value (0.899) and it is appropriate as any program issues will affect directly to what the student can learn from the university and they will impact negatively to the final results of the students in last semester.

3.3. Exploratory factor analysis

The table below described how many components are attracted from survey of questionnaire with 200 students in DNTU regarding to Food Technology teaching and learning.

Table 3: *Rotated component matrix with Varimax rotation technique*

	Component								
Attributes	1	2	3	4	5	6	7	8	9
B6	.943								
B4	.940								
B7	.938								
B5	.836								
B3	.640								
B2	.620								
B1	.599								
C4		.975							
C3		.964							

C6		.948							
C5		.942							
C1		.564							
E3			.972						
E4			.957						
E1			.948						
E5			.929						
D3				.929					
D4				.929					
D2				.782					
D1				.746					
F3					.907				
F4					.883				
F2					.747				
F1					.656				
A5						.869			
A6						.866			
A1						.730			
A4							.779		
A2							.665		
C2								.605	
E2									.677

The table above described about 9 components with factor loading of belong attributes:

- The first component includes 7 attributes of academic aspect, including B1, B2, B3, B4, B5, B6, and B7 with factor loadings of 0.599, 0.620, 0.640, 0.940, 0.836, 0.943, and 0.938. Among 7 attributes, B6 has highest factor loading value so pedagogical behavior of academic staffs will have highest impact on this component. This component is named as academic aspect.
- The second component includes 5 attributes of reputation aspect, including C1, C3, C4, C5, and C6 with factor loadings of 0.564, 0.964, 0.975, 0.942, and 0.948. Among 5 attributes, C4 has highest factor loading value so the specialization of DNTU on Food Technology will have highest impact on this component. This component is named as reputation aspect excepting well-known level of the university.
- The third component includes 4 attributes of program issues, including E1, E3, E4, and E5 with factor loadings of 0.948, 0.972, 0.957, and 0.929. Among 4 attributes, E3 has highest factor loading value so the designation of programs of food technology faculty are designed will have highest impact on this component. This component is named as program issues aspect in excepting flexibility in structure and syllabus of Food Technology learning.
- The fourth component includes 4 attributes of access aspect, including D1, D2, D3, and D4 with factor loadings of 0.746, 0.782, 0.929, and 0.929. Among 4 attributes, D3 and D4 have highest factor loading values so accesses to academic staffs and to laboratory system will have highest impact on this component. This component is named as access aspect.
- The fifth component includes 4 attributes of understanding aspect, including F1, F2, F3, and F4 with factor loadings of 0.656, 0.747, 0.907, and 0.883. Among 4 attributes, F3 has highest factor loading value so the ease to ask for counseling advices from teachers and faculty will have highest impact on this component. This component is named as understanding aspect.
- The sixth component includes 3 attributes of non-academic aspect, including A1, A5, and A6 with factor loadings of 0.730, 0.869, and 0.866. Among 3 attributes, A5 has highest factor loading value so stipulated time provided by non-academic staffs will have highest impact on this component. This component is named as attitude, stipulated time, and connection of non-academic staffs.
- The seventh component includes 2 attributes of non-academic aspect, including A2 and A4 with factor loadings of 0.779 and 0.665. Among 2 attributes, A4 has highest factor loading value so

communication skills of non-academic staffs will have highest impact on this component. This component is named as availability and communication skills of non-academic staffs.

- The eighth component includes C2 as attribute of reputation aspect with factor loading of 0.605.This component is named as well-known level of the university.
- The ninth component includes E2 as attribute of program issues aspect with factor loading of.677. This component is named as flexibility in structure and syllabus of Food Technology learning.

Then, following hypotheses are prepared:

- Hypothesis 1: Academic aspect impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 2: Reputation aspect excepting well-known level of the university impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 3: Program issues aspect in excepting flexibility in structure and syllabus of Food Technology learning impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 4: Access aspect impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 5: Understanding aspect impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 6: Attitude, stipulated time, and connection of non-academic staffs aspect impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 7: Availability and communication skills of non-academic staffs impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 8: Well-known level of the university impacts positively on student satisfaction in Food Technology learning at DNTU
- Hypothesis 9 : Flexibility in structure and syllabus of Food Technology learning impacts positively on student satisfaction in Food Technology learning at DNTU

3.3. Linear regression and hypothesis test

Table 4**:** *Linear regression results between student satisfaction and 9 components*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.785	.617	.598	.287

Coefficients

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	3.175	.020		156.334	.000
	Academic aspect	.170	.020	.376	8.373	.000
	Reputation aspect excepting well-known level of the university	.174	.020	.384	8.543	.000
	Program issues aspect in excepting flexibility in structure and syllabus of Food Technology learning	.137	.020	.301	6.705	.000
	Access aspect	.098	.020	.216	4.814	.000
	Understanding aspect	.142	.020	.313	6.965	.000
	Attitude, stipulated time, and connection of non-academic staffs	.110	.020	.244	5.427	.000
	Availability and communication skills of non-academic staffs	.078	.020	.172	3.821	.000
	Well-known level of the university	-.027	.020	-.059	-1.310	.192
	Flexibility in structure and syllabus of Food Technology learning	.001	.020	.003	.064	.949

The table above showed that all new components can explain for 59.8 percent of total respondents and it is showed through R-Square value in model summary table. Statistically, all new

components can explain for 59.8 percent of changes in student satisfaction as well. Among those components, reputation aspect excepting well-known level of the university as it has estimated beta value of 0.174 compared than estimated beta values of other components.

Hypothesis 1

Academic aspect impacts positively on student satisfaction with estimated beta value of 0.170, meaning that when DNTU improves its academic performance by 1 percent, student satisfaction will be improved by 0.170 percent accordingly. This component has second highest beta value of DNTU has to take into account academic aspect in order to improve its customer satisfaction.

Hypothesis 2

Reputation aspect excepting well-known level of the university impacts positively on student satisfaction with estimated beta value of 0.174, meaning that when DNTU improves this component performance by 1 percent, student satisfaction will be improved by 0.174 percent accordingly. Reputation aspect excepting well-known level of the university has highest beta value compared to other component so that DNTU has to take into account this component in order to improve its customer satisfaction.

Hypothesis 3

Program issues aspect in accepting flexibility in structure and syllabus of Food Technology learning impacts positively on student satisfaction with estimated beta value of 0.137, meaning that when DNTU improves this component by 1 percent, student satisfaction will be improved by 0.137 percent accordingly. Access aspect has second lowest beta value compared to other component so that DNTU does not need to take into account this component in short term.

Hypothesis 4

Access aspect impacts positively on student satisfaction with estimated beta value of 0.098, meaning that when DNTU improves this component by 1 percent, student satisfaction will be improved by 0.098 percent accordingly. Access aspect has second lowest beta value compared to other component so that DNTU does not need to take into account this component in short term.

Hypothesis 5

Understanding aspect impacts positively on student satisfaction with estimated beta value of 0.142, meaning that when DNTU improves this component by 1 percent, student satisfaction will be improved by 0.142 percent accordingly. Understanding aspect has third highest beta value compared to other component so that DNTU does not need to take into account this component in short term.

Hypothesis 6

Attitude, stipulated time, and connection of non-academic staffs aspect impacts positively on student satisfaction with estimated beta value of 0.110, meaning that when DNTU improves this component by 1%, student satisfaction will be improved by 0.110 percent accordingly. Access aspect has third lowest beta value compared to other component so that DNTU does not need to take into account this component in short term.

Hypothesis 7

Availability and communication skills of non-academic staffs impacts positively on student satisfaction estimated beta value of 0.078, meaning that when DNTU improves this component by 1 percent, student satisfaction will be improved by 0.078 percent accordingly. Access aspect has lowest beta value compared to other component so that DNTU does not need to take into account for this component in short term.

Hypothesis 8

This hypothesis is rejected due to estimate beta value that is not statistical significance at 5 percent of confidence interval (Sig. value = 0.192 > 0.05).

Hypothesis 9

This hypothesis is rejected due to estimate beta value that is not statistical significance at 5 percent of confidence interval (Sig. value = 0.949 > 0.05). Indeed, the students often do not have much idea on structure and syllabus of food technology learning at DNTU. This is also recognized as key problem in all local universities whether the students have not been actively involved in program designing. On the other hand, it means that structure and syllabus in DNTU are designed by group of teachers and therefore, their ideas may not be aligned with students' demands.

4. Conclusions

The study is conducted with the application of quantitative research method and it is proposed to use three types of data analyses. The first one is reliability test for the survey scale with the objective of identifying the factors that have meaninglessness in

the scale. The second one is exploratory factor analysis in order to recognizing the underlying constructs between factors used in the conceptual research model. The last one is linear regression of which it helps to test proposed hypotheses based on T-Test at confidence interval of 95 percent. The major weakness is the application of exploratory factor analysis that is determined as not good in case of this analysis is often conducted when there are strong theories and concepts for the relationship between factors in the conceptual research model. Chosen conceptual research model, however, were true in several previous studies but it might not be true in the study. Conclusion, DNTU should establish foreign study teaching community. When the teachers participate in this community, they will be benefited by experiences and expertise from other teachers in teaching foreign study in general and teaching Food technology in particular. Furthermore, the university should reserved the room for teachers to carry their own researches on Food Technology teaching methods as well as supporting for higher degrees.

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Global movements of the black garlic - Research and Business

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Abstract:

Anti-tumor potency of the newly developed black garlic had been first published in 2007 from our laboratory. At that time few researches have been conducted due to less popularity of the products. Bio-functional works of the black garlic have been lately started along with spreading of its popularity. Representative bio-active reports up to now are: 1) enhancement of anti-tumor potency in mouse tumor model, 2) immune system activation, and suppression of allergic symptoms by decreasing IL-4 production, 3) bacteria killing potency, 4) antioxidant activity, 5) reduction of carcinogenic potency, 6) Lowering cholesterol level and preventive effect against obesity, 7) controlling high blood pressure, 8) beneficial effect against diabetics, 9) Regenerative effect to the burned skin (Clinical study case) et al. While on the movement of the black garlic business, the Aomori Black Garlic Association had been established in Japan to offer the citizen high quality, high safety and high security products. When the products passed the quality inspection organized by the third party inspectors, the certification is issued for approval. The quality inspection system is now effectively operating to guarantee quality of the black garlic and differentiate the member's products from others at the markets.

Keywords: Black garlic, Anti-tumor potency, Anti-oxidant, Anti-bacterial act, Aomori Black Garlic Association in Japan, Quality approval inspection

1. Introduction

Globally an upsurge of interest is toward the functional foods in relation to control the lifestyle-related diseases. The Ministry of Health and Welfare in Japan has lately stated that the modification of the daily diet can lower the incidence of variety of diseases, implying the reduction of a large amount of the government budget.

In 1999, the black garlic with sweet taste as a fruit, no pungent odor, and directly edible just after peeling off outer coat was developed by Japanese *Mr. Kamimura* in *Mie prefecture* Japan [1].

Number of the black garlic companies is at the moment counted over 500 in Japan, and some of them build plants in foreign countries and are doing business. The black garlic is easily created just by controlling of temperature and humidity with no use

of additives in the conditioned circumstances.

In 2006 we had first demonstrated presence of the strong anti-tumor potency in the black garlic using a mouse tumor model (Fig. 1). Our report had accelerated the people's healthy preference on foods to control the increasing life style-related diseases themselves. Social movements then stimulated company's management policies and strategies to create new business by handling the eccentric vegetable black garlic.

Since the introduction of our black garlic research in the newspaper, seven years have already passed, and there was a change in situation. It is at the time to overview the worldwide movements of the black garlic from both aspects of researches and business in this paper.

2. Historical background of the black garlic

This chapter will be started by introduction of the following descriptions [2];

Black garlic has gained popularity in Japan after a research showing its health benefits was published in 2006 (Fig 1) by the researcher, *Jiniti Sasaki*, a professor at *Hirosaki University*. The research showed that black garlic reduced cancer cells in mouse. When the same research was conducted using white garlic, it didn't have the same result.

In the first edition of Wikipedia, the black garlic was introduced likely that "Japanese had developed black garlic in 2005".

However in the revised edition this part of descriptions was completely deleted, and started with that "In Korea, black garlic was introduced as a health product." People therefore believe that the black garlic was created by Korean. This incorrect information is now spreading worldwide.

Black garlic creation was attained by Japanese *Mr. Kamimura* in *Mie Prefecture* in 1999 as introduced above, and he had acquired the patent on manufacturing devices of the black from the Japan Patent Office.

3. Survey of the black garlic researches and the related


発酵ニンニク
生より強い
抗がん作用
体内の免疫活性化
動物実験で証明
元行員に無罪判
一共犯者証言 信用

Fig 1. *Anti-tumor activity of the black garlic introduced in the Newspaper Mutsu Shinpo (March 22nd, 2006)*

Since the anti-tumor potency of the black garlic had been reported both in the Newspaper 2006, and in journal of M*edicinal and Aromatic Plant Science and Biotechnology* (Global Science Books) 2007 [3], the citizens and researchers have started to pay concerns to the black garlic (bio-functions). Especially the post-operative cancer patients have been expecting the possible metastasis preventive act due to enforcement of NK cells activity.

Currently the average people are making their own black garlic as a hobby by using the electric-rice cooker.

Fig 2. *The Black garlic filled with multi bio-functions*

3.1. Black garlic ?

Herein a brief introduction of the black garlic's profile appeared in the internet [4].

Fig 3. *Feature of the high quality Japanese Garlic called "White Six Cloves" (right) developed by Japanese researcher, and Chinese garlic as reference (left). Japanese one is easily recognizable by its size and number of cloves inside*

It is learned that the fermented black garlic was made of selected fresh and high-quality raw garlic, using Japanese unique professional approach and carefully processed required for a month which improve the nutrition of the garlic and eliminate the unpleasant odor of garlic to make it easy to accept. Fermented black garlic was researched by Mie Prefecture, Japan in 2003. Professor *Sasaki Jin ichi*, the famous Japanese physician in School of Medicine of *Hirosaki University*, proved through a mice experiment that the active ingredient of black garlic can improve immunity which has a strong inhibitory on cancer cells」.

This is good introduction except for several mistakes such as *"fermented black garlic"*. It is not fermentation but chemical reaction called *"Mailard and Browning"*.

3.2. Bio-functions of the black garlic

Beneficial bio-functions of the black garlic were briefly introduced below for understanding true characters of the black garlic [5].

3.2.1. Tumor therapeutic potency

First report of the bio-functions on the black garlic were independently issued in 2007 from the two universities in Japan, the *Hirosaki University* [3] (Table 1) and the *Fujita Hoken Eisei University* [6]. The *Hirosaki University's* work was on the tumor therapeutic activity and the other was on the anti-carcinogenic potency by the *Fujita Hoken Eisei University* that was briefly introduced in the following 3-2-2 section in this text.

Table 1. *Tumor therapeutic potency of the back garlic (extracts) against Meth A Fibrosarcoma of Balb/c mouse [3]*

Sample	Dosage	No cured/No mice used	Tumor size of the non-cured Against that of control
Black g.	1 mg	5/10	47.5%
Control	(-)	0/5	100%
(Reference)			
Fresh g.	5 mg	0/5	64%
Control	(-)	0/5	100%

** Tumor transplanted mouse was treated by the test samples 3 times injection as therapy after tumor cells transplantation, and left three weeks without further therapy till anti-tumor evaluation*

The boiled water extracts of the black garlic attained 50% cure rate in the mouse model and average tumor size of the non-cured mice was 47.5 % to that of control. By contrast the fresh garlic extracts failed in deletion of transplanted tumor as shown in Table 1, implying surely the presence of therapeutic ingredients in the processed black garlic.

Our experimental results were introduced by Dr. *Joseph Mercola* in his home page as following [4];

In a 2007 mouse study, Japanese researchers found that black garlic was more effective than fresh garlic in reducing the size of tumors. The

study was published in the journal *Medicinal and Aromatic Plant Science and Technology* [3].

3.2.2. Anti-carcinogenic report

In 2007 another report on the anti-carcinogenic activity of the black garlic was published in Journal by the *Fijita Hoken Eisei University* (*Prof. Shimpo's* research group) using the 1, 2-dimethyl hydrazine (DMH)-induced premalignant lesion model in the rat colon [6]. The outcome was:

In the 5% black garlic-fed group for 6 weeks, numbers of premalignant lesion decreased compared with those in the control group given DMH alone. Feeding 5% black garlic to rats did not affect hematological and serum biochemistry parameters in the tested rats.

I am presently considering that one of the major ingredients for cancer therapeutic and anti-carcinogenic activities is the *S-allyl-L-cysteine* (SAC) that was generated during the garlic processing (*Maillard and Browning reaction*). This speculation does not necessary denies other elements association to fight cancer cells *in vivo*.

3.3. Immune system boosting act

Our previous studies on the tumor therapeutic action of the black garlic strongly suggested that its action could be led via enhancement of the immune system. To ascertain our presumption an additional experiment was designed, and it was surely confirmed in the black garlic-fed mouse model. The NK cells activity prepared from the black garlic-fed mouse certainly raised up as shown in Fig. 4, along with increasing generation of the cytokine IFN, TNF, NO, and IL-2 (not shown here) [7].

However cytokine IL-4 took different behavior by decreasing its production in the black garlic-fed mouse. Cytokine IL-4 promotes the proliferation and differentiation of B-cells that eventually result in enforcement of allergic symptoms by increasing production of allergic antibody IgE. Lowering amount of IL-4 production probably represents the allergic disease suppression. Results obtained in our mouse experiment were well corresponded with that of humans clinical symptoms as stopping running nose et al. Daily ingestion of the black garlic appears somewhat improving the allergic symptoms in the patients, even if additional experiments will be required to prove directly anti-allergic efficacy of the black garlic.

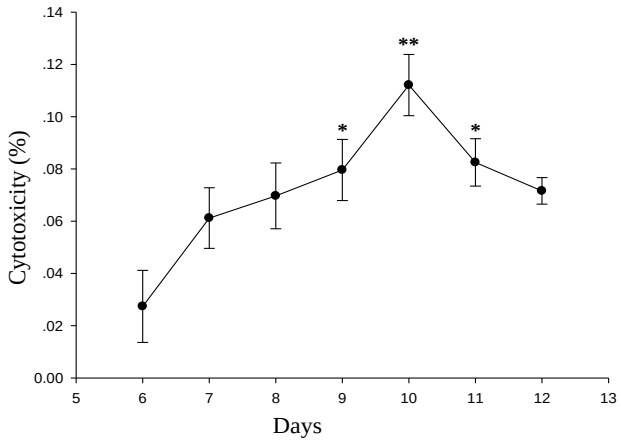


Fig 4. *Increasing NK cells activity in the cell culture system constructed from the black garlic-fed mouse (Asterisk indicates statistically significant to staring activity at day 6)*

Fig 5. *Fighting NK cells (small cells) against tumor cell (large cell) by creating holes that led tumor cell to death*

3.4. Bacteria-killing potency

Anti-bacterial potency was admitted in the extracts of the black garlic against MRSA (Methicillin resistant *Staphylococcus aureus*),

enterohemorrhagic *E. coli* O157, *Pseudomonas spp*. (a causative agent of opportunistic infection), *Candia albicans*. However its potency was less than that of general fresh garlic. Difference in its activity between them might be caused by different amount of volatile *allicin* contained, that was powerful bacteria killing agent. Great benefit of the black garlic is that it had worked against the drug resistant MRSA [3].

3.5. Antioxidant act

Antioxidant either in the form of natural material extracts or chemical constituents are very effective to prevent the destructive process caused by oxidative stress [8]. It is worth noting that the black garlic had the strongest antioxidant properties that effectively work as anti-aging and anti-carcinogenic to protect body from harmful chemical attacks (Table 2) among a large number of medical plants and vegetables

Table 2. *Anti-oxidative potency of the black garlic* [3] *(*RS50%)*

Sample tested	Black Garlic	Fresh Garlic
Japanese	4.1 mg	114.9 mg
Chinese	7.3 mg	88.5 mg

**Milligram used to reduce 50% of 1.1-diphenyl-2 picrylhydrazzy*

3.6. High content of GABA in the black garlic: Improvement of brain disorder?

The *Aomori Prefectural Industrial Technology Research Center* in Japan had lately found high content of the GABA (γ -aminobutyric acid) at 8-70mg/100gr in the processed black garlic. Its amount exceeded that of the germinated brown rice known as the highest content of the GABA at 10mg /100gr.

The compound GABA serves as a neurotransmitter in central nervous system in humans, and deficiency of it can cause various brain disorder such as Parkinson disease et al. [9]. Beneficial bio-functional effects of the black garlic will be expected against the central nervous disorder with no therapeutic or disease preventive ways. Generally it is considered that the GABA will not cross the blood brain barrier, and not carried into the brain. This kind of tasks will be soon started at the above center under the financial support of the local government.

Additional new finding was that the pyroglutamic acid that has the amnesia-preventive action in rats, improvement of learning in age-associated and alcohol-induced memory loss [10] increased in the black garlic. These new findings encouraged the companies related for doing their business.


黒ニンニク 強い抗がん作用に加え
ストレス抑制成分 含有
青森県内の製造者団体
研究成果生かし販促

Fig 6. *Higher content of GABA (γ -aminobutyric acid) in the black garlic that works to reduce stress (March 28, 2014)*

3.7. Additional major bio-functions reported up to now - Just listed the titles

- Detoxification activity (*Japan 2008*)
- Anti-fatigue effect in mouse experiment (Pharmaceutical company *Japan 2008*)
- Effect against acute alcohol-induced hangover and chronic alcohol-induced liver injury (*Korea 2009*)
- Decrease the cholesterol level and obesity of body (*Korea 2009*)

- Control high blood pressure (*Australia 2010*)
- Regenerative of therapy of burn damaged skin (Clinical study case *Malaysia*)
- Liver protective effect (*Korea 2010*)
- Control of diabetics and sugar disease (*Korea 2010, India 2013*)

4. Clinical case reports from the clients

◇ **CASE 1:** Oil painter *Choo Keng Kwang*, 81, has been eating around half a bulb of black garlic for the past few months. *Mr. Choo* who suffers from psoriasis-a skin disease which results in itchy red patches or flaky scales, decided to give the herb a try after seeing a newspaper advertisement for it. He says in Mandarin: "The effect was obvious. After three to four days of consuming black garlic, the red patches disappear. Previously, I've tried many skin creams given by doctors and they were not effective." (www.hardwarezone.com.sg)

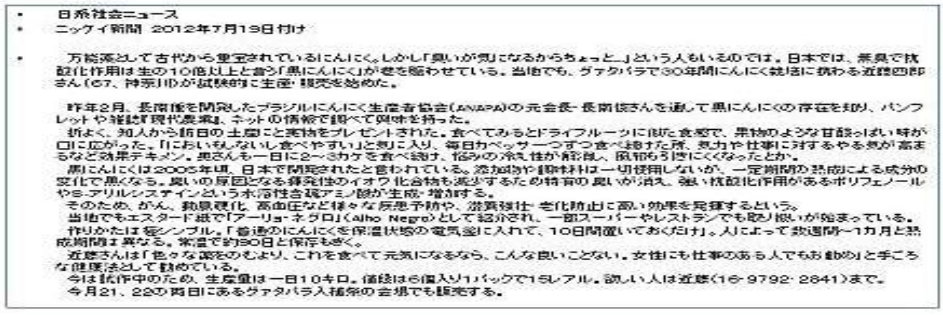
◇ **CASE 2:** Singapore General Hospital dietitian *Tan Ai Shan* says "Unlike fresh garlic, black garlic is sweeter in taste. The pungent smell and spiciness in fresh garlic is removed during the fermentation process. "She pointed to experiments conducted by Japanese researchers in 2007 which suggest that black garlic is more effective in reducing the size of tumor in laboratory mice. The study was published in a Global Science Book Journal about medicinal plant science." (www.sgh.com.sg › Home › About Us › Newsroom)

◇ **CASE 3:** Fermented garlic has greater health benefits than the common varieties, says nutritionist *Velumani Deepapriva* of the Singapore Nutrition and Dietrics Association. "Especially in improving blood circulation and increasing antioxidant levels in the body." She adds. (beforeitsnews.com/.../aged-black-garlic-a-new-superfo)

◇ **CASE 4:** Business consultant and part-time marathon runner *Edward Goh*, 50, started eating black garlic last June to lower in cholesterol level. His cholesterol was high at 250mg/dL. According to the Singapore Heart Foundation's guide lines, an average adult should maintain his total cholesterol level at less than 200mh/dL. (www.sgh.com.sg › Home › About Us › Newsroom)

◇ **CASE 5:** Mr. Kondo in *Guatapara Brazil* made the black garlic at home just to distribute his acquaintance prepared by an electric rice-cooker. Local newspaper introduced the black garlic as *"Alho Negro"* and currently restaurant and supermarket have started to deal in the black garlic. Mr. *Kondo* recently appears started business as the black garlic dealer (Fig 7). (www.nikkeyshimbun.com.br/nikkey/html/show/120719-61colonia.html)

発酵黒にんにくをどうぞ！＝グァタパラ近藤さんが生産・販売
中＝抗酸化作用は生の10倍



日系社会ニュース
ニッケイ新聞 2012年7月19日付け

万能薬として古代から重宝されているにんにく。しかし「臭いが気になるからちょっと…」という人もいるのでは。日本では、無臭で抗酸化作用は生の10倍以上と言う「黒にんにく」が老を脳わせている。当地でも、グァタパラで30年間にんにく栽培に携わる近藤四郎さん（67、神奈川）が試験的に生産・販売を始めた。

昨年2月、長期滞在を開始したブラジルにんにく生産者協会（ANAPA）の元会長・長南保さんを通して黒にんにくの存在を知り、パンフレットや雑誌「現代農業」、ネットの情報で調べて興味を持った。

折よく、知人から新日の土産にと黒物をプレゼントされた。食べてみるとドライフルーツに似た食感で、果物のような甘酸っぱい味が口に広がった。「においもないし食べやすい」と気に入り、毎日カベッサ一つずつ食べ続けた所、見力や仕事に対するやる気が高まるなど効果テキメン。奥さんも一日に2～3カケを食べ続け、悩みの冷え性が解消、風邪も引きにくくなったとか。

黒にんにくは2005年頃、日本で開発されたと言われている。添加物や調味料は一切使用しないが、一定期間の熟成による成分の変化で黒くなる。臭いの原因となる揮発性のイオウ化合物も減少するため特有の臭いが消え、強い抗酸化作用があるポリフェノールやS-アリルシステインという水溶性含硫アミノ酸が生成・増加する。

そのため、がん、動脈硬化、高血圧など様々な疾患予防や、滋養強壮・老化防止に高い効果を発揮するという。

当地でもエスタード紙で「アーリョ・ネグロ（Alho Negro）として紹介され、一部スーパーやレストランでも取り扱いが始まっている。

作りかたは極シンプル。「普通のにんにくを保温状態の電気釜に入れて、10日間置いておくだけ」。人によって数週間～1カ月と熟成期間は異なる。常温で約90日と保存もきく。

近藤さんは「色々な薬をのむより、これを食べて元気になるなら、こんな良いことない。女性にも仕事のある人でもお勧め」と手ごろな健康法として勧めている。

今は試作中のため、生産量は一日10キロ。価格は6個入り1パックで15レアル。欲しい人は近藤（16-9792-2841）まで。

今月21、22の両日にあるグァタパラ入植祭の会場でも販売する。

Fig 7. *Japanese-Newspaper in Brazil reported the black garlic merchandise produced by the emigrant Mr. Kondo (Headline; Kondo's black garlic in Guatapara Brazil showed ten times more in anti-oxidative activity than that of general garlic)*

5. Establishment of the Aomori Black Garlic Association to manufacture high quality and high secure black garlic in Japan

On March 2006, the black garlic had made a sensational debut in the field of foods via the Japanese Newspapers and TV. Thereafter increasing number of companies started to manufacture the black garlic as a new business using advantage of news reported on the black garlic. Some companies had shifted their plants from Japan to the oversea seeking after inexpensive garlic and low-cost of labors.

In connection with increase of companies, the product-related troubles had occasionally occurred by employing garlics cultivated by usage of the illegally agricultural chemicals that were strictly controlled in our country. Japanese are especially sensitive to these kinds of problems that directly affect family health. When purchasing agricultural products at the supermarket they first confirm the country of origin, and read carefully data of chemical analysis placed on for safety. Actually we had experienced in the past severe intoxication affair due to high contamination of the illegal chemicals in the imported products.

Fig 8. *"Authorized Warranty Emblem" issued by "Black Garlic Society of Aomori". This seal secures "Safety and Security of the black garlic"*

Since then, the black garlic companies in Aomori prefecture were considering establishment of "the Aomori Black Garlic Association" to supply the safe products to the citizens [11]. The member's companies in Association were making more of exertions to fulfill the Association's demands to gain citizen's credit. When their products are passed the Association's inspection organized by the third party examinees, the certificate seal will be given to the company to place on their products (Fig. 8).

The quality inspection system is now effectively working to guarantee the Aomori black garlic quality, along with differentiation of the Union member's products from the others. Home-made products currently prevailed among citizens are heterogeneous in quality, color, testy, and they are likely to be a half-raw material (Fig. 9) due to the incomplete processing manners. These sorts of the defectives are hard to eat with the remained pungent odor, lacking of sweetness, and will result in loss of the black garlic customers.

Fig 9. *Insufficient processing created this sort of defective product (by Caroline Hatchett)*

Fig 10. *"Takko Shoji's products" are served at cuisine of the 3 Star Hotel in German*

At that time of the Fukushima Nuclear Power Plant explosion, the radioactive contamination assessment standard on foods had not enacted in our country. After plants explosion the rumor damages rapidly grew and the citizens hesitated to purchase the agricultural and fisheries products shipped from

Fukushima and neighboring prefectures [12]. Foreigners living in Tokyo and around had evacuated immediately to the home countries, and some of them did not come back again. To clear up the rumor damages against radioactive pollution on foods the public inspection center started to measure all foods before shipping to offer people security with “the Radiological testing inspection certificates”. The black garlic was also not exception even in Aomori prefecture, where was 500 km away from Fukushima plants. The Association member’s companies voluntarily measured radioactivity on their products, and shipped to the markets along with the safe certificate as shown in Fig. 12.

Fig 11. *Nuclear power plant explosion due to malfunction of cooling system caused by the Tsunami*

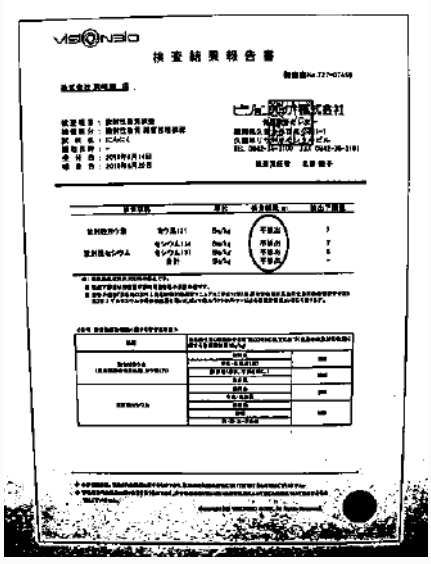


Fig 12. *Safety proof certificate issued by the government authorized inspection center in Japan against the radioactive pollution of the black garlic*

6. Discussion

The novel type of vegetable, the black garlic, rose up its name among the citizen in both domestic and foreign countries because the plenty of beneficial bio-functions as listed in the text are included along with the beneficial actions delivered from the personal experiences. At the moment the black garlic is less recognizable even among the scientific researchers, and most of them know nothing about it.

However its popularity is steadily increasing among the citizen and scientists, and some scientists are initiating experiments on the black garlic currently. One of the latest new findings was report on higher content of the γ -amino-butyric acid (GABA) in the processed black garlic.

This compound is critical important as a neurotransmitter in central nervous system as described above. Alzheimer and Dementia are representative brain disorders at the aged, and deficiency of GABA in brain is considered to causes various mental diseases as anxiety disorders likely to be panic attacks, headaches, Parkinson disease, and can also cause depression, insomnia, and epilepsy. My Japanese colleagues lately initiate bio-functional works of the black garlic to know if it will work to restore damaged nervous systems. Additionally ubiquitous urgent subject on the black garlic is the organization of the national and/or the international association to establish the safety guideline of the products to avoid the unexpected problems.

Researchers in broad areas should co-work trying to fight against the intractable diseases such as cancer, brain disorder et al by finding new bio-functional ingredients in the natural product [13] to contribute for human’s well-being.

7. Conclusion

Newly developed vegetable “Black Garlic” was reviewed from both aspects of scientific and

business fields. Increasing number of the black garlic companies is now competitively cultivating their marketing places. Overheated competition eventually resulted in sale of the defective products ignoring of safe and security of the products. To avoid this kind of the issue(s), the black garlic related companies should tackle conscientiously this problem to provide the safe products for contribution of the nation's health promotion.

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Pilot production of dried porcine intestinal mucosa from waste of sausage casing processing

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Abstract:

In this study, a trial process of dried porcine intestinal mucosa from waste of industrial processing of natural sausage casings has been done with a use of hydrogen peroxide as a strong oxidizing agent. Factors affecting drying process have been studied including temperature ranging from 40 to 60°C, material thickness from 1.0 to 3.0 kg/m^2 and air velocity from 0.5 to 1.5 m/s. Color change index of product (ΔE) and coefficient of rehydration (CR) were observed during the process. The dried product can be used as raw material subtitute in casing sausage. Sensory evaluation was performed to evaluate the smell, taste and structure of sausage. Instrumental texture analysis by Zwick Roell 1.0 has been also conducted to test ability using the dried porcine mucosa in food products. The study results show that dried porcine intestinal mucosa has low color change rate and rehydration capacity with ΔE = 2.06 and CR = 0.78 respectively at temperature of 50°C, material thickness of 1.0 kg/m^2 and air velocity of 1 m/s. The investigated mucosa has dry basic contents of 74.78 % protein, 12.92 % lipid, and low microbiological load (total aerobic bacteria counts of 2.5 x 10^1 cfu/g, negative *E. coli* and negative *Salmonell*a). The dried porcine mucosa can be used to replace as much as 7% lean meat in making sausages without affecting the taste, smell and texture of final products.

Keywords: intestinal mucosa, sausage casing, by-product, dehydration

1. Introduction

According to Lee *et al.* (2001), 34% solution of H_2O_2 (v/v) can be used to treat porcine intestinal mucosa before hydrolysis step. The process begins with heating mucosal solution to 73°C and agitating the solution within 13 minutes, then adds 1.5% of H_2O_2 into the solution and stirs within 10 minutes. The results showed that liquid hydrolyzied product obtains the same color of whey solutions. After 7 days kept at normal storage condition micro-organisms results are obtained with negative Salmonella; 3 cfu/g Coliforms and <3 cfu/g E.Coli. The results are preserved within 20 days. Additionally, H_2O_2 can also used to control the hydrolysis of enriched nutrition solution in order to produce protein concentrate. For bleaching, H_2O_2 is more efficiency than chlorine and lactic acid (Nassar *et al*., 1997).

By-products from slaughtered animals treated with hydrogen peroxide and sodium hydroxide can be futher hydrolyzed by protease and spray-dried to obtain a high protein powder (Woodgate *et al*., 2004).

2. Materials and Methods

Raw porcine intestinal mucosa was collected as the wastes of production processing for sausage casing from Vissan Limited Company (Vietnam).

Chemicals and additives used in the study include food grade 30% solution of H_2O_2 (v/v), sodium and potassium nitrates, polyphosphates and soy protein.

The mucosa was frozen at temperature of -18 ± 3°C until needed. Samples were thawed naturally at room temperature before heated at 80°C with the present of 500 ppm H_2O_2 (30%) as a strong

oxidizing agent within 5 minutes for disinfecting and deodorizing. Treated mucosa was then filtered through a 1mm ID sieve and dried by hot air tray-dryer. Response surface methodology was used to determine optimal process parameters such as drying temperature, material thickness, air velocity for drying porcine intestinal mucosa. The selected response variables were color change index (ΔE) and coefficient of rehydration (CR). The dried porcine intestinal mucosa was used to replace a part of lean meat in making sausages.

3. Results and Discussion

3.1 Optimization of drying process

The study results showed that temperature significantly affects both color change index and rehydration coefficient ($P<0.05$). Air velocity affects only coefficient of rehydration. Meanwhile the material thickness does not have effect on color change and rehydration capacity ($P>0.05$). Nevertheless, there are strong interactions among the investigated process parameters, which affect response variables as represented in the contour plots of response surfaces (Figure 1-3).

In Figure 1, it can be observed that at air velocity of 1.0 m/s an increase of drying temperature from 40 to 60°C and material thickness from 1.0 to 2.0 kg/m^2 increases the color change index from 1.479 to 2.748 and coefficient of rehydration value from 0.6638 to 0.8251. However, when material thickness increases from 2.0 to 3.0 kg/m^2, the color change index and rehydration coefficient values will not increase further.

Figure 2 describes effect of drying temperature and air velocity on color stability and rehydration capacity for experimental condition with material thickness of 2.0 kg/m2. When drying temperature is increased from 40 to 60°C and air velocity is increased from 0.5 to 1.0 m/s, color change index will increase from 1.4793 to 2.7480 and rehydration coefficient values will also increase from 0.6415 to 0.8251. However, when the air velocity is increased further, from 1.0 to 1.5 m/s, the color index and coefficient values will not change.

As observed in Figure 3 with drying temperature of 50°C, increasing material thickness from 1 to 3 kg/m2 and air velocity from 0.5 to 1.0 m/s will decrease color change index from 2.0335 to 1.9719 and increase rehydration coefficient from 0.6908 to 0.7148. A further increasing in air velocity, from 1.0 to 1.5 m/s, will make color index value increase from 1.9811 to 2.1137 and rehydration coefficient increase from 0.7148 to 0.7445.

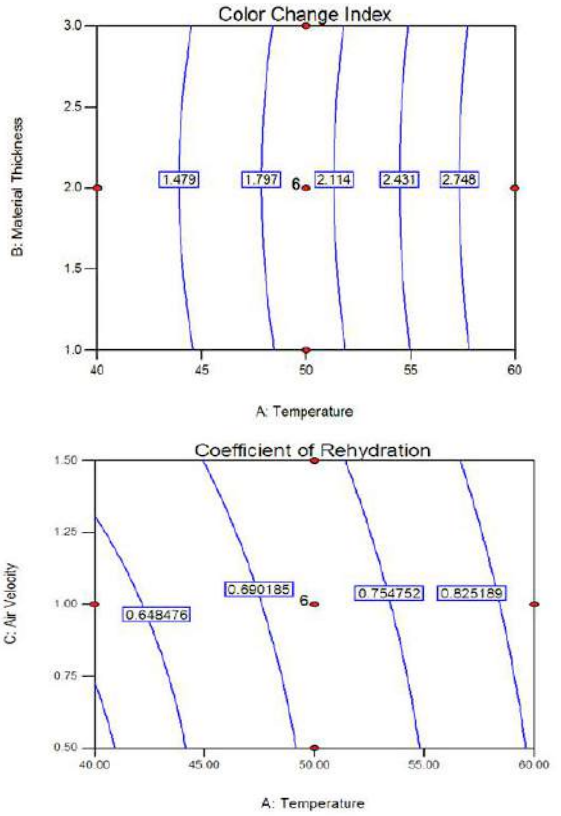

Fig 1. *Effects of drying temperature and material thickness on color change index and coefficient of rehydration.*

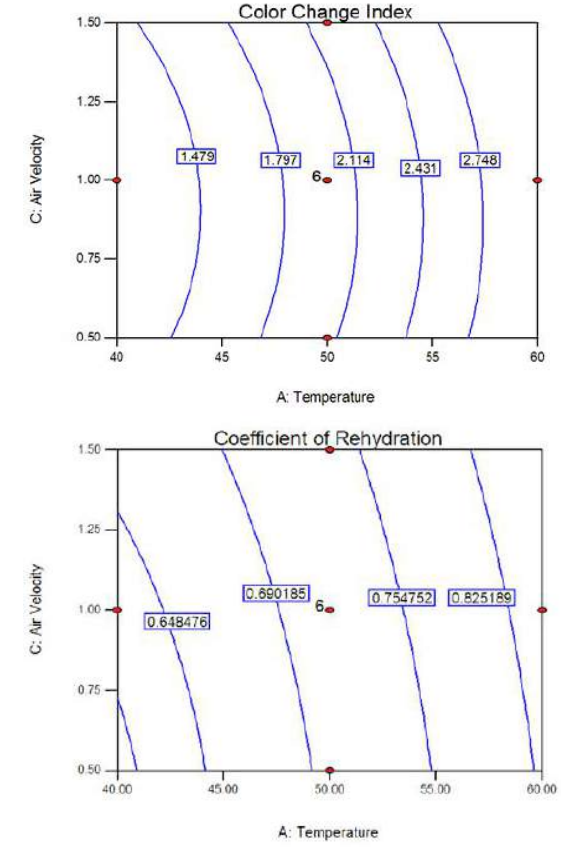

Fig 2. *Effects of temperature and air velocity on color change index and coefficient of rehydration.*

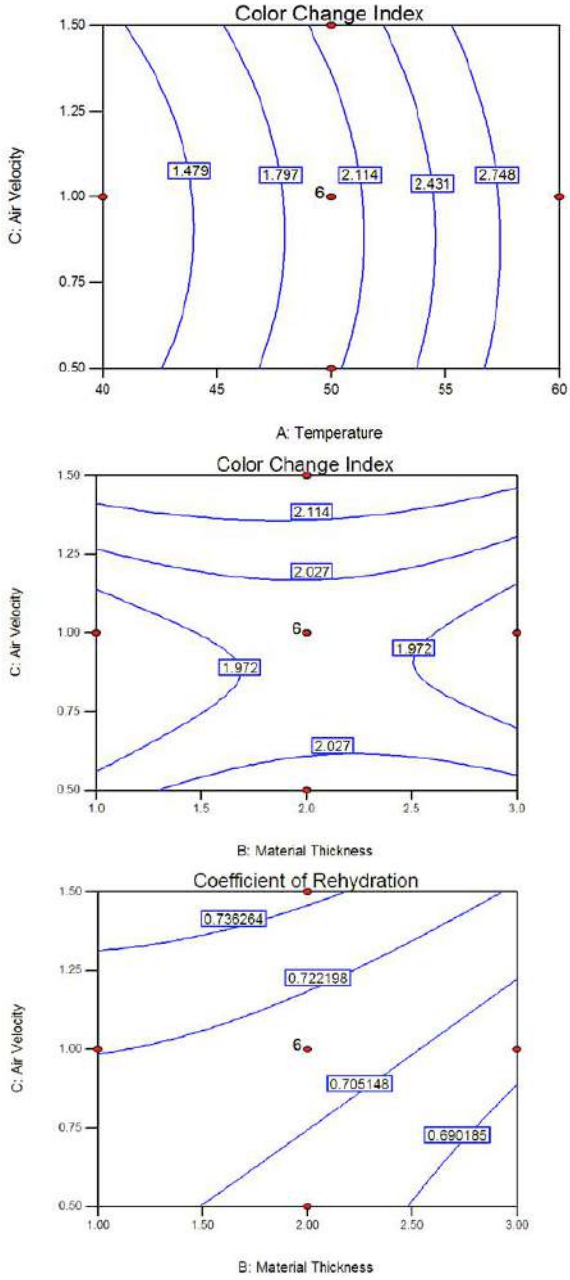

Fig 3. *Effect of material thickness and air velocity on color change index and coefficient of rehydration.*

In general, the study results showed that drying temperature of 50°C, material thickness of 1.0 kg/m^2 and air velocity of 1.0 m/s shall be the optimal conditions to obtain the best color and rehydration capacity for dried procine mucosa.

3.2. Dried porcine mucosa as raw material subtitution in sausage processing

In this study, dried porcine mucosa was used to replace 3 – 7% lean meat in making sausages. It has been found that sausages, which are substitute by 3% or 5% dried mucosa, have significant difference in smell property evaluation ($P<0.05$). There is also strongly significant difference between 5% and 7% substituted ingredient recipes ($P<0.01$). Comparing with a recipe of normal comercial sausage as a blank, there is no significant difference among samples. This is proven that the dried porcine intestinal mucosa can replace a part of lean meat in sausage production without affecting the smell of final products.

About taste property evaluation, there is significantly different among 7% substitution recipe with those of 3% and 5% ($P<0.05$). The others have no significant difference. One more time it shows that dried porcine mucosa can successfully replace lean meat without changing the taste of the sausage.

All sausages produced by using dried porcine mucosa as a part of lean meat substitution have similar structure. Instrumental texture analysis by Zwick Roell 1.0 system also showed no significant different results. It is obvious that the recipes with 7% dried porcine mucosa substitute have no effect on organoleptic properties, especially taste and structure of the final sausages.

4. Conclusion

Drying temperature of 50°C, material thickness of 1.0 kg/m^2 and air velocity of 1.0 m/s, are the best conditions to obtain dried porcine intestinal mucosa with color changing index and coefficient of rehydration values of 2.06 and 0.78 respectively.

The dried mucosa has high nutritional contents, microbiological safety and can be used to replace a part of lean meat in food sausage manufacturing. It is possible to replace up to 7% lean meat by dried porcine intestinal mucosa without any effects on struture and organoleptic properties of final sausages.

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Managing quality in agro - forestry and fisheries in Dong Nai - Situations and Solutions

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Abstract:

The current status of quality management in Agro - Forestry and Fisheries in Dong Nai province are presented, in which proposed some methods to improve the quality management of agro - forestry and fisheries products in Dong Nai province. Five solutions are showed, such as: training, supporting on some main tasks, computerization on managing quality, quantifying assessment of quality management of agro-forestry, fisheries and salt and researching to create a test kit to measure the quality of agro - forestry and fisheries products.

Keywords: Management, Agro – Forestry, Fishery, solution

1. Background

During the years of war and the subsidy period, arranging to have enough food and clothing for people are successful in leadership and management. A delicious meal and beauty clothing seem to be luxury demands. At that time, Dong Nai has only Branch of Standard Measurement and Quality of the Department of Science and Technology with a small part operates in the field of managing quality of all social products in the province.

After the country had advocated opening (1986), Vietnam has developed rapidly from a food deficit countries, which does not guarantee the food security, to become the second largest Country about exporting rice on the world. Our people change from full eating needs and warmly dressing demand to delicious meal and beauty dressing, and now integration into the world economy requires food safety beside fashion is still a necessary demand of civilized era.

The integrating into world economic requires world food production must increase quantity and quality to ensure food safety. Since Prime Minister's instruction 08/1999/CT-TTg on enhancing quality assurance is promulgated, food safety has been proclaimed. It has been marking by the action month about food safety and quality for 15 years until Food Safety and Hygiene Law was proclaimed in 2013 and has been replaced by Food Safety Law in 2011. It presented decision of The Party and State to integrate into Word Economy which associated with ensuring food security, enhancing the management of food quality. On the other hand, the Formation Food Safety of Department of Health, National Agro- Forestry – Fisheries Quality Management Department of Agriculture, and Rural Development Department…. have realized purposes of Party and state in the creation of public power reducing food contamination impact directly on the health of each people, affecting economic development, trade, tour.

Particularly for agriculture, the management of food hygiene and safety of agricultural, forestry, and fishery products specially concerned by the Ministry of Agriculture and Rural Development, the People's Committee of Dong Nai Province, and communities. It has achieved various improvements in past years such as Law system on food hygiene and safety in agriculture, forestry, and fishery; and has been supplemented and perfected step by step. Interdisciplinary collaboration is boosted, institutionalized, and becoming more and more effective. Communication education speeds up and creates positive change on awareness, raises

responsibility of leaders at all levels, sectors and social organizations and consumers, and contributes to the protection of people's health, and socio-economic development.

2. Statement of Need

However, besides achieving many great results, there are a variety of urgent problems of food hygiene and safety such as: non strictly controlling and preventing the situation of contaminated hazardous chemicals in some vegetables; having antibiotics, hormones left over in meat, poultry, and seafood; non effectively managing in food business. And the result can affect badly to people health, impact greatly on economic, tourist development and urban civilization. The situation of fake and poor quality food is not absolutely controlled; violations of the law have not been dealt with promptly and strictly.

Quality Management Department of Agriculture and Forestry and Fishery has responsible in helping Director of Agriculture and Rural Development of Dong Nai with the role of advisor for Province's People Committee in implementing state management function of quality, food hygiene and safety of agriculture, forestry, fisheries and salt in producing process until the food products are on the market or exported. Until now, Department has successfully complete its duties in education, training, popularizing food hygiene and safety policies, developing National target programs in food safety and hygiene; perform the tasks assigned by Province's People Committee which chaired the inspection team and handled violations of food safety in the Lunar New Year, Mid-Autumn Festival and the Elevator Action for quality, hygiene and food safety every year.

Among the above tasks, the ABC classification inspection and evaluating of businesses producing agricultural materials and forestry, fisheries products in conformity with Circular No. 14/2011/TT-BNNPTNT is the most important mission of General department of Agriculture. In 2013, the result of classification inspection and evaluating of 495 per 3,364 businesses producing agricultural materials and forestry, fisheries products in the province showed that: 162 establishments classified as A, accounting for 33%; 191 B-graded establishments, accounting for 38% and 142 C-classified establishments, accounting for 29% (C-classified establishments is not eligible for quality management, Food Hygiene Safety)

In which:

- The number of establishments of terrestrial animal and terrestrial animal products businesses and production chain: 158 establishments, (34 A-classified establishments, 79 B-classified establishments, 45 C-classified establishments, equivalent to 22%, 50%, 28%).
- Number of establishments of fisheries and fisheries products businesses and production chain: 200 establishments, (66 A-classified establishments, 45 B-classified establishments, 88 C-classified establishments, equivalent to 33%, 23%, 45%).
- Number of establishments of flora and flora products businesses and production chain: 32 establishments, (15 A-classified establishments, 14 B-classified establishments, 3C-classified establishments, equivalent to 47%, 44%, 9%).
- Number of establishments of agricultural materials businesses and production: 105 establishments, (47 A-classified establishments, 53 B-classified establishments, 5C-classified establishments, equivalent to 45%, 50%, 5%).

The above figures are not yet reliable enough in evaluating the results of statistical analysis, but somewhat reflects the reality of quality assurance of food safety, and agricultural materials and forestry, fisheries products of production facilities and agricultural, forestry and fisheries in the province is still insufficient.

The cause of this situation is due to the low awareness and sense of responsibility of the business, the consumer; the cultivation, animal husbandry and food processing facilities mainly are still at a low level; organizational and management systems of food safety and hygiene of agriculture, forestry and fishery is imperfect; management capacity is limited, especially the implementation of the management of food hygiene and safety are not given proper attention in commune and district level authorities and some places.

3. Proposal

Identifying the professional capacity of professionals working in quality management of agricultural, forestry and fisheries products have significant and decisive meaning. They at once do propaganda, training knowledge about food safety, food security, and inspect the work conditions to ensure food safety as well as evaluating, analyzing the quality of agricultural forestry and fisheries products in the area to prevent hazards to food safety, food security. Therefore, regular training and re-training in order to update and supplement their knowledge is essential. Suggest Institute, University support and facilitate the professionals of Quality management department of agricultural, forestry and

fisheries to participate in the programs of advanced professional training and vocational skills.

Suggest sectors, localities supporting agriculture sector to perform well a number of key tasks in 2014 and subsequent years as the implementation of Circular 14/2011/TT-BNNPTNT on classified inspection and evaluating of businesses producing agricultural materials and forestry, fisheries products as well as better coordination, supporting the agricultural sector in the implementation of Circular 03/2011/TT-BNNPTNT, 74/2011/TT-BNNPTNT issued regulations on traceability, recovery and processing of agricultural, forestry and fishery products which not guarantee safety; Circular 02/2013/TT-BNNPTNT regulated the risk analysis and food safety management according to chain of agricultural, forestry, aquaculture and salt business production and to gradually put quality management activities of forestry, fisheries into order.

Provide quality management with chemist's news: Quality Management Department of Agriculture and Forestry and Fishery are oriented storing of basis information as well as the sequence of quality management of agriculture, forestry, fisheries and salt business and producing establishments on the basis of geographical information system (GIS). This content is self-organized by Quality Management Department of Agriculture and Forestry and Fishery and meets some financial difficulties. The Department of Technology Science proposed adding this content to Research and Development (R&D) subject list to implement.

Nowadays, the evaluation of quality management of agriculture, forestry, fisheries and salt in local area is only qualitative. It must be quantitative and supplemented with new rural target about food hygiene and safety assurance in the long term. Therefore, the development of a quantitative economic model to evaluate the quality management of agriculture, forestry, fisheries and salt need to be researched carefully with the ways of different management.

There are some test Kit sets to check the quality of agriculture, forestry and fishery products quickly, however, the price of test Kits sets is high and the number of tested products is limited. It is suggested that the Academies and Universities should conduct more researches and apply new technological advances in creating more test Kit sets to detecting toxic chemicals, the residues of pesticides, sick treatment medicines of farming and breeding products at low cost in order that the state agencies and business production facilities have the opportunity to equip themselves with devices to test the quality of agro-forestry in the process of implementing Viet GAP, Global GAP, GMP, HACCP.

4. Conclusion

That is the reality of Quality management of agriculture, forestry and fishery and some solutions are proposed by Quality Management Department of Agriculture, forestry and fishery in order to raise the operation ability of quality management of agriculture, forestry and fishery in Dong Nai Province.

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Risk assessment of veterinary drug residues: The dietary exposure assessment of benzyl penicillin and tetracycline residues in pork consumed on Filipino market

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Abstract:

Biological, physical and chemical agents, recognized as food safety hazards, cause potential risks in modern food production. Although using the antibiotics in intensive animal productions is impossible to avoid the presence and remaining amount of their residues in food, it is possible to reduce them by using the principles of good veterinary, good manufacturing practices, and continuous control of food and risk assessment approach. This study was conducted to determine exposure risk to antibiotic drug residues in pork consumed in the Philippines and aimed to estimate dietary exposure of benzyl penicillin and tetracycline residues by age and gender groups. Dietary modeling, validated method used, combines food consumption data with food chemical concentration data estimate dietary exposure to food chemicals. The results had shown that the dietary exposure of benzyl penicillin and tetracycline residues for children from 1 to <3 years old had the highest exposure, followed by adolescents, adults and infants while males were significantly lower than females ($p<0.05$). This is the initial study to determine risk assessment of dietary exposure to antibiotic residue of different population of Filipino groups.

Keywords: Exposure assessment, pork, antibiotic residues, age-gender group, Philippines.

1. Introduction

Food is needed to consume for maintenance of human functions and it also has to be safe for human consumption. Food safety is imperative for the development and maintenance of a healthy population [1]. Consequently, food safety hazards are important issues for today's consumers. Although consumption of food products available on the market may not necessarily constitute a risk for acute disease or any adverse health effects later in life, consumers are concerned about the safety of their food [2]. There are increasing public health concerns that veterinary drug residues and their metabolites in meat and other foods of animal origin may cause adverse effects on consumers' health [3]. Moreover, there has been concern about carryover of veterinary drug residues in meat, eggs, and milk in people consuming these foods [4,5]. The potential hazards associated with the presence of veterinary drug residues in edible tissues have been reported consist of toxic or allergic reactions, anaphylactic reactions, trembling, headache, malaise, skin rashes, dose-related reversible bone-marrow depression, severe aplastic anemia, organ damage, birth defects, cancer or other chronic illnesses [6,7].

The term "Risk assessment" is generally used to describe the entire process of making a public health decision regarding a specific drug or agent and it is the scientific evaluation of known or potential adverse effects resulting from human

exposure to food borne hazards [8]. Risk assessment offers a formal approach to evaluate the safety of veterinary residues in food of animal origin for the consumer defined by Paustenbach [9]. The Codex Alimentarius Commission [10] defines risk assessment is integrated elements of the structure of risk analysis (Fig. 1) and to be based on the following steps (Fig. 2). The exposure assessment refers to the quantitative evaluation of the likelihood of exposure to drug residues through foods of animal origin, i.e. the estimate of consumer intake [11].

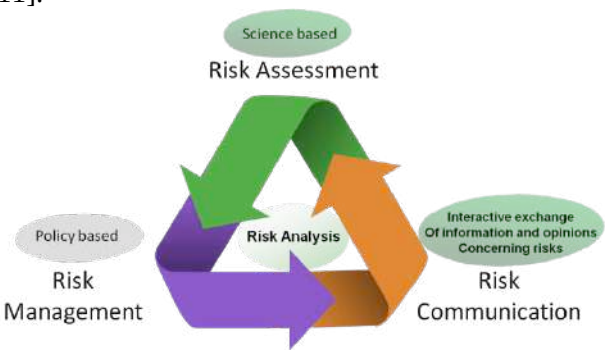

Fig 1. *Structure of risk analysis [10,12]*

Fig 2. *Risk assessment [10]*

A veterinary drug residue means any compound present in edible tissues that results from the use of a drug, and includes the drug, its metabolites, and any other substance formed in or on food because of the drug's use [12]. Landicho [13] stated that the portion of the drug retained in the animal tissues constitutes a drug residue. Ingested edible animal products containing drugs or chemical residues present potential health hazards to human consumers. Adverse effects caused by drug residues may be grouped under any one or more of the three general categories such as toxicological, microbiological, and immunological effects [13]. The potential hazards associated with the presence of veterinary drug residues in edible tissues have been reported in Fig. 3. With the widespread use of veterinary drugs in animal production, there is global concern about the consumption of foods of animal origin that may contain residues and their possible adverse effects on human health [14].

Fig 3. *Potential hazards of veterinary drug residues [6,7]*

Thorough literature search revealed no available study on exposure assessment on veterinary drug residues in the Philippines. With the increase in reports of occurrence of risk of veterinary drug residues in foods of animal origin in the last two decades [15,16], there is a strong need to study exposure assessment of antibiotic residues. The overall objective of the study is to determine exposure risk to antibiotic drug residues in pork consumed on Filipino market. The specific aim is to estimate the dietary exposure of benzylpenicillin and tetracycline residues by ages and gender groups.

2. Materials and Methods

Method used for determination of these antibiotics was validated according to the guidelines laid down by Codex Alimentarius Commission on the amount of antibiotic drug residues, the procedural guidelines on residues of veterinary drugs in food from Joint Expert Committees on Food Additives, and from a report of the 32nd session of the Codex Veterinary Drug Residues in Food 2009.

Dietary modeling combines food consumption data with food chemical concentration data to estimate dietary exposure to food chemicals where food consumption is the amount of food people eating per capita per day expressed in mg/kg/day and amount of drug residue expressed in µg/kg BW/day, respectively. Mathematical equations were used to calculate for Dietary Exposure from amount of drug residue multiplied by the mean food consumption and adjusted by body weight (Fig. 4).

$$\text{Dietary exposure} = \frac{\text{Mean Food consumption} \times \text{Amount of drug residue}}{\text{Adjusted for body weight}}$$

where:
Food consumption is the amount of food people eating per capita per day expressed in mg/kg/day and amount of drug residue expressed in µg/kg BW/day.

Fig 4. *Dietary exposure equation [20]*

3. Results and Discussion

Determining the risk of dietary exposure to benzylpenicillin and tetracycline residues using mathematical model gives an estimate of the risk of having toxicity among the populations. The most accurate estimation of the residual risk and probable quantitative risk assessment is a tool providing information on these aspects. In the present study, it is a first step in determining the most important fields for generating new data to estimate more accurately the proportion of the population at risk of toxicity and antimicrobial resistant due to a given amount of antibiotic residue of dietary intake in pork consumption.

Using equation (Fig. 5), the mean one-day food consumption for different age groups was calculated separately by age (infants, children, adolescents, and adults), gender, average weights at different age groups, and average weight in the Philippines. Table 1 shows the mean one-day per capita pork consumption survey in the Philippines (32g).

Fig 5. *Mean food consumption equation [20]*

Table 1. *Mean one-day per capita pork consumption (gram, raw, as purchased) 2003 food consumption survey*

	PHILIPPINES	LUZON	VISAYAS	MINDANAO
Fresh Meat				
Pork	31.80	39.89	23.07	18.24
Mean	31.76, 31.83	39.84, 39.93	23.01, 23.14	18.19, 18.30
95% CI	108.21	127.31	88.50	57.29
P90				

Source: Percentile Data Released: June 7, 2010 by Glen P. Gironella, FNRI, 2003.

Using the average value in Table 1, the average food intake per capita in the Philippines was respectively computed (Table 2). These were used for the calculation of Dietary Exposure to BENPEN and TCN using equation (Fig. 6 and 7).

Figure 8 shows that Dietary Exposure (DE) of benzyl penicillin residue for children from 1 to 3 years old had the highest value at 0.002923µg/kg bw/day. Likewise, average DE for children (0.002913 µg/kg bw/day) of 4-6 years old was higher than adolescents (0.0029096 µg/kg bw/day), followed by adults (0.002909 µg/kg bw/day) and infants (0.002903 µg/kg bw/day). Also, DE for male was significantly lower than females (p-value = 0.034).

Table 2. *Summary of Mean One-Day Per Capita Pork Consumption Difference Age Groups by Average Weight Difference Age Group, Mean One-Day Per Capita Pork Consumption and Average Weight in the Philippines*

Age/physiologic group[1]	Average weight # age groups[2]	Mean one-day per capita pork consum.[3]	Average weight in the Philippines[4]	Mean one-day per capita pork consum. # age groups[5]
1. Infants, mo				
Birth- <6 (3)	6 kg	32 g	55 kg	3.5 g
6-<12 (9)	9 kg			5.2 g
2. Children, y				
1-3 (2.5)	13 kg			7.6 g
4-6 (5.5)	19 kg	32 g	55 kg	11.1 g
7-9 (8.5)	24 kg			13.9 g
3. Adolescents, males, y				
10-12 (11.5)	34 kg			19.8 g
13-15 (14.5)	50 kg	32 g	55 kg	29.1 g
16-18 (17.5)	58 kg			33.7 g
4. Adolescents, females, y				
10-12 (11.5)	35 kg			20.4 g
13-15 (14.5)	49 kg	32 g	55 kg	28.5 g
16-18 (17.5)	50 kg			29.1 g
5. Adults, males, y				
19-49	59 kg			34.3 g
50-64	59 kg	32 g	55 kg	34.3 g
65 and over	59 kg			34.3 g
6. Adults, females, y				
19-49	51 kg			29.7 g
50-64	51 kg	32 g	55 kg	29.7g
65 and over	51 kg			29.7 g

[1&2] Recommended Energy and Nutrient Intakes, Philippines 2002 Edition, FNRI-DOST

[3] Mean one-day per capita pork consumption (gram, raw, as purchases), 2003 Food Consumption Survey, FNRI-DOST. Percentile data released: June7, 2010 by Glen Melvin P. Gironella and Adrienne S. Constantino

[4] The reference weight for adults, 59 kg for males and 51 kg for females, are the average weights in the Philippines is 55 kg (RENI-DOST, 2002)

[5] Using equation (figure 5)

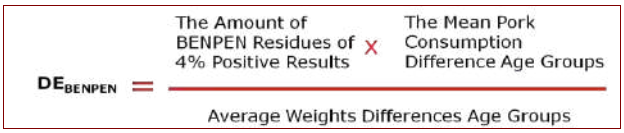

Fig 6. *Dietary exposure of benzylpenicillin equation [20]*

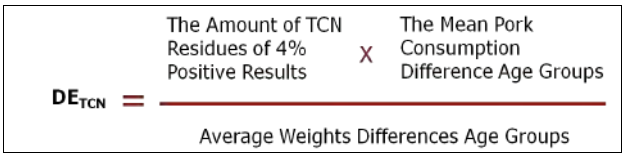

Fig 7. *Dietary exposure of tetracycline equation [20]*

Figure 9 shows that DE of tetracycline residue for children was the highest at 0.1169 µg/kg bw/day from 1 to 3 years old, calculated as 200 µg/kg of amount residues × mean one-day per capita pork consumption of 0.0076 kg ÷ 13 kg of average children weights. The average DE for children (0.1165 µg/kg bw/day) of 4-6 years old was higher than adults and adolescents (0.1164 µg/kg bw/day) followed by infants (0.1161 µg/kg bw/day). However, DE for males was significantly lower than females (p-value=0.043).

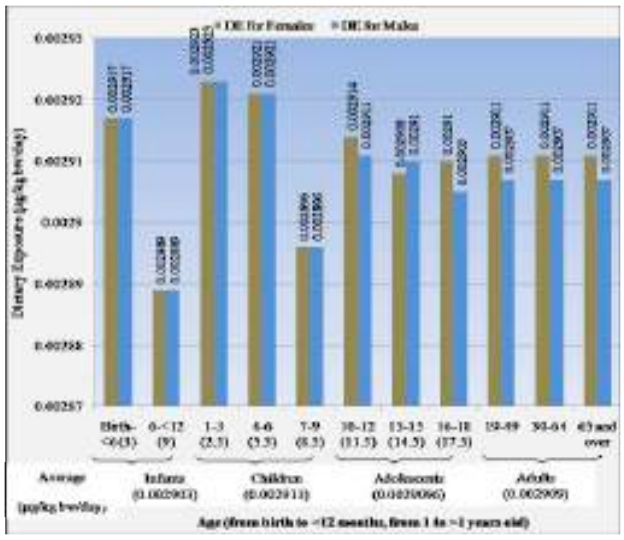

Fig 8. *Dietary exposure to benzylpenicillin residues in the Philippines*

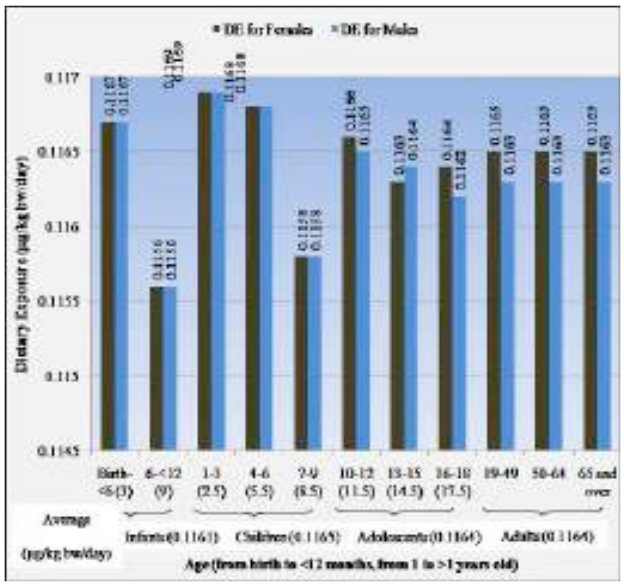

Fig 9. *Dietary exposure to tetracycline residues in the Philippines*

The current study examined the predicted number of dietary exposure of two antibiotic residues in the Philippines. $DE_{BENPEN}$and DE_{TCN} residues for children from 1 to 3 years old had the highest exposure followed by adolescents, adults and infants whilst DE for males was significantly different from females in the Philippines. This result is in agreement with earlier studies, for instance, Kroes et al. [17] concluded that children, because of their higher food consumption rates per kg body weight, were generally expected to have a higher exposure level and is therefore a susceptible subset of the population. In this respect, children may be of special interest because of their higher intake level per kg bodyweight due to growth processes (physical activity).

The use of antibiotics in agriculture and aquaculture is an antimicrobial resistance problem in the Philippines. Based on a 15-year review of infections at the Philippine General Hospital from 1971-1987, it showed that gram-negative bacteria constituted the most commonly isolated organisms with an average of 37-63 percent of antibiotic resistance in 1985. Currently, Research Institute for Tropical Medicine has examined 435 isolates 435 and found that Salmonella sp., *Salmonella typhi*, Shigella sp., and *Vibrio cholera* developed resistance to antibiotics. Thus, the concern about development of antimicrobial resistance, for instance, it is clear that the percentage of penicillin resistance is 25% Salmonella sp. [18].

The present study compared the amount of benzylpenicillin residue intake and dietary exposure of a person per day, which were less than about 10 to 12 times to tetracycline (ARSP-RITM [19]).

4. Conclusion

This is the first attempt to determine risk assessment of dietary exposure to antibiotic residues of different population groups in the Philippines by improving mathematical models used in this study proposing to better prioritized exposure assessment models for veterinary drug residues to reduce the presence of residues in foods of animal origin and by using the risk assessment approach to ensure the safety of food produced from farm to table.

Based on the findings of the present study, it is concluded that benzylpenicillin and tetracycline residues have been detected in pork, where children from 1 to 3 years old are more exposed and more at risk, because of their higher food consumption rates per kg body weight and children have a higher exposure level due to physical activity.

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Harmful effect of insecticides in the population dynamics of spiders on Lady'sfingers *Abelmoschus esculentus*(L.) Moench at field level

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Abstract:

Among the different predators, spider play an important role in the natural suppression of destructive insect pests viz., jassid, aphid, thrips, mites and eggs of many other insect pests infesting vegetable crops. Spider, an important predator in the sub-Himalayan region of north-east India was found very active on different insect pests of lady's fingers Abelmoschus esculentus (L.) Moench throughout the growing period. The level of population varied from year to year depending on their host and prevailing weather conditions. The important species of spider dominated in the lady's fingers field are Argiopeluzona, Cryptophoracicatrosa, Hipassapantherina, Oxyopesjavanes and Lycosapseudoannulata. Peak spider activity (3.94 spiders/plant) was recorded in May (20th standard week), when the average temperature, relative humidity and weekly rainfall were 29.71^{o}C, 80.31 % and 25.35 mm, respectively. However, spider population was very high during April-May and August-September (standard weeks 12-21 and 31-39, respectively). Spider incidence showed insignificant positive correlation (p= 0.05) with average temperature and relative humidity but insignificant negative correlation with weekly total rainfall. This correlation indicated better activity of spider fauna at higher average temperature associated with relative humidity and low rainfall. In early stage of crop growth different insect pests on vegetable crops can be controlled with protective synthetic insecticides but cause harmful effect to the entomophagous insects as well as our environment. From field evaluation of insecticides on lady's fingers it was revealed that insecticides of biological origin were relatively less harmful to spiders than synthetic ones. The botanical extract, Polygonum hydropiper floral part, the pathogens, Beauveriabassiana (Bals.)Vuillemin and Bacillus thuringiensis Berliner caused significant lower killing of the predator (less than 30 %) whereas the synthetic insecticides, profenophos and methomyl caused significantly higher killing (more than 52 %). Botanical and microbial insecticides are bio-pesticides having less or no hazardous effects on bio-agents, human health and the environment, and therefore, they can be incorporated in IPM programs and organic farming.

Keywords: Seasonal Incidence, Predator, Bio-Pesticides, Vegetable IPM, Organic Farming

1. Introduction

Lady's fingers *Abelmoschus esculentus*(L.) Moench is an annual crop belongs to the family Malvaceae and one of the most important vegetable crops grown in various parts of tropical and sub-tropical areas of the globe. Though lady's fingers finds its origin in South Africa, India stands top in area and production, with 3.58 lakh ha area, 35.24 lakh tones production and a productivity of 9.8 t/ha [1]. In the sub-Himalayan region of north east India lady'sfingers is cultivated at a commercial scale but insect and bite pest damage constitutes a limiting factor in successful production [2]. Various predators, parasites and pathogens cause natural suppression of insect pests of vegetable crops. The activity of natural enemy not only depends on prevalent weather conditions but also on the availability and size of host/prey population in crop ecosystem.

In early stage of crop, growth different insect pests on vegetable crops can be controlled with protective insecticides that cause harmful effect to natural enemies of the pests and environment. The control of the pest complex of lady's fingers through the use of synthetic pesticides particularly during the fruit bearing stage is rather difficult as the fruits harvested at frequent intervals, and there is every possibility that, if spray applications are made, harvested fruit would contain toxic residues and may cause health hazards. Usage of pesticides for control of insect pests of vegetables is both extensive and intensive. This created serious upset and imbalance in the arthropod complex and the environment causing resurgence and residues [3, 4, and 5]. The organochlorine and organophosphorus compounds have reported to pose a potential threat to all types of ecosystem [6]. Use of non persistent alternative chemicals with minimum required dosages should be adopted to protect the natural enemies. Moreover, many vegetables have been contaminated with environmentally incompatible poisonous pesticides. Due to these constraints, the research is being done on developing alternative economic and eco-friendly methods of insect control.

Spiders are the most common ubiquitous animals on land, constitute an essential portion of the predatory arthropods in several ecosystems [7-19]. Spiders serve as buffers that limit the exponential growth of pest populations in various ecosystems by virtue of their predatory potency [20-28]. Different species of spider are potential bio-agents, feeding on aphids, thrips, mites and some other insects which are harmful pests of vegetable crops particularly lady's fingers.

Many farmers use chemical pesticides to control pest. Some spiders show tolerance, perhaps even resistance, to some pesticides [29-33]. It was observed that immediately after the application of insecticides the spider population was reduced and subsequently, it increased and attained a peak. The previous results indicated that, the spiders are ideal predators of insect pests in manmade ecosystems. Though insect pest on vegetables have been well studied and catalogued, the spiders received little attention in vegetable field [34]. Hence, the present study was undertaken to record the population of spiders in the vegetable crop viz., lady's fingers and to investigate the harmful effect of insecticides on the population of spiders, and safer measure of bio-pesticides. Under the present investigation an attempt has been made to search some alternative insecticides which are eco-friendly.

2. Materials and Methods

Studies were conducted in the Instructional Farm of Uttar Banga Krishi Viswavidyalaya (State Agricultural University) at Pundibari, Coochbehar, West Bengal, India for two years (2010-2011). The experimental area is situated in the sub-Himalayan region of north-east India. This so called terai zone is situated between $25^0$57' and 27^0 N latitude and $88^0$25' and $89^0$54' E longitude. The soil of the experimental field was sandy loam with pH value 6.9. The climate of this zone is subtropical humid with a short winter spell during December to February.

2.1. Population dynamics of spiders:

The lady's fingers variety 'Nirmal-101' was grown round the year except winter when lady's fingers cultivation is not possible in this area, during 2010-2011 in both years under recommended fertilizer levels (120:60:60 kg NPK/ha) and cultural practices in 4.8 m x 4.5m plots at a spacing of 75 cm x 35 cm. The treatments were replicated five times in a Randomized Block Design (RBD), without adopting any plant protection measures. Observation on the population of spiders as a whole (irrespective of species) was recorded on 5 randomly selected plants from each replicated plots at seven days (Standard Meteorological Week) interval during lady's fingers growing seasons in both the years. Data obtained over two years were presented graphically with important weather parameters viz. temperature, relative humidity. These spider incidence data were correlated (r) with corresponding meteorological data for ambient temperature, relative humidity and rainfall, collected throughout the study period to find out influence of weather on population fluctuation.

2.1. Harmful effect of insecticides on spiders:

This two year (2010-2011) study of the harmful effect insecticides on spiders at field level was conducted at the instructional farm of Uttar Banga Krishi Viswavidyalaya (State Agricultural University) at Pundibari, Cooch Behar, West Bengal, India. The lady's fingers variety 'Nirmal-101' was grown during the post-kharif (early September) season in both years under recommended fertilizer levels (120:60:60 kg NPK/ha) and cultural practices in 4 m x 5m plots at a spacing of 75 cm x 35 cm. The treatments were replicated three times in a Randomized Block Design.

Five bio-pesticides and two synthetic chemical insecticides were evaluated under this program. One botanical insecticide neem, (Neemactin 0.15 EC; 2.5 ml/L), one botanical extract, *Polygonum hydropiper* floral part extract @ 5.0 %, two microbial insecticides *Bacillus thuringiensis* Berliner (Biolep 5 x 10^7 spores/ml; 1.0g/L), and *Beaveria bassiana* (Bals.) Vuillemin

(Biorin 10^7conidia/ml; 1.0ml/L) and one microbial toxin, avermectin (Vertimec 1.9 EC; 0.5 ml/L) were evaluated as compared with synthetic chemical insecticides profenophos (0.05%) (Carina 50EC; 1ml/L) and methomyl (0.05%) (Dunet 40 SP; 1.25 g/L). Four sprayings were made at an interval of 13 days. For 1st and 2nd spray 260 liter water and for 3rd and 4th spray 350 liter water were used to cover one hectare lady's fingers field. The *Polygonum hydropiper* plant's floral parts were extracted in methanol as follows. After washing with water, the plant parts were powdered in a grinder. The powder (50 g) samples of tested plant parts were transferred to a conical flask (500 ml) and dipped in 250 ml methanol. The material was allowed to stand for 72 hours at room temperature with occasional stirring. After 72 h, the extract was filtered through Whatman 42 filter paper and residues were washed twice with methanol.

Observations of spider population on lady's fingers were recorded at 3, 8 and 12 days after each spraying. The total populations per plant from 5 randomly selected plants per replication were recorded. The data were computed as percentage suppression over control and analyzed statistically. The results were expressed as spider population suppression (%) compared to densities recorded on the control treatment. Percent reduction of spider population over control was calculated by the following formula [35]:

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100$$

Where, Pt = Corrected mortality, Po = Observed mortality and Pc = Control mortality.

$$\text{Percent reduction over control} = \frac{\text{Percent reduction in treatment} - \text{Percent reduction in control}}{100 - \text{Percent reduction in control}} \times 100$$

Data were analyzed by using INDO-STAT-software for analysis of variance following randomized block design (RBD) treatment means were separated by applying CD Test (critical difference) at 5 % level of significance.

3. Results and Discussion

3.1. Population dynamics of spider:

Analysis of pooled mean data on spider incidence for both the two years revealed that the predator was active throughout the growing season of lady'sfingers (Fig. 1). The important species of spider found dominated in the lady'sfingers field are *Argiopeluzona*, *Cryptophoracicatrosa*, *Hipassapantherina*, *Oxyopesjavanes* and *Lycosapseudoannulata*. However, from the very beginning occurrences of spider was higher and increased gradually with the rise of temperature and fall down during June- mid July, after that high population was maintained until 1st week of October.

Again, with the fall of temperature (i.e., from middle of October) and with the maturity of crop which promotes low density of prey, spider activity declined up to the end of lady's fingers growing season. Peak spider activity (3.94 spider/plant) was recorded on May(20th standard week), when the average temperature, relative humidity and weekly rainfall were 29.71^0 C, 80.31 % and 25.35 mm, respectively. However, spider population was very high during April-May and August-September (standard weeks 12-21 and 31-39, respectively) (Fig. 1).

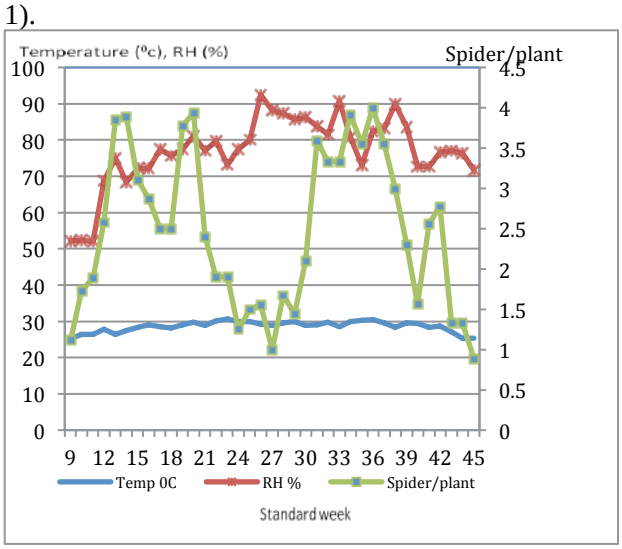

Fig1. *Incidence of spider as influence by temperature and RH*

Table 1. *Correlation co-efficient between weather parameters and incidence of spider*

Environmental parameter		Correlation co-efficient (r)	Co-efficient of determination (R^2)	Regression equation
Temperature ^{0}C	**Maximum**	0.041	0.001	Y = 0.040X+32.54
	Minimum	0.327*	0.107	Y =0.830X+22.50
	Difference	(-)0.333*	0.110	Y=(-)0.788X+10.02
	Average	0.287	0.082	Y= 0.435X+27.52
Relative Humidity (%)	**Maximum**	0.107	0.011	Y=0.778X+80.06
	Minimum	0.185	0.034	Y=2.04X+69.05
	Average	0.158	0.024	Y=1.406X+74.57
Weekly rainfall	**Total**	(-)0.040	0.001	Y=(-)2.310X+64.26

*Significant at 5% level of significance

Correlation between spider incidences with weather parameters (Table 1) showed that spider incidence had an insignificant positive correlation with maximum and average temperature and significant positive (p=0.05) correlation with minimum temperature but significant negative with temperature gradient. The correlation was positive but insignificant with relative humidity (maximum, minimum and average) and negative but insignificant with weekly total rainfall. Very high population of spider was observed in April-May and August-September which indicated that sprays of insecticides should be avoided at that period to save the predator, spider population in lady's finger field. As their population is high they constitute an essential portion of predatory population which is supported by [7,8,9.10]. Spider may serve as buffer that limit the exponential growth of pest in lady'sfinger field by virtue of their predatory potency [20,21,22]. The correlation studies indicated better activity of spider fauna at higher average temperature associated with higher relative humidity and low rainfall.

3.2. Harmful effect of insecticides on spiders

It is revealed that none of the insecticides was found safer to spider population. Although differences in toxicity of insecticides to spider, their relative persistency at different days after spraying was significant (Table-2). Among the seven insecticides evaluated under present investigation the insecticides from biological origin (bio-pesticides) were relatively less harmful to spider population than synthetic ones. The plant extract, *Polygonumhydropiper* floral part extract was found safest to the predator, spider recorded only 23.07 % suppression of the spider population and significantly different from all other treatments. The two microbial insecticides *Beauveriabassiana*and *Bacillus thuringiensis* were less harmful and resulted significant lower killing of the predator (25.30 % and 27.97 % respectively). The two synthetic insecticides methomyl and profenophos were found very harmful and caused significant higher killing (55.33% and 52.57 % respectively) of the predator.

Three days after spraying the plant extract, *Polygonum hydropiper* and the two microbial insecticides *B. bassiana* and *B. thuringiensis* were found less harmful and resulted significant lower killing of the predator (32.16%, 31.36 % and 32.32 %, respectively).Three days after spraying the two synthetic insecticides methomyl and profenophos were found very harmful and caused significant higher killing (72.93% and 68.26 %, respectively) of the predator. Similar type of action of the *P.hydropiper* and the two microbial insecticides was maintained seven days after spraying. Twelve days after spraying the *P. hydropiper* and the two microbial insecticides were found safer to the predators as compared to other sprayings and resulted significant lower killing, 13.13%, 16.34 % and 24.13 %, respectively. In all sprayings the *P .hydropiper* was found safer to the predators as compared to all other treatments and the two synthetic insecticides were most harmful. The organochlorine and organophosphorus compounds have reported to pose a potential threat to all types of ecosystem [6]. Use of non persistent alternative chemicals with minimum required dosages should be adopted to protect the natural enemies. Biopesticides like plant extracts and microbial pesticides may be used as alternatives of chemical pesticides to protect the predators in lady's finger field. *P .hydropiper* was found safer to the predator, spider in lady's finger field recorded very lower killing of the spider.

Table 2. *Mortality of spiders in lady'sfingers field due to action of insecticides*

	Suppression of spider population (%)											
	3 DAS			3 DAS			3 DAS			Grand mean		
	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
Profenophos (0.05%)	69.20 (56.74)	67.32 (55.23)	68.26 (55.98)	45.43 (42.35)	57.56 (49.36)	51.49 (45.84)	32.73 (34.82)	43.17 (41.05)	37.95 (37.93)	49.12 (44.64)	56.02 (48.55)	52.57 (46.59)
Methomyl (0.05%)	76.69 (61.26)	69.18 (56.36)	72.93 (58.81)	48.96 (44.40)	52.87 (46.67)	50.91 (45.53)	39.15 (38.66)	45.15 (42.18)	42.15 (40.42)	54.93 (48.11)	55.73 (48.40)	55.33 (48.25)
Neem (2.5 ml/L)	44.98 (42.12)	39.10 (38.67)	42.04 (40.39)	38.25 (38.16)	36.48 37.10)	37.36 (37.63)	24.52 (29.55)	20.06 (26.51)	22.29 (28.03)	35.92 (36.61)	31.88 (34.09)	33.9 (35.35)
Bacillus thuringiensis (1.0 g/L)	41.01 (39.77)	23.44 (28.93)	32.32 (34.53)	27.76 (31.73)	27.16 (31.35)	27.46 (31.54)	21.21 (27.34)	27.09 (31.27)	24.13 (29.34)	29.99 (32.95)	25.90 (30.52)	27.97 (31.80)
Beauveria bassiana (1.0 ml/L)	39.61 (38.97)	23.12 (28.67)	31.36 (34.01)	32.86 (34.91)	23.52 (28.95)	28.18 (32.00)	16.57 (23.94)	16.13 (23.62)	16.34 (23.78)	29.68 (32.61)	20.92 (27.08)	25.30 (29.93)
Avermectin (0.5 ml/L)	46.18 (42.79)	39.59 (38.96)	42.88 (40.88)	51.48 (45.86)	32.61 (34.79)	42.04 (40.40)	32.48 (34.70)	26.63 (31.02)	29.00 (32.88)	43.38 (41.11)	32.94 (34.92)	38.16 (38.05)
Polygonum (5.0%) 50 ml/L	32.86 (34.92)	31.46 (33.38)	32.16 (34.15)	22.68 (28.36)	25.18 (29.94)	23.93 (29.15)	15.45 (23.0)	10.81 (19.05)	13.13 (21.02)	23.66 (28.76)	22.48 (27.46)	23.07 (28.11)
Untreated check (Control)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)

	2010			2011			Mean		
	A	B	AxB	A	B	AxB	A	B	AxB
S.Em (±)	1.05	1.36	2.53	0.83	1.05	1.92	0.84	1.18	2.05
CD at 5%	3.15	3.93	7.51	2.52	3.03	5.76	2.41	3.40	N.S.

Figure in parenthesis indicate angular transformed values
DAS, Days after spraying
A, Days after spraying; B, Insecticides; A x B, Interaction of DAS with insecticides

4. Conclusion

Pesticide spray should be done carefully to the crop when population of the predator is abundant in the field. Based on their moderate to high efficacy levels, as well as low toxicity to natural enemies and minimum impact on human health and environment, we conclude that microbial and botanical insecticides (both bio-pesticide) can be incorporated in IPM programme and organic farming in vegetable cultivation.

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Effectiveness of a solar pasteurising device in reducing microbial population of pond water in Bangladesh

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Abstract:

Water plays a vital role in the development of healthy human communities. In Bangladesh groundwater between 70 ft and 300 ft is contaminated with arsenic in many areas while surface water is free of arsenic, but contaminated with enteropathogenic microorganisms, including those of diarrhea, cholera, typhoid, paratyphoid, hepatitis-A, etc. Disinfecting water of harmful microorganisms is relatively easy and can be done in each home, or even in the rural areas. The Biomedical Physics Department of Dhaka University has developed a low cost solar pasteurizer for such applications, which can heat about 5L of water to more than 65^0C in about two hours of clear sunshine. The water in this device is also exposed to UV contributing to a synergy of heat and UV. This study was designed to see the effectiveness and feasibility of this device in reducing microbial population of pond water for drinking purposes. Pond water samples from three different ponds in Dhaka city area were subjected to treatment using this device and the highest temperature achieved was 69^0C after about 2 hours of exposure to sunshine. Regardless of sampling pond, highest bacterial population reduction of 3.9±0.45 log CFU/ml was recorded in non-selective medium.

Keywords: Solar pasteurization device, temperature, pond water, Microbial population

1. Introduction

Drinking water is the most important source of gastro enteric diseases. It is estimated that 884 million people lack access to improved water supplies. Many more forced to rely on supplies having unsafe microorganisms, resulting in a higher risk of waterborne diseases including of typhoid, hepatitis, polio, and cholera. Due to poor sanitation and lack of clean drinking water, there are around 4 billion cases of diarrhea each year resulting in 2.2 million deaths, most of these are children under five [1].

Conventional technologies used for disinfection of non-potable water include ozonation, chlorination, and artificial UV radiation. These technologies require capital intensive, sophisticated equipment, and demand skilled operators [5]

Like other developing countries, in Bangladesh, more than 25 million people lack access to an improved water resource and most of them live in the rural areas of Bangladesh [12].

The critical water situation in Bangladesh involves both the groundwater and the surface water. The groundwater is contaminated with arsenic creating health problems that in the end may lead to death [10]. On the other hand, the surface water contains pathogens causing diarrhoeal diseases and being a major cause of child death [9].

Surface water sources of drinking water in

Bangladesh have historically been contaminated by pathogenic microorganisms, which cause a significant burden of disease and mortality. Diarrhoeal disease is the second leading cause of mortality for children under five years old in the world [2]. It is both preventable and treatable, but still diarrhoea kills around 760 000 children under five each year [11]. In developing countries, like Bangladesh, diarrhoea is also a major cause of malnutrition [2].

In the 1970s gastrointestinal diseases was an acute problem; infants and children suffered the most from these grave diseases as the result of pathogenic contamination in pond water, rivers, lakes etc. [11]. Hence, rapid actions were necessary and tube wells began to be installed [7]. The installation of tube wells spread fast and Bangladesh shifted from drinking surface water to drinking groundwater. Unfortunately, the water turned out contaminated with arsenic.

Tube wells have been used in Bangladesh since the 1940s, but only recently has the problem with arsenic-contaminated water come to light [10]. This is due to the increasing installation of tube wells during the past 30 years and the consequential rising number of persons drinking from them. During the 1970s, the United Nations Children's Fund (UNICEF) and the Department of Public Health Engineering installed tube wells around the country to intentionally provide safe drinking water [7]. At this time arsenic in water supplies was not known as a problem and hence standard testing of water did not include arsenic tests.

In 1993 the first case of arsenic contaminated water was detected and further testing was done in the following years, including investigations of the Department of Occupational and Environmental Health of the National Institute of Preventive and Social Medicine. Results from various laboratories were gathered in a country report of World Health Organization (WHO) in 1996. In about half of the measurements, the arsenic concentrations were above 50 µg/l [10]. This did not meet the guidelines from WHO which the recommended maximum level is 10 µg/l. Even worse was that cases with concentrations higher than 50 µg/l were identified in Bangladesh.

According to survey data from 2000 to 2010, it estimated that about 35 of 77 million people in the country have been chronically exposed to arsenic [7]. This has been described as the largest mass poisoning in history [10]. The mechanism of controlling water borne contaminants by sunlight exposure is due to both the effect of UV radiation and heat.

In order to improve the water situation in rural areas of Bangladesh, a research group at Biomedical Physics and Technology Department, University of Dhaka has been developing a low cost domestic method to remove pathogens from surface water by pasteurization of water using free solar energy. Pasteurization, which destroys all diarrhoeal pathogens, is a process in which water is heated to 60 °C and maintained for 30 minutes [3], or heated to 70 °C and maintained for 15 seconds. Some types of bacteria may still survive, but these are usually harmless [8]. The device involves the use of polyethylene sheets or polyethylene bags filled with water and other available materials in the rural area in order to set up a simple device that creates 'Green House Effect'-conditions. This is essentially a flat plate solar water heater, which provides the safe drinking water to user. In this method, the water is also exposed to UV-light being available in the sunshine, which causes destruction of diarrhoeal pathogens at temperatures somewhat lower than that required in normal pasteurization.

This study was designed by understanding the prevailing conditions and urgency of safe water supplies in flood prone rural communities. To assess the feasibility of solar disinfection of small quantities of drinking water that would satisfy the daily needs of individuals or a family, these experiments essentially consist of subjecting natural and/or artificially contaminated water in small, transparent, 1 to 5 liters in volume, exposure with direct sunlight for varying periods.

2. Materials and Methods

2.1. Sample collection

Water samples were collected in 250ml sterile bottles from approximately 4-5 cm below the pond water surface. This was usually done in between 08h00 to 09h00 hours of the day and transported to the laboratory in an insulated box immediately. In each case, the water was initially examined for bacteriological content just before sunlight exposure. The standard plate count of selective and nonselective microbiological medium was used for the estimation of total bacterial counts, total coliform counts and E. coli counts. Identical batches of water in similar containers were kept at the room in which the light is controlled to compare and assess the effect of sunlight.

2.2. The Experimental Work

Collected water was filtered through eight fold cotton clothes typically known as "sharee" in South Asia. The filtered water was then poured in transparent polyethylene bags (120cm x 120cm). Water was poured up to one third of the polyethylene bags and ensured that the depth of water is within 2.0 cm when the polyethylene bags was laid down on the blackened bamboo tray with the open end placed over the raised edge of the tray. The air bubbles were removed by lightly pushing with fingers. The 9-storied roof of the Center for Advanced Research in Sciences buildings of the University of Dhaka served as the site for these experiments. The hay was spread to a thickness of at least 10 cm on the roof and the bamboo tray with water containing bags was placed on it. Then another transparent polythene sheet was spread over the water bags and few strands of rice straw was spread over the second polyethylene sheet to create an air layer to prevent escaping heat. Then the third polyethylene sheet was spread over the straw and then weight was put in the edges of the polyethylene sheet to prevent wind blowing the polyethylene sheet up. The above-mentioned procedure was named as "Gadget" for water disinfection and shown in Figure 1.

2.3. Preparation of Inoculum

Strains of E. coli isolated from surface water were used in this study. The test strains were adapted to grow in Tryptic Soy Broth (Sigma Chemical Co Ltd, St Lewis, UK) (PH 7.3) supplemented with rifampicin (TSB-Rif ; 50µg/ml). Cultures were transferred to TSB-Rif by loop at three successive 24-hr intervals before they were used as inoculant. Then cells were collected by centrifugation (3000 rpm, 5 min) and re-suspended in sterile normal saline. The lower inoculums with initial concentration of 103 CFU/ml and higher inoculums with initial concentration of 10^8 CFU/ml were maintained at 25°C ±1°C and applied to the pond water within one hour of preparation. Introduction of drug resistant mutations into test strains previously has been used effectively since the selection of bacteria could successfully recover from water environment (Beuchat et al., 2003). Plating on media containing rifampicin greatly minimized the interference of colony development by naturally occurring microorganisms and thus facilitated the detection of test pathogen on recovery media.

2.4. Sample preparation and exposure to sunlight

One ml of lower initial inoculum (10^3 CFU/ml) and higher initial inoculum 108 CFU/ml was added to 1.5 L of "saree" filtered pond water in transparent polyethylene bags separately and exposed to direct sunlight. In every one-hour interval bacteriological count was conducted up to 4 hours. The experiments were generally run from 9h00 to 14h00, when the solar intensity reaches its highest levels. Both the inoculated and non-inoculated filtered pond water was exposed to sunlight separately.

2.5. Microbiological Analysis

During the exposure to sunlight, in every one hour, one plastic bag was picked and decimal diluted (10^{-1} to 10^{-6}) with 0.85% saline water. One hundred microliter (100 µl) of the treated samples were surface plated onto both selective and non-selective medium for non-inoculated pond water.

On the other hand, for inoculated pond water, non-selective Tryptic Soy Agar and selective Coliform Agar were used for total aerobic bacterial count and coliform counts respectively and Sorbitol MacConkey Agar medium supplemented with 50µg/ml rifampicin was used to recover the inoculated bacteria. In addition, Chromogenic Chromocult Agar (MERCK Germany) supplemented with ceftazadime, and pentahydrate were also used as selective medium for the confirmation of *E. coli.* The entire supplement including rifampicin was added to the molten agar before pouring the medium into petri plates. For that reason, only rifampicin resistant bacteria were grown in that medium. All the plates were then incubated at 37°C for 16 to 24 hours before presumptive colonies were counted. All the experiment was repeated three times to confirm the reproducibility.

2.6. Statistical Analysis

All experiments were repeated four times, and duplicate samples were analyzed at each sampling time. Significant differences in plate count data were evaluated using the least significant difference test at the 5% level of significance.

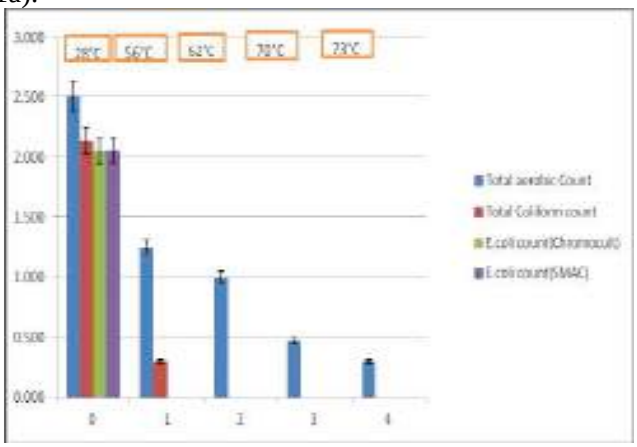
3. Results and Discussion

3.1. Effect of sunlight on different pond water

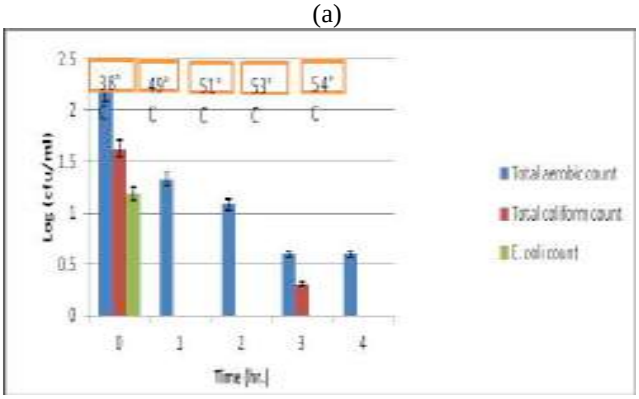
In case of Shahidullah Hall pond water, the initial aerobic bacterial load was recorded as 2.6 log CFU/ml, and coliform count and E. coli counts was recorded as 2.2 and 2.0 log CFU/ml, respectively after saree filtered water (Fig 1a). The physical properties of the treatment day was recorded and observed as clear sunny day, high humidity and sunlight intensity was recorded as 1410 LUX/mn. The initial day temperature was recorded as 28°C at 9h00 and temperature reached to maximum 73˚C at the treatment point of pond water at 14h00. As the temperature increased, the initial aerobic population declined to 1.2 logs CFU/ml after 1h of exposure and decreased further in 2h and 3h and

finally reached to 0.2 log CFU/ml at 4h of sunlight exposure in the gasket.

Total coliform and *E.coli* population decreased significantly after 1 hour of exposure and no survivors were recorded after 2 h of exposure and thereafter (Fig 1a).



(a)



(b)

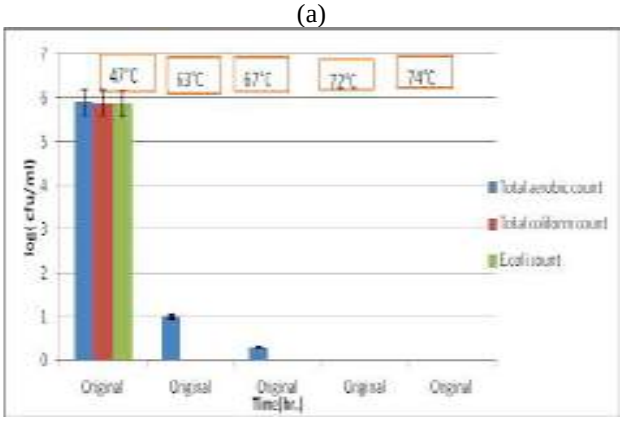
Fig 1 *(a,b) Effect of sunlight on total aerobic, total coliform and E. coli count of Shahidullah Hall pond water. Because of temperature and solar radiation difference of the experiment day, so, different curves (a,b) have been done.*

On the other hand, same pond water were used in treatment on another day (Fig 1b) The physical properties of the treatment day was recorded and observed as cloudy day, with higher humidity and sunlight intensity was recorded as 880 LUX/mn. The initial temperature was recorded as 38^{o}C at the point of treatment at 9h00 and temperature reached to maximum 54˚C at 14h00. The initial aerobic bacterial load was recorded as 2.4 CFU/ml and as the temperature increased, the initial aerobic population declined to 1.4log CFU/ml after 1h of exposure and decreased further in 2h and 3h and finally reached to 0.5 log CFU/ml at 4h of sunlight exposure in the gasket (Fig 1b).

Total coliform and *E.coli* population decreased significantly and after 1 hour of exposure no survivors were recorded. However, there was an increase of total coliform after 3 hours of exposure for unknown reason and no survivor was found at 4 hours of exposure (fig 1b)

In case of artificially inoculated *E. coli* in Shahidullah Hall pond, the initial aerobic bacterial load was recorded as 5.897 log CFU/ml, and coliform count and *E. coli* counts was recorded as 5.892 and 5.875 log CFU/ml, respectively after saree filtered water (Fig 2). The physical properties of the treatment day were recorded and observed as clear sunny day; high humidity and sunlight intensity was recorded as 1660 LUX/mn. The initial day temperature was recorded as 47°C at 10.30 hr and temperature reached to maximum 74°C at the treatment point of pond water at 14.30 hr. As the temperature increased, the initial aerobic population decline to 1 log CFU/ml after 1h of exposure and decreased further in 2h and 3h and finally reached to lower than detection limit.

(a)



(b)

Fig 2. *Effect of pasteurizing device on total aerobic, total coliform and E. coli count of Rifampicin resistant Bacteria (marker Bacteria) of Shahidullah Hall pond water. As because this is not temperature resistant bacteria rather than antibiotic resistant bacteria. So solar radiation has an effect to destroy it.*

In the case of Dhanmondi Lake water, the initial aerobic bacterial load was recorded as 4.84 log CFU/ml, and coliform count and *E. coli* counts were recorded as 3.32 and 3.1 log CFU/ml, respectively

after saree filtered water (Fig 3). The physical properties of the treatment day was recorded and observed as clear sunny day, high humidity and sunlight intensity was recorded as 1645LUX/mn. The initial day temperature was recorded as 33°C at 9.40 hrs. and temperature reached to maximum 74°C at the treatment point of pond water at 13.40 hrs. As the temperature increased, the initial aerobic population declined to 2.54 logs CFU/ml after 1h of exposure and decreased further in 2h and 3h and finally reached to 1.9 logs CFU/ml at 4h of sunlight exposure in the gasket.

Total coliform and *E.coli* population decreased significantly after 1 hour of exposure and no survivors were recorded after 2 h of exposure and thereafter (Fig 3).

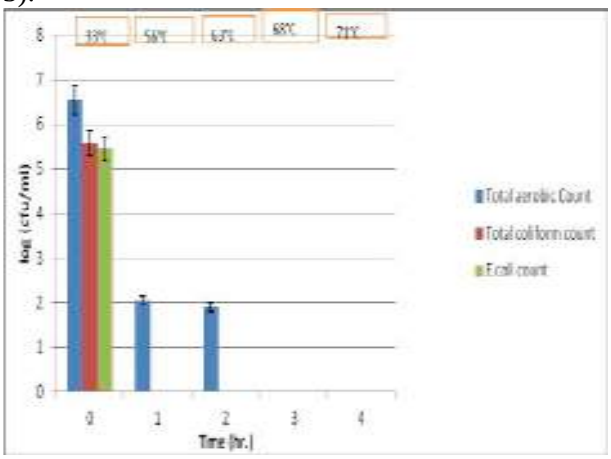


Fig 3. *Effect of sunlight on total aerobic, total coliform and E. coli count of Dhanmondi lake water*

In the case of artificially inoculated *E. coli* in Dhanmondi Lake, The initial aerobic bacterial load was recorded as 6.54 log CFU/ml, and coliform count and E. coli counts were recorded as 5.56 and 5.44 log CFU/ml, respectively after saree filtered water (Fig 4). The physical properties of the treatment day was recorded and observed as clear sunny day, high humidity and sunlight intensity was recorded as 1870 LUX/mn. The initial day temperature was recorded as 33°C at 10.40 hr and temperature reached to maximum 71°C at the treatment point of pond water at 14.40 hrs. As the temperature increased, the initial aerobic population declined to 1 log CFU/ml after 1h of exposure; decreased further in 2h - 3h; and finally reached to below the detection limit (Fig 4)

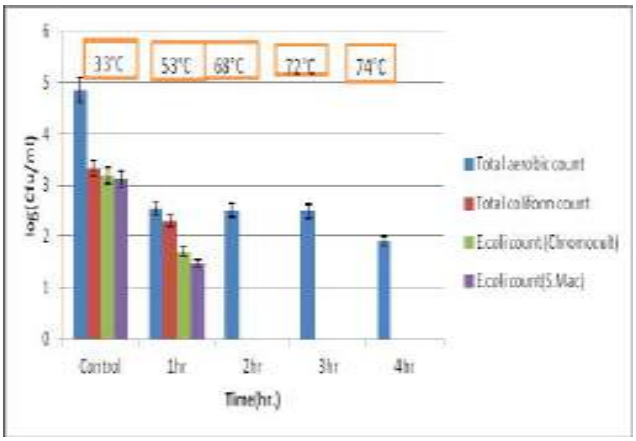
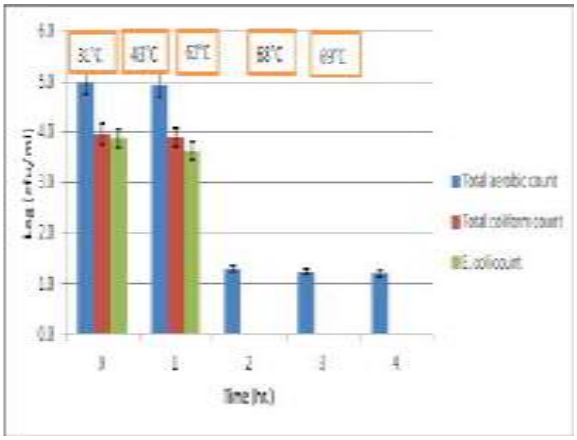


Fig 4. *Effect of pasteurizing device on total aerobic, total coliform and E. coli count of Rifampicin resistant Bacteria (marker Bacteria) of Dhanmondi lake water. As because this is not temperature resistant bacteria rather than antibiotic resistant bacteria. So solar radiation has an effect to destroy it.*

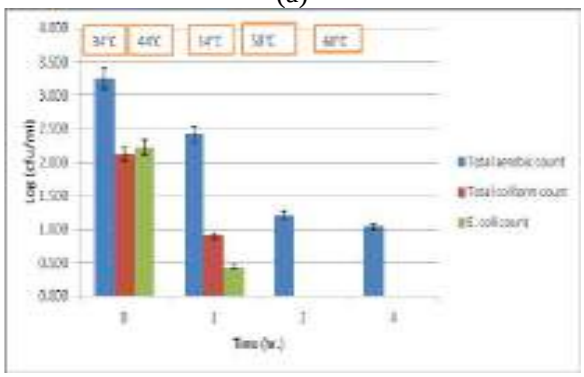
In the case of Bangla Academy pond water, the initial aerobic bacterial load was recorded as 5.0 log CFU/ml, and the coliform count and E. coli counts were recorded as 4.0 and 4.0 log CFU/ml, respectively after saree filtered water (Fig. 5). The physical properties of the treatment day was recorded and observed as clear sunny day, high humidity and sunlight intensity was recorded as 1610 LUX/mn. The initial day temperature was recorded as 31°C at 9h00 and temperature reached to maximum 69°C at the treatment point of pond water at 13h00. As the temperature increased, the initial aerobic population declined to 1.1 log CFU/ml after 2h of exposure and thereafter no decrease in 3 and 4 h of exposure in the gasket.

Total coliform and *E.coli* population decreased significantly after 2 hour of exposure and no survivors were recorded after 2 h of exposure and thereafter (Fig 5a).

On the other hand, same pond water in another treatment day was conducted. The physical properties of the treatment day was recorded and observed as cloudy day, with higher humidity and sunlight intensity was recorded as 1620 LUX/mn. The initial temperature was recorded as 34°C at the point of treatment at 10h00 and temperature reached to maximum 60^0C at 14h00. The initial aerobic bacterial load was recorded as 3.25 log CFU/ml and as the temperature increased, the initial aerobic population decline to 1.2log CFU/ml after 3h of exposure and little decreased further at 4h of sunlight exposure in the gasket (Fig 5b).



(a)



(b)

Fig 5 (a,b). *Effect of sunlight on total aerobic, total coliform and E. coli count of Bangla Academy pond water. Because of temperature and solar radiation difference of the experiment day, so, different curves (a,b) have been done.*

Total coliform and *E.coli* population decreased significantly after 1 hour of exposure no survivors were recorded after 2 h of exposure and thereafter (Fig 5b).

In case of artificially inoculated *E. coli* in Bangla Academy pond, the initial aerobic bacterial load was recorded as 5.596 log CFU/ml, and coliform count and E. coli counts was recorded as 5.35 and 5.34 log CFU/ml, respectively after saree filtered water (Fig. 5). The physical properties of the treatment day was recorded and observed as clear sunny day, high humidity and sunlight intensity was recorded as 1730 LUX/mn. The initial day temperature was recorded as 32°C at 11.10 hr and temperature reached to maximum 69°C at the treatment point of pond water at 15.10 hr. As the temperature increased, the initial aerobic population decline to 2.11 log CFU/ml after 1h of exposure and decreased further in 2h and 3h and finally reached to 0.3log CFU/ml. (Fig 5)

Total coliform and *E.coli* population decreased significantly after 1 hour of exposure no survivors were recorded after 2 h of exposure and thereafter (Fig 5).

Conclusion

In this study, effectiveness of solar pasteurizing device on reducing microbial population has to be determined. Two of the most significant factors are the solar radiation and time to have a safe water quality. Since insulation and air gap thickness, as well as time duration in the sun, have a major impact on temperature, these have been the main focus during the study. Solar radiation is the general source of heat, and necessary to achieve an adequate water treatment, but if the insulation of the device is lacking, the heat loss would be considerable and the rate of temperature increase will be reduced significantly. Double thickness of insulation showed capacity of retaining the solar energy longer and thus water temperature would be higher.

One of the greatest difficulties while performing the solar radiation test was the maintenance of the same initial temperature among all tests, since the outdoor temperature was not as constant as the indoor temperature. The preparation time in the sun before starting the tests varied slightly each time, and also affected the starting temperature of the water. Besides the variation in time for preparation, other impacts such as the initial temperature of the table, where the device was placed during the test, also fluctuated due to the solar radiation. These issues could explain why the results are different.

The microbiology studies show that the low cost solar pasteurization device can purify pond water to supply safe drinking water. To extend the study further, more microbiology samples should be taken from rivers and other ponds in rural areas. The pond near the institute is not entirely representative of the rural areas and the day when the test was made the sun had pre-heated the water in the pond. This might have affected the water quality and reduced pathogens already before treatment with the pasteurizing device. This could be the reason for the low value of pathogens in the reference samples.

In a clear sunshine typical day in Bangladesh (during February to July), when the solar radiation is the strongest, the device could be able to disinfect three water volumes per day. This will give 4 liter of water in late spring and during summer. In the winter (December to February) when the solar radiation is less strong to water volumes per day will be possible to obtain, which provides 3 liter soft water.

The costs per liter water using the solar water pasteurizer would be around 400 taka, which is around US Dollar 5. Which is very low cost to achieve. Otherwise, this device is made of such things (Black color bamboo tray, straw, polyethylene sheets, Polyethylene), which is available in the rural areas, if people aware before flood and arrange these materials, then they can use this device to during flood.

Acknowledgement

UNESCO Bangladesh National Commission for the financial assistance to this work. Also thankful to CARS, University of Dhaka for their logistic and technical support and thanks to all members of the Department of Biomedical Physics and Technology, University of Dhaka for their help and cooperation to conduct this Research work.

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Enterohemorrhagic *Escherichia coli* (EHEC) infection and beef consumption in Japan

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Abstract:

There were some infection events occurred associated with the consumption of beef in Japan over the last decade. After the first bovine spongiform encephalopathy (BSE) case found in the United States, the Japanese government imposed a ban on U.S. beef imports in December 2003. The ban was lifted in December 2005, but the amounts of U.S. beef imports were still smaller compared with those before the ban. In April 2011, enterohemorrhagic *Escherichia coli* (EHEC) O111 and O157 outbreak occurred associated with the consumption of raw beef in Japan. This incidence triggered to set new standard for preparing raw beef in October 2011, and serving raw beef liver has banned since July 2012. The aim of this study was to evaluate the relationship between EHEC infection and beef consumption in Japan, by using the statistical and epidemiological data from 2000 to 2013.

In 2004 and 2005, importing U.S. beef was almost stopped and then, the amount of U.S. beef imports were gradually increased but were not recovered to the level before the ban. The amounts of the total imported beef and domestic beef were almost constant during this period. On the other hand, the number of food-borne EHEC patients and cases, and the total number of EHEC patients (not only foodborne cases) were fluctuated but relatively constant from 2000 to 2013. It is thought that the U.S. beef might not have a great effect on the situation of EHEC infection in Japan, and it is too early to estimate whether the new standard for raw beef preparation or the ban of raw beef liver is effective or not.

Keywords: Enterohemorrhagic *Escherichia coli* (EHEC), beef consumption, Japan, US

1. Introduction

There were occurred some events concerning with enterohemorrhagic *Escherichia coli* (EHEC) infection and beef consumption in Japan over the last decade. The aim of this study was to evaluate the relationship between EHEC infection and beef consumption in Japan, by using the statistical and epidemiological data from 2000 to 2013. Previously, we reported the relationship between U.S. beef imports and EHEC infection from 2000 to 2006, elsewhere [1].

2. Materials and Methods

The amounts of total beef consumption (domestic + imported), the amount of domestic beef, and the amount of imported beef were cited from "Chikusanbutsuryutsutoukei (Statistics on Livestock Products Marketing) [2]" and "Shokunikuryutsutoukei (Statistics on Meat Products Marketing) [3]" provided by the Ministry of Agriculture, Forestry and Fisheries. The amount of U.S. beef imports and the amount of Australian beef imports were cited from "Boekitoukei (Foreign Trade Statistics) [4]" provided by the Ministry of Finance. The amounts of beef were expressed as portion meat weight.

The number of food-borne EHEC cases and patients and the summary of food-borne EHEC cases were cited from "Shokuchudokuhasseijoukyo (Investigation on Food Poisoning) [5]" and "Shokuchudokuhasseijirei (Summary on Food Poisoning Cases) [6]" provided by the Ministry of

Health, Labour and Welfare. The number of EHEC patients with symptoms (not only food-borne cases) was cited from the Infectious Agents Surveillance Report [7] provided by the National Institute of Health Sciences.

3. Results

3.1. Beef consumption in Japan

Fig. 1 shows the amounts of total beef consumption (domestic + imported), domestic beef, imported beef, U.S. beef imports, and Australian beef imports. The amounts of total beef consumption decreased in 2001, because of bovine spongiform encephalopathy (BSE) problems in Europe and Japan. (The first BSE case was found in Chiba, Japan in September 2001.) Then, the first BSE case was found in the United States in December 2003, and the Japanese government imposed a ban on U.S. beef imports immediately thereafter. The ban was lifted in December 2005, but in January 2006, the spine, a region whose removal was required as a material designated at risk of containing infectious agent of BSE, was found in imported U.S. beef and the ban was imposed again. The ban was lifted again in July 2006, but the amounts of U.S. beef imports were still smaller, although recovered gradually, compared with those before the ban. On the other hand, the amount of domestic beef production has been stable around 350 kilo tons from 2000 to 2013, and the amount of imported beef and total beef consumption have been stable around 500 kilo ton and 800 - 900 kilo ton, respectively from 2002 to 2013.

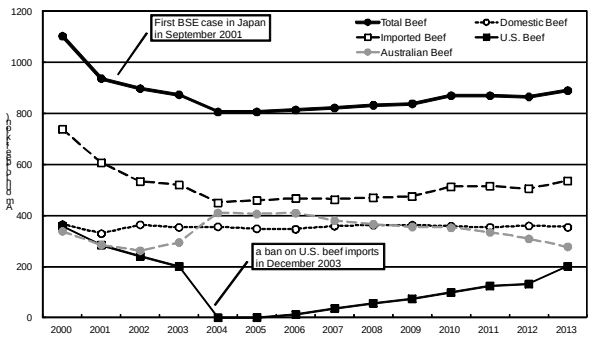

Fig 1. *The amounts of domestic and imported beef*

3.2. EHEC infection in Japan

Fig. 2 and Fig. 3 show the number of food-borne EHEC cases and patients, respectively. These numbers are reported to the Ministry of Health, Labour and Welfare based on the Food Sanitation Act. On the other hand, Fig. 4 shows the number of EHEC patients with symptoms. This number is reported to the Ministry of Health, Labour and Welfare based on the Infectious Diseases Control Law. The formers are the number of confirmed food-borne EHEC cases and patients, and the latter is the total number of confirmed EHEC patients with symptoms, but not only food-borne patients.

The number of food-borne EHEC cases was fluctuated from 12 to 27 cases, but relatively constant from 2000 to 2013. The number of food-borne EHEC patients shows the peaks in 2007 and 2011. On the other hand, the total number of EHEC patients with symptoms was also relatively constant around 2500 people from 2000 to 2013.

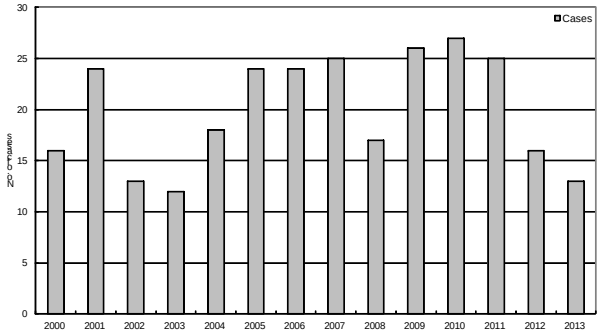

Fig 2. *The number of food-borne EHEC cases*

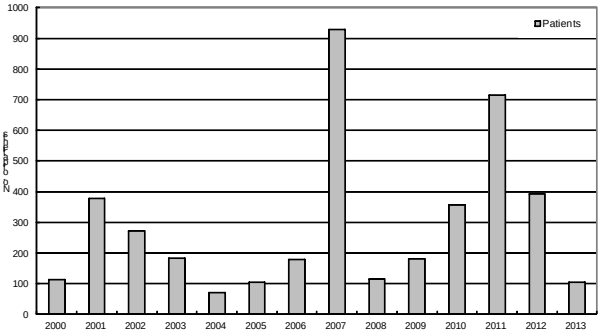

Fig 3. *The number of food-borne EHEC patients*

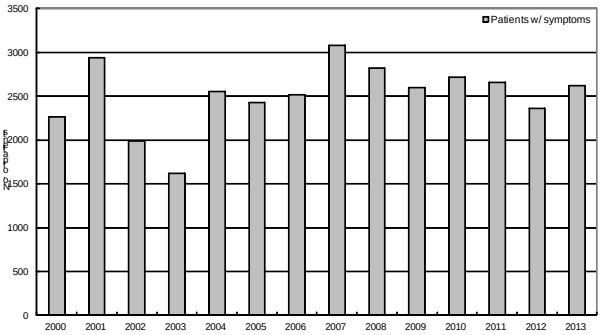

Fig 4. *The number of total EHEC patients with symptoms*

3.3. Food-borne EHEC cases in Japan

The summaries of foodborne EHEC cases in the past 14 years (2000 - 2013) were shown in Table 1. From 2000 to 2013, 12 to 27 foodborne EHEC cases were reported. The causative foods were unknown in most of the cases, but barbecue, raw beef liver, molding steak and etc. were confirmed or suspected as causative foods in many cases.

3.4. EHEC O111 and O157 outbreak and the new standard for raw beef and ban on raw beef liver in Japan

In April 2011, EHEC O111 and O157 outbreak caused by raw beef consumption occurred at branches of a barbecue restaurant in Kanagawa, Toyama and Fukui Prefectures, Japan. This outbreak involved 181 infected patients, including 34 hemolytic-uremic syndrome (HUS) patients. Among the 34 HUS patients, 21 developed acute encephalopathy and 5 died. The causative dish was yukhoe, Korean-style raw beef dish. From the patients, 2 serotypes of EHEC, that is, O111:H8 and O157:H7, were isolated but EHEC O111 was considered the main causative agent of this outbreak, because the patients showed higher anti-O111 antibody titer.

The Ministry of Health, Labour and Welfare set new standard for preparing raw beef in October 2011 and a ban on serving raw beef liver since July 2012, triggered by this incidence. The most important point of this standard is "meat wrapped under aseptic conditions is heated to 60ºC to a depth of 1 cm or more for at least 2 minutes".

3.5. Raw or undercooked Japanese-style beef dishes

Raw or undercooked Japanese-style beef dishes are explained in this section. The explanation was mainly cited from wikipedia.

"Gyu sashi" means raw beef sashimi (raw beef meat). "Sashimi" is a Japanese delicacy. It consists of very fresh raw meat, most commonly fish, sliced into thin pieces. This is restricted by the above-mentioned new standard for preparing raw beef (Fig.5a).

"Gyu tataki" means raw beef tataki (undercooked beef meat). "Tataki" is a manner of preparing fish or meat in Japanese cuisine. The meat or fish is seared very briefly over a hot flame or pan, briefly marinated in vinegar, sliced thinly and seasoned with ginger (which is ground or pounded into a paste) This is also restricted by the above-mentioned new standard for preparing raw beef (Fig.5b).

"Reba sashi" means beef liver sashimi, raw beef liver. Serving raw beef liver is prohibited at restaurant in Japan now, as mentioned above (Fig.5c).

"Yukhoe" is so-called Korean steak tartare. "Yukhoe" refers to a variety of raw dishes in Korean cuisine, which are usually made from raw ground beef seasoned with various spices or sauces. The beef is thinly julienned with the fat removed, then mixed with seasoning. A raw egg yolk is usually added, either on top of the dish or separately (Fig.5d).

4. Discussion

The amount of U.S. beef imports showed drastic changes from 2000 to 2013. It was almost zero in 2004 and 2005 and gradually increased from 2006. On the contrary, the amount of Australian beef showed compensatory changes for the changes of U.S. beef imports, and the amount of total imported beef were almost constant from 2002 to 2013. The amount of domestic beef production was almost stable from 2000 to 2013, and the amount of total beef consumption was also stable from 2002 to 2013.

a) *"Gyu sashi"*

from http://shingama.com/hpgen/HPB/entries/51.html

b) *"Gyu tataki"*

from http://www.recipe-blog.jp/profile/16094/blog/1732547

c) *"Reba sashi"*

from http://www.mytokachi.jp/sigejirou/entry/43

d) *"Yukhoe"*

from http://en.wikipedia.org/wiki/Yukhoe

Fig 5. *Raw or undercooked Japanese-style beef dishes*

The number of foodborne EHEC cases and patients, based on the Food Sanitation Act, are the number of cases and patients when the doctors

diagnose (or suspect) them and report to the local public health centers as food-borne EHEC infection and the bacteria are confirmed. These numbers are thought to be “the tip of the iceberg”. On the other hand, the number of EHEC patients with symptoms, based on the Infectious Diseases Control Law, is the number of patients when the doctors confirm the symptom and the bacteria are isolated. This number is thought to be quite reliable because all the patients that EHEC were isolated from must be reported. The number of foodborne EHEC cases, based on the Food Sanitation Act, fluctuated but relatively constant. The number of foodborne EHEC patients, based on the Food Sanitation Act, showed some peaks but no trends were seen. And the total number of EHEC patients with symptoms was also relatively constant.

Compared to the drastic changes in the U.S. beef imports, the numbers of EHEC patients and cases, regardless of foodborne or not, were relatively constant between 2000 and 2013. It is thought that the U.S. beef might not have a great effect on the situation of EHEC infection in Japan. It was reported that the prevalence of EHEC was 3.49% (61/1750) and that of *E. coli* O157 was 1.14% (20/1750) in the ground beef in the U.S. [8]. On the other hand, the prevalence of *E. coli* O157 were 0.00% in beef meat (0/262) and minced beef (0/82), and 0.61% in beef liver (5/826) between 2005-2010 in Japan (“Shokuchudokukinosenjittaichosa (Surveillance of Food-Poisoning Bacterial Contamination on Food) [9]” by the Ministry of Health, Labour and Welfare).

Before 2011, there were several cases caused or suspected to be caused by raw beef, raw beef liver and yukhoe (Table 1). For these dishes, domestic beef is often used rather than imported beef. So, the numbers of EHEC patients and cases might be related with the amounts of domestic beef production or total beef consumption, rather than the amounts of U.S. beef imports.

In 2012 and 2013, no cases were reported caused by raw beef, raw beef liver and yukhoe (Table 1). The number of foodborne EHEC cases and patients decreased in these 2 years but the total number of EHEC patients with symptoms did not decrease. It is thought to be too early to estimate whether the new standard for raw beef preparation or the ban of raw beef liver is effective or not, and further investigations are needed to apply the proper countermeasures.

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Factors affecting the drying process of shrimp powder from black tiger shrimp (*Penaeus monodon)* head meat

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Abstract:

The most important waste material in shrimp processing industries is shrimp head, comprising about 30÷35% of whole shrimp weight. This waste is rich in nutrients are being wasted. This waste is mainly dried to powder and use for food and feed industries. This study was designed to evaluate the factors affecting the drying process of shrimp head meat. The water activity (a_w) value, color parameters, and the protein content were the main investigations of this research. In this study, minced shrimp head meat was heated at temperature ranging from 60 ºC to 70, 80 and 90 ºC for 5 to 50 minutes with 10 minutes interval. The water activity value and the color parameter at respective heating temperature were also investigated. The results showed that drying at 65 ºC has low a_w value and high soluble protein content is suitable for product. In addition, thermal inactivation of protease at temperature ranging from 60 to 90 ºC of shrimp head meat resulted in a fractional conversion model. When minced shrimp head meat was treated at 80ºC for 30 minutes before drying at 65ºC to reach 6 % moisture was the most suitable value to grind into powder. The rate of powder production through sieve (Size: 1mm x 1mm diameter) is over 90%, and showed special color of dried shrimp, low water activity (0.38) and high soluble protein content with approximately 13 % of dry material. Therefore, the study results demonstrated that drying and grinding shrimp head meat with above condition has potential application in feed and food industries, which could increase economic efficiency and reduce the waste in the environment.

Keywords: drying, moisture, shrimp head meat powder, thermal inactivation, water activity

1. Introduction

Vietnam has advantage for the development of the fisheries industry because of its natural conditions and became one of the world's top ten seafood exporters to the world market in more than ten years. Every year, about 65.000 tons of shrimp head is discharged from the factories (34% of raw materials) in Vietnam [1]. For discharging these by-products into river or soil involved higher cost while treating with modern technology and cause environmental pollution.

M.S Heu *et al.* [2] have studied the composition and nutritional quality of waste products such as shrimp head, shell and tail. The study results indicated that they contain protein (9.3 ÷ 11.6%), lipid (0.7%), minerals (Ca, P, Na, Mg) and small amount of heavy metals (Hg, Pb and Cd). It contains also a small amount of valuable carotenoids. Proteins represent the major component of shrimp heads [3]. Currently, the shrimp head is mainly use as a source of protein in processing animal feed [4] and shrimp shells to recover chitin, or chitosan by chemical procedures [5]. However, the production of chitosan requires removing meat from the shrimp head that leads to a huge impact on the environment. Therefore, the study using shrimp meat in food processing to improve the commercial value of black tiger shrimp while reducing the environmental impact shrimp waste is needed. The studies of shrimp meat are mainly in the extraction and purification of protease in shrimp head. [6,7]. Muoi *et al.* [8] studied the additional processing of shrimp head meat to produce sausage. Khan and Nowsad [9] also use shrimp head meat powder in processing biscuit.

Shrimp head meal production is a new approach to solve the problem. However, enzyme

protease in shrimp mostly concentrates in digestive system, which is located in the head [7]. This enzyme will quickly cause spoilage during the processing if not inactivated. Therefore, the main objective of the study was identifying thermal inactivation kinetics of protease in shrimp head meat and also to determine the other factors affecting drying process during the production of shrimp meat powder.

2. Materials and Methods

2.1. Sample collection and preparation

Shrimp head meat (already shelled) as a by-product of black tiger shrimp (*Penaeus monodon)* were purchased in Thoi Binh district, Ca Mau province, the south of Viet Nam (ensure samples were refrigerated below 4 ºC and 12 hours maximum from separation head to collect head meat). After collection, shrimp head meat was transported to the laboratory of Food Technology department, Can Tho University in iced condition not more than 4 hours. The by-product then was washed under running water, packed in plastic bags containing 1.0 kg in each bag and stored at -25 ºC until use.

2.2. Preparation protease extracts of black tiger shrimp head meat

Protease extraction method is based on the research results of Ha N.L [10].

Frozen shrimp head meat is mixed with water (cooled to 2 to 4°C) the ratio of 1:3. Next, the mixture was crushed by a blender (rotational speed of the motor at 2000 to 3000 rpm) for 3 minutes before extracting enzyme. During the grinding process, the temperature does not exceed 5 ºC. After grinding, the sample is poured into a glass, annealed at different temperature and time to extract enzymes with each 5 minutes stir. The extract obtained by the filter has size of 1 x 1mm to remove large sections of insoluble dry substance (shrimp residue), then cooled 15 minutes before centrifugation in 20 minutes with 3000 rpm speed to remove the residue, obtained extracts, known as crude protease extract.

2.3. Determination of protease activities

Protease activity in crude enzyme extracts was determined according to the modified Anson's method. 1.0 ml enzyme solution was mixed with 5.0 ml substrate (1% casein in 0,133 M Sorensen's phosphate buffer) and incubated at 37 ºC for 30 minutes. At the end of 30 minutes, 10.0 ml of 10% TCA (trichloroacetic acid) was added to stop the reaction. The precipitated casein was then filtered off and 1.0 ml of the filtrate was taken in a test tube. To this 2.0 ml of 0.5 N NaOH solutions and then 0.6 ml of the folin ciocalteu reagent (one ml diluted with 2.0 ml of distilled water) were added. Final readings were taken in a spectrophotometer at 660 nm. Blanks of the samples were prepared by adding the TCA before the addition of substrate.

Standard curve is created by using a graphing program changing absorbance of standards on the Y axis, versus the amount in micromoles for each of 5 tyrosine standards (from 0 to 1 micromole each 0.2 micromole) on the X axis.

Protease activity (UI/ml) = x.V/t.v

With x: micromole tyrosine equivalent from the standard curve; V: Total volume (in milliliters) of assay (11 ml); v: Volume of enzyme (in milliliters) of enzyme used (1 ml); t: Time of assay (in minutes) as per the unit definition (30 min);

1 UI (Anson) = 1 µmol Tyrosine/ml/mi or 1 µmol/mg/min.

2.4. Thermal inactivation enzyme protease in shrimp head meat

The effect of time and temperature on inactivate enzyme protease were studied by heating 50g shrimp head meat which contain in a plastic tube in water bath. The temperature was set at different level (60, 70, 80 and 90°C). Each temperature had 6 rates of time (5, 10, 20, 30, 40, 50 minutes). After thermal inactivated, sample was cooled rapidly under running water to finish the process, conducted protease extraction using method presented above and assayed the residue protease activity by modified Anson's method.

2.5. Determination of thermal inactivation enzyme protease to the color and quality of shrimp head powder

The sample with suitable inactivation time in each temperature above would be dried at 60°C until reached 6% final moisture before grinding into powder by a blender (2000rpm in 1 minute) and jigged through sieve (1mm x 1mm diameter). Water activity and color of the powder were measured by water activity meter and color meter.

2.7. Determination of final moisture to shrimp head powder

Choose one value of thermal inactivation enzyme protease from the result above; begin dry at 60°C until reach different final moisture (4, 6, 8, 10 and 12%). Continue the grinding and jigging process as mention.

2.8. Chemical analysis

All the analyses were performed in triplicate. Moisture content was determined by oven drying samples at 105 °C until constant weight [11]. The total nitrogen content of the raw material was determined using the Kjeldahl method [12]. Crude

protein was estimated by multiplying the total nitrogen content (%N) by 6.25.

2.9. Color and water activity

Each sample was measured three times and the average values were reported. The color (L*, a*) of the shrimp head powder was evaluated by a colorimeter (Shenzhen, model SJ-0520-C, China). The water activity of shrimp head powder was measured by a water activity meter (HANNA, model HI9564, Romania)

2.10. Kinetic data analysis

The inactivation kinetics of enzyme protease was analyzed by using a fractional conversion model as Eq (1):

$$A = A_\infty + (A_o - A_\infty)\, e^{-kt} \quad (1)$$

Where A is residual enzyme activity at time t; A_∞ is the residual enzyme activity after thermal treatment; A_o is the initial enzyme activity and k is the reaction rate constant (min−1).

The value of standard deviation and reliability of thermal inactivation kinetics of enzyme protease were measured by (SAS, 1990):

$$R^2 = 1 - (m-1)\frac{1 - \frac{SSQ_{regression}}{SSQ_{total}}}{(m-j)}$$

$$SD = \sqrt{\frac{SSQ_{residual}}{(m-j)}}$$

Where m is the observations; j is parameters; SSQ is sum of squares and SD is standard deviation.

2.11. Statistical analysis

All data presented are means ± standard deviations. Analysis of variance (One way ANOVA) was performed by Statgraphics centurion XVI version 16.1.11 (Manugistics Inc., USA). The method used to discriminate among means was Fisher's least significant difference (LSD) procedure. Mean were accepted as significantly different at 95% level ($p \leq 0.05$).

3. Results and Discussion

3.1. Basic moisture content, total nitrogen and pH of black tiger shrimp head meat

Processing of shrimp head meat to get shrimp head powder was influenced by many factors, including the initial moisture content, total nitrogen and pH, play an important role that directly affect the product quality. Basic moisture content, total nitrogen and pH of shrimp meat are showed in Table 1.

The results in Table 1 indicated that black tiger shrimp head meat contains higher moisture content (85.42 ± 0.69) %, near neutral pH (7.70 ± 0.07) value, while the pH value of shrimp meat fluctuate from 6.8 ÷ 6.9 [13]. In addition, shrimp head contains a small amount of fat [2] and higher protein content (approximate 12.62 % wet average material). According to Adams and Moss [14], the spoilage microorganisms' optimal conditions (neutral pH, environment, humidity > 80 %) for growth could be easily spoiled the shrimp head meat therefore, thermal treatment along with good storage method could be able to prevent the declining in quality of shrimp head meat.

Table 1. *Basic moisture content, total nitrogen and pH of black tiger shrimp head meat*

Components	Contents
Moisture (%)	85.42 ± 0.69
Total Nitrogen (% wet material)	12.62 ± 0.31
pH	7.70 ± 0.07

3.2. Effect of thermal treatment on inactivation enzyme protease result

The processing of shrimp could be interfered by enzyme protease, which is contained in shrimp head, and become a major spoilage cause during storage. Therefore, inhibition temperature to inactivate the enzyme required being determined. Therefore, protease activity at different temperature was carried out to evaluate the changing patterns using Anson method. The results of the kinetic parameters were shown in Table 2; the changes of kinetic of protease activity were summarized in Table 3

Based on the result in Table 2, the kinetic equation showed high reliability due to low SD value and very high, nearly 1 R^2 value. Therefore, shrimp head meat protease activity patterns in different temperature follow fractional conversion model (Table 3). The residue protease activity decreased with temperature from 60 ºC to 90 ºC while the inactivation rate constant k (1/min) increased. Beside, the ratio A/A_o (ratio of residue enzyme activity after thermal treatment compared with the initial activity) decreased from 5 to 50 minutes thermal treated (Table 3). This proves that inactivated speed increased with time and temperature; the higher the temperature, the more decreased the residue protease activity.

At 60 ºC and 70 ºC, residue protease enzyme activity after thermal treatment is still high compared with the others as the result from heat durability of this type of enzyme in about 52-67 ºC [10]. At the same level of time when increasing temperature from 60 to 90 ºC, the enzyme protease activity decreased. At a temperature of 80 ÷ 90 ºC, residue protease activity showed the lowest value. This result could explain that enzyme is basically protein; if processed at high temperatures would denature protein leads to the inactivation enzyme.

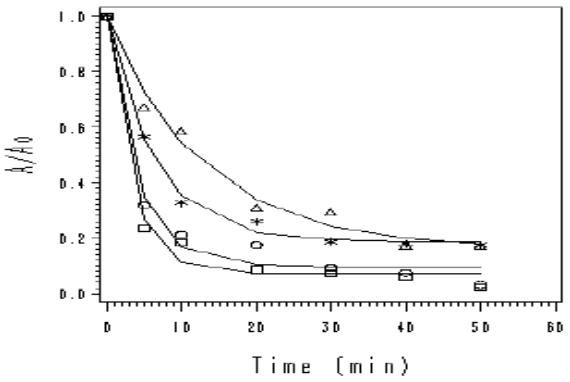

Fig 1. *Thermal inactivation kinetics of shrimp head meat protease: 60 °C (△); 70 °C (✳); 80 °C (○); 90 °C (□).*

Thermal inactivation protease in a long period of time, enzyme activity rapidly decreased in the beginning and then slightly decreased but not decreased to 0 values (Fig.1). This can be explained because protease in shrimp head includes 2 categories, endo-protease and exo-protease. The thermal treatment conditions could fully inactivated exo-protease enzyme however, was not able to inactivated endo-protease [6]. The result in this study confirm the hypothesis that only partial enzyme protease was inactivated.

Moreover, determination of the suitable time for inactivation at each temperature was done and recorded in Table 4. Due to the inactivation of high temperature of any enzyme, thermal treatment at 90 ºC showed lowest residue enzyme protease. Thermal treatment at 60 ºC for 40 minutes, the residue protease activity decreased from 1.03 IU/g to 0.19 IU/g, however, it took only 30 minutes to decrease into this value at 70ºC. On the other hand, the residue protease activity after treating at 80 ºC declined to 0.1 IU/g and 0.09 IU/g and treating at 90 ºC in 20 minutes, achieved the similar protease activity, respectively. Therefore, thermal treatment at 60 ºC, 70°C, 80 ºC and 90 ºC for 40 minutes, 30 minutes, 30 minutes and 20 minutes, respectively required inactivate the protease.

3.3. Effect of thermal inactivation enzyme protease on the drying kinetics and head shrimp powder

Table 5 showed the statistical difference in drying time between samples using heat treatment and no heat treatment. The drying time of heat treatment of sample 2 and 3 were the shortest (460 minutes) compared with other two remaining treatment samples, 486 and 526 minutes for heat treatment 1 and 4, respectively. It is explained that the inactivation before drying makes the tissue softer and the outer membrane broken helps water escape easily. But when the temperature rises up to 90°C, materials clumping lead the difficulty in draining, prolong the drying time. Beside, with the higher treating temperature, the lower water activity was because at higher temperature, protein denatured decrease free water in material.

Some relevant research and trial experiment proved that 60 ºC is suitable temperature for drying shrimp head. The drying time gradually decreased along with increasing inactivation temperature at temperature (60°C) and final moisture (6%) (Fig 2).

The survey showed that the color have significant improvement through its brightness and special red after pretreatment comparison with the sample without treating. At 80 and 90°C, the color improved better than the rest temperature (see in Table 6).

This can be explained by the pre-treatment at higher temperature, protein denatured limited chemical change as well as the inhibition of protease activity. Besides, the drying process make astaxanthin which is sensitive from heat and turn red, represented by a * value. Heat treatment process prevents Maillard reaction occurring during the drying process, will limited brown color and helps product become brighter [15].

The survey showed that the pretreatment *temperature starting materials for improved product quality better than the untreated sample can be expressed through* color and improved for shorter drying time.

3.4. Effect of product's moisture on the grinding into powder

Moisture contents of the shrimp head meat (% db) decreased with time. The moisture decreased rapidly in the early hours of the drying process and lower in the next hour. Therefore, the slope of the drying curve decreases with time. Because the samples are dried at the same temperature (60 ºC), so the drying curves with different final moisture had nearly similar shape. The product moisture was lower, the drying time was longer. The drying time was 340 minutes to achieve the 12% moisture products, while 10%, 8%, 6% and 4% moisture products, the drying time is also increased respectively in 350 minutes, 360 minutes, 400 minutes and 430 minutes.

Based on Table 7, statistical results showed that the water activity of the product had significant difference 5%. Specifically, the water activity of 12 % moisture product (0.580 ± 0.013) reduced to 0.456 in product 4% moisture. The grinding process is hard if product has higher moisture content.

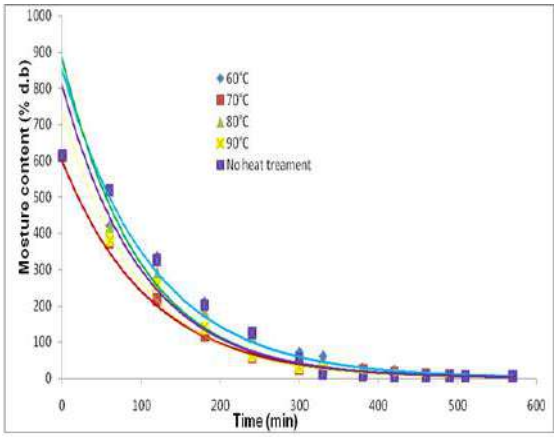

Fig 2. *Drying curve of different thermal treatment*

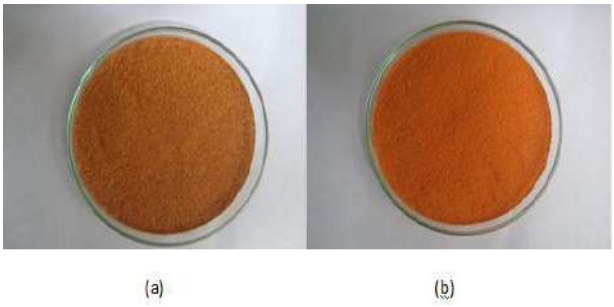

Fig 3. *Shrimp head meat powder with different thermal treatment before drying (a) not using heat treatment, (b) treating at 90°C in 20 minutes*

Table 2. *Estimated kinetics parameters for thermal inactivation of shrimp head meat protease at different temperature*

Temperature	SD	k (1/min)	R2	A_∞
60°C	0.0420	0.0792 ± 0.0107	0.994	0.1695 ± 0.0367
70°C	0.0224	0.1588 ± 0.0106	0.998	0.1913 ± 0.0128
80°C	0.0510	0.2544 ± 0.0405	0.987	0.1001 ± 0.0261
90°C	0.0417	0.3122 ± 0.0450	0.990	0.0743 ± 0.0208

Table 3. *Kinetics equations for thermal inactivation of shrimp head meat protease at different temperature*

Temperature	A_o (UI/g)	Kinetic equation
60°C	1.0267	$A/A_o = 0.1651 + 0.8349\exp(-0.0792t)$
70°C	1.0267	$A/A_o = 0.1863 + 0.8137\exp(-0.1588t)$
80°C	1.0267	$A/A_o = 0.0975 + 0.9025\exp(-0.2544t)$
90°C	1.0267	$A/A_o = 0.0724 + 0.9276\exp(-0.3122t)$

Table 4. *Effect of thermal treatment on inactivation of protease enzyme*

Time (min)	Residue protease activity in shrimp head meat at different temperature (UI/g)			
	60°C	70°C	80°C	90°C
0	1.03^{d}	1.03^{f}	1.03^{d}	1.03^{c}
5	0.69^{c}	0.58^{e}	0.33^{c}	0.24^{b}
10	0.60^{c}	0.34^{d}	0.22^{b}	0.19^{b}
20	0.32^{b}	0.27^{cd}	0.18^{b}	0.09^{a}
30	0.30^{b}	0.19^{ab}	0.10^{a}	0.08^{a}
40	0.19^{a}	0.19^{ab}	0.08^{a}	0.06^{a}
50	0.18^{a}	0.18^{a}	0.06^{a}	0.05^{a}

Different letters indicate significant differences ($P < 0.05$).

Table 5. *Effect of thermal treatment on drying time, water activity and rate of powder through sieve*

	Drying time (min)	a_w (%)	Rate of powder through sieve (%)
No heat treatment	523.33^{a}	0.550^{c}	82.067^{a}
Heat treatment 1 (60°C-40 min)	486.67^{b}	0.583^{c}	85.957^{ab}
Heat treatment 2 (70°C-30 min)	460.0^{c}	0.472^{b}	90.327^{bc}
Heat treatment 3 (80°C-30 min)	460.0^{c}	0.413^{a}	92.467^{c}
Heat treatment 4 (90°C-20 min)	526.67^{a}	0.436^{ab}	93.077^{a}

Different letters indicate significant differences ($P < 0.05$).

Table 6*. Effect of thermal treatment on color value of the powder*

	Color value	
	L*	a*
No heat treatment	80.77^{a}	20.63^{a}
60°C, 40 min	82.67^{ab}	27.89^{c}
70°C, 30 min	83.55^{b}	27.33^{bc}
80°C, 30 min	86.72^{c}	26.95^{bc}
90°C, 20 min	86.24^{c}	26.29^{b}

Different letters indicate significant differences ($P < 0.05$).

Table 7*. Effect of moisture's product on water activity and rate of powder through sieve*

Moisture (%)	Water activity (a_w)	Powder through sieve rate (%)
4	0.456^{a} ± 0.014	90.467^{a} ± 0.929
6	0.469^{a} ± 0.019	90.200^{a} ± 1.054
8	0.513^{b} ± 0.012	85.900^{b} ± 1.308
10	0.542^{c} ± 0.016	81.867^{c} ± 1.914
12	0.580^{d} ± 0.013	79.233^{d}±0.723

Different letters indicate significant differences ($P < 0.05$).

4. Conclusion

Protease activity was affected by temperature and time. Shrimp head powder with pre-cooking gives better color. Moisture content had a strong influence on drying time, color and water activity. The rate of powder production from shrimp head through sieve is over 90%, showed special color of dried shrimp, low water activity and high soluble protein content. Therefore, the study results demonstrated that drying and grinding shrimp head meat with above condition has potential application in feed and food industries, which could increase economic efficiency and reduce the waste in the environment.

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Isolation and characterization of *Escherichia coli* O157:H7 in raw goat meat in Dhaka city using conventional and molecular based technique

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Abstract:

A total of 40 raw goat meat samples were collected from different markets in Dhaka city during September to December, 2011 and analyzed for the presence of *Escherichia coli* O157:H7, in this study. *E. coli* O157:H7 strains were isolated from 24 samples (60%) of 40 goat meat samples. From the 24 goat meat samples, total 86 presumptive *E. coli* O157:H7 colonies were isolated using different selective agar medium, however, after biochemical and immunological analysis, only 11 isolated colonies were found to be *E. coli* O157:H7, and rest were identified as different strains of *Enterobacter spp*. Further characterization using agglutination reaction with anti-O157 sensitized latex showed that only four isolates has strong agglutination reaction against anti-H7 sensitized latex. The biochemically and serologically confirmed isolates were then screened for major virulence factors include *eaeA*, *rfbE*, *fliC*, *stx1* and *stx2* genes by polymerase chain reaction. Most clinical signs of disease arise as a consequence of the production of Shiga toxin 1 (Stx1), Stx2 or combinations of these toxins. Among the 11 immunological positive isolates, 10 isolates showed *eaeA* gene positive, which is involved in the attaching and effacing adherence phenotype. These 10 isolates were then subjected to PCR against *rfbE* and *fliC* gene**,** which encodes the ***E. coli*** O157 serotype and **E. coli** flagellum H7 serotype, respectively. All the 10 isolates showed positive result with *rfbE* gene, however, only six *E. coli* strains were fliC gene positive, indicating that these isolates were genetically H7 with flagellum antigens that were either not expressed or not detectable in serotyping tests. A multiplex PCR analysis for *eaeA, stx1* and *stx2* genes of the isolates showed similar results. Therefore, out of presumptive 86 positive *E. coli* O157:H7 isolates only 6 (7%) isolates were confirmed to be shiga-toxin producing *E. coli* O157:H7. Thus the detection/screening methods greatly influenced the estimation of *E. coli* O157:H7 and its virulence factors in goat meat samples.

Keywords: *E. coli* O157:H7, virulence factors, Conventional and molecular technique, raw goat meat, Dhaka city.

1. Introduction

Food borne illness is a major concern of the modern world. Illness associated with the consumption of fresh foods, vegetables and meat have increased in the United States during past decades [27].

The recognition of EHEC as a particular class of pathogenic *E. coli* resulted from two observations. It was reported in 1983 by Riley who investigated two outbreaks of a particular gastrointestinal illness characterized by severe crampy abdominal pain, watery diarrhea followed by bloody diarrhea, and little or no fever [25]. The second observation was made by Karmali, also in 1983, who reported the association of sporadic cases of hemolytic uremic syndrome (HUS) associated with cytotoxin producing *E. coli* in stools [18].

Escherichia coli O157:H7 is one of the hundreds serotypes of the bacterium *Escherichia coli*. *E. coli* O157:H7 has several characteristics uncommon to most other *E. coli*. The O157:H7 serotype is negative for invasiveness [24]. The ability to produce Shiga toxin, possession of attaching and effacing *(eae*) gene and 60-MDa plasmid, inability to ferment sorbitol within 24 h, inability to grow well at temperatures >44°C, inability to produce P-glucuronidase (i.e. in ability to utilize 4-methylumbelliferyl-D-glucuronide) and ability to utilize raffinose and dulcitol [13,26].

E. coli O157:H7 is the cause of many outbreaks of severe illness throughout the world. At least 30 countries in six continents have reported *E. coli* O157:H7 infection in humans [12]. The data of CDC shows that the annual disease appearance of *E. coli* O157:H7 in the United States to be more than 20,000 episodes and as many as 250 deaths [3]. In the United Kingdom, especially in England, Wales, Scotland and Northern Ireland, there has been a significant increase in the isolation rate of *E. coli* O157:H7 over the last decade. There is also report of *E. coli* O157:H7 outbreak in Turkey [31]. In most of the Asian countries, EHEC is not yet a major health problem, except in Japan, where 29 outbreaks were reported between 1991 and 1995. Few reports are available on the isolation of EHEC from Hong Kong, Thailand, Malaysia, India and Sri Lanka [19].

Cattle are thought to be the main source of *E. coli* O157:H7. This bacterium has also been isolated from other domestic and wildlife animals, such as sheep, goats, deer, dogs, horses, swine, cats, seagulls and rats [21]. In many studies, it is found that, young animals are more likely to be colonized by *E. coli* O157:H7 compared with older ones of the same herd. Prevalence of *E. coli* O157:H7 in cattle increases during warmer months of the year, which is related to the seasonal variation in human diseases [9].

A variety of foods are identified as vehicles for *E. coli* O157:H7 transmission which include ground beef, roast beef, cooked meats, mayonnaise, unpasteurized apple juice, salami, raw milk, pasteurized milk, yoghurt, cheese, cheese curds, ice cream, cake, lettuce, potatoes, radish sprouts, alfalfa sprouts and fruit/vegetable salad [21]. In one study, *E. coli* O157:H7 was isolated from 3.7% of retail beef, 1.5% of pork, 1.5% of poultry, and 2.0% of lamb samples [8]. The first documented outbreak of *E. coli* O157:H7 infection occurred in Oregon in 1982, which was associated with eating undercooked hamburgers [29]. Beef donar kebabs sold in cars are also source of *E. coli* O157:H7 in Turkey [31].

Generally direct transmission from bovines to humans has been documented in very few cases. In Canada a case of transmission of *E. coli* O157:H7 between calves and a human has reported [22] and in Cornwall and West Devon transmission of vero cytotoxin producing *E. coli* O157 has reported [28]. However, such transmission appears to be rare.

Four water-borne outbreaks of EHEC infection were reported in the United States during 1982 to 1994 [19]. Of those, two each were associated with swimming pool water and contaminated drinking water respectively. Drinking water, probably contaminated with bovine feces, has been associated to outbreaks in Scotland [6], sothern Africa [15], and subsequently with well water in Japan [2]. EHEC has been found to be in large number in seawater of Ohio [11], lake Michigan [14], Huron [1], Great lake [5].

The fecal carriage of *E. coil* O157:H7 by humans is the most common routes for person-to-person transmission of the pathogen. Fecal shedding of *E. coli* O157:H7 by patients with hemorrhagic colitis or HUS usually lasts for no more that 13 to 21 days following onset of symptoms. However, in some cases, the pathogen can excrete in feces for weeks [17]. There is a very chance of secondary transmission, which may involve direct hand-to-hand contact (e.g. among children in day care centers) or indirect contact (e.g. via contaminated water used for swimming).

The major virulence factor and a defining characteristic of *E. coli* O157:H7, is the production of Shiga toxin. This potent cytotoxin is the factor, which leads to death and many other symptoms in patients infected with *E. coli* O157:H7 [22].

In *E. coli* O157:H7 a 35-kb pathogenicity island termed locus of enterocyte effacement (LEE) is located on chromosome, which confers the ATE phenotype. EHEC may have acquired this pathogenicity island by horizontal gene transfer from other species. The AiE lesion is characterized by intimin attachment of the bacteria to intestinal cells with effacement of the underlying microvilli and accumulation of filamentous actin in the subjacent cytoplasm. *E. coli* O157:H7 produces A/E lesion in the large intestine [10].

The LEE region consists of three segments. The middle segment includes the *eae* gene, which encodes intimin and the *tir* gene, which encodes a translocated receptor for intimin. Downstream of *eae* are the *esp* genes, which encode secreted proteins responsible for inducing epithelial cell signal transduction events leading to the A/E lesion. Upstream of *eae* and *tir* are several genes (*esc* and *sep*) those encode a type III secretion system that is involved in extracellular secretion of proteins encoded *esp* genes.

Intimin is a 94-kDa to 97-kDa outer membrane protein encoded by *eae* (which stands for *E. coli* attaching and effacing) [7]. Intimin is the only potential *E. coli* O157:H7 adherence factor that has been demonstrated to play a role in intestinal colonization in vivo in an animal model. O157:H7 strains produce extensive A/E lesions in the large intestine, featuring intimate adherence of the bacteria to the epithelial cells. O157:H7 strains with mutation in the *eae* gene no longer produced A/E lesions and, did not appear to colonize any intestinal site [22]. Additional support for a role in human disease is seen with the anti-intimin immune response in HUS patients [20]. EHEC and EPEC intimins share only 49% identity [30].

In Bangladesh, no *E. coli* O157:H7 associated infection has been reported yet officially. The reasons might be the lack of proper surveillance for *E. coli* O157:H7 or this pathogen may be present but the infections due to this occur in very few numbers because of the acquired immunity in the population. Only non-O157:H7 Shiga toxin-producing *E. coli* associated diarrhea in Bangladesh has been investigated among hospitalized patients with diarrhea including children and the urban slum community of Dhaka city. Shiga toxin genes were detected by multiplex PCR in 2.2 % of hospitalized patients and 6.9% of community patients [16].

In this study an attempt has been taken for designing of a complete protocol for the detection and isolation of *E. coli* O157:H7 from meat sources (goat meat).

2. Materials and Methods

2.1. Sample

A total of 40 sliced, raw goat meat samples (approximately 100g) were collected randomly from different markets around Dhaka city. Samples were collected weekly from September to December 2011 and were transported to the laboratory in a cooling box and processed as early as possible.

2.2. Isolation and biochemical identification

Tweenty five gram of meat sample was homogenized with 225 mL of Tryptic Soy broth (Oxoid, England) containing 20mg/L novobiocin in a stomacher (400 CIRCULATOR; Seward, England) and enriched for 6-8 h at 37 ºC. After enrichment, the broth was subjected to a series of tenfold dilution, and spread plated onto Sorbitol MacConkey Agar (SMAC; Oxoid, England) containing 0.05mg/l cefixime and 2.5mg/l tellurite (Merck KGaA, Germany). The colorless colonies were subcultured onto Eosine methylene blue (EMB; Oxoid, England) and 4-methylumbelliferyl-β-D-glucuronide [(MUG) (Difco, USA)] agar plates. The isolates showing metallic sheen on EMB agar plates and did not produce fluorescence on MUG media were subjected to other biochemical tests (Citrate, Methyl red, Voges-Proskaure, Indole, TSI and oxidase tests). The isolates, biochemically identified as *E. coli* O157:H7 were further confirmed by testing with Analytical Profile Index kits (API 20E, bioMeriuex Inc.). Eight other isolates, which were not identified as *E. coli* O157:H7 (as gave citrate positive reaction) but belong to Enterobacteriacae family (as per other biochemical reactions) were randomly picked and also tested with API kits.

2.3. Serological identification

Culturally and biochemically identified isolates were serologically analyzed using commercial 'Wellcolex *E. coli* O157:H7 rapid latex agglutination test kit' (Ramel, USA). To determine the specificity of serological test performed, 4 culturally and biochemically identified non-*E.coli* isolates were also randomly picked (from the isolates which were tested with API kit) and tested by the same kit.

2.4. Identification by amplifying specific genes

To amplify genes for virulence of *E. coli* O157:H7 were amplified either singly or in combination. Template DNA was prepared from the isolates, which are culturally, biochemically and serologically identified as *E. coli* O157:H7. DNA was prepared by boiling method and 2µl of

extracted template DNA was subjected to PCR amplification for the detection of virulent genes *eaeA, rfbE, fliC, stx1* and *stx2* by using specific primers and thermal condition (table-1). Isolates those gave bands of expected size were considered to carry these genes. For *eaeA, rfbE, fliC, stx1, stx2* gene specific primers 150 bp, 259 bp, 625 bp, 348 bp, 584 bp band were expected on agarose gel. Four *E. coli* non- O157:H7 were also selected to amplify the *eaeA* gene.

In order to lessen the time requirements of PCR amplification of each gene individually, we have attempted to set a multiplex PCR for amplification of three genes typical for *E. coli* O157:H7. For this, genes for attaching effacing and shiga-toxins were chosen. Primers were same and thermal conditions used were initial denaturing at 94^{o}C for 10 min, 35 cycles of denaturing at 94^{o}C for 1 min, annealing at 56^{o}C for 1 min, extension at 72^{o}C for 1 min and final extension at 72^{o}C for 5 min.

Table 1: *Primer pair and thermal condition used for the detection of eaeA, rfbE, fliC, stx1 and stx2 genes*

Target gene	Primer name	Primer Sequence (5'→3')	Reference	Thermal condition
rfbE	O157F O157R	CGGACATCCATGTGATATAGG TTGCCTSTGTACAGCTAATCC	Paton and Paton, 1998	Initial denaturing at 94^{o}C for 10 min, 35 cycles of 94^{o}C for 1 min; 56^{o}C for 1 min; 72^{o}C for 1 min and final extension at 72^{o}C for 7 min
fliC	FLICH7-F FLICH7-R	GCGCTGTCGAGTTCTATCGAG CAACGGTGACTTTATCGCCATTC	Gannon *et al.* 1997	Initial denaturing at 94^{o}C for 10 min, 35 cycles of 94^{o}C for 30 sec; 65^{o}C for 30 sec ; 72^{o}C for 75 sec and final extension at 72^{o}C for 7 min
eaeA	VS8 VS9	GGCGGATTAGACTTCGGCTA CGTTTTGCCACTATTGCCC	Kawasaki *et al.* 2005	Initial denaturing at 94^{o}C for 10 min, 35 cycles of 94^{o}C for 20 min; 60^{o}C for 30 sec; 72^{o}C for 30 sec and final extension at 72^{o}C for 7 min
stx_1	LP30 LP31	CAGTTAATGTGGTGGCGAAGG CACCAGACAAATGTAACCGCTC	Vidal *et al.* 2004	Initial denaturing at 94^{o}C for 10 min, 35 cycles of 94^{o}C for 1 min; 56^{o}C for 1 min; 72^{o}C for 1 min and final extension at 72^{o}C for 5 min
stx_2	LP41 LP42	ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	Vidal *et al.* 2004	Initial denaturing at 94^{o}C for 10 min, 35 cycles of 94^{o}C for 1 min; 55^{o}C for 1 min; 72^{o}C for 1 min and final extension at 72^{o}C for 5 min

3. Results and Discussion

A total of 40 goat meat samples were included in this study. Of 40 samples, 1523 Sorbitol non-fermenting colonies were selected and proceed for further investigation.

3.1. Identification of suspected *E. coli* O157:H7 isolates by cultural methods

Of 1523 sorbitol non-fermenting suspected *E. coli* O157:H7 isolates, 107 showed growths with typical green metallic sheen on EMB plates. On the other hand, the isolates grown on MUG plates 86 of them showed growth without blue fluorescence which is typical for *E. coli* O157:H7. All the MUG negative isolates were also EMB positive. The MUG negative and EMB positive isolates are presumed to be *E. coli* O157:H7 and followed up for further investigations by biochemical tests distinguishing for *E. coli*. Isolates showed pattern of biochemical reactions typical for *E. coli* O157:H7 were selected for further confirmation by a series of biochemical test, which are typical of *E. coli*. A total of 86 isolates were subjected to biochemical characterization. All of them fermented glucose and lactose with the production of gas (TSI). Eleven of 86 isolates were citrate negative which is the characteristic of *E. coli*. All the isolates were indole and methyl red positive but Voges-proskaure negative. So, it is very much likely that although most of the biochemical properties are similar to *E. coli*, but citrate positive isolates might be another member of Enterobacteriaceae.

3.2. Analytical Profile Index (API)

The isolates, which were biochemically identified as *E. coli* O157:H7 were further tested with API kits. Among them, 11 isolates were conformed as *E. coli* in API test as ′Good identification′. Randomly picked 8 isolates were identified as different species of *Enterobacter*.

3.3. Identification of *E. coli* O157:H7 by serological test

Among the biochemically positive 11 samples, 10 showed agglutination with O157 anti-sera. The remaining one sample did not show any agglutination**.** On the other hand, only 4 of 11 isolates showed

agglutination against H7 anti-sera. None of the four randomly picked isolates gave agglutination reaction against O157 or H7 anti-sera.

3.4. Detection of *E. coli* O157:H7 by PCR amplification of *eaeA, rfbE, fliC, stx1* and *stx2* genes

Among the biochemically identified eleven isolates, ten were positive for *eaeA* gene amplification**.** All the *eaeA* gene amplification positive isolates showed positive result with *rfbE* gene amplification. In case of *fliC* gene amplification, six isolates showed positive results. Other isolates are possibly *E. coli* O157:H-. In PCR amplifications of *stx1* and *stx2* genes, 4 of 6 isolates which were positive for *eaeA*, *rfbE* and *fliC* showed positive results for both *stx1* and *stx2* genes amplification. The remaining two isolates showed positive results with only for *stx2* gene amplification. Three isolates positive for *eaeA, rfbE* but negative for *fliC* gene amplification, showed negative results with both *stx1* and *stx2* gene amplification. The other isolates which was positive for *eaeA, rfbE* but negative for *fliC* gene amplification, showed positive result only with *stx1* gene amplification.

The isolates, which were randomly picked *E. coli* non- O157:H7, none showed positive results in *eaeA* gene amplification, so no other gene amplification by PCR was done.

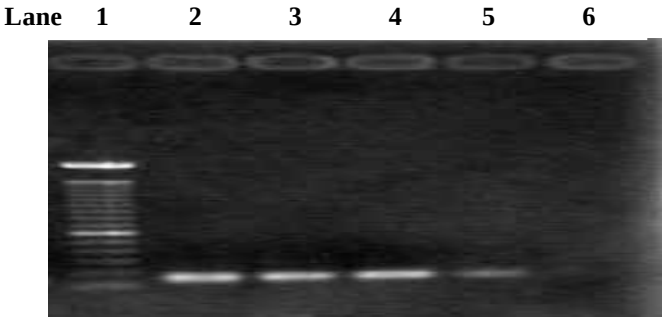

Fig. 1. *150 bp amplification products of* ***eaeA*** *gene specific primers. Lane 1: 100 bp marker, Lane 2: Positive control, Lane 3: Isolate, Lane 4: Isolate, Lane 5: Isolate, Lane 6: No template control.*

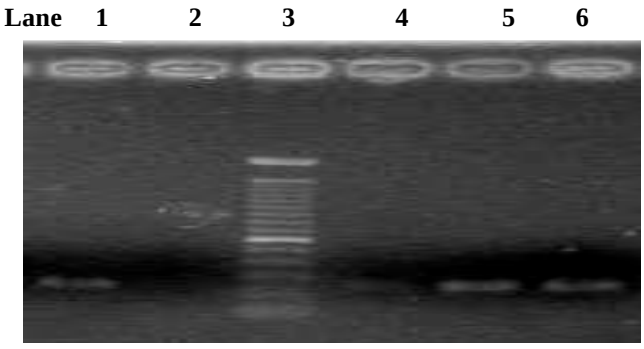

Fig. 2. *259 bp amplification products of* ***rfbE*** *gene specific primers. Lane 1: Positive control, Lane 2: No template control, Lane 3: 100 bp marker, Lane 4: Isolate, Lane 5: Isolate, Lane 6: Isolate.*

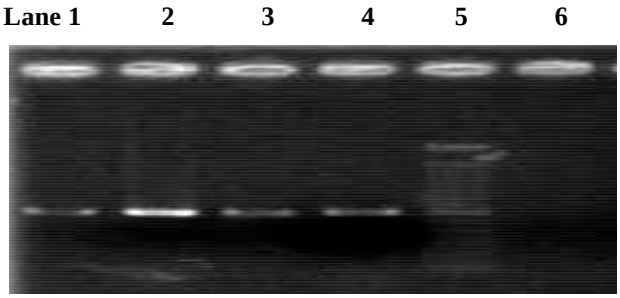

Fig. 3. *625 bp amplification products of* ***fliC*** *gene specific primers. Lane 1: Isolate, Lane 2: Isolate, Lane 3: Isolate, Lane 4: Positive control, Lane 5: 100 bp marker, Lane 6: No template control.*

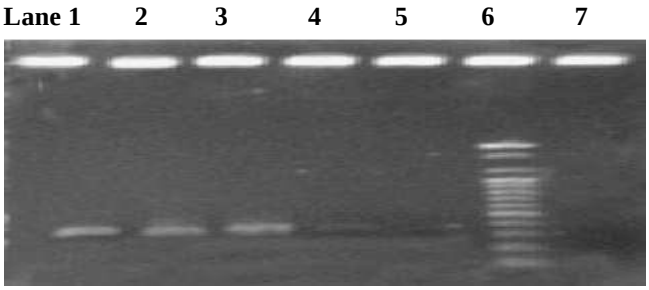

Fig. 4. *348 bp amplification products of* ***stx1*** *gene specific primers. Lane 1: Positive control, Lane 2: Isolate 3v, Lane 3: Isolate 8b, Lane 4: Isolate SN-2g, Lane 5: Isolate RM-5k, Lane 6: 100 bp marker, Lane 7: No template control.*

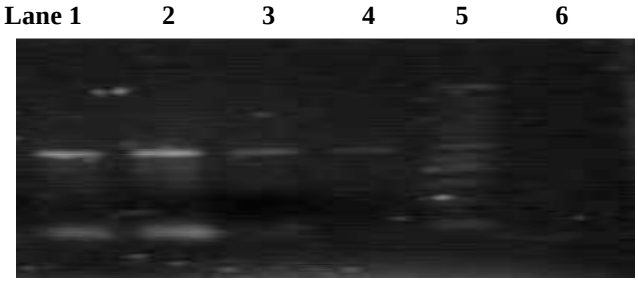

Fig. 5. *584 bp amplification products of* ***stx2*** *gene specific primers. Lane 1: Positive control, Lane 2: Isolate, Lane 3: Isolate, Lane 4: Isolate, Lane 5: 100 bp marker, Lane 6: No template control.*

Multiplex PCR

In multiplex PCR for *eaeA, stx1* and *stx2*, the isolates showed similar result as showed in the PCRs for each gene individually.

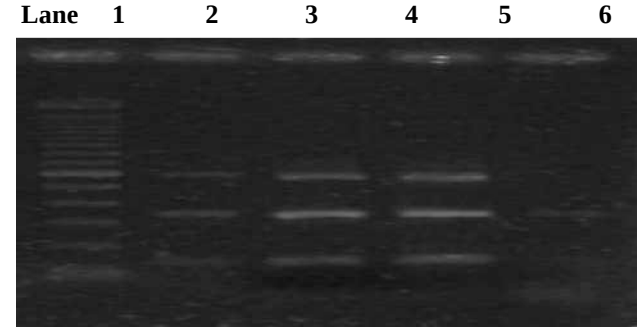

Fig. 6. *150 bp, 348 bp and 584 bp amplification products of* ***eaeA, stx1*** *and* ***stx2*** *genes specific primers in multiplex PCR. Lane 1: 100 bp marker, Lane 2: Isolate, Lane 3: Isolate, Lane 4: Positive control, Lane 5: No template control.*

4. Conclusion

Most identified strains of *E. coli* are able to ferment sorbitol but *E. coli* O157:H7 cannot. So isolation of *E. coli* O157:H7 is based on the inability of this organism to ferment sorbitol. After recovering the sorbitol negative colonies, the biochemical characteristics of the isolates are determined. For further confirmation, the isolates are checked by agglutination reactions to identify the O157 somatic and H7 flagellar antigens. Characterization based on nucleic acid is done by PCR procedures that target the shiga toxin genes (*stx-1* and *stx-2*), the attaching and effacing gene (*eaeA*), the O157 antigenic gene (*rfbE*) and the flagellar gene (*fliC*). To minimize the time, effort and the cost a multiplex PCR has also been designed. From all the isolates, the identified *E. coli* O157:H7 was low in number though the overall hygiene practices of the shoppers and the household concern is alarming. The cooking practice and the strong immunity of the people of Bangladesh may be playing a part regarding the pathogenic effect of this very organism.

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Novel microbial fingerprinting of fruits by PCR-DGGE: An application to determine the geographical origin of Shea tree fruits from Senegal

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Abstract:

Food traceability is essential to preserve the identity of unique quality traits against fraud or commercials disputes. Therefore, there is growing demand of new traceability systems for the collection of information related to units/batches of food ingredients and products. A rapid method based on a molecular technique employing 28S rDNA profiles generated by PCR-DGGE was used to detect the variation in fungal community structures of Shea tree fruit (*Vitellaria paradoxa*) from Senegal. When the 28S rDNA profiles were analyzed by multivariate analysis, distinct microbial communities were detected. The band profiles of Shea tree fruit fungi from different countries were specific for each location and could be used as a bar code to discriminate the origin of the fruits. This method is a new traceability tool, which provides fruit products with a unique biological bar code and makes it possible to trace back the fruits to their original location.

Keywords: Molecular markers, Shea tree fruits, Fungal communities, Origin

1. Introduction

In the past years, the development of biological identification technologies has greatly contributed to support and check traceability systems. In parallel, computer technology provided many new and innovative tools for tracing products [1]. Biological, analytical and informatics tools have been synergistically proposed and utilized for traceability in the wine industry [2]. Currently, there are not molecular biology techniques for determining the geographical origin of food.

The Shea tree has been exploited by African communities for about 3000 years and offers an opportunity for sustainable development in Sudanian countries [3]. Shea trees produce fruits, which form an important part of the diet of Africans. It is a perennial tree which starts producing fruits at the age of 10 - 15 years. It takes 20 - 30 years for the complete growth of Shea trees. The botanical name of Shea is *Vitellaria paradoxa*. The region where Shea trees grow extends from eastern Senegal to northern Uganda. Shea tree seeds or nuts are used in the preparation of Shea butter products, which have a great commercial value. Shea tree is very important from the point of sustainable development of the African rural economy [4]. The estimated yield of dry kernel is about 600,000 tons per year, and exports have increased over the past decade, reaching 350,000 tons today, mainly to USA and Europe. Total production potential has been estimated at over 2.5 million MT kernels [5].

Shea fruit is often consumed as fresh fruit for its sweet edible pulp. The fruit pulp is a particularly rich source of ascorbic acid, i.e. 196.1 mg/100g compared with 50 mg/100 g for oranges. The iron content compares favorably with raspberries: 1.93 mg/100g compared with 0.92mg/100 g. The solidified Shea oil is called ‘butter’. It is constituted primarily of heavier carbon molecules and is more or less solid at room temperature. Each kernel contains a fat for about half its weight. Shea butter is completely natural and very rich in vitamins (A and E) and essential fatty acids [6].

In Africa, Shea tree fruit is also nicknamed "The Gold of Women", because of the Shea butter is not only used as a cosmetic by women, but also used as a fat for cooking, mainly in rural areas which account for 80% of total consumption [4]. Demand for Shea butter produced in West Africa has increased by over 1200% over the last 10 years. The increased demand has led to new businesses. There are now more companies operating at every level of the Shea value chain. In Europe, Shea butter is used mainly (95%) by the chocolate industry. The quantities exported to Japan, the United States or Switzerland would be mainly used for cosmetic or pharmacological [5].

In Senegal, Shea butter (kare in Pulaar and karité in French) is an extremely popular product, people use it as a lip balm and a body moisturizer and they also cook with it. Making Shea butter is astoundingly hard work. The Shea nuts have to be gathered, dried, shelled, pounded, ground, soaked, strained, boiled, re-strained, melted down, and poured into containers. The women's groups often get together to make and sell Shea butter. Usually they just pour it into old water bottles or whatever containers happen to be on hand, and a half-liter goes for around 1,000 CFA, or about $2.00 USD [6].

Certification of Shea kernel and butter has become increasingly important for a number of reasons – The EU started demanding that all agricultural products are traceable from source from 1st January 2005. A number of cosmetic companies are asking for organically certified Shea butter for formulation of organically labelled 'botanical' products and the demand for consistent 'Quality @ Quantity' from rural producers is increasing the need for quality assurance [5]. The idea was to create a 'biological bar code' [7] based on the analysis of the DNA of microorganisms present on the products. This method is based on the assumption that the microbial communities of the fruits are specific for a geographical area [6, 8, 9, 10, 11, 12].

The main objective of this study is to apply polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) method to analyze in a unique step all the fungi present on the fruit in order to create an analytical technique that will permit the linkage of fungi communities to the geographical origin and avoid the individual analysis of each fungus strain. To the best of our knowledge, there are few papers published by our team describing a molecular method of fungi ecology, the PCR-DGGE that will permit the certification of Shea tree fruit using 28S rDNA fingerprinting of fungi.

2. Materials and Methods

2.1. Fruit samples

Mature fruits of Shea tree (*V. paradoxa*) were collected in three different locations from Senegal (two different districts for each location). These locations were: Kenioto, Saraya and Samecouta, Tambacounda region in Senegal. Table 1 gives the geographical coordinates of the sampling sites. Additionally, Tambacounda region follows the Sudano-Guinean area climate; these forests are very large and dense savannah. The trees are varied: baobab, kapok, palm, casuarina and Roni. August is the wettest with a total rainfall of 308 mm. Temperatures are also experiencing significant seasonal variations. It records the hottest temperatures in April (T_{max} = 40 °C) and coldest in January (T_{min} = 18 °C).

The fruits were gathered to preserve their initial flora. They were collected directly on the tree using gloves and put in sterile bags in July 2008. These bags were kept into a refrigerator then transferred by plane to CIRAD Montpellier (France) where the fungal DNA was extracted immediately from the fresh fruits. The origin of the samples was defined by country, site and date of harvest.

2.2. Fungal DNA extraction from Shea tree fruit samples

We applied a new protocol created by El Sheikha [6] and takes into account the methods of Karakousis et al. [13] developed for fungal DNA extraction and El Sheikha et al. [11] developed for yeast DNA extraction. Two fruits of Shea tree were randomly taken and put in sterile Stomacher bag containing 6 mL peptone. The two Eppendorff 2 mL vials contained the resulting suspension with 0.3 g of 0.5 mm Aldrich Chimie S.a.r.l., Lyon, France). The mixture was vortexed vigorously for 30 min in a bead beater instrument (Vortex Genie 2 SI–A256, Bohemia, NY, USA) then centrifuged at 12 000 × *g* for 15 min and the supernatant discarded. The cell pellet was re-suspended in 300 mL of breaking buffer [2% Triton X-100 (Prolabo, Fontenay-sous-Bois, France); 1% sodium dodecyl sulphate (SDS, Sigma); 100 mM NaCl (Sigma); 10 mM Tris-HCl pH 8.0; 1 mM EDTA pH 8.0 (Promega, Charbonnie`res-les-Bains, France)]. Then, 100 mL TE (10 mM Tris-HCl; 1 mM EDTA; pH 8.0, Promega) and 100 mL of lysozyme solution (25 $mg.mL^{-1}$, Eurobio, Les Ulis, France) and 100 mL of proteinase K solution (20 $mg.mL^{-1}$, Eurobio) were added and incubated at 42 °C for 20 min. Then 50 mL of 20% SDS were added to each tube, and the tubes were incubated at 42 °C for 10 min. Four hundred microlitres of mixed alkyltrimethyl ammonium bromide (Sigma) were added to each tube, and the tubes were incubated at 65 °C for 10 min. The tubes were vortexed vigorously for 5 min after each addition. The lysates were then purified

by twice repeated extraction with 700 mL of phenol–chloroform–isoamyl alcohol (25:24:1, Carlo Erba, Val De Reuil, France) and the tubes were vortexed for 5 min and then centrifuged at 12 000 × *g* for 15 min. The aqueous layer was transferred to an Eppendorff vial and the residual phenol was removed by extraction with 600 mL of chloroform–isoamyl alcohol (24:1) and centrifuged at 12 000 × *g* for 15 min. The aqueous phase was collected and the DNA was stabilized with 30 mL of sodium acetate (3 M, pH 5), followed by precipitation by adding equal volume of ice-cold isopropanol and stored at -20 °C for 12 h (overnight). After centrifugation at 12,000 × *g* for 15 min, the supernatant was eliminated, DNA pellets were washed with 500 mL 70% ethanol, and tubes were centrifuged at 12,000 × *g* for 15 min. The ethanol was then discarded and the pellets were air dried at room temperature for 45–60 min. Finally, the DNA was re-suspended in 50 mL of ultra-pure water and stored at -20 °C until analysis. In order to check DNA extraction, an electrophoresis on agarose gel was done. The entire DNA was loaded into 0.8% agarose gel in 1 × TAE buffer (40mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0 mM Na2–EDTA, Eppendorff, Hamburg, Germany) with molecular weight ladder 16.21 kb as reference (Supercoiled DNA ladder, Invitrogen, Carlsbad, CA, USA). After running at 100 V for 30 min, the gels were stained for 30 min with ethidium bromide solution (50 $mg.mL^{-1}$, Promega), rinsed for 20 min in distilled water, then observed and photographed on a UV transilluminator using black and white camera (Scion Company, Bethesda, MD, USA) and Gel Smart 7.3 system software (Clara Vision, Les Ulis, France).

2.3. PCR-Denaturing gradient gel electrophoresis (DGGE) analysis

For fungi, a fragment of region of the 28S rDNA was amplified using eukaryotic universal primers U1 (5- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTG TTG AAA GGG AA-3, Sigma) and the reverse primer U2 (5-GAC TCC TTG GTC CGT GTT-3, Sigma) amplifying an approximately 260 bp fragment [6, 12, 14, 15, 16, 17, 18, 19, 20, 21]. A 30-bp GC-clamp (Sigma) was added to the forward primer (the GC-clamp is underlined) to ensure that the fragment of DNA will remain partially double-stranded and that the region screened is in the lowest melting domain. PCR was performed in a final volume of 50 µL containing 2.5 µL DMSO, 0.4 µM each primers, all the deoxyribonucleotide triphosphate (dNTPs) at 200 µM, 3 mM $MgCl_2$, 5 µL of 10 × of reaction Taq buffer $MgCl_2$ free (Promega), 1.25 U of Taq DNA polymerase (Promega), and 2 µL of the extracted DNA. The amplification was carried out as follows: An initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 45 sec, 50 °C for 50 sec and 72 °C for 90 sec, and a final extension at 72 °C for 5 min. Aliquots (5 µL) of PCR products were analyzed first by conventional electrophoresis in 2% (w/v) agarose gel with TAE 1 × buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0 mM Na2-EDTA), stained with ethidium bromide 50 µg.mL–1 in TAE 1 × and quantified by using a standard (DNA mass ladder 100 bp, Promega).

The PCR products were analyzed by DGGE by using a Bio-Rad DcodeTM universal mutation detection system (Bio-Rad Laboratories, USA), using the procedure first described by El Sheikha [6]. Samples containing approximately equal amounts of PCR amplicons were loaded into 8% (w/v) polyacrylamide gels (acrylamide/N,N'-methylene bisacrylamide, 37.5/1, Promega) in 1 × TAE buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0 mM Na2-EDTA).

All electrophoresis experiments were performed at 60 °C using a denaturing gradient ranging from 30–40 to 60–70%, and were finally standardized at 40–70% (100% corresponded to 7 M urea and 40% [v/v] formamide, Promega). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 16 h. After electrophoresis, the gels were stained for 30 min with ethidium bromide and rinsed for 20 min in distilled water and then photographed on a UV transilluminator with the Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

2.4. Image and statistical analysis

Individual lanes of the gel images were straightened and aligned using Image Quant TL 7.0 (GE Healthcare, USA). This software permitted to identify the bands and their relative position. The DGGE fingerprints were manually scored by the presence and absence of co-migrating bands, independent of intensity. Analysis of data was done using the Dice similarity coefficient (SD), which is calculated according to the formula $SD = 2\ N_c/N_a + N_b$ (1) [22], where N_a represented the number of bands detected in the sample A, N_b represented the number of bands in the sample B, and N_c represented the numbers of bands common to both sample. Similarity index were expressed within a range of 0 (completely dissimilar) to 100 (perfect similarity). Genogram was constructed by using software of Primer version 6 (PRIMER-E Ltd, United Kingdom).

2.5. Sequence analysis of DNA bands and fungi identification

DNA bands from DGGE gel were carefully selected and excised from the gel using sterile razor blades. The pieces of gel were soaked in 100 mL of TE buffer overnight at 4°C. Eluted DNA for each band was purified by kit Wizard PCR Preps DNA

Purification system (Promega), and then the purified DNA was re-amplified by the same PCR conditions as described above using the primers without GC-clamp. The amplicons were sequenced by GATC Biotech (Konstanz, Germany). DNA base sequences were analyzed by comparison with the GenBank databases of the National Centre for Biotechnology Information. Searches in GenBank with BLAST program were performed to determine the closet known relative of partial 28S rDNA sequences [23].

3. Results

3.1. Performance of fungal DNA extraction methods

Eight µL of genomic DNA extraction of the fungal communities were verified on a 0.8% (w/v) agarose gel and achieved an admirable success. On the gel, the bands with a molecular weight greater than 16 kb corresponding to genomic fungal DNA were clearly observed.

3.2. Verification of the PCR amplification of the extracted DNA

The fungal genomic DNA obtained after extraction were amplified by PCR using the protocols first described by El Sheikha [6]. In order to verify the efficiency of this fragment, five µL of the PCR amplicon were electrophoresed on 2% (w/v) agarose gel at 100 V for 30 min in the TEA buffer as described above. All of the bands were clearly observed and had a molecular weight of 260 bp, the expected size of the amplicon. Successful amplification permits one to continue to analyze these amplicons by the DGGE method.

3.3. DGGE pattern of fungal DNA from Shea tree fruits among different districts of Senegal

On DGGE gel, the observed bands had sufficient intensities to analyse samples of fungal DNA extracted from Shea tree fruits from various geographical locations in Senegal (Fig. 1), so the total quantity of DNA deposited in the wells of DGGE gel was sufficient to consider that fungal DNA could be used as potential markers to ensure the determination of Shea tree fruits origin.

The reference DNA of *Mucor racemosus* and *Trichoderma harzianum* indicates that DGGE was done successfully. Each vertical line represents a fruit and each spot represents a fungus. The PCR-DGGE patterns of duplicate Shea tree fruits for each location were similar and revealed the presence of four to twelve bands for each Shea tree fruit (Fig. 1). Some spots appeared double or smear because of the presence of single-strand DNA [24].

Clusters analysis by Statistica version 6 software (StatSoft, Maisons-Alfort, France) of the DGGE gel patterns for the duplicate Shea tree fruit samples from three different districts showed a community similarity among the geographical locations where the fruit samples were collected (Fig. 2). At 89% similarity level, two main clusters were observed: the first cluster included the samples from Saraya and Kenioto and the second cluster comprised the samples from Samecouta.

3.4. Identification by sequencing of dominant fungi on Shea tree fruits

To our knowledge, there is no information on the populations of fungi from Senegal by culture dependent methods. The bands cut from DGGE gel profiles of extracted fungi DNA from Shea tree fruits were sequenced in order to know the identity of strains of fungi present in the fruit samples. Each sequenced band corresponds to a unique sequence which has a sufficiently long to allow an identification by comparison between the sequences and those listed in GenBank (Results not shown).

4. Discussion

There are only a few publications published by our team that analyzed the fungal communities in fruits and described the linkage between the fungal communities and the geographical origin of fruits [12, 19, 20, 21]. Flórez and Mayo [25] used 26S rDNA to detect the fu ngal species in Cabrales cheese during the manufacture and ripening. Durand et al. [26] used 28S rDNA to improve the knowledge about fungi dynamics and biodiversity of potentially OTA producing fungi during coffee processing.

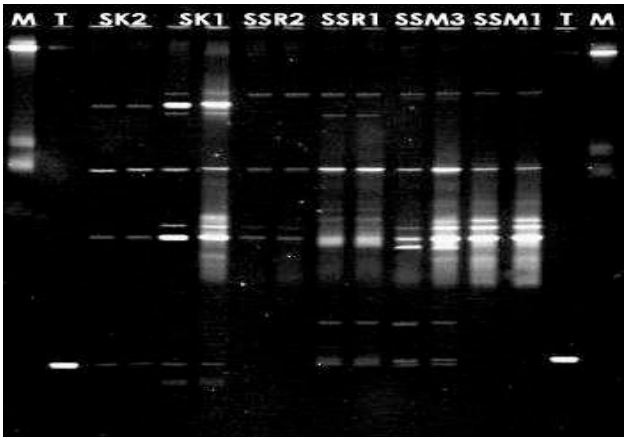

Fig 1. *PCR-DGGE band profiles of 28S rDNA of Shea tree fruits from three different locations of Senegal. Kenioto (SK2, SK1); Saraya (SSR2, SSR1); Samecouta (SSM3, SSM1); M: Mucor racemosus; T: Trichoderma harzianum.*

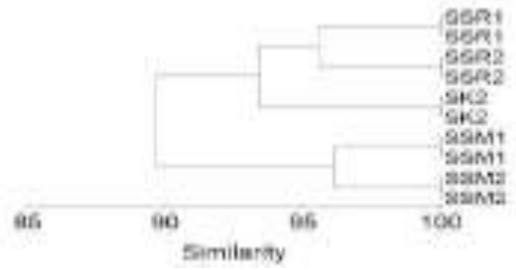

Fig 2. *Cluster analysis of 28S rDNA banding profiles of Shea tree fruits from three different locations of Senegal (two different districts for each location). Kenioto (SK2, SK1); Saraya (SSR2, SSR1); Samecouta (SSM3, SSM1).*

Previous work on Shea tree has focused on morphological, genetic diversity and technological studies [3, 27, 28, 29]. In our study, we proved that the DGGE pattern that the band pattern of the fungal communities isolated from Shea tree fruits obtained by PCR-DGGE was strongly linked to the microbial environment of the fruits. For Shea tree fruits, the DGGE gel showed some significant differences in the migration patterns. However, the duplicates for each sampling location gave statistically similar DGGE patterns throughout the study.

The differences in the band profiles can be attributed to the differences in environment between districts. In the gel some common bands appeared in all of the samples independently to the location. These bands could be common fungi for all of the Shea tree fruit samples. The fungi strains were identified from the environment, such as *Fusarium oxysporum* could be found on many plants [30] (Anaissie et al., 2001). The presence of fungi on the fruit is the good reflection of the environment of the sampling areas thus they could serve as markers of the geographical origin of the fruit. These results can give an idea of the biodiversity of the fungi according to the geographical origin.

Table 1*. Geographic description of sampling sites*

Country	Region	Site	GPS		Altitude (m)
			Longitude	Latitude	
Senegal	Tambacounda	Saraya	12° 49' 53.62" N	11° 45' 21.10" W	189
		Samecouta	12° 36' 10.00" N	12° 07' 80.13" W	120
		Kenioto	12° 34' 20.11" N	12° 09' 60.16" W	120

5. Conclusion

We could conclude that the analysis of Shea tree fruit fungi communities by PCR-DGGE could be applied to differentiate geographical locations. We showed that the biological markers for the specific locations were sufficient statistically to discriminate regions. These meanings also as there were enough environmental differences between the districts where the Shea tree fruits were harvested to obtain a major effect on the fungal ecology, whereupon we could create a statistical link between the fungi populations and the geographical area. This global technique is quicker (< 24 h) than all of the classical microbial techniques and avoids the precise analysis of fungi by biochemistry or molecular biology (sequencing). This method can thus be proposed as a rapid analytical traceability tool for fruits and could be considered as a provider of a unique biological bar code for each country. Furthermore, the ecological study of fungi in many other products in which they occur provide another area for future study.

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The optimized condition of ochratoxin a production for reference material production

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Abstract:

Ochratoxin A (OTA) is a significant mycotoxin that causes nephrotoxicity in animal and human. Since toxin is contaminated in foods at very small amounts as nanogram per gram (ng/g), thus the precision and accuracy of toxin analysis is required. This research aims to produce OTA in maize in order to develop as OTA reference material. The optimum conditions for fungal growth and toxin production was investigated. Comparing the toxin production by 4 strains of *Aspergillus spp*., isolated from brown rice and 2 reference strains were done under various substrates, temperatures, moisture contents and incubation times. Preliminary results showed strains I1012/1, 2277, K6723, P15723 including *A. ochraceus* TISTR 3557 and *A.alliaceus* TISTR 3218 produced toxin well on MEA agar at 28 °C for 7 days, measured by TLC method. Confirmation test on laboratory media showed only I1012/1 had ability to produce toxin well on CCA and PDA as 172 and 559 ng/g. However when cultured on maize and rice all five strains grew well, but did not produce toxin. Similar results was found when inoculated *A. ochraceus* TISTR 3557 on three different types of maize, Suwan 5, ATS 5 and popcorn grain, high mycelium production but none of toxin production was found. Changing strain to *A. alliaceus* TISTR 3218 was done and found very well toxin production on maize and rice at 20 %, 30 % and 40 % moisture, 28°C for 14 days as 330-500 ng/g, and produce better on MEA as 2000 ng/g, detected by HPLC method. While at this mentioned conditions *A. ochraceus* TISTR 3557 did not produce toxin. In conclusion the optimal condition for OTA production by *A. alliaceus* TISTR 3218 was on maize 30 % moisture (a_w 0.97) incubated at 28 °C for 7 days which could produce as high as 3200 ng/g.

Keywords: Mycotoxin, *Aspergillus alliaceus*, OTA reference material

1. Introduction

Ochratoxin A (OTA) is significant mycotoxins among five mycotoxins that are considered to be important in human health [11]. OTA is mainly produced by some species of *Aspergillus* and *Penicillium*, particularly *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum*. Although these moulds can easily contaminate foodstuffs, but occurrence of OTA in foods may depend on climatic condition. The critical factors that affect fungal growth on food commodities are temperature, moisture content and time [3].

Toxicity of OTA is well documented in many animal species and in 1993 The International Agency for Research on Cancer (IARC) has classified OTA as Group 2B, a possible human carcinogen [5, 10]. In animal models, OTA was shown to produce a wide toxicological effect, including nephrotoxicity and nephrocarcinogenicity, neurotoxicity and immunotoxicity [12]. Since OTA is often not rapidly removed from the body and significant amounts may accumulate in the blood and other selected tissues. In March 2001, the commission of the European Communities has set OTA maximum levels of 5 ppb for raw cereal grains (including raw rice and buckwheat) and 3ppb for all

processed cereal products derived from cereals (including processed cereal products and cereal grains intended for direct human consumption).

Commonly used analytical methods for the determination of OTA are high-performance liquid chromatography (HPLC) with fluorescence detection or thin layer chromatography (TLC) with a cleanup step [13]. In the development of new analytical methods for OTA the analytical methods must be fully validated particular if they are to be used for control, monitoring and risk assessment studies [2]. Thus, the reference materials (RMs) or (CRMs), the materials containing a known or certified content of analyte(s) along with its uncertainty, are essential tools in achieving comparability and trueness of analytical data. Aside from that, the use of (CRMs) is a major requirement for an accreditation according to ISO/IEC 17025 [6]. This research aims to produce OTA in maize in order to develop as OTA reference material. The optimum conditions for fungal growth and toxin production was investigated.

2. Materials and Methods

2.1. Growth and toxin production on media

Fungal strains I1 / 1012, 2277, K6723, P 1572 isolated from brown rice and two reference strains from Institute of Scientific and Technological Research (TISTR), *A. ochraceus* TISTR 3557and *A. alliaceus* TISTR 3218, were used in this study. The isolates were cultured on CCA, CY20S, PDA and MEA then incubated at 28° C for 7 days. The analysis for the amount of toxin OTA was done by Thin Layer Chromatography (TLC) (Camag TLC scanner 3, Switzerland).

2.2. Ability of the toxin production on culture media and grain

Fungal strains I1 / 1012, 2277, K6723, P15723 and *A. ochraceus* TISTR 3557were cultured on CCA, CY20S, PDA and MEA. Replicate plates were incubated at 28° C for 7days then the center of agar colonies were drilled, put into a test tube. Pipetted 5 ml of saturated solution of ethanol - formic acid (25:1) then the mixture was shaken for 5 min, filtered through filter paper No 1. Then pipetted 2 ml of filtrate into a dark bottle, dried with nitrogen gas then dissolved with 200 µl of benzene : acetic acid (99:1). The amount of OTA in the extract was analyzed by TLC-densitometry method

2.3. Culture preparation and inoculation

Spore suspension was prepared by culture fungal isolates on malt extract agar (MEA) at 28°C for 7 days, then added 10 ml and wiped the upper layer of media with sterile hockey stick. Pipette spore suspension and mixed with vertex. Maize 100 g were added with sterile water to achieve 20%, 30% and 40% moisture, placed in sterile plastic and inoculated with 10 ml of spore suspension. Growth was determined after maize was incubated at 28 °C and 35 °C and measured the diameter of colony at 7 days. The experiment was carried out twice with two replicates per conditions.

To compare OTA production on maize and rice, sterile water was added to maize and rice as 10, 20 and 30 ml of water to achieve 20%, 30% and 40 % moisture. Five isolates I1012/1, 2277, K6723, P15723 including *A. ochraceus* TISTR 3557, was chosen and mixed with 100 grams of maize and rice left overnight in sterile plastic, then autoclaved for 30 min before inoculated at 28°C and 35°C for 7 and 14 days.

2.4. TLC-densitometry detection of OTA

High performance TLC sheet was baked at 105 °C for 1 h then washed (predevelop) by using chloroform - methanol (1:1) which will be used mobile phase. Let solvent moves to the left of a sheet as 80 mm. When the plate is dry, immediately used to analyze, but if it is not used immediately plate should store in a desiccator at 30 ° C. Extract sample 20l was spot onto TLC plate added develop solution of toluene - ethyl acetate - formic acid (6:3:1) used as mobile phase let solvent moves to 70 mm. Read plate with Dennis, wavelength of 333 nm using a mercury lamp as a light source. Then confirmed by spectroscopy photometer (Camag TLC scanner 3) at a wavelength of 200-500 nm using fluorescent as a light source. Standard solution was prepared at concentrations of 2.5, 7.5, 10.25, 17.5 and 22.5 ng/g, respectively, and spot same time of sample detection.

2.5. HPLC detection of ochratoxin A

After 7 and 14 days of incubation, two replicates per conditions were blended and dried at 70 °C for 12 h, stored at 4°C until OTA analysis was carried out. 50g of sample was weighed and ground with 5 g of NaCl in blender jar, added 100 mL methanol: water (80:20), and blended at high speed for 1 minute. Extract was filtered through filter paper no. 4 and filtrate was collected in a clean vessel. Pipetted filtrate 10 mL and diluted with 40 mL PBS. Mixed well and filtered again through 1.5 µm glass microfiber and collected. 10 mL Filtrate was filtered through column (VICAM OchraTest^{TM}, USA) at a rate of 1-2 drops/second then the column was washed with 10 mL PBS containing 0.01% tween 20, and then rinse twice with 5 mL distilled water. OTA was eluted from the column using methanol (HPLC grade) as a solvent, at a flow rate of 1–2 drops per second. Each treatment was performed by HPLC (Water, USA).

3. Results and Discussion

Among five isolates from rice I1012/1 has shown the high ability to produce OTA when cultured on CCA and PDA at 28°C for 7 days (Table 1) comparing to other isolates. Reference culture *A. alliaceus* TISTR 3218 produced OTA better on CCA, CY20S, PDA and produced the highest amount of OTA as 2090 ng/g on MEA comparing to *A. ochraceus*, which was commonly isolated from maize. Then *A. alliaceus* was inoculated on maize and rice at different moisture contents and incubated for 7-14 days.

Table 1 *Concentration of OTA in agar (ng/g) extracted from each isolates cultured on laboratory media at 28 °C for 7 days*

Culture	OTA (ng/g)[c]			
	CCA	CY20S	PDA	MEA
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A.ochraceus [a]	131.41 ± 0.63	-	300.28 ± 0.27	400.35 ± 2.76
A.alliaceus [b]	1678.00 ± 1.03	1350.85 ± 2.30	1245.85 ± 1.54	2090.00 ± 1.16
2277	-	-	94.36 ± 1.34	-
I1012/1	172.00 ± 0.23	-	559.00 ± 0.99	-
K6723	-	-	-	-
P15723	44.78 ± 0.15	-	0.68 ± 0.54	-

[a] *Reference culture A. ochraceus TISTR 3557,* [b] *Reference culture A. alliaceus TISTR 3218*
[c] *detected by TLC*

Fig. 1 showed *A. alliaceus* has potentially produced high amount of toxin production on both maize and rice at 20%, 30% and 40 % moisture, 28°C for 7 days as 260-400 ng/g, and 14 days as 300-400 ng/g, the selected condition *A. alliaceus* for OTA production was on 30% moisture maize and incubate for 7 days.

The growth and toxin production of *A. ochraceus* TISTR 3557 and four isolated from rice were observed on maize and rice at 20%, 30% and 40% moisture. Although all five strains shown the potential to produce OTA on laboratory media and grew very well on maize and rice but none of those produce toxin at any conditions (Table 2), including reference strain *A. ochraceus* TISTR 3557.

Similar results was found when cultured *A. ochraceus* TISTR 3557 on three different variety of maize, Suwan 5, ATS 5 and popcorn grain, high mycelium production was shown but none of toxin production was found (Table 3). Although maize was crushed, or added yeast extract as a nutrient to promote OTA production and this strain showed high growth on Suwan 5 and ATS 5 but none of OTA was found. Further study we decided to use reference strain *A. alliaceus* as the OTA producing strain to produce OTA reference material, and Suwan 5 was use as the material.

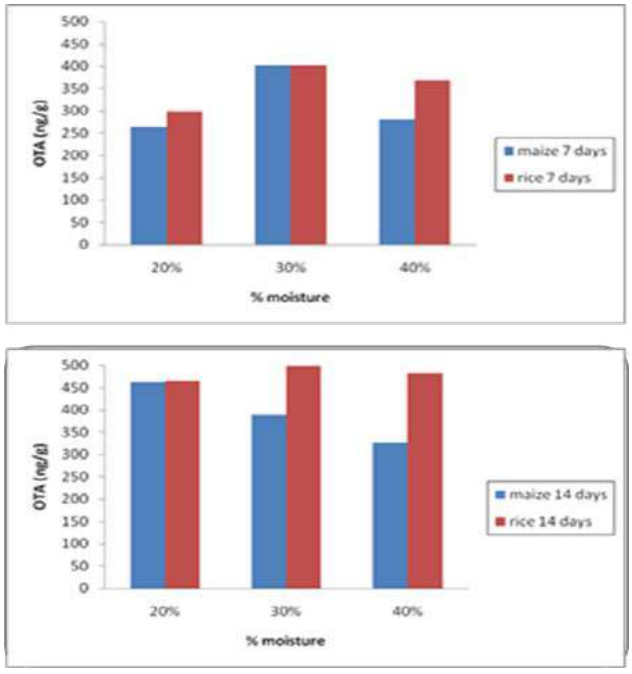

Fig. 1 *OTA concentration (ng/g) produced by A. alliaceus TISTR 3218 strains on maize and rice at different % moisture and incubation at 28 °C for 7 and 14 days*

Table 2 *Ochratoxin production by Aspergillus stains under various growth conditions*

Culture	Sample	% moisture (wet wt.)												OTA (ng/g)											
		20%				30%				40%				20%				30%				40%			
		28°C		35°C		28°C		35°C		28°C		35°C		28°C		35°C		28°C		35°C		28°C		35°C	
		7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14
A. ochraceus	Maize	+++[a]	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-[*]	-	-	-	-	-	-	-	-	-	-	-
	rice	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
I1012/1	Maize	++[b]	+++	++	+++	+++	+++	+++	+++	++	+++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	rice	++	+++	++	+++	+++	+++	+++	+++	++	+++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
2277	Maize	++	+++	++	+++	++	+++	++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
	rice	++	+++	++	+++	+[c]	+++	++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-

Culture	Sample	% moisture (wet wt.)												OTA (ng/g)									
		20%				30%				40%				20%				30%				40%	
		28°C		35°C		28°C		35°C		28°C		35°C		28°C		35°C		28°C		35°C		28°C	
		7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14
K6723	Maize	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
	rice	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
P15723	Maize	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
	rice	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-

+++[a] = *excellent mycelium production (71-100%),* ++ [b] = *good mycelium production (31-70%),*
+ [c] = *low mycelium production (0-30%),*
* = *less than limit of detection (LOD) 0.87 ng/g by HPLC/FLD detection*

Table 3. *OTA concentration (ng/g) produced by A. ochraceus TISTR 3557 on three different variety of maize at 30 %moisture incubation at 28 °C for 14 days*

Type of maize	Suwan 5		ATS 5		popcorn grain	
	growth	OTA (ng/g)	growth	OTA (ng/g)	growth	OTA (ng/g)
Grain	+++	-*	+++	-	++	-
Crushed	+++[a]	-	+++	-	++	-
Crushed: grain (1:10)	+++	-	+++	-	++	-
Yeast extract: grain(1:100)	++[b]	-	++	-	+[c]	-

+++ [a] = *excellent mycelium production (71-100%),* ++ [b] = *good mycelium production (31-70%),*
+ [c] = *low mycelium production (0-30%),*
* = *less than limit of detection (LOD) 0.87 ng/g by HPLC/FLD detection*

Table 4*. OTA concentration (ng/g) produced by A. alliaceus TISTR 3218 on maize 30 %moisture at 28 °C for 7 days*

Toxin	Culture	20%	30%	40%
		OTA (ng/g)	OTA (ng/g)	OTA (ng/g)
OTA	*A. alliaceus*	1099.05 ± 131.03	3200.24 ± 61.43	908.14 ± 120.82

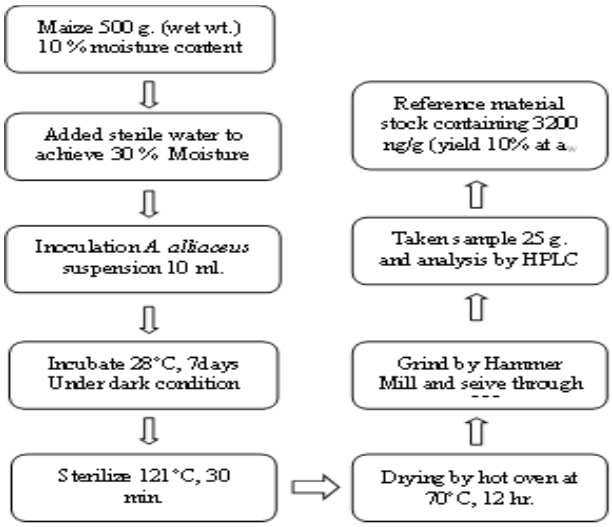

Fig 2*. Flow chart showing the step of OTA reference materials production*

Fig.2 concluded the scheme of OTA production in our laboratory which achieve the final product of OTA reference material containing 3200 ng/g (a_w 0.97) and has yield 10% from 500g maize.

This study detailed the optimum conditions for fungal growth and toxin production of A. alliaceus TISTR 3218. This study has shown the interactions of substrate, temperature and time to optimize the condition producing OTA and further scale up to produce the OTA reference material.

Our five isolates from brown rice and reference culture *A. ochraceus* TISTR 3557 could grow and produced OTA well when cultured on laboratory media MEA, while lost ability to produce toxin when cultured on maize and rice. Actually OTA producing fungal *A. ochraceus* is mostly used to study the production of toxins OTA, similar result was found that *A. ochraceus*, isolates from natural source such nut and fig, produced OTA less than (LOD) 0.01 μg/ml [4], while *A. alliaceus* isolated from the source produced substantial OTA as high as 30000 ng/g. Our reference strain *A. alliaceus* TISTR 3218 exhibit high OTA production up to 3200 ng/g on maize and thus at appropriate conditions for growth and OTA production, 30% and incubated at 28 °C, could be used in the manufacture of reference materials.

Optimal conditions for growth and OTA production according to Ali *et al.* (2013) showed that *A. ochraceus* , obtained from NIBGE Pakistan, could produce toxin well on corn, rice and wheat as 1600-1870 ng/g at 30 °C incubated for 3 weeks, while this strain produce less OTA production at 20 °C and 40 °C as 1000-1380 ng/g. OTA production decreases as temperatures increase [1, 8]. The different ability to produce toxin may differ from strain to strain. Individual fungal species also differ in their growth responses to the water activity (a_w) and temperature of the food such as grain [7].

4. Conclusion

Based on Bayman *et al*. (2001) suggested that future studies of ochratoxin production and contamination by the aspergilli should focus on *A. alliaceus* rather than *A. ochraceus* [4]. Studies of the *A. ochraceus* group are complicated by difficulties in distinguishing *A. ochraceus* from related species.

Corn grain (Zea maize) is an important food for large numbers of people in the developing world. Corn and products from corn are frequently contaminated with ochratoxin A (OTA) and associated with OTA producer fungi (Caldas *et al*., 2002). Apart from OTA producing fungal, corn have been concerned with the other contaminated fungal. *Fusarium* spp. are generally considered to be field fungi although this fungal require high water for growth, but they can sometimes grow in stored grain [9] while *Aspergillus* and *Penicillium* spp. are typical storage species that are able to thrive at relatively low water activities [7].

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State's roles in ensuring food safety in Vietnam

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Abstract:

People are more and more interested in safety food, because food affects life and existence of human. Nowadays, unsafe food supply is killing the Vietnamese. The big problem is processed food. We're being killed not by *E. coli, Salmonella*, or *Campylobacter*, but the nutritionally hollow contents of the bags, boxes, and fast-food clamshells that pass as nourishment.

Legislation has been drafted giving the VFA authority to help prevent foodborne illnesses and deaths. But much of our food supply remains unsafe; with at least 35 Vietnamese annually going to early graves from diet-related conditions and the VFA doesn't have the clout or will to help to change it.

So which solutions for food issues in Vietnam today?

Keywords: Food safety, current situation, reasons, breakthrough solutions, Law on Food Safety

1. Introduction

From past to present, food plays a very important role for the growth and survival of human beings. Food provides the essential nutrients for human life: vitamins, minerals, energy, and other nutrients [1]. However, if we use unsafe food, it will be the cause being harmful to humans and race.

At present, the world in general and Vietnam in particular, a large number of population strata is very interested in the issue of food safety: from consumers to scientists and managers. So, the management of food safety is both urgent and long-term, this is large, complex task and involves many agencies [3]

Food safety to protect the health of the people is one of the important tasks, so it needs steering drastic by Party, Governments. It is the responsibility and right of organizations, individuals and every citizen in producing and trading food. Last time, the Party and State have many policies for Food Safety. June 17th, 2010, The National Assembly promulgated the Law on Food Safety No.55/2010/QH12. To implement the Law of Food Safety, the Government issued the National Strategy on Food Safety for the period 2011-2020 and vision to 2030.

2. Materials and Methods

2.1. Documents synthesizing method: Documents are synthesized from some resources such as: reports of Dong Nai People's Committee, of Vietnam Food Agency (VFA), and of National Agro-Forestry-Fisheries quality assurance sub-department of DongNai province (NAFIQASD).

2.2. Investigating some companies, firms in Dong Nai province and other provinces: Authors chose some companies, firms that produce, buy and sell or exchange food to investigate: products, trade, process of producing,…

3. Results and Discussion

3.1. Current situation of food safety in Vietnam

Ensuring food safety has been concerned by the local government, however the current situation show that food safety issue are worrisome, food poisonings are still occurring and affecting the lives and health of people.

Through the survey, the authors overview the current situation of food safety in Vietnam, such as:

- *Unsafe of food alarming in the present.*

In recent times, food poisoning is taking place consecutively that is harmful to consumers' health. Products such as rice spirits 29% vol of the Ha Noi 29 Import-Export Company, the ingredients of this product is 30% alcohol, 70% water, and the other flavors [3]. Or poisoned spinach in specialized areas in Thu Dau Mot city (Binh Duong province, Vietnam) and areas of specialized vegetable in Northern region. Department of Plant Protection said the cause is due to the vegetable growers have used a large number of pesticide to spray the vegetables and pesticide residues in vegetable is higher 100 times than the permitted level [7].

- *The use of unsafe flavors in manufacturing and processing food are now very popular, and tends to increase.*

Across the country, the area of safe fruit and vegetable is very few, about 8.5% of the total area of vegetables, and 20% of the total area of fruit trees [8]. Foods containing toxic substances, preservatives, additives, food coloring flavors are being circulated in the market, such as: soy sauce containing 3-MCPD, fish sauce containing urea, fresh seafood marinated by urea to preserve, eggs and milk containing melamine, pig skin whitened by bleach [4]; melon seeds, chili powder and coloring flour containing Rhodamine B, dried fruit from China poisoning lead, sausage containing carcinogenic substances, formaldehyde used to soak rice noodles, spring rolls containing borax , horticulture residues of plant protection; spirits made at home[8].

- *The phenomenon of food poisoning still occurs regularly in industrial Zones and at the collective kitchens, and no sign of reducing.*

According to statistics of the Bureau of Food Safety - Ministry of Health (VFA), the period from 2009 to 2013, on national average there are 161 food poisoning cases occur a year, with an average of 5.271 people infected a year [3].

***Table 1**. The infected and died human during 2009 – 2013 by unsafely food*

Indicators	2009	2010	2011	2012	2013	Sum	Average/year
Total (times)	152	175	148	168	160	803	161
Number of infected people (People)	5.212	5.664	4.700	5.541	5.238	26.355	5.271
Number of died people (People)	35	51	27	34	28	175	35

(Source: Bureau of Food Safety - Ministry of Health, 2014)

In the first months of this year, food poisoning occurred in many places (Lao Cai, Binh Duong, Chau Thanh, Hau Giang, Ba Ria - Vung Tau, Dong Nai,...) [3], in which typically a food poisoning occurred in Panko Vina Co., Ltd. (My Phuoc 1 Industrial Zone, Ben Cat District, Binh Duong) at 15:30 pm 17th January, 2014 and as a result there were 159 worker that were hospitalized [7]. Or food poisoning from collective kitchen of Lac Ty Co., Ltd (Hau Giang) made 46 people were hospitalized [6].

- *Food smuggling, unknown origin, still circulate widespread in our country*

In spite of strict control of the authorities, but the phenomenon of food smuggling still complicated, increasing the risk of unsafe food hygiene. Smuggled food unknown origin was not only risks a major influence on consumers' health but also affect the production and circulation of production. The smuggled foods include: organ toxic materials, all kinds of seafood, foods cooked sausages, honey, sausages, ... [5].

Besides smuggled food, smuggled food additives is also a worried problem because it affects food safety and hygiene. The Ministry of Health said that at present, 90% of food additives consumed in Vietnam are smuggled [4]. Currently, the amount of food additives produced in the country only account for 5-10% of the total consumption of food additives on the market, over 90% are imported. Of these, only handful of import are under quota, the rest is ... smuggled [3]. Moreover, the Ministry of Health also said, more than 90% of food additives being consumed in the domestic market are imported from 20 companies from: the U.S, France, Switzerland, Thailand, Indonesia and especially China (accounting for approximately 30% of total imports food additives) [3].

Currently, Vietnam allows to use 23 groups of food additives with 337 substance, including aromatherapy [2]. In fact, the number of official import food additives only supplies a small part of the consumer demand. Food additives are very popular but they are not been statistically, especially additives smuggled from China across borders, sold in retail at counters. The use of toxic food additives, smuggled food additives with unknown origin is very common; this causes serious damage to the health of consumers. The test results on the use of food additives in recent times show that, up to 15.6% of food samples are positive for borax, 13% of sample contain preservatives exceeding permitted level and 12% of food samples contain alkaline color [4]. The medical experts warn that the using of toxic food additives with small amount can cause poisoning chronically; high dose will cause poisoning acute [9]. For example, with 1 gram of borax can cause diarrhea, vomiting, prolonged use of the substance will accumulate and impact serious on the health, with 15g would be died ... [9]. Therefore, the management and control food

additives will limit poisoning food for generations.

3.2. Causes of the situations above

Unsafe food in Vietnam is at alarm level. People say that all food from meat, dairy, fruit, and even rice contain toxins being harmful to human health. Current situations can be derived from the following main reasons:

- *Lack of uniformity in implementing cleaner production processes in the food production chain*

In order to have food safety, the food production must meet safety requirements: using the safe feed, safe livestock/crop processing, safe storage …However, in Vietnam, food production is just good at some phase above. For example, stages of rearing do well but lack of the facilities to do harvest stages: the danger of infection will start from here. Or processing phase is implemented well but it is not good at preservation, distribution, sale because of risk of bacterial infections. Therefore, when food poisoning occur, consumers or agency is difficult to determine the main cause.

In addition, the application of good manufacturing process is also very slow. Of the total area of fruit trees, and vegetables, the safe production area is very limited. Number of farmers trained Good Agricultural Practices (GAPs) is not much and not effective. Although some exporters companies apply GAPs such as EUREPGAP, Vietnam have not built GAP, GVP, GHP, GMP,… for each industry yet.

- *Organize structure to control food safety and hygiene, unreasonable, overlapping equipments both insufficient, and poor.*

Currently in Vietnam there are 5 management agencies on food safety: Ministry of Agriculture and Rural Development, Ministry of Health, Ministry of Industry and Trade, Ministry of Science and Technology and the Ministry of Natural Resources, Environment, and People's Committees of provinces and cities. Due to the overlap management, it is difficult to blame the agency. For the provincial level, they established the Food Safety and Hygiene Department of Health, but no guidelines on organizational structure. So every unit have a different organizational structure and staffing quantity whether the functions and duties are a similar.

In addition to the restrictions on the organizational structure, the lack of equipment and expertise is one of the causes of food insecurity: confirming the safety of food, testing residues of plant protection drugs, nitrates, heavy metals and bacteria present in food. For qualitative and quantitative hazard, they need a laboratory to test. Vietnam only has 11 national analysis units to test food safety, even some provinces have not lab yet. Moreover, most of these facilities are located in Hanoi and Ho Chi Minh City; so sending samples away will increase the cost and time of testing.

On the other hand, the inspection and testing are not regular, continuous because of limited funding, so they only inspect and test the high-risk food businesses.

- *Restrictions of the law, the guidelines documents related to food safety.*

Although there is a lot of overlap document and no clear delineation of responsibilities between the management, the industry has shortcomings, not cover all field, there are gaps between the stages of management responsibilities associated keep a product. Some emerging field (such as functional foods, a number of toxic substances and micronutrients) is not clear in management guidelines, local details so they are difficult to make.

Besides, there are regulations that do not apply at the current time, such as commune can not have enough professional staff to perform physicals, evaluation units, and supply certificates. They prescribe a scale feasts on 200 people at granting county level, but the festival communes or hamlets is often over 200 people so district staff can not manage, and communes, hamlets is not competent to manage. The feast of the social organization, religion (churches, temples, pagodas) without specialized personnel permission cannot be inspected. The rapid tests are not sufficient legal basis to sanction and processed immediately. In order to avoid food poisoning occur, wait for the official results (usually long duration), the food was be consumed; the violation was not handled in accordance with the scale of the facility and its many other shortcomings that need to be adjusted, logical addition.

- *The enforcement of regulations on inspection and monitoring food safety has not been taken seriously.*

According to Decree No. 178/2013/ND-CP dated 14-11-2013 of the Government on sanctioning of administrative violations of food safety, the level of penalties for violations of regulations on the use of additives, Food additives, processing aids in food production, food processing is quite high, up to 100 million or higher. However, there are many offenders, but implementing penalties is not seriously.

In fact, during the inspection, sanitary inspection and food safety exist negative officers. Many officers "ignored" for the violations.

- *Awareness of people about food safety are limited, even consumers.*

Final reason, and as a consequence of the above reasons is people's awareness about food safety is still weak, business units ready violate regulation of food safety because of profit.

3.3. Breakthrough solutions for food safety in current period

Stemmed from food hygiene and safety and reasons presented above, a number of breakthrough solutions to ensure food safety in the future as follows:

- *Completion of legislation on food hygiene and safety systems:*

Congress should issues Law on Food Safety (still draft) and the guidelines under Law timely, appropriate (specified standards and regulations). That is the legal framework and basis for the construction of the local policy, planning and quality management plan. Besides, the laws enacted to unify, to avoid duplication of content, and implementation.

- *Planning zones to cultivate and farm safety.*

Each local must plan to cultivate and farm, based on geography and soil, economic characteristics - social, ... Promote establishment convergent areas for slaughtering livestock, poultry, construction food safety markets. Plan well will create proper orientation to propose other management solutions.

- *Consolidate the organization structure*

The organization structure must be organized throughout the nation from the central to the grassroots (existing provincial now). At the ward level, to strengthen specialized inspection systems (medical, veterinary, agricultural, market management) to inspect cargo, inspect product (currently this network is now very thin, hard to able to assume full responsibility). Establish a committee (not the steering committee) in each local joint coordination headed by a Vice Chairman for unity of action in the management of product quality goods, avoid spreading, shirking of responsibility, waste of money and manpower.

- *Increase resources and facilities:*

Have enough manpower, strong quality. Basis, the working facilities and equipment to be focused investment, creating favorable conditions for continuous operation 24/24 (the same as in hospital emergency direct or direct prevention) . Promote to invest capacity of the test facility locally. Allocate funding sufficient for operations and their assigned tasks. It should be permitted the units to retain a portion fines to invest more infrastructure to serve.

- *Promote professional training, and communication*

Strengthening training, educating, improve staff quality. Promote communication, education and healthcare to everyone and this is one of the fundamental solution, long term. Implement Physical examination, evaluation, supply certification. Strengthen inspection, testing and handling of all serious violations. Enhance international cooperation is absolutely necessary.

4. Conclusion

While we wait for the slow gears of government to step in with stronger regulation of what the food industry is allowed to package and sell as food, the way we eat is making too many Vietnamese people sick and overweight. What can we do in the interim?

In brief, survey above shows that unsafe food in Vietnam is taking place more and more complicating and there is no sign to stop. In order to prohibit unsafe food, central government and local authority need to carry out five solutions more and more soon.

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Comparison of the effect of different composting materials on the yield of paddy straw mushroom (*Volvariella volvacea*) cultivation in Vietnam

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Abstract:

Paddy straw mushroom (*Volvariella volvacea*) has high demand due to its deliciousness and nutritive value, but there was no suitable method of cultivation since it needs high temperature (33 - 35°C) and 85 - 90 % relative humidity in the growing environment; and there was no suitable composting material, which give good yield but reuse the waste to help the green environment development.

In this study, different compositing materials were used with different ratio, such as: cotton waste (CW), paddy straw (PS), and the waste of Lingzhi (LW) and Ear mushroom (EW) growing compositing materials after harvest. All surveys were done indoor process.

The results showed that the highest yield of straw mushroom was when using 50% CW and 50% EW. The following compositing materials gave lower yield but also good result, such as: 50%CW and 50%LW; or 100% CW. In the market, the prices of EW and LW are much lower than CW. So it is also good way for mushroom farm development as environment – friendly and sustainable solution for Vietnam

Keywords: *Volvariella volvacea*, paddy straw mushroom, cultivation, compositing material

1. Introduction

Paddy straw mushroom (*Volvariella volvacea*) is one kind of edible mushroom which very delicious and nutritious. The composition of Paddy straw mushroom contain sufficient protein, sugar, fat, fiber and minerals needed for the human body. Specially, It contain many essential amino acids such as lysine, tryptophan, methionine and most of the group vitamin B [5]. In the world, paddy straw mushroom third in six popular edible mushrooms. They are grown in many countries such as: China, Taiwan, Hong Kong, Singapore, Indonesia,….. Several subtrates in rich of different materials such as straw, cotton waste, dried bananas, sugarcane bagasse, water hyacinth have used for mushroom cultivation. Nowadays, there are two methods for growing paddy straw mushroom: in vivo (outdoor) and in vitro (indoor) growing methods. Paddy straw mushroom cultivated in vitro which can control the temperature, light, humidity, ventilation and high industrialization capabilities have high yield and stable than in vivo method [3, 4].

Otherwise, the climate condition in Vietnam are absolutlely suitable for the cultivation of paddy mushroom (*Volvariella volvacea*). But how can paddy straw mushroom (PSM) cultivate following the industrial cultivation method? So the study of comparison of the effect of different composting materials on the yield of paddy mushroom (*Volvariella volvacea*) cultivation as improve the essential development of paddy mushroom in the future.

2. Materials and Methods

Materials: Straw mushroom (*Volvariella* spp.) from Sai Gon Green Company

Cotton waste (CW), paddy straw (PS) (Fig. 2), and the waste of Lingzhi (LW) and Ear mushroom (EW)

(Fig. 1) are the growing compositing materials.

(a) (b)

Fig 1*. The waste of (a) Ear mushroom (EW) (b) Lingzhi mushroom (LW) after harvesting*

(a) (b)

Fig 2. *(a) Cotton waste (CW), (b) paddy straw (PS)*

Methodology

Design and survey the effect of different materials on the straw mushroom growing with different rate

Table 1. *The formulas of each experiment*

Formula	Materials (%)			
	LW	EW	PS	CW
CT1	100	-	-	-
CT2	-	100	-	-
CT3	-	-	100	-
CT4	-	-	-	100
CT5	50	-	50	-
CT6	50	-	-	50
CT7	-	50	50	-
CT8	-	50	-	50
CT9	-	-	50	50
CT10	40	-	40	20
CT11	80	-	-	20
CT12	-	80	-	20
CT13	-	-	80	20

Note: All formulas are supplemented: Urea 1%, Phosphor 2% and Potassium 0.8%.

***The basic processing of straw mushroom cultivation*:**

LW and EW were incubated with 1% lime during 3 days. The CW was shredding and incubated with 5% of lime in 3 days (Fig. 4).

After 3 days the materials were mixed and heated at 80^oC in 12h in the mushroom house (Fig. 3, Fig. 5)). When it was cooled to the room temperature, the mother straw mushroom culture was mixed (Fig. 6). The mushroom was growing in 2 phases. During the breeding phase, the following environment should be kept: the dark annealing temperature: $35^oC \pm 2^oC$, 70% of humidity substrate, 80-90% of air humidity. The second phase, when the mushroom began to forming (Fig.7), the growing environment should be in $30^oC \pm 2^oC$, with 70% of air humidity, 80-90% humidity of substrate, light diffusion and aeration intension.

(a) (b)

Fig 3*. (a) Paddy straw mushrrom house, (b) The shelf*

Fig. 4*. The treatment of the substrate for paddy straw mushroom growing*

Fig. 5*. The substrate of the paddy straw mushroom on the shelf*

(a) (b)

Fig 6. *(a) The mother paddy straw mushroom culture, (b) Mixture of the substrate and the mother paddy straw mushroom culture*

Fig 7. The harvesting paddy straw mushroom

The impact factors for the formula of materials that is using in straw mushroom growing:

- The total time of mycelia growth fully in incubated package
- The time of fruit body development
- The time period before the first day of mushroom harvesting
- Total days from mycelial to harvesting
- Biological state of mushroom during cultivating

3. Results and Discussion

3.1. Effect of substrates on the mycelia growth of paddy straw mushroom

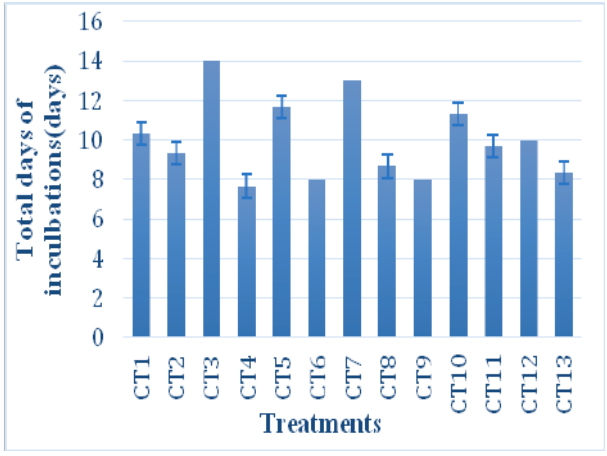

***Figure 8**. The total time of mycelia incubation of 13 treatments*

In the figure 8 showed the growth of mycelia longest in the spawn belong to the treatment CT3 (14 days) that contains of only paddy straw (PS) and shortest belong to the treatment CT4 (8 days) that contains of cotton waste (CW). This is absolutely suitable with the research of Akinyele B.J. and Adetuyi F.C (2005) in the growth of mycelia fungi on cotton waste substrate to be the strongest and this also helps to reduce time for cultivation.

3.2. Research on period of the paddy straw mushroom fruiting

During the survey time of fruiting body forming in 13 treatments had a perceptible different between each other. The parameter after 8 days (CT4) to 15 days (CT3 and CT7) of cultivation and remaining of 10 formula treatments from 9 to 11 days do not have significantly different (Fig 9, Table 2).

Table 2: The period of the paddy straw mushroom fruiting (days)

Treatments	Total day formed fruit body	%CV
CT1	$10.67^{de} \pm 0.58$	5.41%
CT2	$10.33^{de} \pm 0.58$	4.68%
CT3	$15.00^{g} \pm 0.00$	5.41%
CT4	$8.33^{a} \pm 0.58$	0.00%
CT5	$13.00^{f} \pm 0.00$	6.19%
CT6	$9.00^{ab} \pm 0.00$	5.59%
CT7	$14.67^{g} \pm 058$	0.00%
CT8	$10.00^{cd} \pm 0.00$	6.93%
CT9	$9.00^{ab} \pm 0.00$	0.00%
CT10	$12.33^{f} \pm 0.58$	0.00%
CT11	$10.67^{de} \pm 0.58$	3.94%
CT12	$11.00^{e} \pm 0.00$	0.00%
CT13	$9.33^{bc} \pm 0.58$	0.00%

Note: Mean ± SD. In the same column: the figures differ statistically with 95% reliability.

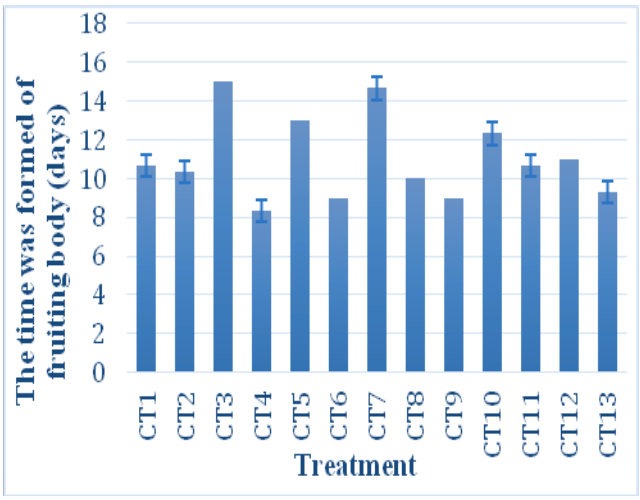

***Figure 9**. The total time of fruiting body formed in 13 treatments*

3.3. Study on the time period before the first day of mushroom harvesting

The result showed that the time of paddy straw mushroom (PSM) cultured on CW is shorter than on other substrates. Because of the incubate time in PSM was slowed each other thus on the first harvest of PSM as similar with above surveys (table 3).

Table 3: *The time period before the first day of* mushroom harvesting

Treatment	The time period before the first day of harvest (days)	%CV
CT1	$15.33^{bc} \pm 0.58$	3.77%

CT2	$15.00^{abc} \pm 0.00$	16.54%
CT3	$18.67^{d} \pm 0.58$	4.33%
CT4	$13.00^{a} \pm 0.00$	0.00%
CT5	$15.33^{bc} \pm 0.58$	4.03%
CT6	$13.00^{a} \pm 0.00$	0.00%
CT7	$16.33^{c} \pm 0.58$	3.09%
CT8	$13.33^{ab} \pm 0.58$	0.00%
CT9	$15.00^{abc} \pm 1.00$	3.77%
CT10	$16.00^{c} \pm 2.65$	0.00%
CT11	$13.33^{ab} \pm 0.58$	3.53%
CT12	$15.00^{abc} \pm 0.00$	4.33%
CT13	$14.33^{abc} \pm 0.58$	6.67%

Note: Mean ± SD. In the same column: the figures differ statistically with 95% reliability.

3.4. Total harvesting days (day)

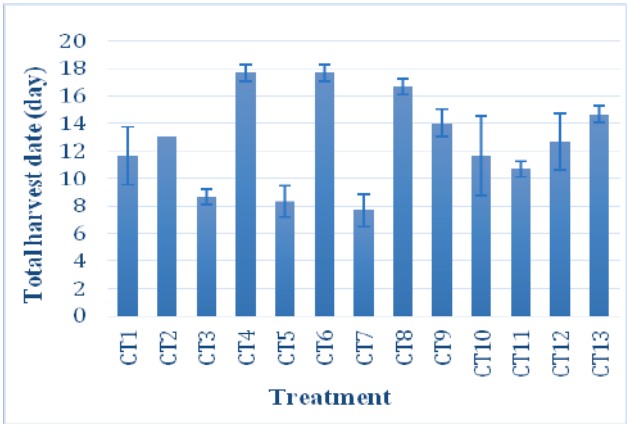

***Figure 10**: Total harvesting day of PSM*

As the figure 10 show that the highest total harvesting day of PSM was in the CT4 treatment (18 days) and lowest was in the CT3, CT5 and CT7 (8 days). This has significant different in the comparing between experiment that used cotton waste.

3.5. Biological efficiency of different treatments on yield of PSM (%)

There are differences in biological efficiency in 13 treatments. Biological efficiency ranged from 26.81% to 15.02%. Thelowest biological efficiency in CT5 only 15.02% and 100% cotton formulas (CT4) for the highest performance in bio-treatments with biological efficiency reached 26.81%. It is followed by the CT6 (24.19%), the CT8 (22.88%) and CT2 (21.21%). (Table 5, Fig.11)

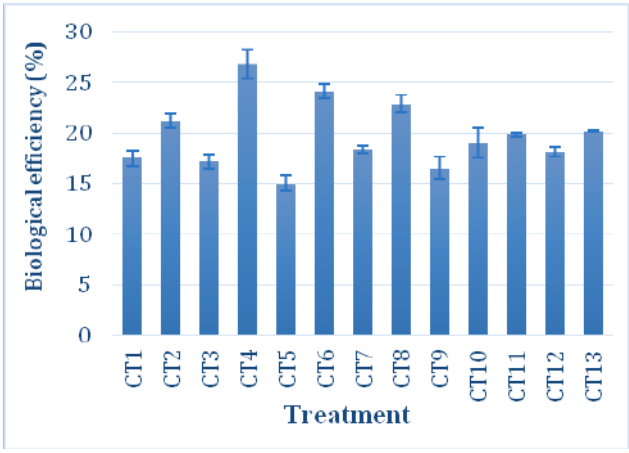

***Figure 11:** Assessment of biological state*

***Table 5:** Biological efficiency of different treatments on yield of PSM.*

Treatment	Biological efficiency (%)	%CV
CT1	$17.52^{fg} \pm 0.80$	4.56%
CT2	$21.21^{c} \pm 0.74$	7.58%
CT3	$17.19^{fg} \pm 0.70$	0.97%
CT4	$26.81^{a} \pm 1.43$	2.56%
CT5	$15.02^{h} \pm 0.77$	0.32%
CT6	$24.19^{b} \pm 0.70$	3.48%
CT7	$18.37^{ef} \pm 0.34$	4.08%
CT8	$22.88^{b} \pm 0.88$	5.32%
CT9	$16.55^{g} \pm 1.09$	5.16%
CT10	$19.00^{de} \pm 1.44$	2.92%
CT11	$19.86^{cd} \pm 0.19$	1.85%
CT12	$18.14^{ef} \pm 0.47$	3.85%
CT13	$20.20^{cd} \pm 0.07$	6.53%

Note: Mean ± SD. In the same column: the figures differ statistically with 95% reliability.

4. Conclusion

The result from the target track in experiments show that the formula contains of 100% cotton waste is the best for mushroom cultivation. This is suitable for cotton waste ratio C/N 40.90, this ratio is in the range suitable for mushroom growing. Besides, cotton waste containing high levels of cellulose, the carbohydrate molecule with a low molecular weight, nitrogen and other mineral oils are suitable for mushroom growth and development [1].

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Research the application oligochitosan/silver nano preservation on pomelo (*Citrus maxima* merr.)

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Abstract:

In this paper, we studied chitosan degradation obtained oligochitosan (OCTS) with hydrogen peroxide (H_2O_2) agent and applied of antifungal of the OCTS contain nano-Ag (nAg) to preserve post-harvest Citrus maxima. The results showed that when used H_2O_2 2% for 6 hours (reaction time), the OCTS have molecular weight d from 50 - 60 kDa and DDA from 75 – 77%. With concentrations OCTS 0,5% containing nAg 5 ppm, Citrus maxima is preserved after 3 months that the external appearance and internal quality little change compared with the starting material.

Keywords: OCTS/nAg, oligochitosan, nano silver, pomelo

1. Introduction

Chitosan film has interesting properties are non-toxic, anti-transpiration, high resistance to microorganisms, capable of biodegradation, do not harm the environment and humans. The molecular weight of chitosan produced from shrimp shells in the range of 380-460 kDa [1]. Many recent research results demonstrated that chitosan inhibits the growth of microorganisms. This feature depends on the Mw of chitosan and microorganisms, chitosan (Mw = 1106 kDa) influence on Gram-negative bacteria such as *E. coli, Pseudomonas fluorescens, Salmonella,...* chitosan (Mw = 470 kDa) inhibits Gram-positive bacteria are very good, as *Lactobacillus sp, L. monocytogenes, B. cereus, ...* [2]. Meanwhile chitosan (Mw = 30-50 kDa) resistant *S. aureus* bacteria and fungus *Candida, Aspergillus Niger* [3].

Silver has been known to have strong anti-bacterial properties, inhibits and kill the growth of mold, the silver atoms at the nanoscale, antiseptic activity increased about 20-50 times higher than silver ions [4]. Wen-Li Du researched activity against *E. coli, Salmonella and S. aureus* of chitosan - tripolyphosphate with many different nanoparticles metals such as Ag^+, Cu^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+} results showed chitosan nanoparticles combine Ag^+ is the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) is the lowest [5].

2. Materials and Methods

2.1. Materials and equipment

- Chitosan (DDA 75 – 77%, M_w = 480 kDa)
- Silver nano (d_{tb} = 8-25 nm) was provided by the Institute of Applied Materials Science.
- Pathogenic fungus *Phytophthora sp* was provided by Center for Test Variety and Plant - Department of Crop Production.
- Pomelo was bought in the same garden, the same breed and the same time in Ben Tre province.
- Spectrophotometer UV-*vis*, UV-2401PC, Shimadzu; equipment infrared spectroscopy (IR); X-ray diffraction spectrometry: ADVANCE 8–Bruker; Transmission electron microscopy (TEM), JEM1010.
- Saccharometer (Model: RHB – 18ATC; 0 – 18% brix)

2.2. Experimental method

2.2.1. Degradation chitosan to oligochitosan with

H_2O_2 agent at room temperature

Take 20 g of chitosan in the reactor, adding 1% lactic acid solution for chitosan absorbed. For solutions containing H_2O_2 concentrations available in (2%, 4%, 6%, 8%) and 1 g of iron salts $FeSO_4.7H_2O$ (catalytic reaction) in container chitosan (ratio of liquid/chitosan = 1/10). Reaction time turn 2 hours, 4 hours, 6 hours, 8 hours of receiving, the product by precipitation in alcohol (ratio of ethanol/chitosan = 2/1) and dried at 45^0C. Measured molecular weight (Mw) and degrees deacetyl (DDA) after the reaction.

2.2.2. Prepare oligochitosan (OCTS) containing silver nanoparticles (nAg).

Dilute 500 ppm colloidal silver nanoparticles to achieve the test concentration, respectively 30 ppm, 40 ppm, 50 ppm and 60 ppm. Each concentration of nAg prepared 4 bottle and 1 liter nAg solution per a bottle. For OCTS respectively concentration 4%, 4.5%, 5% and 5.5% in the silver nanoparticles bottle was prepared. OCTS/nAg solution stir for 30 minutes (200 rpm), stabilize the solution for 4 hours. Measured average size of silver nanoparticles, structure OCTS/nAg by UV-*vis*, XRD.

2.2.3. Test antifungal activity for Phytophthora sp of OCTS/nAg film.

Phytophthora sp strain cultured on potato dextrose agar (PDA) in petri dishes (which has been sterilized by autoclave at 121^0C for 30 min) and incubated at 37°C for 4 days, the filaments diameter of 30 mm film set OCTS/nAg (d = 20 mm) at 0.2%/nAg 2 ppm, OCTS 0.4%/nAg 4 ppm, OCTS 0.6%/nAg 6 ppm, antifungal track within 7 days after the experiment. Inhibit of the target film via IC_{50}, (50% inhibitory concentration).

2.2.4. Preservation pomelo by OCTS/nAg

Prepare 6 plots, each plot consists of 7 pomelo has shape and dimensions are equivalent. Pomelo is washed, drained and dipped in a solution of OCTS/nAg in surveys levels of 2 times (time 1: dipped in the 2 minutes to air dry for 15 minutes, 2 times continue to dipped for 2 minutes to dry 60 minutes in the air) to the normal carton storage at room temperature (T = 30-32^0C, RH = 75-80%). Measured weight loss (WL), Brix, Vitamin C (Vit C) following preserved for 3 months.

2.2.5. Statistical analysis

Using JMP 4.0 software (statistical discovery software from SAS) in response surface method for ANOVA, LSD, t-test

3. Results and Discussion

3.1 Degradation chitosan to oligochitosan with H_2O_2 agent at room temperature

With $FeSO_4.7H_2O$ catalyst, H_2O_2 oxidize Fe^{2+} to Fe^{3+} by the following reaction [6, 7]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{2+}O + H_2O$$

Under light reaction reconstituted Fe^{2+}:

$$Fe^{3+} + H_2O \rightarrow Fe^{2+} + OH\cdot + H^+$$

When the concentration of H^+ dissociation increases (H^+ ions are agents cation catalyzed process degradation chitosan), H^+ create links for getting to pair electrons of oxygen atoms in the linked β-(1-4)-glucoside of chitosan. Molecule H_2O attacked link β-(1-4)-glucoside and stretch off forming low Mw chitosan. Experimental results were analyzed by response surface method (Fig 1).

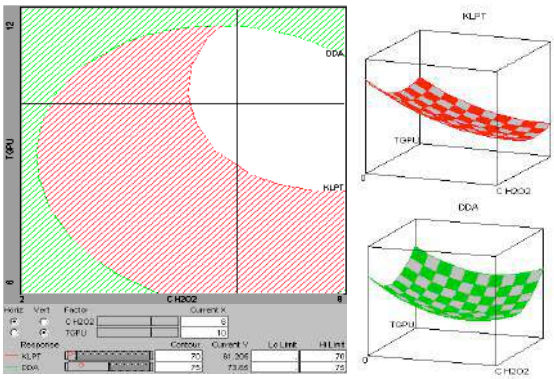

Fig 1. *Plot response surface show the influence of the concentration of H_2O_2 and t_{PU} (reaction time) to Mw, DDA of OCTS*

Figure 1, when the concentration of H_2O_2 increased from 2-6% and t_{PU} increased from 6-10 hours, Mw of OCTS plummeted to around 50-70 kDa, but when the concentration of H_2O_2 increased to 8% and t_{PU} increased to 12 hours, the Mw of OTCS no significant reduction and range 50-60 kDa. After the reaction, DDA of OCTS does not change significantly from the initial raw chitosan (from 75-77%), angle of diffraction characteristics of chitosan remained at 20 ($2\theta = 20^0$), indicating that the following reaction degradation, structure single molecule of OCTS not change does not affect the functional groups in chitosan only create OCTS with Mw smaller than chitosan (Fig 2).

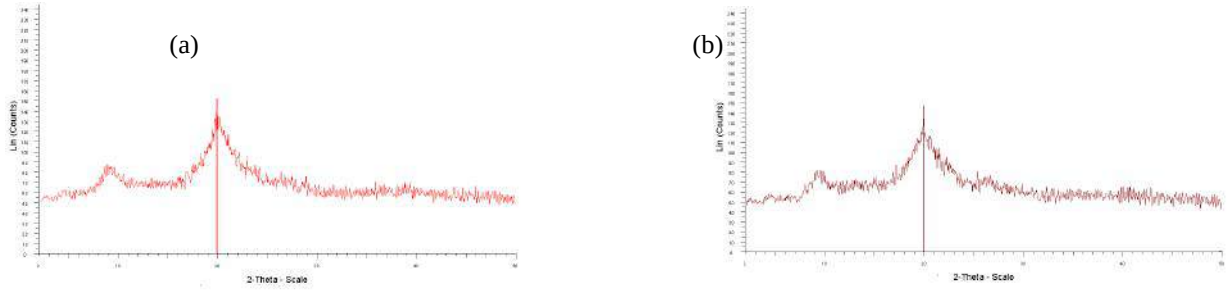

Fig 2. *(a) XRD spectrum of initial chitosan, (b) XRD spectrum of chitosan after degradation reaction with H_2O_2*

3.2. Prepare oligochitosan (OCTS) containing silver nanoparticles (nAg).

OCTS concentrations (C_{OCTS}) and silver nano-particles content (C_{nAg}) affect the average size of silver nanoparticles, viscosity of OCTS/nAg and the stability of the silver nanoparticles. Survey results were analyzed by response surface method (Fig 3).

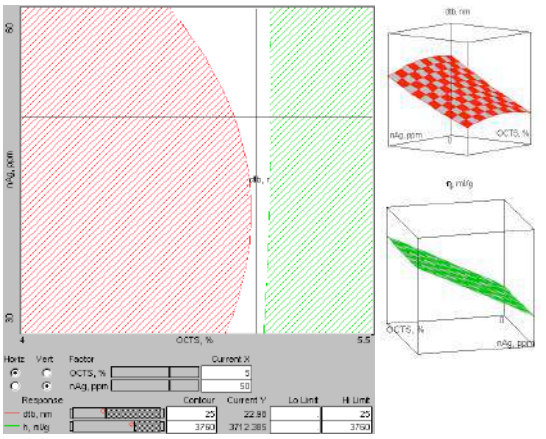

Fig 3*. Plot response surface show the influence of the concentrations of C_{OCTS} và C_{nAg} to d_{tb} và [η] of OCTS/nAg*

The average size of silver nanoparticles decrease and stability by way of C_{OCTS}, when C_{OCTS} increased from 4-5%, the d_{tb} of the silver nanoparticles decreased to about 15-20 nm and the viscosity increases to a including (viscosity increases linearly with concentrations OCTS). Silver nanoparticles content increased agglomeration phenomena as the average size of silver nanoparticles increases but does not increase more than the size of the starting material (d_{tb} = 18.2±2.4 nm) (Fig 4, Fig 5). Experi-mental results showed little effect C_{nAg} properties (viscosity, water-soluble OCTS) of OCTS/nAg. According to author N. Velmurugan (2009) content of silver nano-particles in the CTS film from 50-100 ppm (d_{tb} = 100-200 nm) effectively destroy pathogens best [8]. Author Bui Duy Du (2009) pointed out that just nano silver content 5-10 ppm (d_{tb} ~ 10 nm) has the ability to kill the fungi plants diseases effectively.

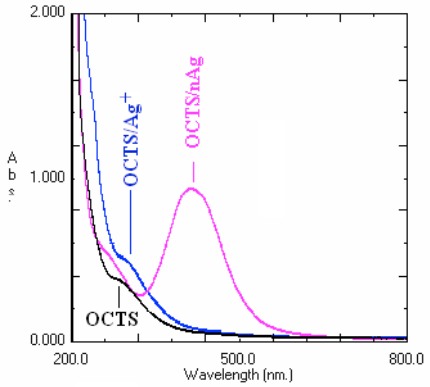

Fig 6. *UV–vis spectrum of CTS, CTS/Ag^+, OCTS/nAg*

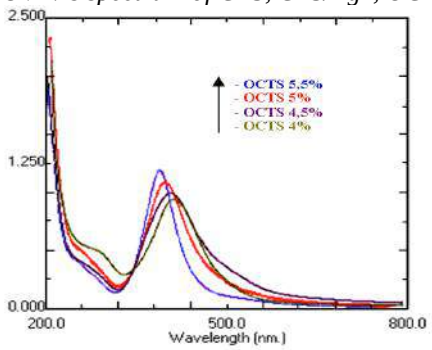

Fig 7. *UV–vis spectrum of OCTS/nAg according to different concentrations of OCTS.*

UV-*vis* spectrum has shown in Figure 7, when C_{OCTS} increased from 4% to 5.5%, λ_{max} of OCTS/nAg shifted from 410.5 to 399.5 nm, optical density (E) increased from 0.82 - 1.2.

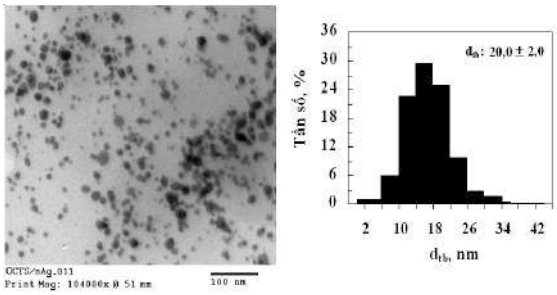

Fig 4. *TEM images and distribution d_{tb} of silver nanoparticles in OCTS/nAg (5%/50 ppm)*

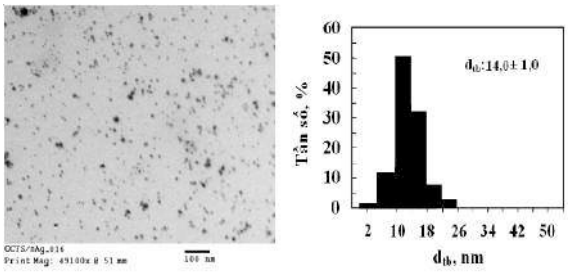

Fig 5*. TEM images and distribution d_{tb} of silver nanoparticles in OCTS/nAg (5.5%/50 ppm)*

3.3. Effect of pH on the average size of the silver nanoparticles in OCTS/nAg

Chitosan is only soluble in the acid environment by CTS has large Mw. OCTS have Mw much lower solubility of OCTS should be improved, low pH affects the solubility of OCTS, the ability to stabilize the nanoparticles average size silver. Survey results are presented in Table 1.

***Table 1**. Effect of pH on the average size of silver nanoparticles*

pH	4	5	6	7	8	9
d_{tb} (nm)	26.8±2.4 a	24.1±2.8 ab	20.0±1.4 ab	19.5±1.3 b	21.4±2.1 ab	24.6±4.6 ab

Note: In the same row different letters showed statistical differences in the level of significance α = 0.05. (t-test)

The results in Table 1 show that pH is not significantly affected by d_{tb} silver nanoparticles in solution OCTS/nAg. Specifically, OCTS/nAg made from acid pH (pH ~ 4), has d_{tb} = 26.8 nm, the average size of silver nanoparticles is higher than OCTS/nAg was adjusted to pH = 6-8 (d_{tb} = 19.5 to 21.4 nm). At the same time, samples OCTS/nAg at pH = 6 - 7 with a narrow size distribution (from 18.6 to 22.4 nm) than the sample OCTS/nAg at pH = 4 and pH from 7 to 9.

3.4. Effect antifungal activity for *Phytophthora sp* of OCTS/nAg film

Antifungal diameter (D_K) of *Phytophthora* sp OCTS/nAg membrane after 7 days of culture on PDA is presented in Table 2.

***Table 2.** Antifungal diameter (D_K) and IC_{50} of Phytophthora sp OCTS/nAg film*

STT	Pattern	d_K (mm)	IC_{50}
1	OCTS 0.2%/nAg 2 ppm	14.0 ± 2.0 a	OCTS 0.46%/nAg 4.6 ppm
2	OCTS 0.3%/nAg 3 ppm	29.0 ± 2.7 b	
3	OCTS 0.4%/nAg 4 ppm	47.3 ± 2.1 c	
4	OCTS 0.5%/nAg 5 ppm	52.7 ± 3.1 d	
5	OCTS 0.6%/nAg 6 ppm	51.3 ± 1.2 cd	
6	CTS 1%	14.3 ± 1.5 a	
7	OCTS 1%	19.0 ± 2.6 a	

Note: In the same column different letters showed statistical differences in the level of significance α = 0.05 (t-test)

Table 2 shows that, treatments at OCTS 0.2%/nAg 2 ppm, 14 mm diameter, the concentration increased OCTS 0.4%/nAg 4 ppm, D_K is 47.3 mm. D_K is 52.7 mm at treatments OCTS 0.5%/nAg 5 ppm and the difference was statistically compared with the other treatments. In treatments OCTS 0.6%/nAg 6 ppm, D_K = 51.3 mm statistically equivalent OCTS 0.5%/nAg 5 ppm. The regression equation represented *Phytophthora* sp resistance of the OCTS/nAg film as follows: D_K = 4.8832 [OCTS/nAg] – 0.1664 (R^2 = 0.93). From the regression equation to calculate the IC_{50} *Phytophthora* sp = OCTS 0.46%/nAg 4.6 ppm.

3.5. Preservation pomelo by OCTS/nAg

***Table 3**. Wight lose (WL), Vitamin C (Vit C) and degrees Brix of pomelo preserved by OCTS/nAg*

Storage time	Pattern	WL (%)	Degrees Brix (%)	Vit C (mg%)
After 1 month	OCTS 0.45%/nAg 4.5 ppm	5.23 ± 0.32 bc	9.66 ± 0.09	37.83 ± 0.10
	OCTS 0.5%/nAg 5 ppm	4.49 ± 0.85 c	9.51 ± 0.10	38.64 ± 0.15
	OCTS 0.55%/nAg 5.5 ppm	4.56 ± 0.67 c	9.64 ± 0.11	38.73 ± 0.29
	CTS 1%	5.82 ± 0.17 b	9.75 ± 0.03	37.86 ± 0.25
	OCTS 1%	5.45 ± 0.16 bc	9.62 ± 0.07	37.69 ± 0.16
	ĐC	7.40 ± 0.57 a	10.71 ± 0.41	37.85 ± 0.05
After 2 months	OCTS 0.45%/nAg 4.5 ppm	9.61 ± 0.24 b	9.76 ± 0.10	37.74 ± 0.09
	OCTS 0.5%/nAg 5 ppm	8.69 ± 0.70 c	9.76 ± 0.06	38.26 ± 0.07
	OCTS 0.55%/nAg 5.5 ppm	8.63 ± 0.70 c	9.67 ± 0.07	38.23 ± 0.08
	CTS 1%	9.83 ± 0.36 b	9.94 ± 0.05	37.44 ± 0.16
	OCTS 1%	9.15 ± 0.51 b	9.83 ± 0.25	37.41 ± 0.13
	ĐC	12.53 ± 0.47 a	11.39 ± 0.14	37.08 ± 0.17
After 3 months	OCTS 0.45%/nAg 4.5 ppm	13.52 ± 0.35 a	10.27 ± 0.08	37.11 ± 0.12
	OCTS 0.5%/nAg 5 ppm	11.81 ± 0.75 b	10.06 ± 0.08	37.58 ± 0.13
	OCTS 0.55%/nAg 5.5 ppm	11.90 ± 0.78 b	10.16 ± 0.09	37.55 ± 0.14
	CTS 1%	-	-	-
	OCTS 1%	-	-	-
	ĐC	-	-	-

Note: In the same column different letters showed statistical differences in the level of significance α = 0.05 (t-test); - :

pomelo was damaged.

From the results obtained (Table 3) showed OCTS/nAg have limited effect change pomelo quality during storage ($p < 0.05$). Through analysis and statistical treatment we choose OCTS 0.5%/nAg 5 ppm is most suitable for preservation pomelo during 3 months which the nutritional value and organoleptic qualities remains maintain than the original material.

4. Conclusion

Studied using H_2O_2 as oxidizing agent in the degradation reaction into oligochitosan chitosan with Mw = 50-60 kDa, make preparations OCTS/nAg active antifungal, antibacterial and tested preserved pomelo for good results

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Research on the change of biochemical characteristics in processing traditional vermicelli prepared from Vietnamese and Korea rice

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Abstract:

Beef rice vermicelli (Bun bo Hue) is a popular soup rice vermicelli dish in Vietnam. It was originated from Hue City, the old imperial capital of Vietnam. The main material in Bun bo Hue is completely prepared from Vietnamese rice. In the production process of vermicelli, rice quality and its different duration of soaking in water definitely influenced on quality and specific flavor of end-product. It means that soaked process made change of biochemical constituents in rice leading to change the quality of the end- product eventually.

In this study we analyzed the changes of biochemical characteristics of raw materials (rice) consisting of nitrogen, protein, gel consistency, content amylose, and number of lactic acid bacteria before and after soaking in water for 2, 3, and 5 days,. Preliminary results of the research on three rice varieties of Vietnam: Dakmil, Long An 110, Long An aroma, and two Korea rice varieties: Chucheong (milled rice), Black rice (mixture of several varieties) showed that most biochemical constituents have been significantly changed. Based on that result, the soaked duration in production process will be adjusted accordantly.

Keywords: Biochemical constituents, nitrogen, protein, gel consistency, content amylose, number of lactic acid bacteria

1. Introduction

During 2009-2010, preliminary documents of some traditional products prepared from Vietnamese rice were collected by observations, interviews, and investigations from manufacturers, specialists, and looking up from different areas in Vietnam such as Hue, Ha Noi, Ho Chi Minh, Can Tho, Long An Province, My Tho -Tien Giang, Ben Tre, Sadec-Dong Thap….. Based on this, to thoroughly understand scientific basis and recipe formulation in processing these traditional rice foods, the changes of biochemical properties of raw materials, semi-products in the key steps of the process have been analyzed and evaluated with a view to well establish a model technology to produce the Vietnamese traditional rice foods meeting both domestic and international quality standards.

Vermicelli was selected as a typical product for this research. It is the main material to prepare the dish “Hue Beef rice vermicelli” (Vietnamese name “Bun bo Hue”), a very famous and really beloved in Vietnam. Bun Bo Hue was originated from Hue city, the old imperial capital of 2009). In the production process of vermicelli strands, rice quality and its different duration of the soaked step definitely influences on quality and flavor of the vermicelli-end-product. It means that soaked step actually made change most biochemical properties

of rice material leading to finally change quality of the end- product.

In order to clearly understand scientific base of this biochemical changes. nitrogen, protein, gel consistency, amylose, and number of lactic acid bacteria before and after the soaked step for 2, 3, and 5 days have been analyzed. Preliminary research on three rice varieties of Vietnam: Dakmil, Long An 110, Long An aroma, and two rice varieties from Korea: Chucheong (milled rice), black rice (mixture of several varieties) will be presented in this paper.

2. Materials and Methods

2.1. Materials

- **Vietnamese** rice: Dakmil, Long An 110, Long An aroma (Long an thom).
- **Korean rice**: Chucheong (milled rice), Black rice (mixture of several Korea rice varieties)

2.2. Analysis methods

Investigation of total nitrogen and protein change in rice by Kjeldahl method (TCVN 4328-1:2007, TCVN 4328-1:2007), the amylose of rice with standard amylose solution (TCVN 5716-1993). Analyze the gel consistency (10 TCN 424-2000). Identify and quantify the Lactic bacteria exiting in the process (52 TCN 0013:2006)
(TCVN and TCN are methods under Vietnamese standard)

All experiments were triple repeated.

3. Results and Discussion

3.1 Research on the Vietnamese rice

***Table 1**. Investigating the change of biochemical constituents in Vietnamese rice soaked in water in vermicelli processing.*

N^0	Sample Code (variety & soaked duration)	Nitrogen content	Protein content	Acid Lactic Bacteria N^0	Gel consistency (mm)	Amylose content (%)
Analytical methods		TCVN (*) 4328-1:2007	TCVN(*) 4328-1:2007	52 TCN(*) 0013:2006	10 TCN (*) 424-2000	TCVN (*) 5716-1993
1	*Dakmil not soaked (control)*	1.12	6.65	$2.4.10^7$	68.5	17.65
2	*Dakmil soaked 2 days*	0.84	5.00	$3.8.10^8$	67.0	20.06
3	*Dakmil soaked 3 days*	0.85	5.03	$6.6.10^7$	76.0	19.92
4	*Long An 110 not soaked*	1.13	6.72	$1.8.10^7$	55.5	22.94
5	*Long An 110 soaked 2 days*	0.88	5.27	$1.1.10^8$	62.0	24.87
6	*Long An 110 soaked 3 days*	0.85	5.03	$2.9.10^8$	57.5	25.75
7	*LongAn aromatic not soaked*	1.18	7.00	$1.2.10^7$	73.0	19.18
8	*LongAn aromatic soaked 2 days*	0.85	5.07	$2.1.10^8$	76.5	20.84
9	*Long An aromatic soaked 3 days*	0.83	4.49	$2.9.10^8$	78.5	21.59

Note: (*) *Analysis methods under Vietnamese standard (TCVN)*

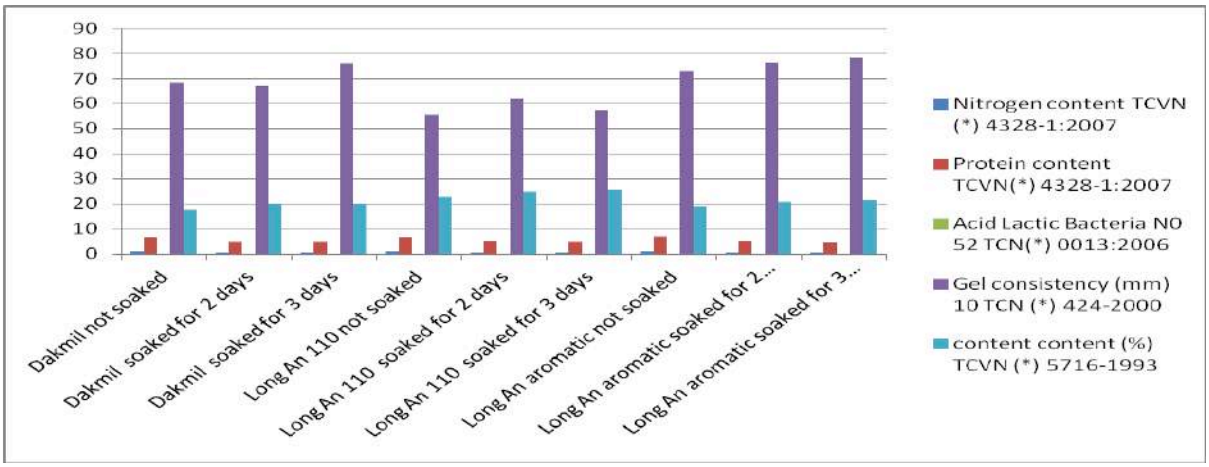

***Fig 1 .** The change of Biochemical properties in bun processing from Vietnamese rice*

(i) For the Dakmil rice: when soaked in water 2,3and 5 days:

- Nitrogen and protein content decreased after soaked 2-3 days
- Number of lactic acid bacteria increased after 2 days, and decreased after 3 days
- Gel Consistency: dropped after 2 days, increased after 3 days

- Amylose up after 2 days, decreased after 3 days

(ii) ***For Long An 110 and Long An aroma:*** number of acid lactic bacteria, gel consistency and amylose increased after 2, 3 days.

Therefore in the natural climatic conditions in HCMC and southern provinces of Vietnam, this study demonstrated and recommends that in the process of vermicelli production prepared from Vietnamese rice, the best duration of the soaked step should be not over two days and nights (48 hrs).

3.2. Research on Korea rice

Table 2. *The change of biochemical constituents in the soaked step in processing vermicelli from Korea rice*

No	Sample code	Total nitrogen	Total protein	N^0 lactic bacteria	Gel consistency (mm)	Amylose (%)	Status
Analytical methods		TCVN 4328-1:2007	TCVN 4328-1:2007	52TCN 0013:2006	10TCN424-2000	TCVN 5716-1993	
1	A: milled rice not soaked	0.91	5.43	$1,3.10^7$	35.0	19.53	
2	A soaked for 3 days	0.81	4.83	$2,8.10^6$	70.0	24.26	Sour smell
3	A soaked for 5 days	0.86	5.12	$4,2.10^8$	59.5	22.59	Rotten smell
4	B: Black rice not soaked	1.21	7.18	$3,1.10^7$	39.0	16.82	
5	B soaked for 3 days	1.18	7.03	$1,4.10^8$	35.0	21.02	Sour rotten
6	B soaked for 5 days	1.05	6.25	$6,4.10^8$	43.5	18.57	Stench rotten

- Nitrogen and protein content decreased after 2-3 days (0.91 down to 0.86) but highly increased after 5 days due to the fermentation process made damaged materials, too rotten, (1.21: N; 5.12: protein).
- Number of lactic acid bacteria increased after 2 or 3 days (from $1,3.10^7$ to $2,8.10^6$), but highly increased after 5 days due to high fermentation has peaked and made sour for products ($4,2.10^8$)
- Gel Consistency: strongly increased after 3 days (from 35 to 70)
- Amylose increased after 3 days (from 19.53 to 24.26), but it decreased after 5 days (22.29) and very stench smell

Therefore, in the natural climatic conditions in Ho Chi Minh City and southern region of Vietnam in processing bun from Vietnamese rice or Korea rice in the step of soaking rice in water should not exceed 2 days. If the soaked duration is longer than 2 days the rotten and sour fermentation process will be strongly developed that make rotten the end product.

From these results, it can be observed that lactic fermentation takes an important factor in the process.

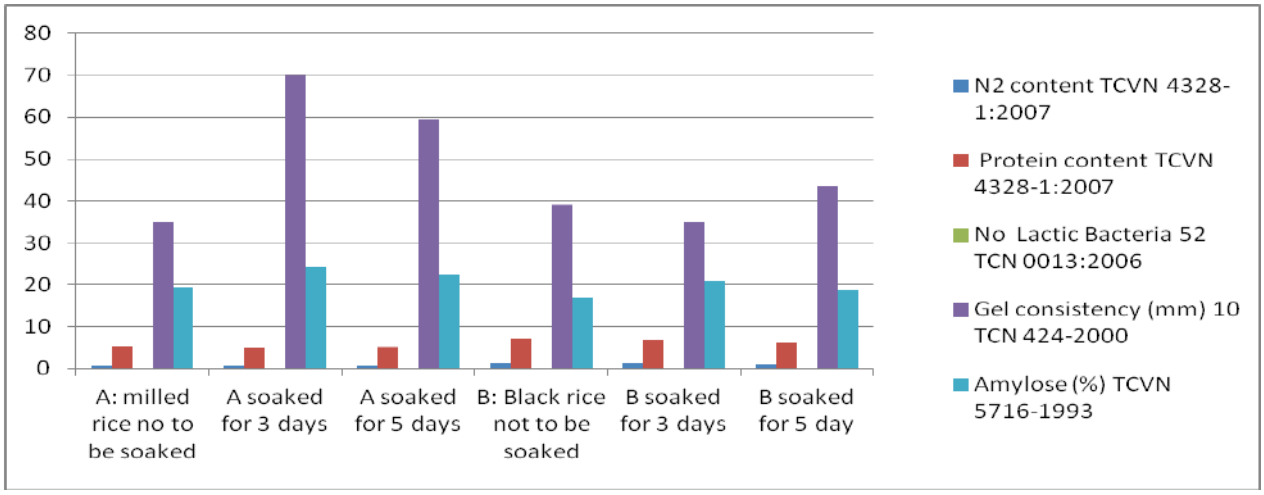

Fig 2*. The change of Biochemical properties in bun processing from Korea rice*

4. Conclusion and recommendation

In traditional process of producing vermicelli (bun) from Vietnamese or Korea rice, the step of soaking rice in water is very important. Preliminary researches on analyzing the changes of biochemical properties of rice materials indicated that total nitrogen, total protein, gel consistency, amylose content and lactic acid fermentation have been significantly changed after soaked rice in water for 2, 3, 5 days.

Initial results of the study also found that the best duration of the soaked step in water of both Vietnam and Korea rice should not be longer than 2 days in natural weather conditions in HCMC and southern regions in Vietnam. If soaked longer than that duration the rice materials will be strongly reduced or completely damaged by rotten and sour fermentation process.

These are only initial findings, in the near future further research will be continued carrying out.

Acknowledgements

We would like to highly appreciate and sincerely thank The Korea Food Research Institute (KFRI) to completely fund and effectively cooperate with us to carry out the project on" Traditional products prepared from Vietnamese rice" during 2009-2012 in Vietnam.

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Survey of production a milky like beverage from purple rice that is grown in Vietnam

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Abstract:

Purple rice *(Oryza sativa)* is the special rice, which is planted in Vietnam and in some Asian countries. In Vietnam the purple rice is planted in organics way. This rice consists of much anthocyanin and other components that is good for health, such as: protein, lysine, vitamin B1, vitamin B2, iron, zinc, selenium, ….

The aim of this study is to find the method to produce the milky like drink from purple rice. Purple rice was collected from the organics farm of Vietnam. The survey was played in two stages. In the first stage, the purple rice seed was treated into the solution. The experiment was conducted with the following variables: purple rice dried time (DT, 0-16 min), purple rice-to-water ratio (P/W, 1–1:10), purple rice treated time (PT, 20–40 min) in hot water (95-100^{o}C). In the second stage, the purple rice solution was prepared to the milky like liquid with natural sweet taste. The experiment was conducted with the following variables: enzyme concentration (EC, 0.1–0.4%), solution treated time (ST, 15–60 minutes), milky like liquid prepared temperature (MT, 35–50^{o}C), diluted to % saccharose (DB, 0–16%) and mixed with suitable pectin to (MP, 0 – 0.01%) to find the most suitable taste for this drink.

The milky like purple rice beverage had the best taste and structure value when the rice was treated in DT, 8min; P/W, 1:7; PT, 20 min; EC, 0.01%; ST, 30 min; MT, 55^{o}C; DB, 8%. The final product was acceptable in sensory evaluation test with affective testing (n= 120). This product can be sold in market and should be make more advertising for people to be more popular.

Keywords: *Oryza sativa*, purple rice, anthocyanin, beverage, milk.

1. Introduction

The purple rice that is harvested in Soc Trang area is one of the most nutritious in Vietnam. This rice consists of much protein, Ca, K, Fe, anthocyanin and GABA, which are good for human health (Sutharut, Sudarat, 2012; PiebiepGoufo and Henrique Trindade, 2014). At present, there are not much products from this rice, there are only some products can be found such as: boiled rice, flour, ….

For human health to find the new product from purple rice, which is suitable for every ages of people is right way. This study was conducted to find out the best method to produce the instant milk from the red rice, which is rich of nutrient and good for human.

2. Materials and Methods

The purple rice was collected from Soc Trang province, Vietnam. After collecting, the sample was storage in the dry place for using in the experiments.. The purple rice was studied to find out the suitable drying time (0, 4, 8, 12, 16min.). The liquid was treated from the dried purple rice solution. The ratio purple rice and water (1:6, 1:7, 1:8, 1:9, 1:10 (w/v)), pretreated time (20, 25, 30, 35, 40 min) was surveyed. Then the liquefied purple rice was treated with alpha amylase to prepare the sweet liquid via the transforming of the part of purple rice starch into glucose.

The treated liquid was mixed with saccharose (0, 4, 8, 12, 16 %) and pectin (0, 0.0025, 0.005, 0.0075, 0.01 %) to receive the suitable product.

The desired milky purple rice should be the purple color, thick, light sweet, milky taste and rich of anthocyanin.

The rice was analyzed the moisture, protein content, carbohydrate content, Ca and Fe content by AOAC (2000) method. The total anthocyanin content was tested by the method that was mention of some scientists like Huynh Thi Kim Cuc et al, 2004 and Fuleki, Francis, 1968. The testing milky purple rice samples were evaluated via affective sensory evaluation test (n= 80) with structure, color, smell and taste factor. The experiment testing data were analyzed by using ANOVA and TUKEY. (Nguyen, 2010; Morten et al., 2007).

3. Results and Discussion

The researched purple rice was 12.58% of moisture, 10.1% protein, 12mg/100gCa, 1.43mg/100g Fe, 6.4 ppm Gamma Acetyl Butyric Acid (GABA), 0.4% anthocyanin, 73.3% carbohydrate.

3.2. Effect of drying time on the quality of the dried purple rice seed

When the purple rice was dried, many reaction was performed, such as: maillard reaction, caramel reaction,.. Those reactions improved the quality of the treated liquid, such as: taste, color and smell. The un-dried purple rice seed made the extracted solution become uncooked starch taste. So, for pretreatment, the drying method should be used. From the results we can see that after 8 minutes of drying, the dried purple rice seed had the best quality with sensory evaluation score of 15.4 (table 1 and Fig.1).

When the ratio of purple rice and water was 1:6, the extracted liquid also was acceptable, but uneconomic. With the ratio 1:7 of purple rice and water, the extracted liquid was the best quality, and more economic than the above. (table 2, fig 2).

The results were showed that, after 8 minutes of drying the purple rice gave the best characteristics with good aroma, dark brown purple color. Then the dried rice was liquefied. The water was mixed with dried rice with ratio 1:7 (w/v). The liquid was purple color, attractive flavor and give the after-taste.

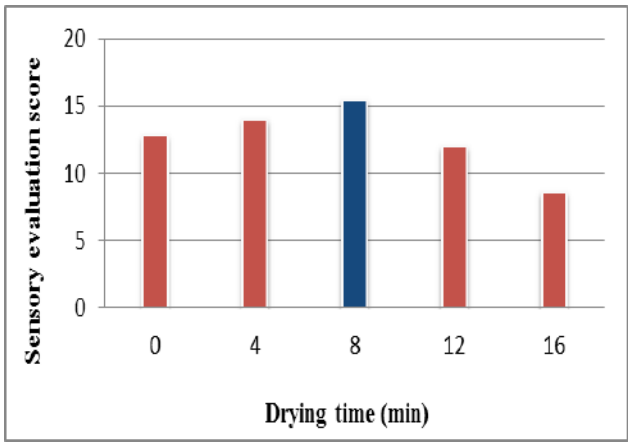

Fig. 1. *Effect of drying time on the quality of purple rice product*

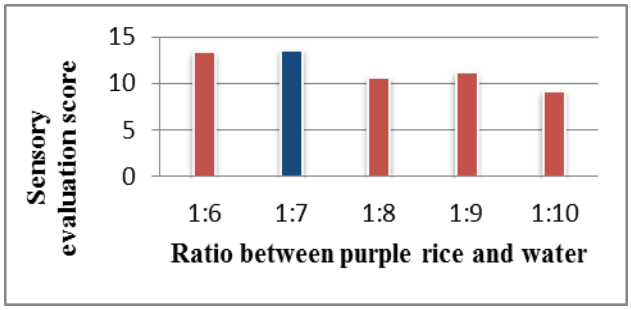

Fig. 2*. Effect of ratio between raw material and water on the quality of purple rice product*

Table 1.*Compare the quality of the purple rice seed after different drying time*

Factor	**Drying time (minutes)**			
	0	**4**	**8**	**12 - 16**
Color	purple	brown purple	Dark brown purple	brown purple seed with some black seed
Smell	bran smell	light smell	good special smell	good smell mixed burnt smell
Structure	Hole seed	Seed with some cracks	Seed with many cracks	Seed become like popcorn

3.3. Effect of the ratio of purple rice and water on the quality of the product.

Table 2. *Compare the quality of the liquid of purple rice with different ratio of purple rice and water*

Factor	**The ratio of purple rice and water (w/v)**			
	1:6	**1:7**	**1:8**	**1:9 - 1:10**
Color	Brown purple	Purple	Light purple	Light whitening purple
Smell	Strong smell	Good special smell	Light smell	Unidentified
After taste	Strong	Good taste	Unidentified	Unidentified

3.4. Effect of the liquefied time on the quality of the product

According to the result from table 3, fig 3 after 35 minutes treatment, the liquid was the best quality in the color, smell and taste to get the highest of sensory evaluation (15.4). That's why this extracted time was chosen for the other experiments.

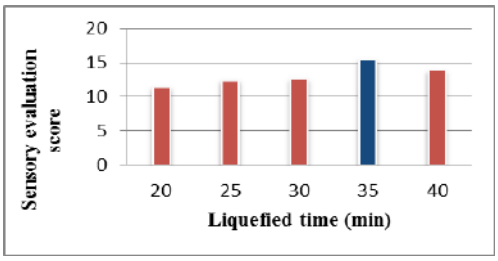

***Fig. 3**. Effect of liquefied time on the quality of purple rice product*

***Table 3.**Compare the quality of the researched liquid of purple rice after different liquefied time*

Factor	**Treated time (minutes)**				
	20	25	30	35	40
Color	Light purple	Light purple	Light purple	Good Light purple	Dark purple
Smell	light smell	light smell	good special smell	good special smell	Strong smell
After taste	Light tart taste	Unidentified	Unidentified	Good sweet taste	Strong taste
Structure of liquid after treatment	Too much water, much turbid	Separate out	Separate out	Homogenous	Too liquid, separate out

3.5. Effect of the adding saccharose on the quality of purple rice milk.

The sensory evaluation showed that the product, which was mixed with saccharose to 8% was the good quality with harmonious sweet of taste and after taste and got the highest sensory evaluation score (15.8) (table 4, fig 4).

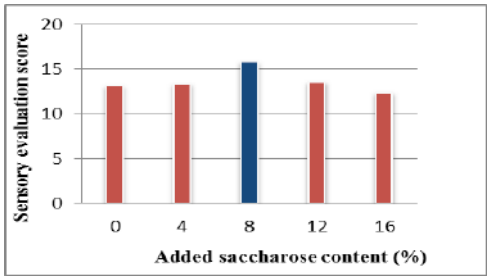

***Fig. 4.** Effect of the added saccharose content on the quality of purple rice milk*

***Table 4.**Compare the quality of the purple rice milk with different ratio of saccharose*

Factor	**The quantity of adding saccharose (%)**			
	0	4	8	12-16
Taste	Slight sweet	Slight sweet	Harmonious sweet	Strong sweet
After taste	Unidentified	Harmonious	Harmonious sweet	Sweet

3.6. Effect of the adding pectin on the quality of purple rice milk.

The sensory evaluation showed that the product, which was mixed with pectin to 0.005% was the good quality with harmonious homogenous of taste and structure and got the highest sensory evaluation score (16.2) (table 5, fig5).

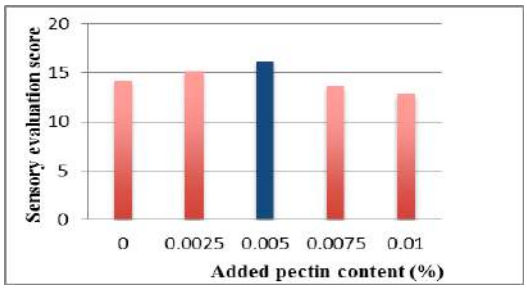

***Fig. 5**. Effect of the added pectin content on the quality of purple rice milk*

***Table 5.**Compare the quality of the purple rice milk with different ratio of saccharose*

Factor	**The quantity of adding pectin (%)**				
	0	0.0025	0.005	0.0075	0.01
Taste	Slight milky	Slight milky	Milky	Heavy	Strong heavy
Structure	Little liquid	homogenous	Harmonious homogenous	Thick	Too thick

4. Conclusion

The instant purple rice milk consists of much protein, amino acid, GABA, iron and calcium. This is natural product with harmonious flavor, structure and taste that is consisting much of anthocyanin, which is good for human health. The process is easy to do with following parameters, such as: 8 min. of drying time for pretreatment of seed, 1:7 of ratio between seed rice and water in 35 min. of liquefied process; then mixed with 8% saccharose and 0.005% pectin to improve the taste and structure of final product. The product should be storage under 15^{o}C; in this condition its shelf life will be in 14 days. The product can be distributed in the market with suitable marketing program.

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Anaerobic technology for fish processing wastewater treatment: A cost saving approach

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Abstract

Fish processing wastewater treatment is a common issue in Vietnam. There are several techniques have been proposed. However, not all of them are appropriate, because of the cost and operation conditions. This paper, in general, provides an introduction of fish processing wastewater characterization and comparison the various types of treatment that could be applied. In developing countries, anaerobic technology for wastewater treatment systems are used widely. Anaerobic digestion (AD) is a well-established treatment technology suited to treating high-strength wastes, or wastes containing high levels of solid matter. Treatment systems such as anaerobic ponds, up-flow anaerobic sludge blanket (UASBs), anaerobic filters and continuous stirred tank reactors (CSTR) provide a high removal of organic loads, but limited removal of pathogens and other pollutants (nitrogen, phosphorus, etc.). These are low energy processes that generates relatively low volumes of sludge, making it cheaper and simpler to operate than aerobic processes. In addition, the temperature range in which the bacteria operate most efficiently is suited to application in tropical, or sub-tropical climates. With all factors shown above and practical applications, this technology will continuously be a sustainable solution for treating fish processing wastewater treatment in Vietnam.

Keywords: Fish processing wastewater, anaerobic treatment, organic loads, low energy

1. Introduction

In recent years, Vietnam is one of the most important countries in fishery industry. However, accompanied with the developing in industry, polluted wastewater has risen every year and become the common environmental problems.

Wastewater from seafood processing operation can be very high in dissolved and suspended organic materials (Nguyen et al, 2010). This results in high biological oxygen demand (BOD) and chemical oxygen demand (COD). Fats, oil and grease also present in high amounts. Suspended solid and nutrients such as nitrogen and phosphate often can be high. Seafood processing wastewater was noted to sometimes contain a high concentration of sodium chloride from boat unloading, processing water and brine solutions.

The major types of waste found in seafood processing wastewater are blood, offal products, viscera, fins, fish heads, shell, skins and meat fine (A. Palenzuela-Rollon et al, 2002). The major process operation include product receiving, boat unloading, sorting and weighing, preparation (butchering, scaling, filleting, skinning, evisceration), inspection and trimming,…Organic material in the wastewater are produced in the majority of these process. However, most of it originate from the butchering process, which generally produces organic materials such as blood and gut materials (Hicks et al, 2007).

A few fish processing wastewater treatment techniques as biological treatment had been established with many problems such as the high cost of treatment for meeting discharge standard and instability of the treatment system. The effective and economical wastewater treatment of fish processing become an important issue for the beginning development of fish industry (Johns et al, 1995).

In the case of a relatively low strength wastewater such as fish processing, the hydraulic retention time and organic loading rate are the most important parameters for successful operation of an anaerobic reactor (Nair et al, 1990).

Water consumption in a fish-processing industry and high-strength wastewater from such an industry are of great concern world-wide. Liquid effluent regulations are becoming more stringent day by day. Biological treatment is the best option for such a wastewater. Anaerobic processes such as up-flow anaerobic sludge blanket (UASB) reactor, anaerobic filter (AF) and anaerobic fluidized bed (AFB) reactor can achieve high (80-90%) organics removal and produce biogas. Aerobic processes such as activated sludge, rotating biological contactor, trickling filter and lagoons are also suitable for organics removal. Anaerobic digestion followed by an aerobic process is an optimal process option for fish processing wastewater treatment (Chowdhury P et al, 2010).

2. Materials and Methods

2.1. Fish processing wastewater characterization

Fish processing wastewater characteristics that raise concern include pollutant parameters, sources of process waste, and types of wastes. The wastewater of seafood-processing wastewater can be characterized by physicochemical parameters, organics, nitrogen, and phosphorus contents. Important pollutant parameters of the wastewater are five-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), fats, oil and grease, and water usage. As in most industrial wastewaters, the contaminants present in fish processing wastewaters are an undefined mixture of substances, mostly organic in nature. It is useless or practically impossible to have a detailed analysis for each component present; therefore, an overall measurement of the degree of contamination is satisfactory (P. Battistoni et al, 1995).

Nitrogen and phosphorus are nutrients that are of environmental concern. They may cause proliferation of algae and affect the aquatic life in a water body if they are present in excess. However, their concentration in the fish processing wastewater is minimal in most cases. It is recommended that a ratio of N to P of 5:1 be achieved for proper growth of the biomass in the biological treatment.

Table 1. *Vietnamese fishery production 1990-2010 (adapted from Nguyen Minh Duc, 2011)*

Year	Catch (tons)	Aquaculture (tons)	Total production (tons)	Export value (1000 USD)	Export growth (%)
1990	709,000	310,000	1,019,000	205	
1991	714,253	347,910	1,062,163	262,234	27.92
1992	746,570	351,260	1,097,830	305,630	16.55
1993	793,324	368,604	1,161,928	368,435	20.55
1994	878,474	333,022	1,211,496	458,200	24.36
1995	928,860	415,28	1,344,140	550,100	20.06
1996	962,500	411,000	1,373,500	670,000	21.80
1997	1,062,000	481,000	1,543,000	776,000	15.82
1998	1,130,660	537,870	1,668,530	858,600	10.64
1999	1,212,800	614,510	1,827,310	971,120	13.11
2000	1,280,590	723,110	2,003,700	1,478,609	52.26
2001	1,347,800	879,100	2,226,900	1,777,485	20.21
2002	1,434,800	976,100	2,410,900	2,014,000	13.31
2003	1,426,223	1,110,138	2,536,361	2,199,577	9.21
2004	1,923,500	1,150,100	3,073,600	2,400,781	9.15
2005	1,995,400	1,437,400	3,432,800	2,738,726	14.08
2006	2,001,656	1,694,271	3,695,927	3,357,960	22.61
2007	2,075,000	2,123,000	4,197,000	3,763,000	12.40
2008	1,850,000	3,399,000	5,249,000	4,509,000	19.82
2009	2,277,700	2,569,900	4,847,600	3,488,000	11.12
2010	2,450,800	2,706,800	5,157,600	5,034,000	18.40

2.2. The differences of aerobic and anaerobic technology

Aerobic, as the title suggests, means in the presence of air (oxygen); while anaerobic means in the absence of air (oxygen). These two terms are directly related to the type of bacteria or microorganisms that are involved in the degradation of organic impurities in a given wastewater and the operating conditions of the bioreactor. Therefore, aerobic treatment processes take place in the presence of air and utilize those microorganisms (also called aerobes), which use molecular/free oxygen to assimilate organic impurities i.e. convert them in to carbon dioxide, water and biomass. The anaerobic treatment processes, on other hand take place in the absence of air (and thus molecular/free oxygen) by those microorganisms (also called

anaerobes) which do not require air (molecular/free oxygen) to assimilate organic impurities. The final products of organic assimilation in anaerobic treatment are methane and carbon dioxide gas and biomass.

Table 2. *Major differences in aerobic and anaerobic treatment (adapted from Arun Mittal, 2011)*

Parameter	Aerobic Treatment	Aerobic Treatment
Process Principle	• Microbial reactions take place in the presence of molecular/ free oxygen. • Reactions products are carbon dioxide, water and excess biomass.	• Microbial reactions take place in the absence of molecular/ free oxygen. • Reactions products are carbon dioxide, methane and excess biomass.
Applications	• Wastewater with low to medium organic impurities (COD < 1000 ppm) and for wastewater that are difficult to biodegrade e.g. municipal sewage, refinery wastewater etc.	• Wastewater with medium to high organic impurities (COD > 1000 ppm) and easily biodegradable wastewater e.g. food and beverage wastewater rich in starch/sugar/alcohol.
Foot-Print	• Relatively large.	• Relatively small and compact.

2.3. Types of anaerobic wastewater treatment

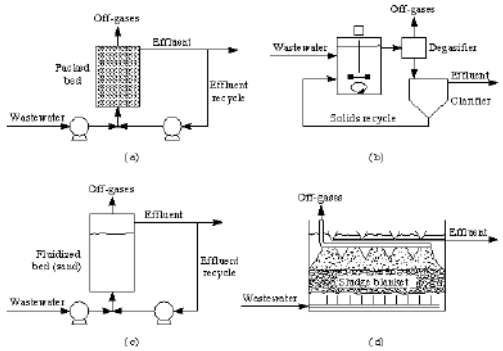

Fig 1. *Anaerobic wastewater treatment processes: (a) anaerobic filter reactor; (b) anaerobic contact reactor; (c) fluidized-bed reactor; (d) upflow anaerobic sludge blanket (UASB).*

The fermentation process in which organic material is degraded and biogas (composed of mainly methane and carbon dioxide) is produced, is referred to as anaerobic digestion. Anaerobic digestion processes occur in many places where organic material is available and redox potential is low (zero oxygen). This is typically the case in stomachs of ruminants, in marshes, sediments of lakes and ditches, municipal landfills, or even municipal sewers.. There are many reactor configurations are used for the anaerobic treatment wastewaters, (Mustafa Evren Ersahin, 2011).

- Anaerobic Filter: the anaerobic filter is similar to a trickling filter in that a biofilm is generated on media. For very high strength wastewaters, a recycle can be employed.
- Anaerobic Contact: this process can be considered as an anaerobic activated sludge because sludge is recycled from a clarifier or separator to the reactor.
- Fluidized Bed: this reactor consists of a sand bed on which the biomass is grown.
- Up-flow Anaerobic Sludge Blanket (UASB): under proper conditions anaerobic sludge will develop as high-density granules.

3. Results and Discussion

Compared to conventional aerobic treatment systems, the most advantages of high-rate anaerobic for fish processing wastewater treatment are (Pankaj Chowdhury et al, 2010):

- Reduction of excess sludge production up to 90%; excess sludge has a market value.
- No use of fossil fuels for treatment; production of energy in the form of methane gas at a theoretical value of 3.8 kWh/kg COD removed.
- Up to 90% reduction in space requirement.
- High applicable COD loading rates. No or very little use of chemicals.
- Rapid start-up (< 1 week), using granular anaerobic sludge as seed material.
- Anaerobic sludge can be stored unfed; reactors can be operated during agricultural campaigns only (e.g. 4 months per year in the sugar industry).
- Plain technology with high treatment efficiencies.
- High rate systems facilitate water recycling.

4. Conclusion

Anaerobic biotechnology has a significant potential for the recovery of bio-methane by the treatment of medium and/or high strength wastewaters especially produced in agro-industries. By using this technology, ~ 250-300 m^3 bio-methane can be recovered per ton COD removed depending on the inert COD content of the substrate. COD removal rates are generally between 65-90% in

these systems. Anaerobic biotechnology, when used in the first treatment stage, provides the reduction of aeration energy and excess sludge production in the followed aerobic stage, thus increasing the total energy efficiency of the treatment plant. Besides, it contributes to the increase in the treatment capacity of the aerobic stage.

Anaerobic systems are well suited to the treatment of fish processing wastewater because a high degree of BOD5 removal can be achieved at a significantly lower cost than comparable aerobic systems and generate a smaller quantity of highly stabilized, and more easily dewatered, sludge. Furthermore, the methane-rich gas, which is generated, can be captured for use as a fuel.

Acknowledgements

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Evaluation of localized corrosion in food industry by electrochemical methods

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Abstract:

A wire-beam electrode (WBE) fabricated from 100 316L stainless steel wires has been used to study the localized corrosion processes of stainless steel exposed to simulated food corrosive environments. Results indicate the possible application of a WBE in simulating and monitoring localized corrosion in food industry. A new method of localized corrosion estimation has been developed using a WBE. In addition, other electrochemical techniques such as potential dynamic polarization, potential static and electrochemical impedance spectroscopy also used for confirming the occurrence of localized corrosion.

Keywords: Food Industry, localized corrosion, wire beam electrode, electrochemical measurement

1. Introduction

Food processing industry is one of the largest manufacturing industries in both Viet Nam and worldwide. Based on the requirements of food quality, stainless steels have been tremendously used as common material in beverage production, food machinery, cutlery, utensils, commercial and restaurant equipment and other appliances because of their superior surface finish, good hardness, relatively high chemical stability, high strength, low corrosion rate and low gas permeability [1]. The food industry highly contents chloride, which mixed with significant concentrations of organic acids. In addition, sulfating agents forming sulfur dioxide that helps in treating food processes. They are highly corrosive to food-processing equipment. The under deposit corrosion, galvanic corrosion and stress corrosion cracking could be counted for corrosion problems in the food industry. Among them, localized corrosion is one the most important problems. Salas et al. [2] also suggested the following three types of foods are recognized according to their corrosion: (i) non corrosive: milk, meat, fish, oil, fat, cereals; (ii) mild corrosion, foods with pH 6 - 7 and less than 1% of salt: dairy products, fruit syrups, wine, carbonated sweet drinks, beer, soups, canned meat; (iii) high corrosion, foods with pH 3 - 5, such as citric fruit juices, jams and acidic canned fruits or hot gravies, sauces and dressings, vegetables and fish pickled in brines with 1 - 3 % salt. In addition, many cleaning, disinfection and sanitation agents (including alkaline, acidic, strong or weak oxidizing and reducing chemicals) are employed to remove bacteria, scale, fouling and corrosive biological and. These great corrosive environments and aggressive chemical agents demand the use of high corrosion resistance materials such as stainless steels for the food industry to resist equipment damage and food contamination.

The electrochemical techniques have been widely used in a corrosion science and engineering for several decades. However a comprehensive understanding of (i) how localized corrosion rates can be determined quantitatively and quickly, (ii) how the patterns of localized corrosion can be monitored and (iii) how suitable it is for traditional electrochemical techniques to be used in studying the corrosion kinetics of practical corrosion systems has not been answered properly and neglected yet [3].

WBE is a newly developed electrode consisting of a metal wire beam embedded in epoxy

resin. The metal wire beam is made by binding a large number of 316L stainless steel wires together. Each wire is insulated from its neighbors and constitutes a mini sensor. WBE divides a large electrode area into several tiny individual parts and measures the electrochemical properties of each part using an individual wire sensor. The WBE was initially designed for detecting localized defects in organic coatings [4-6], but it was applied later to the study of crevice corrosion [7]. These preliminary studies have shown that use of the WBE is a promising method for studying localized electrode processes.

Based upon the previous findings, the present work was designed to test the capability of WBE in simulating and monitoring corrosion processes and to develop an electrochemical method that could be used to study localized corrosion in the food industry.

2. Materials and Methods

2.1. Chemicals and materials

The working electrodes used for the electrochemical tests were machined from a cylindrical 316L stainless steel rod to a 12 mm diameter of 2 mm thickness. The 316L SS electrodes were coated with an epoxy resin and attached to a Teflon holder. The stainless steel compositions were checked by Optical Emission Spectroscopy and results obtained are as follows: 0.02 % C, 0.76 % Si, 1.66 % Mn, 17.16 % Cr, 2.2 % Mo, 10.81 % Ni, 0.41 % P, 0.025 % S, 0.085 % N and Fe balance. The structure of the 316L SS specimen showed in Fig. 1 was examined by X-ray diffraction (XRD) using Cu Kα radiation. XRD data was measured by Model D/MAX-RC equipment. The scan range of the 2 theta was from 30º to 100º. The 316L SS specimens for corrosion tests were finished by grinding with 4000-grit silicon carbide paper. The reagent grade sodium chloride purchased from Sigma Aldrich, ≥ 99 % pure and distilled water were used for making 0.01, 0.1 and 0.6 M NaCl solutions containing 50ppm vinegar. In these solutions, the Cl^- ion additive induces pit initiation but the vinegar additive retards the incorporation of Cl^- ions into the oxide film on the alloy surface so that they practically impede pit initiation.

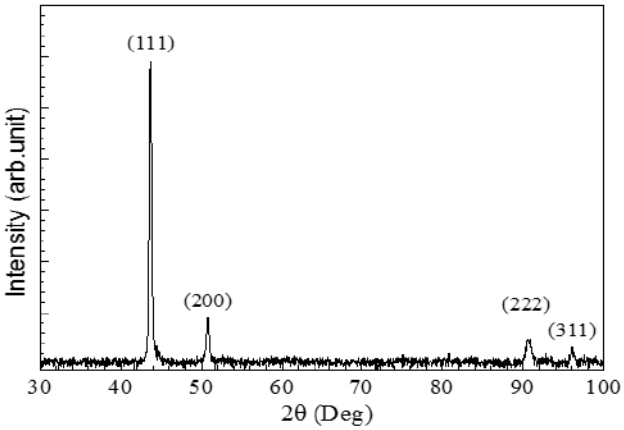

Fig 1. *XRD patterns of 316L SS.*

2.2. Electrochemical tests

All experiments were performed at room temperature. The exposed area was 1.13 cm^2. The electrochemical polarization tests were performed using a VSP system (BioLogic Scientific Instruments). Saturated calomel and pure graphite were used for the reference and counter electrodes, respectively. Prior to the potential dynamic polarization test, the samples were kept in the solution for 3 h to establish the open-circuit potential. The potential of the electrodes was swept from an initial potential of -250 mV versus corrosion potential (E_{corr}) to a selected anodic potential at a rate 0.166 mV/s. Electrochemical impedance spectroscopy (EIS) tests were conducted using a VPS system with a commercial software program for the AC measurements. Peak-to-peak amplitude of the sinusoidal perturbation signals of 20 mV was used. The frequency ranged from 100 kHz to 10 mHz. The model VSP system of BioLogic scientific instruments was also used to apply + 650 mV_{SCE} as near pitting potential of the specimen immersed in 0.01 M NaCl solution containing 50 ppm vinegar.

2.3. Wire beam electrode

The wire beam electrode was used to study the tendency of localized corrosion of 316L stainless steel (316L SS) in the test solutions. The WBE was made from one hundred identical 316L SS wires embedded in epoxy resin, insulated from each other with a thin epoxy layer. Each wire had a diameter of 0.19 cm and acted both as a sensor and as a corrosion substrate. The working area was grinded using 4000-grit silicon carbide paper, rinsed with deionized water and ethanol before being exposed to three liters of solutions. The procedure is shown in Fig. 2. Corrosion processes were monitored by mapping galvanic currents between a chosen wire and all the other wires shorted together using a pre-programmed Autoswitch device and an ACM AutoZRA. Galvanic current data were obtained and analyzed by procedures similar to that described in previous publication [8]. The measurements were taken regularly to examine the changes taking place.

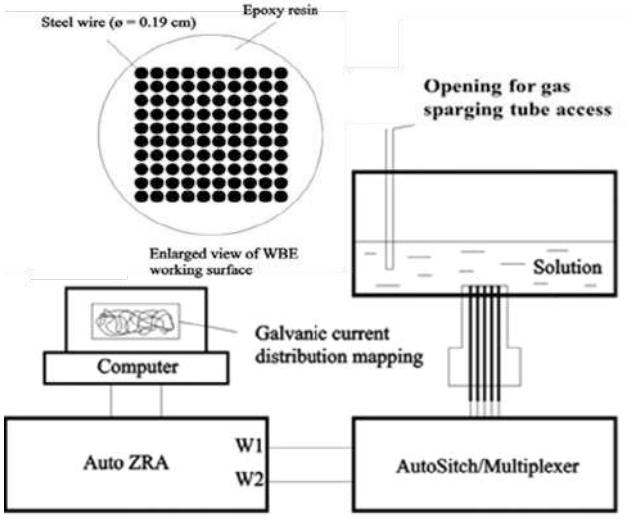

Fig 2. *Schematic diagram shows new electrochemical technique of WBE experiment setup to supply galvanic current distribution maps for localized corrosion tests [8]*

3. Results and Discussion

The potential dynamic polarization curves for the 316L SS immersed in 50ppm vinegar solution

containing different Cl^- concentrations are shown in Fig. 3 (a). All conditions were well passivated with a low passive current density and the range of the passive potential increased with decreasing chloride concentration in solution. In addition, lower corrosion potentials and higher pitting potentials occurred for 316L SS in the investigated solution containing lower chloride concentration as shown in Fig. 3 (b), indicating that the range of passive potential and pitting resistance of the 316L SS was accelerated by the increase of chloride concentration.

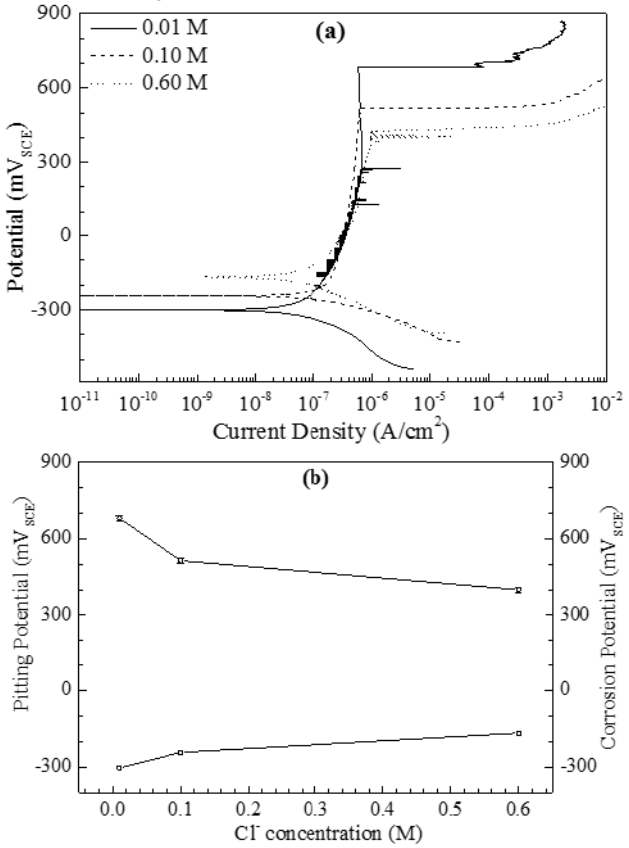

Fig. 3. *(a) Potential dynamic polarization curves of 316L SS as a function of Cl^- content and (b) Effect of Cl^- on corrosion and pitting potentials of 316L SS in 50 ppm vinegar solution at room temperature.*

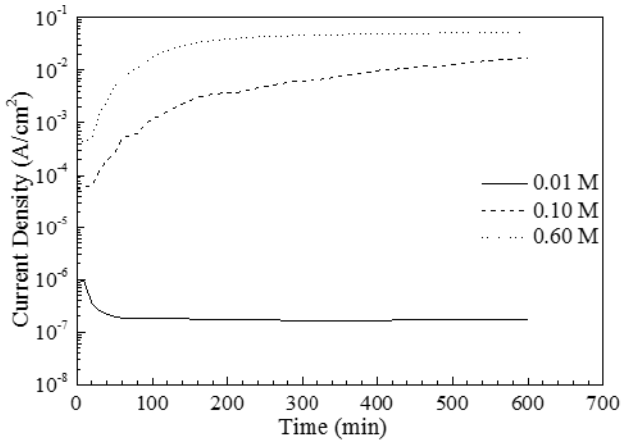

Fig. 4. *Current density as a function of time under a constant applied potential of + 650 mV_{SCE} in 50 ppm vinegar solution containing different Cl^- concentrations*

Fig. 4 exhibits the results of potentiostatic test performed at a constant potential of + 650 mV_{SCE} for 5 mins to determine the stability of the passive film. The applied potential was based upon the data from polarization curves in Fig. 3(a), corresponding to a potential nearby pitting potential of the specimen immersed in solution containing 0.6M NaCl. The high current density is related to the initiation and propagation of pits. The decrease in chloride concentration increases the pitting corrosion resistance. This corresponds to the results of the polarization test.

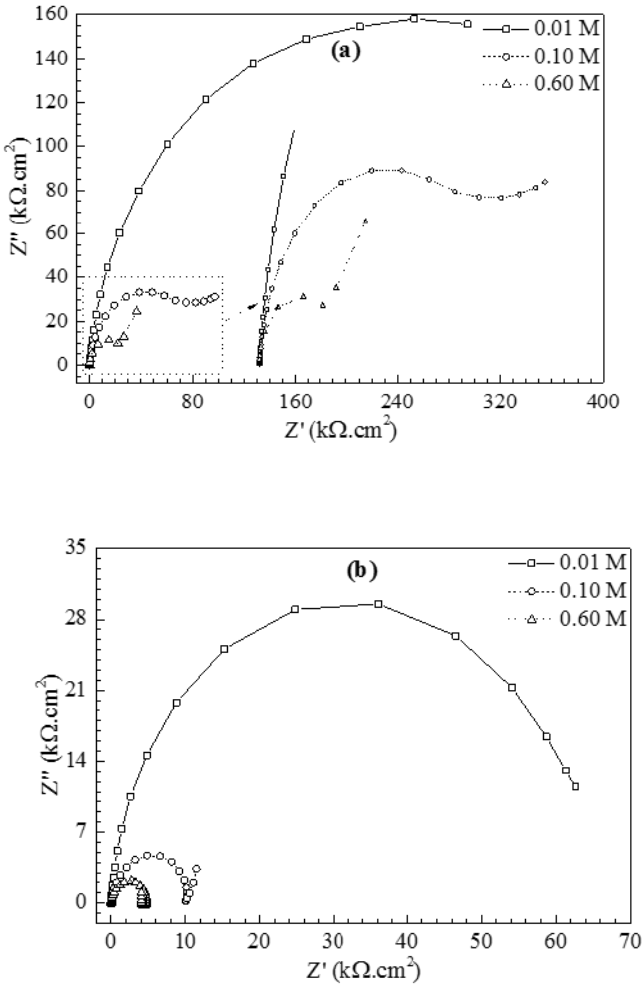

Fig. 5. *Nyquist plots of 316L SS (a) before and (b) after + 650 mV_{SCE} application.*

Fig. 5(a) shows the impedance spectra in the form of the Nyquist plots obtained from the 316L SS specimens in a 50ppm vinegar solution containing different Cl^- concentrations. The semicircular depression in the Nyquist diagram was attributed to the surface heterogeneity, surface roughness and the existence of two different processes with similar relaxation times. The increase in the diameter of the arc suggests the improvement in corrosion resistance. Furthermore, the high spectrum detects local surface defects, whereas the medium and low frequency spectradetects the process within the film and at the metal/film interface, respectively. The impedance increases with decrease in Cl^- content, suggesting higher

corrosion resistance.

Fig. 5(b) shows the Nyquist plot in the pitting region. + 650 mV_{SCE} was applied to all specimens. The impedance diagram spectra obtained in the pitting region represents a single semicircle. Scully [9] reported that the R_p value and depression angle are strongly related to the pitting resistance. Therefore, the surface modification introduced by the formation and propagation of localized pits should be related to the depression angle. This figure shows both higher R_p and the depression angle related to the pitting resistance with lower Cl^- containing solutions.

The electrochemical response to the impedance tests for the materials under consideration was best-simulated using equivalent circuits. Fig. 6 shows the equivalent circuits used for fitting (a) the breakdown film and (b) the passive film, where R_s represents the solution resistance, CPE is the constant phase element, R_p is the polarization resistance, R_{film} is the film resistance, and R_{ct} is the charge transfer resistance. The high- (R_{film}) and low- (R_{ct}) frequency resistance components were affected by the Cl^- ions. In this case, the capacitor was replaced with a CPE to improve the fitting quality, where the CPE contains a double-layer capacitance (C) and phenomenological coefficient (n). The n value of the CPE indicates its meaning: n = 1, capacitance; n = 0.5, Warburg impedance; n = 0, resistance; and n = -1, inductance. In this study, n was maintained consistently near 0.8 as a result of the deviation from ideal dielectric behavior. The Zsimpwin program was used to fit the EIS data and determine the optimized values for the resistance parameters (R_{total}). The fitting results indicated that the total resistance increased with decreasing Cl^- ions in the investigated solutions. This is important because a high R_{total} value indicates good corrosion resistance.

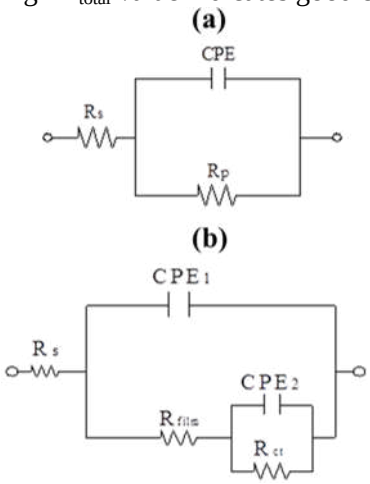

Fig. 6. *Equivalent circuit model for fitting of: (a) the breakdown film, and (b) the passive film on 316L SS.*

A WBE has been used to determine the kinetics of non uninform corrosion of 316L SS in a 50 ppm vinegar solution containing different Cl^- concentrations. Local electrochemical parameter such as galvanic current was measured from local areas of a WBE surface. This parameter was used to calculate the kinetics of local electrochemical corrosion processes. A map showing instantaneous galvanic current distribution over a WBE surface has been produced. The galvanic current distribution maps are given in Fig. 7(b-d), the WBE current distribution maps are characterized by a small number of minor anodes distributed over the WBE surface. The maximum anodic current density can be used to describe localized corrosion dissolution of the most active anode, while the total anodic current density can be used to describe overall corrosion of the specimen. Only one or two locations behave as anodes and the current density values remain very high and increased with increasing Cl^- content, suggesting the implying Cl^- as an induced pit initiation that promotes localized corrosion rather than general corrosion.

4. Conclusion

This paper reports the advantage of the WBE for examining the localized corrosion. This is a convenient new method which, helps in monitoring the localized corrosion processes in food industry. Furthermore, excellent agreement was observed between the WBE and other electrochemical methods. In addition, the pitting resistance of 316L stainless steel increased with a decrease of chloride concentration. Thus, automation of the experimental process and data acquisition and handling is very important for WBE's industrial application.

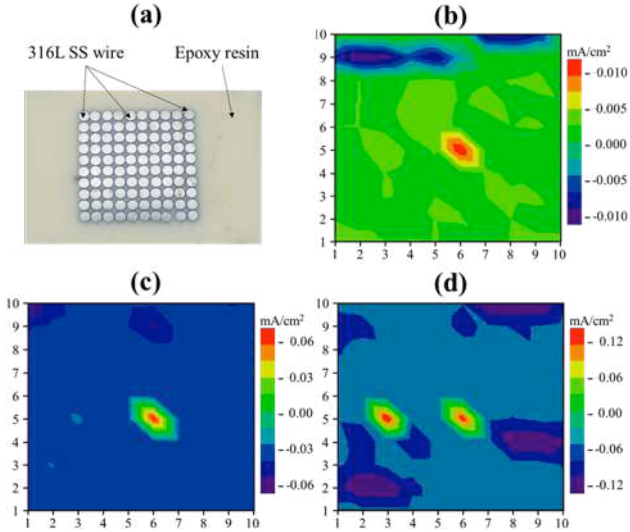

Fig. 7. *(a) WBE surface after 4000-grit silicon carbide paper grinding and galvanic current distribution maps measured over a WBE surface in 50 ppm vinegar solution containing (a) 0.01 M, (b) 0.1 M and (c) 0.6 M Cl^-.*

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Factors affect to extraction process of lipase from snakehead fish offal

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Abstract

This study was to discover a new source of lipases for potential use to meet the growing demands for enzymes in commercial applications. In this connection, the appropriate conditions for enzyme lipase extracted from the offal of snakehead (*Channidae*) were investigated. Homogenized offal with different ratios was extracted using various buffers and pH adjusted solutions at buffers and pH adjusted solutions at different temperatures and time. Based on the result, the best lipase extraction effect achieved when using phosphate buffer solution (pH 6.0) with the ratio of material and extraction solvent was 1:4, extraction process during 3 hours at 50ºC. In this optimal condition, the highest enzyme activity obtained was 71.28 ± 1.92 U/g of dry material. The study results have demonstrated the potential acquisition lipase from offal of snakehead fish, increase economic efficiency and reduce waste in the environment.

Keywords: extraction temperature, extraction time, phosphate buffer, lipase, snakehead fish offal

1. Introduction

In recent years, the production of all kinds of enzyme preparations has a significant development. There are thousands of enzyme preparations sold in the world market; these preparations are produced with the purity based on industrial standards and applications. The common enzyme preparations such as amylase, protease, catalase, cellulase, lipase, glucosidase not only apply in medicine but also used in many different industries, in agriculture [1].

In the last 20 years of the twentieth century and the early years of the twenty-first century there are many different enzymes have been commercialized and applied in many fields of life. In Vietnam, the applications of enzymes in the processing of agricultural products and foodstuffs, especially in the manufacturing beer, wine, starch [1].

Lipase (glycerol ester hydrolase, EC 3.1.1.3) is used in many industries such as food industry, chemical industry, cosmetics, leather, medicine and other industries due to the ability to catalase hydrolyze triglycerides into di-, mono glycerides or glycerol and fatty acids by interfacial activity on the water and oil phases. In the oil and fat industry, the use of lipase is very popular. There are more than 100 different lipase is used to convert lipid into other substances [2].

Currently, Vietnam uses large amounts of lipase in food, detergent, chemical and medical industry... Using imported enzyme preparations is costly; therefore, confirming product of enzyme from natural origin is a critical requirement for many countries around the world including Vietnam. So the purpose of study focus on lipase enzyme obtained from snakehead offal by extraction method to obtain the highest enzyme activity.

This research mainly concentrate in determining: (i) the ratio of raw material and solvent (distilled water) for suitable extraction and lipase obtained efficiency, (ii) the effect of pH and type of buffer solution used in the stability of obtained lipase, (iii) effects of temperature and extraction time on lipase activity. The extraction temperature was maintained room temperature.

2. Materials and Methods

Material

Snakehead offal were purchased from fish market in Can Tho City in the early morning, packed by polyethylene bags and stored in tanks at a temperature from 0 – 4°C with ice, then brought to the laboratory of Food Technology department (Can Tho University) within 30 minutes. Raw materials are removed fat and stored at -18°C until needed for extraction.

Lipase extraction

Minced offal firstly were extracted with distilled water at different ratio of the ratio of raw material to extraction solution changing from 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 (w/v) during 2,5 hours. The sample was pressed in a cheesecloth bag before centrifuge at 4500 g. The solution was applied to determine lipase activity. Similarly, lipase was extracted using various solutions (pH 3 and 4 using glycine – HCl buffer; pH from 5 to 8 using phosphate buffer; pH 9 and 10 using glycine NaOH buffer).

After that, influence of extraction time (from 1 hour to 4 hours) and temperatures (from 20°C to 70°C) on lipase activity were also determined.

Lipase analysis: The titrimetry method was used to determined lipase activity with 0.05 N NaOH and using phenolphtalein as an indicator [3].

Data collection and Statistical analysis

The experiment was conducted based on the change a in factor and remaining factors were fixed. The results of the previous experiments were used as fixed parameters for the next experiment. The experiments were assigned randomly triplicate.

The data are collected and processed by statistical Stagraphics Centrution 15.2, Copyright (C) PP, USA and Excel software. Analysis of variance (ANOVA) and LSD to test the difference between the treatments.

3. Results and Discussion

3.1. Influence of the ratio of raw material to extraction solution for lipase extraction

Table 1 showed that a significant difference of lipase activity depend on the ratio of raw material to extraction solution (using distill water).

The activity of lipase was increased when this ratio changed from 1:1 to 1:4. At a low ratio of raw material and extraction solution, lipase activity was a low value. This can be explained by low amount of solvent used not fully penetrate into the material [4], not enough for the diffusion of the solute (enzyme) in solvent [5].

Table 1. *Effect of ratio of raw material and distill water on lipase activity*

Rate of raw material : distill water	Enzyme lipase activity (U/g, dry basic)
1: 1	6.24^{a} ± 1.32
1: 2	12.84^{b} ± 0.72
1: 3	15.36^{bc} ± 1.92
1: 4	17.76^{c} ± 2.76
1: 5	17.52^{c} ± 0.48
1: 6	17.64^{c} ± 0.48

When the ratio of solvent increased, the difference between the solute concentration and solvent materials rise, increasing the solubility, diffusion leads to the so increasing in enzyme activity [6,1]. However, using high ratio of solvent (1:5 and 1:6), the enzyme activity would decrease due to the increasing lipase dilution in the extract [4]. With a ratio of buffer solution using higher, extraction collected much, while the amount of enzyme produced from the extraction process is not changed.

The rate of raw material : solvent used to extract 1:4, lipase activity reach the peaked when compared to the higher or lower level (relative value lipase activity response was 17.76 ± 2.76 U/g , according dry basis). However, lipase activity differences not statistically significant with two rate 1:5 (17.52 ± 0.48 U/g of substrate) and 1:6 (17.64 ± 0.48 U/g of substrate). In addition, the enzyme extracted at the rate of 1:4 economical than 1:5 and 1:6 by saving solvent used in the extraction process and reduce chemicals used in the purification of the enzyme [6].

3.2. The change of pH on lipase activity

When the influence of pH (in the range 3-10) was studied (see Table 2), a significantly increase in extraction yield was produced. This was explained that the pH control is also one of the effective solutions to help regulate the ability of the enzyme dissolved in a solvent, thereby increasing obtained enzyme yield [7]. The best extraction conditions were achieved at pH 6.0, corresponding lipase activity reached to 51.36 ± 0.48 U/g, dry basic, higher 3 times than enzyme activity obtained in blank samples (extracted by water).

Table 2. *Effect of pH on enzyme activity from snakehead fish offal*

pH	Enzyme lipase activity (U/g, dry basic)
Blank	$17,64^{a}$ ± 2.76
3.0	23.52^{b} ± 0.12
4.0	23.64^{b} ± 0.12
5.0	27.60^{c} ± 0.96
6.0	51.36^{e} ± 0.48
7.0	42.24^{d} ± 3.36

8.0	$26.28^{bc} \pm 3.00$

3.3. Influence of extraction time on lipase activity from snakehead fish offal

Times under 2 hours were found to be too short, but an extraction time of 4 hours did not entail a dramatic increase in the amount of lipase extracted, rather it kept constant after 3 hours of extraction. Extraction time of 3 hours is enough to ensure the equilibrium was reached and to get a high extraction yield (see Table 3). At the same ratio of solvent used, short extraction time would be not enough for solvent leach to raw material, reduced enzyme forming [7]. Conversely, if the extraction time is too long will reduce the activity of the enzyme [8].

Table 3. *Effect of time extraction on lipase activity from snakehead fish offal*

Time (hours)	Enzyme lipase activity (U/g, dry basic)
1.0	$45.60^{a} \pm 0.48$
1.5	$48.36^{bc} \pm 0.36$
2.0	$49.08^{c} \pm 1.08$
2.5	$51.48^{d} \pm 0.84$
3.0	$52.68^{d} \pm 0.60$
3.5	$47.52^{b} \pm 1.44$

3.4. Effects of extracted temperature on lipase activity

Temperature has a great influence on the activity of lipase. A summary of optimum temperature for lipase is displayed in Table 4. The optimum temperature of lipase ranges from 50 to 60ºC. When the temperature deviates from the optimum value, the activity of lipase drops due to the the losing catalytic role of enzyme, the reaction rate will decrease [9].

Table 4. *Effect of temperature on lipase activity extracted from snakehead fish offal*

Temperature (°C)	Enzyme lipase activity (U/g, dry basic)
20	$46.20^{a} \pm 1.20$
30	$52.56^{b} \pm 0.48$
40	$64.32^{c} \pm 0.72$
50	$71.28^{d} \pm 1.92$
60	$70.56^{d} \pm 0.48$
70	$54.24^{b} \pm 2.04$

However, when the temperature is higher than 60ºC, enzyme activity started declining. The decrease in activity is explained by the inactivation of central lipase enzyme activity leads to the losing catalytic role of enzyme [9]. At the lower temperature, the lipase has slower reaction due to the less energy than the other temperatures (20 – 40ºC). Each enzyme was active best at certain optimal temperature. When the temperature deviation laterally, optimum temperature of the enzyme activity decreases and the reaction rate will decrease. The temperature rose make the molecules move faster, the complex enzyme-substrate formed more rapidly than reaction. If the temperature is too high, enzyme is denatured no longer suitable for substrate, inactivated enzyme. When the temperature is lower than the optimum temperature, enzyme molecules slow motion. Enzyme complex - formed at the substrate and the reaction rate decreases.

The results match with the general properties of lipase from animals. Pahojar and Sethar (2002) [10] recorded the activity of lipase, extracted lipase conditions from animals closely related to the location of the enzyme in organs, affects extracted conditions or lipase activities. Knospe and Plendi (1997, cited by Pahojar and Sethar, 2002 [10]) have found conditions lipase extracted from goats organs is about 5.6 – 6.5 pH, 43 – 60°C. This again confirms, in surveying conditions, the temperature suitable for the extraction of lipase from snakehead offal was 50 – 60ºC in 3 hours corresponding period extracted 3 times with the phosphate buffer pH 6.

4. Conclusion

The study results showed that the prospect of receiving the enzyme lipase from snakehead fish offal. The highest lipase activity obtained when minced snakehead fish offal was extracted at the ratio 1:4 (w/v) of raw material and extraction solvent, using phosphate buffer pH 6. Lipase activity reached to 71.28 ± 1.92 (UI/g, dry basic) after 3 hours extraction at 50°C.

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Survey the betacyanin extraction from the flower of purple *Bougainvillea* of Vietnam and the stability of this pigment

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Abstract:

Betacyanins has potential applications as a natural color, antioxidant component, and many other healthy benefits. This pigment obtained good application in the food industry. *Bougainvillea* is a very common plant in Vietnam, flowering all year around. The flower of this plant contains high Betacyanins. The betacyanin extraction was surveyed, and the best method of the extraction was optimized. The experiment was conducted according to a central composite design, with the following variables: solvent pH (pH, 3.0–7.0), solvent-to-*Bougainvillea* ratio (S/B, 4:1–10:1), solvent temperature (ST, 10–60 °C) and extraction time (ET, 10–60 min). *Bougainvillea* were ground with the solvent, filtered and evaluated for betacyanin contents. A highly significant model was obtained for betacyanin extraction efficiency (BEE), which was positively affected by ST and S/B, and inversely affected by pH. The most adequate extraction conditions were pH, 6.0; S/B, 5:1; ST, 30 °C and ET, 30 min. The betacyanin extraction parameters were optimized. The result showed that the best adequate extraction conditions were pH, 5.97; S/B, 5.25:1; ST, 28.8 °C and ET, 30.6 min. At such condition, 53.86 mg / 100g raw material was extracted. The obtained pigment should preserve at 15^0C with 0.15 % vitamin C addition, in the pH range from 4-7. The method is simple and produces a bright red–purple extract to be later dried or concentrated for use as a food colorant.

Keywords: *Bougainvillea*, betacyanin, food color, pigment stability, optimizing extraction.

1. Introduction

Betacyanin is a natural color, pink to red-violet; the pigment is belonging to betalain group. Betacyanin has many applications in the food [1] like E162 which is extracted from *Beta vulgaris*; the color is red or purple. Betacyanin is also resistant to oxidation, prevent chronic leukemia [15], protecting nerve cells [16], prevent cancer [17]. Betacyanin capable of staining foods with high intensity colors, beautiful colors. Betacyanin from *Bougainvillae* flower with high content is a subject of potential research and high economic efficiency.

Bougainvillea is suitable for growing in hot, dry climate. That's why *Bougainvillea* is grown everywhere in Vietnam, mainly grown for ornamental.

The aim of this research was focusing on the optimality of the betacyanin extraction from *Bougainvillea*, which is grown in Vietnam, and surveyed the optimality of storage condition for the color stability of the extracted pigment.

2. Materials and Methods

2.1. Materials: After plucking, selecting the flower, cleaning and drying, *Bougainvillea* flower was mixed with distilled water (1:1 ratio) and frozen at (-16^oC). Before doing the survey, the frozen *Bougainvillea* flower was defrozed in 2 hrs. The sample was defrosted at room temperature for 2 hours. Bougainvillae flowers was ground in the solvent with 5:1 ratio of solvent and raw material,

then the pigment was extracted at 20^{o}C for 30 minutes, pH 5. The extracted solution was centrifuged (5000 rev/min) in 10 min to remove the sediment. Used the UV/Visible scanning spectrophotometer to measure the content of the extracted pigment. To get the higher content of extracted pigment, we used the rotary evaporator system [22].

2.2. Preliminary survey the extracted betacyanin content from the type of materials*:* using different age of the flower including: bud flowers, bloomed flowers and fade flowers in the rainy season and dry seasons.

2.3. Survey the optimized betacyanin extraction: The ground flowers were treated with different solvent (water, water : methanol (v/v:1/1), water : ethanol (v/1:1/1)) at 20^{o}C, in 30 min, with pH = 5. From the experiments, the most suitable extracted solvent was selected. Then the productivity of betacyanin of Bougainvillea flower was surveyed with different ration of selected solvent and raw material (4:1 – 10:1), at different temperature (10^{o}C – 60^{o}C), in different extraction time (10 – 60 min), with different pH (3 – 7) (Castellar et al, 2003).

The optimized betacyanin extraction according to a central composite design was studied with the following variables: solvent pH (5.0–7.0, center value is 6), solvent-to-*Bougainvillea* ratio (4:1–6:1, center value is 5), solvent temperature (20–40°C, center value is 30^{0}C) and extraction time (20–40 min, center value is 30 minutes).

2.4. Survey the optimized storage conditions: The extracted pigment was studied the fast color under the effect of different temperature (20 – 95^{o}C), time (5 – 20 min), pH (2 – 10) and using vitamin C (0 – 0.2% (v/v)) [7]. *The optimized storage condition* was studied with the following variables: storage temperature (15-35^{o}C, center value is 25^{o}C), Vitamin C (0.1-0.2%, central value is 0.15%)

2.5. Spectrophotometer analysis: Color changes of the treated samples were measured at 538 nm with an UV/Vis spectrophotometer [13]. Colorimetric method is used L*a*b color space system (Technical Services Department Hunter Associates Laboratory, Inc.)

2.6. Statistical analysis: All measurements were done in triplicate, except the center value was repeated 5 times. Mode 5 software is used to design experiment and analyse data for experiments. Analyses of Variance (ANOVA) were conducted by using SPSS Version 13.0 for Windows (SPSS). Turkey tests were performed to test the significant differences between the mean values for treatments ($p<0.05$).

3. Results and Discussion

3.1. Preliminary survey the extracted betacyanin content from the type of flower

Surveyed 3 kinds of flower: bud flowers, bloomed flowers and fade flowers in the rainy season and dry seasons. The results showed the bloomed flower, which was harvested in dry season, gave the highest content of betacyanin.

Bougainvillea is well grown in the tropical climate, sunshine preferred. So, in the dry season, flowers are brilliantly and have dark colors. In the rainy season, flowers are sparsely and have light color. Bloomed flowers just have "physiological ripeness" and the highest pigment content. The solute pigment from bud flowers hasn't absolutely formed yet (or not converted yet). The solute components from fade flowers are decomposed or transformed to a new substance, reduced amount of pigment in flowers.

3.2. Survey the optimized betacyanin extraction condition

When the solvent was water, the received productivity of betacyanin extraction is highest (0.032%). The results showed that, water could extract betacyanin from *Bougainvillea* flower better than other researched solvent. This result is similar to the research of Castellar et al (2006) to extract betacyanin from the fruit of *Opuntia*; and Lim et al. (2011) to extract betacyanin from pitaya fruit pulps, with *water* as solvent [21].

The ratio solvent and raw materials is 5:1 and 6:1 giving the highest extraction efficiency (0.036% and 0.035%, respectively) and there was no big difference between the two rates. So that, the ratio of 5:1 was chosen for the other studies.

In the range pH = 3-6, the amount of obtained betacyanin increased gradually from 0.03% pigment content and reached the optimum at pH = 6 with 0.037% pigment. When pH was higher than 6, the amount of received betacyanin was decreased; this may be due to degradation of betacyanin when it was outside the optimal pH conditions. Comparing the received betacyanin with couple of pH, pH = 3 and pH = 3.5, pH = 4 and pH = 4.5, pH = 4.5 and pH = 5, pH = 5 and pH = 5.5, pH = 6.5 and pH = 7, the changes of betacyanin content were no difference. According to some other studies, the extracted betacyanin content depends on the pH, and stable in a wide range of pH = 3-7 (Jackman & Smith, 1996). The optimal pH range for betanin stability is 5 - 6 [3,7,8]. Lim et al., 2011 also reported that the highest concentration of extracted betacyanin from pitaya fruit pulps in the water solvent when the value of pH of solvent was 6 [21]. Therefore, the results showed that the optimum pH

at 6 is quite consistent.

The optimum extraction time was 30 minutes with the highest received betacyanin (0.038%). If the extraction time was shorter it could be not enough to all betacyanin extraction, but if longer, the pigment could be destroyed and reduced [2]. According to Von Elbe et al, preserving betanin liquid in the air, the regression of the pigment increases to 15% and depends on the atmospheric conditions. Betanin react with oxygen molecule, the result is reducing pigment [2].

The optimum extraction temperature was 30^{o}C with the highest received betacyanin (0.041%). The lower temperature was not enough to all betacyanin extraction; and the higher extraction temperature was affected on the quality of pigment and decreased the amount of received betacyanin [5].

3.3. Survey the optimizing of ratio of solvent and raw material and extraction pH.

The experiment was conducted according to a central composite design, with the following variables: solvent pH (5.0–7.0, center value is 6), solvent-to-*Bougainvillea* ratio (4:1–6:1, center value is 5). The extraction time was fixed at 30 min and the extraction temperature was fixed in 30^{o}C. Conducting experiments and results are in the table 1.

Table 1: *Results of optimizing the ratio of solvent with raw materials and pH extraction effect of extracted betacyanin pigments from Bougainvillae flower.*

Samples	M1	M2	M3	M4	M5	M6	M7	M8	M9
The ratio of sovent and raw material (w/w)	4:1	6:1	4:1	6:1	3.586:1	6.414:1	5:1	5:1	5:1
pH	5	5	7	7	6	6	4.586	7.414	6
Betacyanin content (mg)	8.34	8.95	8.54	9.29	8.52	9.59	8.72	8.26	10.40
The ratio of Betacyanin and raw material (%)	0.042	0.045	0.043	0.046	0.043	0.048	0.044	0.041	0.052

The optimal result was by Modde 5 (Fig.1) with $Q^2 = 0.849 > 0.7$ and $R^2 = 0.976 > 0$. The results are absolutely suitable with the optimal model. The regression equation of betacyanin pigment extracted efficiency depending on the ratio of solvent with raw material and extraction pH was as following:

$$Y = 0.0522 + 0.0016\ X_1 - 0.0034\ X_1^2 - 0.0049\ X_2^2$$

(X_1 is the ratio of solvent with raw material value, X_2 is the extraction pH value).

Optimal results obtained after empirically verifiable were: the ratio of solvent with raw material was 5.2471 and extraction pH was 5.9669; predictable efficiency was 0.0524%.

According to theory, the betacyanin content change following to pH, however, betacyanin is relatively stable in a wide range of pH = 3-7 [20]. Optimal pH range for betanin stability maximum is 5 - 6 [3. 7. 8]. Therefor, the surveyed results showed that the best of pH value to betacyanin extraction was approximate 6.

The more solvent was used, the more solute was extracted. However, low a_w (moisture) will improve the stability of betacyanin pigment, the stable pigment will decrease when a_w increased from 0.32 to 0.75 [4]. Therefore, the moisture content must be not too high because the betacyanin content will be destroyed.

3.4. Survey the optimizing of extraction time and extraction temperature

The experiment was conducted according to a central composite design, with the following variables: solvent temperature (20–40°C, center value is 30^{o}C) and extraction time (20–40 min, center value is 30 minutes).

The optimal result was treated by Modde 5 (Fig.2) with $Q^2 = 0.759 > 0.7$ and $R^2 = 0.960 > 0.8$. The results are absolutely suitable with the optimal model. The regression equation of betacyanin pigment extracted efficiency depending on the extraction time and extraction temperature was as following:

$$Y = 0.0532 + 0.0007X2 - 0.0021X_1^2 - 0.0031X_2^2$$

(X_1 is the extraction time, X_2 is the extraction temperature).

Table 2: *Results of optimizing the extraction time and extraction temperature effect of extracted betacyanin pigments from Bougainvillae flower.*

Sample	M10	M11	M12	M13	M14	M15	M16	M17	M18
Time (min)	20	40	20	40	15.86	44.14	30	30	30
Temperature (^{0}C)	20	20	40	40	30	30	15.86	44.14	30
Betacyanin content (mg)	9.57	9.76	9.36	9.11	9.8	10.01	9.63	9.39	10.63
The ratio of Betacyanin and raw material (%)	0.048	0.049	0.047	0.046	0.049	0.050	0.048	0.047	0.053

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Optimal results obtained after empirically verifiable were: the extraction time was 30.6146 min, extraction temperature was 28.8512^{o}C, and predictable efficiency was 0.0532%.

To extract out all pigment, the enough time was necessary. However, the sample exposure to the oxygen in the air when extending the extraction time will change the structure of betacyanin molecule, and reduce the amount of the obtained color [19]. Therefore, the extraction time must not be too long.

Betacyanin is destroyed at high temperature [12]. But the extraction process also needs a sufficient temperature to speed up the diffusion betacyanin out of materials. Therefore, if the temperature is too low, it will decrease the extraction effect and extend the extraction time; while high temperatures will degrade betacyanin pigments due to un-stability by the heat and reduce the extraction effect.

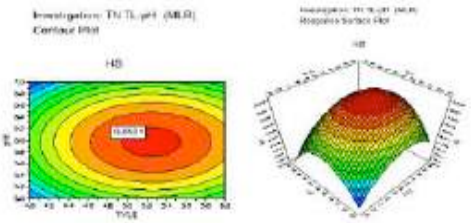

Fig.1*: Contour pilot and response surface pilot of survey the optimization of ratio of solvent and raw material and extraction pH*

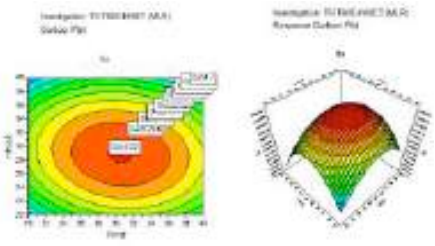

Fig.2*:Contour pilot and response surface pilot of survey the optimization of extraction time and extraction temperature*

3.5. Survey the optimized storage conditions

pH affected the color stability of betacyanin. The color of extracted betacyanin was stable in the pH range 4-7. Temperature also affected the color stability of betacyanin. At higher temperatures, the betacyanin content decreased faster. The heating time was longer, the color stability of betacyanin decreased faster. It can be caused because of the heat treatment process; betanin can be degraded due to isomerization; or by releasing CO_2 during reactions; or by cleavage of the molecule. The result was to reduce the intensity of the final color and appear red or light brown color [8].

The presence of ascorbic acid increased the color stability of betacyanin. This may be due to ascorbic acid has the ability to prevent pigment degradation or improve betalain regeneration. The amount of obtained color is gradually increased when the intake of vitamin C was added to the pigment liquid from 0.05% to 0.15%. However, when 0.2% vitamin C was added, the amount of pigment was decreased. Additional amount of ascorbic acid is 0.15% would be resulted in the best.

3.6. Survey optimum of storage temperature and the concentration of added vitamin C

Fixed the optimal parameters as follows: ratio of solvent and raw material was 5.2471, extraction pH was 5.9669, extraction time was 30.6146 min and extraction temperature was 28.8512^{o}C

The experiment was conducted according to a central composite design, with the following variables: storage temperature (15-35^{o}C, center value is 25^{o}C), added Vitamin C (0.1-0.2%, central value is 0.15%).

The increasing of time, temperature, light, pH changes, and oxygen-air impacted on the degradation of betanin (the main ingredient in betacyanin). The more storage time increased, the more oxygen penetrated. The oxidation reaction and the betacyanin pigments decomposition reaction were under the influence of oxygen and light, which penetrated before. Therefore, the longer storage time was, the more decreasing of betacyanin concentrations increased.

Table 3: *Results measured betacyanin content in the samples over time (mgL^{-1})*

Sample	Temperature (^{o}C)	VitaminC (%)	Storage time					
			0 day	1 day	3 days	7 days	10 days	15 days
M19	15	0.1	480.7	471.5	461.2	455.1	441.8	415.4
M20	35	0.1	480.7	480.9	465.0	428.0	363.9	233.6
M21	15	0.2	480.7	473.7	463.8	445.9	439.8	423.4
M22	35	0.2	480.7	416.5	401.0	394.8	333.3	254.9

M23	10.86	0.15	480.7	463.0	463.7	455.6	444.2	432.0
M24	39.14	0.15	480.7	369.8	292.9	249.2	175.3	66.6
M25	25	0.0793	480.7	424.2	411.0	393.4	354.8	251.4
M26	25	0.2207	480.7	462.0	433.5	416.1	347.8	287.3
M27	25	0.15	480.7	452.0	432.4	420.4	362.5	299.3
M0	25	0	480.7	425.2	417.5	399.3	359.7	270.6

Sample M24 was added 0.15 % vitamin C and kept at a temperature of 39.14^{o}C. This temperature was relatively high combined with long preservation time led to the decline betacyanin concentration. During storage at high temperatures, betanin could be degraded by the isomerization reaction (isomerization), decarboxylation - chemical reaction which liberated CO_2 (rejection of a C atom in a carbon chain of molecule) or molecular cleaved (by heat or acid). The results led to decrease in red and eventually appeare light brown [8]. The reduction of betanin also led to form neobetain, so the solution turned to yellow. The cleavage of isobetanin and betanin could also be caused by the base [23, 24], leading to the creation of the bright yellow of betalamic acid and cyclo- dopa-5-O-colorless glycoside. Besides, the color of betanin was maintained when occurring isomer reactions or chemical reactions, which liberated CO_2 on C15. When occurring the C17 removed reaction in molecule to form CO_2, it made a change spectral position in the absorption, reflection, transmission or emission spectrum of a molecule to create a shorter wavelength from 538nm to 505nm, the result was red orange appearance [5].

Concentration of pigment, which was added vitamin C of 0.15%, had limited regression speed of betacyanin by high temperature because vitamin C is a good antioxidant agent [5].

When the storage temperature of samples M19, M21, M23 were 15°C (M19, M21) and appropriate 11oC (M23), the stability of betacyanin pigment was inversely proportional to the temperature increasing. Thus, at low temperature (cool temperature), pigments were relatively stable, additional the support of antioxidants like vitamin C; betacyanin content was relatively stable during 15 days of storage.

Sample M0 had lower storage temperature than samples M20, M22, but it was not added vitamin C so that the content was more decreased. Therefore, vitamin C support to the stability of betacyanin during the preservation process

4. Conclusion

The fresh blooms of *Bougainvillae* flowers, which were harvested in the dry season, gave the highest quantity of betacyanin content. The optimal parameters of the extraction condition with distilled water were: the ratio of solvent and raw material was 5.2471; extraction pH was 5.9669, extraction time was 30.6146 min, extraction temperature was 28.8512^{o}C. The obtained pigment was preserved at temperature of 15^{o}C, added vitamin C of 0.15%. The pigment content was 53.86 mg/100g raw materials. This result was higher than previous studies from other materials. For example, Wu and colleagues studied extracted betacyanin from dragon fruit (*H. polyrhizus*) in Taiwan was 10.3 ± 0:22 mg/100 materials; Tang and Norziah studied the color stability of betacyanin pigments in red dragon fruit was 10.1± 0.6mg/100 materials with methanol solvent or 9.8 ± 0.6 mg/100g raw materials with water solvent; Wybraniec and Mizrahi extracted betacyanin from fruits which were grown in Israel was 28 ± 1.9 mg/100 materials. From the above results, it is necessary to continue researching to improve the process of extraction, changing obtained betacyanin from aqueous solution into easy form to use [10].

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Response of different banana cultivars (AAA and AA group) to 1-MCP microbubble immersion

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Abstract

1-MCP microbubble immersion is a novel and effective technology for maintaining quality, antioxidant properties and chemical composition of banana. However, a response of different banana cultivars to 1-MCP microbubble immersion (500 ppb) has not yet been reported. The changes of quality and some selected antioxidant properties in banana cv. 'Hom Thong' (AAA group) and cv. 'Khai' (AA group) to 1-MCP microbubble immersion were investigated. Untreated banana from both cultivars served as the control. The bananas were sampled at two days intervals for 8 days during storage at 25±2°C and evaluated for changes in physical properties and chemical composition. The ethylene production rates of both banana cultivars showed the same trends during the initial stage of storage and especially, in 'Hom Thong', it was suppressed strongly by 1-MCP microbubble immersion. The respiration rate and weight loss in 'Hom Thong' were also suppressed significantly by 1-MCP microbubble immersion compared with those of 'Khai' banana. Total soluble solids concentration continuously increased during storage in untreated 'Hom Thong' banana while only a slight change was observed in the other treatments. Hue angle value of untreated 'Hom Thong' banana sharply declined during storage while it only slightly decreased in the other treatments. Fruit firmness showed the same trends among treatments at the initial stage of storage but it subsequently decreased in 'Hom Thong' faster than 'Khai' but at slower rates in both cultivars when treated with 1-MCP. Total phenolics content of 'Hom Thong' banana was significantly higher than 'Khai' bananas throughout storage but was consistently lower in 1-MCP microbubble treated than in untreated fruits. In contrast, total ascorbic acid content in all treatment increased during storage especially when either banana cultivar was immersed in 1-MCP microbubbles. Overall, 1-MCP microbubble immersion maintained quality and some antioxidant components of banana cv. 'Hom Thong' and 'Khai'. 'Hom Thong' responded more to 1-MCP microbubble immersion compared with cv. 'Khai' indicating that the response of fruit to 1-MCP treatment is cultivar dependent.

Keywords: Banana, 1-MCP microbubble, Qualities, Antioxidant

1. Introduction

For a number of years, the gaseous anti-ethylene compound 1-methylcyclopropene (1-MCP) has been reported to have inhibitory action on the effect of ethylene [1]. Recently, 1-MCP has been used commercially as a postharvest tool for maintaining the quality of numerous horticultural products including banana [2]. Many researchers have found that 1-MCP fumigation maintained the quality of banana [2,3,4,5]. Furthermore, 1-MCP fumigation controlled changes in the chemical composition including some antioxidant properties in different fruits such as pineapple [6], mandarin [7], strawberry [8,9,10], tomato [11,12], apricot [13] and apple [14,15].

Microbubble technology is a new technology, which is being used in a number of different fields in the food and postharvest sectors. Microbubbles are small bubbles with 10-50 μm diam. and under

this technique can dissolve some gases in- to different solvents successfully due to the high pressure inside of the bubbles [16,17]. This technique has been explored for various sectors successfully with O_2 [18], CO_2 and O_3 [19,20]. This technology is also being use currently in the postharvest sector for 1-MCP immersion treatments [21]. However, response of different commodities to 1-MCP treatment depends on many factors [2]. Cultivar is a vital factor the responsiveness to 1-MCP in apple [22,23], grapefruit [24], tomato [25] and avocado [26]. However, information is very limited about the responsiveness of different banana cultivars to 1-MCP treatments, including 1-MCP microbubble immersion. The present work aimed to study the responsiveness of two Thai banana cultivars ('Hom Thong', AAA group and 'Khai', AA group) to 1-MCP microbubble immersion treatment with specific regard to quality and some antioxidant properties.

2. Materials and Methods

2.1. Preparation of plant materials

Banana fruits (Musa sp., AAA group, cv. 'Hom Thong' and Musa sp., AA group, cv. 'Khai') were obtained from a commercial market in Bangkok at the 80% mature stage. De-handed bananas were transported to the laboratory and fruit samples were selected for uniformity based on green color, size and freedom from defects. Thereafter, selected fruit hands were cut into clusters with 3-4 fruit fingers each, washed in 0.5% $MgSO_4$ solution to remove the latex from the cut surfaces [27], rinsed in tap water, and then dipped in 100 ppm sodium hypochlorite solution as a disinfection treatment.

2.2. 1-MCP microbubble treatments

Prepared samples of each banana cultivar were randomly divided into two lots with one lot being immersed with 500 ppb 1-MCP microbubble solution for 15 min with continuous microbubble generation at 25±2°C. The 1-MCP microbubble solution was prepared using a microbubble generator that used the decompression method [28]. Bananas without 1-MCP treatment were used as the controls. Bananas treated with 1-MCP microbubbleS were allowed to air dry before storage. All treatments were stored at 25±2°C, RH 85% for 8 days and samples were withdrawn for analysis at 2 day intervals.

2.3. Measurements of parameters

To determine fruit weight loss the weight of fruit (g) was measured using an electronic scale with three decimals and the percentage of weight loss was calculated. Peel color changes of banana fruits were measured with a colorimeter (Model CR-400, Konica Minolta, Japan) and fruit firmness was measured using a fruit texture analyzer (Model; TA-XT2, Stable micro- system, England) incorporating a 3.5 mm diam cylinder probe.

To determine respiration rate and the rate of ethylene production, four banana fruits were selected randomly and fruit were put into 1,000 mL air tight plastic chambers for 3 h at 25ºC. Gas samples (1 mL) were taken and injected in-to at gas chromatograph ('Shimadzu' GC–8A Japan) for determination of the amount of CO_2 and C_2H_4 production. TSS concentration was measured on a fresh juice sample by using a digital refractometer (Brix 0- 32%; ATAGO, Model NI, Japan) while total ascorbic acid concentration was measured following the DNPH method [29]. Absorbance was determined at 540 nm using a UV visible spectrophotometer (Shimadzu, UV-1601, Japan). A standard curve was prepared using standard ascorbic acid with concentrations of 20, 40, 60, 80 and 100 ppm.

Total phenolics concentration was measured using the Folin-Ciocalteau method [30]. Absorbance of the resulting blue colored solution was determined at 765 nm using a UV visible spectrophotometer. A standard curve was prepared using standard ascorbic acid with concentrations of 20, 40, 60, 80 and 100 mg L-1. The experiment was conducted with three replicates in a completely randomized design. Analysis of variance (ANOVA) was performed using the statistical analysis system program version 9.0. Duncan's multiple range test was used for determining means separation. The means were compared using the least significant differences (LSD) test at a significance level of 0.05.

3. Results and Discussion

Ethylene production of the controls increased rapidly during storage but the ethylene production of 1-MCP microbubble immersed bananas of both cultivars was suppressed compared with the controls (Fig. 1). The suppression of ethylene production of both 1-MCP treated bananas was similar in initial stage of the storage but it was more marked in 'Hom Thong' in the stages storage. Respiration rate also rapidly increased in the control fruits of both cultivars and it was suppressed successfully in both 1-MCP treatments. However, the suppression of respiration rate in 1-MCP treated 'Hom Thong' banana was about two times greater than that in 1-MCP treated 'Khai' bananas (Fig. 1). 1-MCP is considered to be an effective treatment for maintaining the quality of banana. It retards ethylene production, respiration, ripening, senescence and metabolic changes [31,4,32,33]. Fan et al. [34]

observed that the efficacy of 1-MCP depends on cultivar and storage conditions in apples and Ambuko et al. [35] reported that the efficacy of 1-MCP is affected by various pre-harvest factors including species, cultivar and maturity stage. Present results provide good evidence that response of 'Hom Thong' to 1-MCP was greater than that for 'Khai'. These results showed that 1-MCP microbubble immersion (500 ppb for 15 min) suppressed ethylene production in both banana cultivars. Similar results have been reported by Golding et al. [36]. 1-MCP may block the normal feedback regulation of ethylene production and the associated ethylene biosynthetic enzymes, such as ACC oxidase, ACC synthase and associated mRNA accumulation [37]. The suppression of the ethylene production rate was similar in the initial stages of storage for both banana cultivars but it was more marked in banana cv. 'Hom Thong' after day 4. Previous studies have found that cultivar is an important factor in responses to 1-MCP and that the efficacy of 1-MCP depends on cultivar in many fruits [34,22,24].

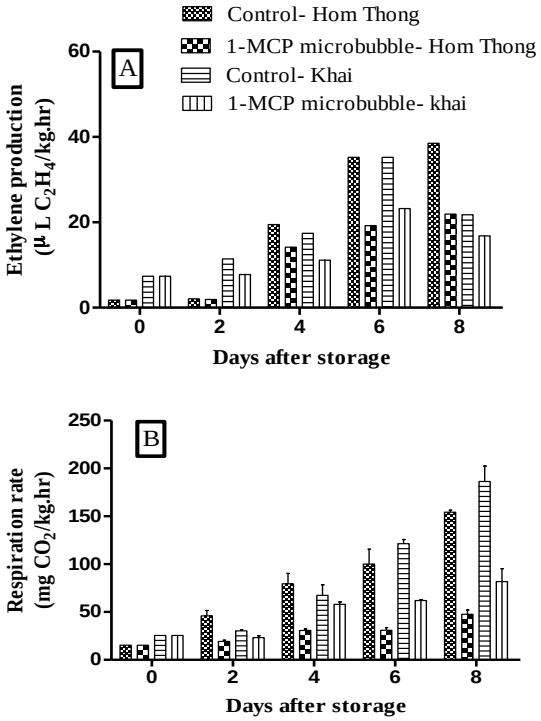

Fig. 1 *The response of banana cv. 'Hom Thong' and cv. 'Khai' to 1-MCP microbubble immersion on ethylene production (A) and respiration rate (B).*

The weight loss of 1-MCP microbubble immersed bananas of both cultivars was lowered compared with the controls. The rate of weight loss was suppressed more successfully in 'Hom Thong' than in 'Khai' (Fig. 2). Present results showed that 1-MCP microbubble immersion reduced weight loss in both banana cultivars. This reduction of the weight loss could be the result of reduced respiration rate as 1-MCP microbubble treatments suppressed respiration rate in both banana cultivars. In general, 1-MCP has been shown to reduce or delay the increase in respiration rate in other species [2]. Previous studies have reported that 1- MCP treatment inhibited the increase of ethylene-induced respiration in both tomato [12] and banana [4]. However, the suppression of weight loss was more marked in 'Hom Thong' than in 'Khai' during the overall which could have been the result of a stronger reduction in the respiration rate in 'Hom Thong' which was about two times higher than those in 'Khai' bananas.

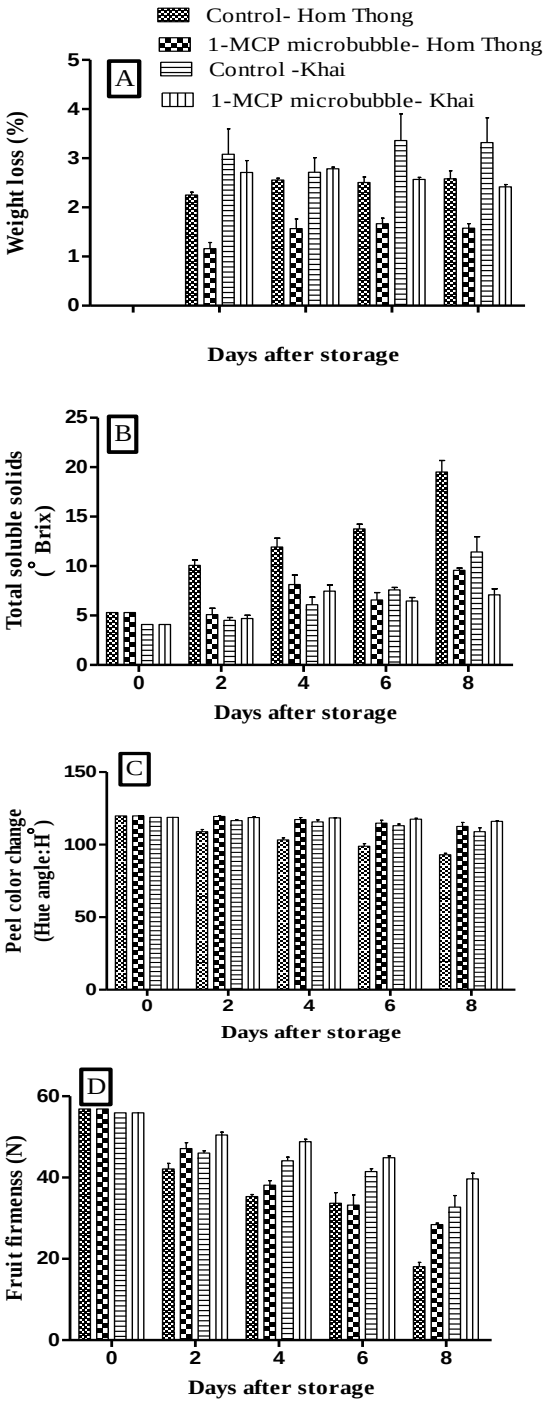

Fig. 2 *Response of banana cv. 'Hom Thong' and cv. 'Khai' to 1-MCP microbubble immersion (500 ppb) on weight loss (A), TSS (B), peel color change (C) and fruit firmness (D).*

Hue angle value and fruit firmness both

declined during storage but in 1-MCP treated fruits of both cultivars the values were well maintained compared with the controls (Fig. 2). TSS concentration in the controls of both banana cultivars rapidly increased during storage. The 1-MCP microbubble treatment restricted this increase in TSS concentration, especially in 'Hom Thong' compared with 1-MCP treated 'Khai'. Hue angle in 1-MCP treated fruits of both cultivars was well maintained compared with the controls. Many previous studies have found similar results with different fruits, including bananas [38,32]. The level of hue angle value maintained in 1-MCP treated 'Hom Thong' banana was significantly higher from days 2 to 8 which coincide with the lower ethylene production level in this cultivar. Similarly, fruit firmness of 1-MCP microbubble treated fruits in 'Hom Thong' was also significantly higher after day 6 compared with 1-MCP treated 'Khai'. The strong maintenance of fruit firmness in 'Hom Thong' was directly correlated with the good maintenance of hue angle value and these were the main factor associated with delayed ripening in 1-MCP treated 'Hom Thong' compared with 'Khai'.

Total ascorbic acid concentration of all treatments with both banana cultivars increased during storage. The 1-MCP treated bananas of both cultivars exhibited higher ascorbic acid concentration values during each stage of storage compared with the controls. Banana cv. 'Hom Thong' treated with 1-MCP microbubble had the highest ascorbic acid concentration after day 6. The trend of increasing in ascorbic acid content was similar in both 1-MCP treated cultivars during the initial stages of the storage (Fig. 3). Total phenolics concentration of 1-MCP microbubble immersed fruits in both cultivars was lower than in the control fruits. 1-MCP treated bananas of both cultivars exhibited a suppression of total phenolics from day 2 to day 8 but it was more marked in 'Hom Thong' over this period (Fig. 3). In general, 1-MCP increases the total ascorbic acid concentration in banana [39,40] and inhibits or delays the production of free phenolic compounds of many fruits during ripening [6,8,9]. This present study also exhibited similar results as 1-MCP treatment of both cultivars increased total ascorbic acid concentration and suppressed the total phenolic concentration during storage, especially in 'Hom Thong'.

Information is not readily available, however, on the efficacy of 1-MCP microbubble immersion with different cultivars of bananas. Previous studies with many different fruits have demonstrated the efficacy of 1-MCP including differences across different cultivars [34,15,25]. Beaudry and Watkins [41] showed that the response of apple fruit to 1-MCP depended upon cultivar which they termed 'cultivar sensitivity'. Furthermore, many papers are available about this cultivar sensitivity in apple [34,15,42]. However, Ambuko et al. [35] reported that no varietal differences in response to 1-MCP between 'Tommy Atkins' and 'Apple Mango'. In the present study, we observed a difference in efficacy of 1-MCP on two cultivars of banana where 'Hom Thong' was more sensitive to 1-MCP microbubble treatments than 'Khai'.

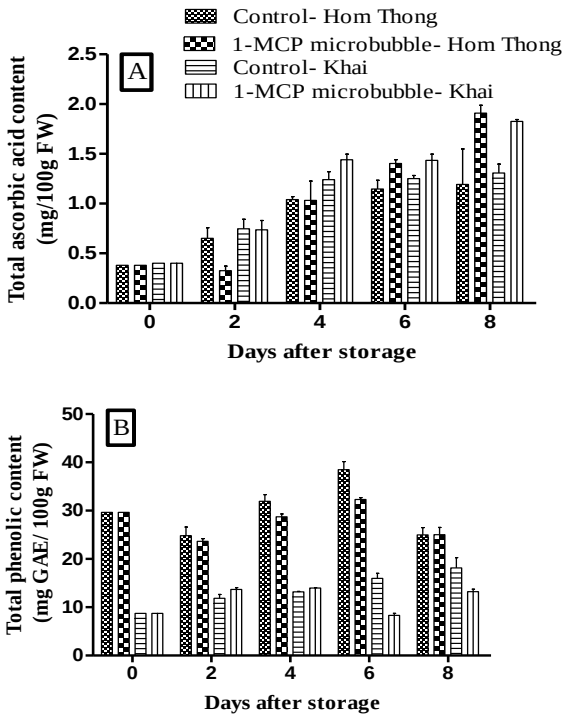

Fig. 3 *Response of banana cv. 'Hom Thong' and cv. 'Khai' to 1-MCP microbubble immersion (500 ppb) on total ascorbic acid concentration (A) and total phenolics concentration (B).*

4. Conclusion

1-MCP microbubble treated fruits of banana cv. 'Hom Thong' and cv. 'Khai' had suppressed ethylene production, respiration rate and weight loss and had successfully maintained peel color, firmness and total ascorbic acid concentration. The suppression of ethylene production, respiration rate and weight loss in the cultivar 'Hom Thong' was higher than in the 'Khai'. Maintenance of peel color, fruit firmness and total ascorbic acid concentration were also higher in 'Hom Thong'. Hence overall sensitivity of 'Hom Thong' to 1-MCP was higher than for Khai. We conclude that the efficacy of 1-MCP microbubble immersion depends upon the cultivar of banana.

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Survey the quality of the banana (*Musa troglodytarum*) that is in different growing condition in Lam Dong, Vietnam

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Abstract:

Banana (*Musa troglodytarum*) is the tropical plant, which is grown much in Vietnam and in other Asian countries. In Vietnam this tree is grown well at the temperature from 25 – 35oC. For the general development the banana requires much of water (15 – 20L/day).

The aim of this study is survey the general method to grow the special type of banana (Laba) in different garden of Lam Dong, Vietnam, than to find out the best condition for this banana growing. The experiment was conducted with the following variables: forms of farming, fertilizer regime, irrigation method, kinds of fertilizer, fertilizer using, harvested time. The results are followed to the quality of the products, such as: reduce the level of fruit weight, fruit color, soluble solids, starch, total sugar, reducing sugar, fruit firmness, total acid concentration, concentration of ethylene gas.

Keywords: *Musa troglodytarum*, Banana, plant growing, banana fruit, quality

1. Introduction

Banana (*Musa troglodytarum*) is tropical tree, which is grown much in Indian, China, Malaysia, Vietnam, Philippine, Ecuador, Brazil, Indonesia, Colombia and some of East Africa countries.

Banana grows well in the temperature range 25 – 35^{o}C. When the temperature dropped to 10^{o}C, the fruits will be small, poor quality and slow growth. In conditions of prolonged frost, banana leaves will be gray and wilt. The water content of banana trees is in high such as: 92.4% in elder tree, 96% in roots, 82.6% in leaves and 96% in fruits. The water evaporation of leaves is high; under the sun, it is about 40-50mg/dm^2/min. Water demanding of banana depend on the shady or sunny weather (15-20 liters/day). Bananas have the ability to adapt in the large range light intensity.

Bananas chemical composition is mainly water, sugar and polysaccharide, organic acids, nitrogen compounds, aromatic substances, pigments, polyphenols and vitamin compounds. The chemical composition of bananas is obviously difference between green banana (green bananas after harvest) and completely ripened bananas. Because bananas contain a high amount of water, so the physiological and biochemical reactions are easy formation to increasing respiration and digestion of nutrients, resulting in heat and water evaporation during storage, causing chemical composition changes.

Laba banana is one of the few agricultural products of Vietnam that has pretty much conquered the difficult markets in the world. Laba banana has beautiful shape, long and slightly curved, thin skin when ripening, bright yellow, yellow pulp, flexible, sweet and aromatic characteristics. Laba banana is an agricultural product that is exported to the Chinese market, the Middle East and especially the Australian market with high prices. The aim of this study was focusing on the growing condition for the special type of banana (Laba) in different garden of Lam Dong, Vietnam, than to find out the best condition for this banana growing.

2. Materials and Methods

2.1. Materials: The banana (*Musa troglodytarum*) fruits that were harvested from different farms in Lam Dong province after 55 days (still green) and after 60 days (the ripening is beginning) when fruit is forming.

2.2. Survey the conditions of banana farms: the quality of the banana was tested after harvesting 10 days, from different banana farms, which were under different growing condition, such as: forms of farming, fertilizer regime, irrigation method, kinds of fertilizer, fertilizer using, harvested time.

Table 1: *The growing condition of 1st banana group (GP01)*

Parameter	**Farm**									
	F04	**F05**	**F 14**	**F 15**	**F 21**	**F 23**	**F 24**	**F 25**	**F 28**	**F 30**
Area (m^2)	500	800	800	700	800	1000	1000	2000	1500	1500
Number of grove	50	80	60	50	80	100	100	200	150	120
Tree height (m)	3 - 4	3 - 4	3	3.5	3	3.5	4	3 - 4	4	3 - 4
Density of grove (m)	3x3	3x3	3x4	3x4	3x3	3x4	3x4	3x3	3x3	3x4
Time of reducing small tree/year	12	9 – 12	12	12	12	9 – 12	12	12	9 – 12	9 – 12
Fertilizer using (kg/grove/ year)	Cattle manure = 1, NPK= 0.5	Organic fertilizer = 1, NPK= 0.5	Cattle manure = 2, NPK= 0.2	Organic fertilizer= 2, NPK= 0.3	Cattle manure = 1, NPK= 0.2	Organic fertilizer = 2, NPK= 0.3	Organic fertilizer = 1, NPK= 0.5	Organic fertilizer = 2, NPK= 0.3	Organic fertilizer = 2, NPK= 0.2	Cattle manure = 2, NPK= 0.3

The 1st banana group (GP01) from the small farm (500-2000m^2), with low density of banana grove (3x3 – 3x4 m/grove), in the bazan soil, only banana growing, 1 harvesting time/year; the schedule of fertilizer regime (1-2kg cattle manure or organic fertilizer and 0.2-0.5kg NPK/grove) is 3 times during growing of tree (young, adult, before fruit forming); full irrigation is 2 times/year in dry season; the area of covering of grove is 3m^2; reducing small tree is 9-12 times a years; harvesting time is after 60 days when fruit is forming.

Table 2: *The growing condition of 2nd banana group (GP02)*

Parameter	**Farm**									
	F02	**F06**	**F 07**	**F 11**	**F 11**	**F 12**	**F 16**	**F 18**	**F 20**	**F 29**
Area (m^2)	5000	2000	7000	5000	2000	5000	9000	4000	7500	10000
Number of grove	100	50	100	100	80	120	150	100	150	200
Tree height (m)	4	3	3.5	3.5	4	3.5	3-4	3.5	4	4
Time of reducing small tree/year	4-6	3-4	3	3 - 4	4-6	4	4-6	3	3-4	4-6
Fertilizer using (kg/grove/ year)	NPK = 0.2	Organic fertilizer = 1, NPK= 0.5	Cattle manure = 1, NPK= 0.1	Organic fertilizer = 2, NPK= 0.3	NPK = 0.3	Urea = 0.05, Potassium = 0.05	Organic fertilizer = 1, NPK= 0.1	NPK = 0.3	Organic fertilizer = 1, NPK= 0.1	NPK = 0.5

The 2nd banana group (GP02) from the medium farm (2000-10000m^2), with low density of banana grove (50-200 grove/farm), in the bazan soil, with other tree growing, 1 harvesting time/year; the schedule of fertilizer regime (0-1kg cattle manure or organic fertilizer and 0.05-0.5kg NPK/grove) is 1 times during growing of tree (before fruit forming); full irrigation is 2 - 4 times/year in dry season; the area of covering of grove is 3m^2; reducing small tree

is 3-6 times a years; harvesting time is after 60 days when fruit is forming.

Table 3: *The growing condition of 3rd banana group (GP03)*

Parameter	Farm									
	F01	**F03**	**F 08**	**F 09**	**F 13**	**F 17**	**F 19**	**F 22**	**F 26**	**F 27**
Area (m^2)	10000	12000	20000	15000	10000	25000	15000	20000	25000	15000
Number of grove	150	200	100	200	100	200	150	150	250	200
Tree height (m)	4	3	3.5	3.5	4	4	3-4	3.5	4	4

The 3rd banana group (GP03) from the large farm (10000-25000m^2), with low density of banana grove (100-250 grove/farm), in the bazan soil, with other tree growing, 1 harvesting time/year, no using fertilizer, no using irrigation system; the area of covering of grove is 3m^2; reducing small tree is not happened; harvesting time is after 60 days when fruit is forming.

2.3. Analysis: The bananas, which were storage in 10 days in the moisture 60-65% at the temperature of 25 – 35^oC, were tested with parameters: reduce the level of fruit weight, fruit colour (comparing with colour standard), soluble solids [12], starch [8, 2], total sugar [2], reducing sugar [7, 2], fruit firmness (with Instron machinery and software), total acid concentration [3], concentration of ethylene gas (via FID (Flame Ionisotion Detector) theory with GC-8A machinery of Japan)).

2.4. Statistical analysis: All measurements were done in triplicate. Using Modde 5 software to perform optimization. Analyses of Variance (ANOVA) were conducted by using SPSS Version 13.0 for Windows (SPSS). Turkey tests were performed to test the significant differences between the mean values for treatments ($p<0.05$).

3. Results and Discussion

3.1. Effect of the condition of growing on the soluble solids of banana

In the nutrient composition of the fruit, the soluble solids content is an important property, which represents the concentration of dissolved nutrients in fruit storage. Soluble solids content decides the quality, taste, and structure of fruit ... If the soluble solid content in fruits increases rapidly, the ripeness level of the fruit will increase, and the damaging time also will increase rapidly. In contrast, if the soluble solids content increases slowly, the ripening of the fruit will grow slowly, the damaging time will be prolonged, the storage time increases. In all the treatments of studies, the soluble solids content of fruit increased during the storage period. The reason is that during ripening, under the action of endogenous enzymes like pectinase, the protopectin changed into pectin, along with the conversion of starch, cellulose and hemicellulose into sugars to make the concentration of juice increased significantly, while the evaporation occurs naturally in the preservation process also to make the insoluble solids concentration increasing.

Table 4*.Effect of the condition of growing on the soluble solids of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	**2**	**4**	**6**	**8**	**10**	**11**
GP 01	60 days	3.63^{a}	5.27^{bc}	7.57^{d}	14.95^{d}	17.33^{e}	24.07^{c}	
	55 days	3.32^{cd}	5.10^{de}	6.89^{f}	14.19^{f}	17.70^{c}	25.60^{b}	
GP 02	60 days	3.58^{b}	5.17^{cd}	7.82^{c}	14.55^{e}	17.48^{d}	23.37^{d}	
	55 days	3.17^{e}	5.00^{e}	6.93^{e}	15.12^{c}	17.82^{c}	24.14^{c}	Destroyed
GP 03	60 days	3.44^{bc}	5.34^{ab}	7.87^{b}	15.79^{a}	19.28^{b}	26.26^{a}	
	55 days	3.11^{ef}	5.42^{a}	7.92^{a}	15.45^{b}	19.59^{a}	26.44^{a}	

3.2. Effect of the condition of growing on the starch content of banana

Starch plays an important role in the ripening of the fruit. Starch is the substrate for respiration of the fruit. Starch amount is reduced, because the banana is climacteric fruit that have ability to change starch into sugar through a typical reaction. In 1987, V.A.Adisa and E.N.Okey studies showed that banana starch amount decreased significantly from 34% to 10%, at the same time, glucose concentration increased from 8.6 to 40%, after 1 week storage at 28^oC [1]. The speed of starch amount decreasing will determine the storage time.

If the amount of starch decreased rapidly, breathing process of fruit will be faster, so the hydrolysis and transformation process play faster, the fruit will be damaged faster. The survey results of the effect of the growing conditions on the changing of starch amount during storage time in the garden are shown in Table 5.

***Table 5**. Effect of the condition of growing on the starch content of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	**2**	**4**	**6**	**8**	**10**	**11**
GP 01	60 days	24.13^{a}	18.06^{b}	12.64^{e}	9.26^{d}	6.46^{d}	5.73^{c}	Destroyed
	55 days	23.70^{b}	18.01^{c}	12.01^{f}	8.89^{e}	5.80^{e}	5.20^{e}	
GP 02	60 days	23.25^{c}	18.02^{c}	14.80^{c}	9.67^{c}	7.48^{b}	**5.34^{d}**	
	55 days	23.11^{d}	17.23^{e}	13.12^{d}	8.11^{f}	6.21^{d}	**4.88^{f}**	
GP 03	60 days	23.16^{d}	18.33^{a}	17.67^{a}	12.10^{a}	8.16^{a}	6.05^{a}	
	55 days	23.19^{cd}	17.92^{d}	15.00^{b}	10.56^{b}	7.01^{c}	5.99^{b}	

Starch content of all surveyed treatments decreased during storage. The reason is that after harvesting, the phosphorylaza enzyme, α-amylase, β-amylase still react in banana fruit, catalyze the hydrolysis of starch into sugars, dextrin, maltose and glucose, which are the materials for the process of cell respiration. The amount of starch hydrolyzed slowly, and when fruit fully ripe, the remaining amount of starch is very little [13]. Fruits of the group GP 02 has the lowest amount of decreased starch

***Table 6**. Effect of the condition of growing on the total sugar content of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	**2**	**4**	**6**	**8**	**10**	**11**
GP 01	60 days	1.85^{c}	4.45^{b}	6.49^{c}	12.45^{c}	15.54^{b}	19.41^{d}	Destroyed
	55 days	1.99^{a}	4.50^{b}	7.01^{a}	13.60^{a}	15.77^{a}	20.11^{a}	
GP 02	60 days	1.93^{b}	4.01^{c}	6.71^{b}	11.70^{d}	14.74^{c}	**19.28^{de}**	
	55 days	1.92^{b}	4.02^{c}	6.98^{a}	11.90^{d}	15.90^{a}	**19.89^{b}**	
GP 03	60 days	1.82^{c}	4.70^{c}	6.54^{a}	12.28^{c}	14.06^{e}	19.24^{e}	
	55 days	1.79^{d}	4.60^{a}	6.34^{d}	13.01^{b}	14.30^{d}	19.55^{c}	

3.3. Effect of the condition of growing on the total sugar and reduced sugar content of banana

In banana, when the starch converse into sugar, the starch content will reduce and the sugar content increase to help the fruit ripening to be more sweeter and getting more aroma. In addition, the accumulation of sugar in maturity not only by the saccharification of starch hydrolysis but also by other substances such as sucrose, celluloses, hemicelluloses, pectin, lignin that are forming sugar like xylose, mannose, galactose, ... making the increasing of total sugar content during storage. The increasing speed of sugar content affect on the shelf life of fruit. If the sugar content increased quickly, the respiration of fruit will react strongly to the hydrolysis and the metabolism process faster, so the fruit will be damaged faster and the shelf life will be shorter.

Total sugar and reduced sugar content in all treatments were increased. In the first period time the total sugar increased more than reduced sugars. After short time, a reduced sugar increased sharply over the first time. The sugar content increases in storage time when the fruit is ripening because the starch is hydrolyzed into sugars by enzymes α-amylase, and β-amylase. Some of polyol transform to the reduced sugar because of acid and invertase reaction; therefore, reduced sugar in the fruit will increase [6]. Sugar content in the fruit will gradually increase during storage, in the end of the preservation process, the total sugar and reduced sugar of fruit will be higher [11].

***Table 7**. Effect of the condition of growing on the reduced sugar content of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	**2**	**4**	**6**	**8**	**10**	**11**
GP 01	60 days	0.81^{c}	1.74^{e}	3.48^{d}	7.92^{b}	11.04^{d}	16.78^{c}	Destroyed
	55 days	0.81^{bc}	2.22^{a}	3.67^{b}	8.23^{a}	12.00^{b}	17.54^{a}	
GP 02	60 days	0.82^{b}	1.89^{c}	3.02^{f}	6.06^{e}	**10.99^{d}**	**16.45^{e}**	
	55 days	0.80^{c}	2.01^{b}	3.56^{c}	7.28^{c}	**10.62^{e}**	**17.00^{b}**	
GP 03	60 days	0.82^{ab}	1.81^{d}	3.18^{e}	5.82^{f}	11.64^{c}	16.24^{f}	
	55 days	0.84^{a}	1.90^{c}	4.38^{a}	6.71^{d}	12.37^{a}	16.66^{d}	

The sugar transformation is not the same between treatments. In the 3 gardens, the fruits were

taken care with different modes (different fertilizer levels and different types of fertilizers) and harvested at different times so the preservation process, speeds of metabolism and accumulation of sugar will not be the same. When the plants get enough nitrogen, the color and flavor of fruits were improved, so the nutritional quality was better and storage time was longer.

3.4. Effect of the condition of growing on the total acid content of banana

Total acid content of the samples in the experiment were slightly higher during the first 04 days of storage, then decreased gradually in the following days. The reason is in the preservation process, the fruits continued respiration, metabolising or combinating the substances including acids [10]. According to Nguyen Thi Bich Thuy (2010), in the first phase of the preservation process, the physiological disorders makes respiratory rate increasing to convert a number of substances into fatty acids and unevaporative esters making the concentration of total acid growing in fruit [9]. On the final stage of the preservation process, the fruits start to degrade, the total acid is hydrolyzed and oxidised. Simultaneously, the amount of vitamin C in fruits was also significantly reduced by the tissue reduction process and air entering, making the total acid decreasing in the fruit. Therefore, in the fruits the acidity is reduced and sweetness increased.

***Table 8**. Effect of the condition of growing on the total acid content of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	2	4	6	8	10	11
GP 01	60 days	311.65^{d}	423.49^{d}	506.16^{b}	459.67^{a}	375.83^{a}	306.17^{b}	Destroyed
	55 days	311.55^{e}	430.00^{c}	489.01^{e}	444.64^{c}	310.91^{f}	292.38^{e}	
GP 02	60 days	318.67^{a}	497.65^{a}	522.46^{a}	444.90^{b}	374.68^{b}	305.40^{c}	
	55 days	3.12.45^{c}	487.67^{b}	500.23^{c}	401.01^{f}	329.82^{d}	299.23^{d}	
GP 03	60 days	315.60^{b}	408.53^{e}	491.69^{d}	443.79^{d}	371.67^{c}	310.37^{a}	
	55 days	309.09^{f}	390.01^{f}	414.17^{f}	434.78^{e}	317.00^{e}	278.00^{f}	

3.5. Effect of the condition of growing on the firmness of banana

The firmness of the fruits helps to assess the level of physical damage or mechanical harvest. During storage, the fruit is more ripening; the hardness is decreasing because of the loss of water in the skin and the transformation of the chemical composition in fruit. Part of the insoluble solids converted into soluble substances, such as: starch was hydrolyzed into sugars; insoluble protopectin was converted into soluble pectin.

***Table 9**. Effect of the condition of growing on the firmness of banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	**2**	**4**	**6**	**8**	**10**	**11**
GP 01	60 days	24.16^{a}	21.41^{a}	17.64^{a}	11.51^{a}	6.17^{a}	1.05^{c}	Destroyed
	55 days	23.13^{c}	20.11^{d}	16.22^{c}	10.00^{d}	5.66^{b}	0.97^{cd}	
GP 02	60 days	**23.52^{b}**	**20.93^{b}**	**17.55^{a}**	**11.41^{b}**	**6.15^{a}**	**1.30^{a}**	
	55 days	**23.00^{d}**	**20.56^{c}**	**17.00^{b}**	**11.32^{c}**	**6.15^{a}**	**1.20^{b}**	
GP 03	60 days	21.58^{e}	19.96^{e}	8.81^{d}	3.83^{e}	2.50^{c}	0.85^{d}	
	55 days	21.23^{f}	19.56^{f}	8.43^{e}	3.67^{f}	2.14^{d}	0.60^{e}	

The firmness decreases with storage time. In this study, after harvesting the banana of GP 02 achieve the best structure. Under the same conditions and storage time, to ensure the quality of the fruit, the good fruit should be chosen that contains high levels of nutrients and harvests at the right time when fruit is maturity; if harvesting time is later or earlier, the quality of the fruit will drop rapidly during storage [4]. The results obtained that the fruit in the group GP 02 will give the most stable firmness.

3.6. Effect of the condition of growing on the weight loss of banana

During storage, the mass of all the surveyed samples were reduced. This means that the mass of the fruits were decreased by storage time. The evaporation process occurred the mass reducing. The main cause of the water loss is due to the difference pressure between surface of fruit and

external environment and the results led to the evaporation process. The dehydration negatively affects on the metabolic processes to breaking the normal metabolism of the cells. The dehydration also reduces the cell growth, brittleness and mass; and makes the wither of the fruit to reducing the commercial value. During storage, the nutrients loss more when the respiration is higher. According to Grierson and Wardowsky (1978), after the post-harvest the dehydration reduces the mass of fruit that is ripening [5]. The evaporating of water in the plant cells can stimulate the production of ethylene, resulting in an increasing in respiratory of the cells [14]. The reducing of the mass is not the same between experiments. The reason is that there are differences method of planting banana tree and harvesting time. With suitable watering mode, right and enough fertilizing, while preserving the fruit mass reducing would decrease greatly. The results showed that the fruit from the GP 02 farms reduced the weight at the lowest volume.

Table 10. Effect of the condition of growing on the weight loss of banana during storage

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	2	4	6	8	10	11
GP 01	60 days	0.00	2.62^{b}	5.38^{e}	7.18^{f}	9.87^{e}	11.91^{f}	Destroyed
	55 days	0.00	2.76^{a}	5.67^{d}	8.00^{c}	11.00^{c}	12.78^{b}	
GP 02	60 days	0.00	2.43^{d}	5.68^{d}	7.35^{e}	9.64^{f}	12.35^{e}	
	55 days	0.00	2.55^{c}	5.99^{c}	7.68^{d}	9.98^{d}	12.67^{c}	
GP 03	60 days	0.00	2.35^{e}	7.55^{b}	9.30^{b}	11.86^{b}	12.45^{d}	
	55 days	0.00	2.39^{d}	7.76^{a}	10.01^{a}	12.19^{a}	12.89^{a}	

3.7. Effect of the condition of growing on the ethylene producing from banana

During the storage period, the process of respiration is still ongoing in the fruit. When the fruit is ripe the intensity of respiration is stronger and the ethylene producing is higher. Then, the amount of ethylene producing stimulates the respiratory intensity to continue the increasing the amount of ethylene producing, to continue the effect on the stimulating. If the respiratory intensity is strong, the shelf life of fruit will be short. These are factors that adversely affect on the process of preserving bananas. In the fruit the intensity of ethylene production in all experiments increased day-by-day during storage and increased quickly after from 4 to 6 days. The reason is that after 6 days, the fruits would be ripening

***Table 11**. Effect of the condition of growing on the ethylene producing from banana during storage*

Group of farm	Time of harvesting after fruit forming	Storage days						
		0	2	4	6	8	10	11
GP 01	60 days	0.00	0.70^{b}	0.90^{e}	**1.80^{f}**	2.24^{ef}	2.84^{f}	Destroyed
	55 days	0.00	0.68^{a}	0.89^{d}	**2.00^{c}**	2.38^{c}	3.07^{b}	
GP 02	**60 days**	**0.00**	**0.80^{d}**	**0.90^{d}**	**1.70^{e}**	**2.22^{f}**	**2.90^{e}**	
	55 days	**0.00**	**0.87^{c}**	**0.92^{c}**	**1.84^{d}**	**2.32^{d}**	**3.02^{bc}**	
GP 03	60 days	0.00	0.70^{f}	0.80^{b}	**1.80^{b}**	2.44^{b}	2.95^{d}	
	55 days	0.00	0.79^{e}	0.90^{a}	**1.86^{a}**	2.52^{a}	3.15^{a}	

3.8. Effect of the condition of growing on the color changing of banana

Color is one of the indicators that shows the maturity and ripening of the fruit. The fruit skin color is one of the key features that the consumers determined to fruit ripening level. For banana during storage, fruit skin color in all experiments gradually changed from green to yellow on 6th day and then the yellow-brown spots and deep black areas were exited after 10th day. This is one of the first signs of ripening and occurring the damage. This process occurs due to the resolution when the chlorophyll structure was breaking down, under the action of the chlorophyllase. When the chlorophyll content decreased, the skin of the fruit will gradually revealed the carotenoids and flavonoids pigments to making the skin color changing from green to yellow and appearing the dark spots appearance on the surface of fruit due to the oxidation of phenolic compounds in the skin.

Storage days	GP 01	GP 02	GP 03
1			
2			
4			
6			
8			
10			

***Fig 1**. Effect of the condition of growing on the ethylene producing from banana during storage*

4. Conclusion

During cultivation, there are many factors that impact on the quality of banana. Through the control indicators such as: reduce the level of fruit weight, fruit color, soluble solids, starch, total sugar, reducing sugar, fruit firmness, total acid concentration, concentration of ethylene gas. The results show that after harvesting, the quality of fruits is good and stability at the best, when the fruits were grown in the GP 02 (medium farm (2000-10000m^2), with low density of banana grove (50-200 grove/farm), in the bazan soil, with other tree growing, 1 harvesting time/year; the schedule of fertilizer regime (0-1kg cattle manure or organic fertilizer and 0.05-0.5kg NPK/grove) is 1 times during growing of tree (before fruit forming); full irrigation is 2 - 4 times/year in dry season; the area of covering of grove is 3m^2; reducing small tree is 3-6 times a years; harvesting time is after 60 days when fruit is forming).

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Use of hazardous chemical, coating materials, coloring substances in fruits and vegetables on human health and the environment

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Abstract:

Fruits and vegetables are highly nutritious and form as key food commodity in the human consumption. They are highly perishable due to their low shelf life. These food commodities are reported to be contaminated by toxic and health hazardous chemicals. Chemicals like calcium carbide; higher doses of ethylene etc. are reportedly being used in fruit for artificial ripening of fruits and for increasing the size of fruits etc. Moreover, formalin also used for extending the shelf life of fruits vegetables also causes several health problems. Calcium carbide more commonly was known, as 'masala' is a carcinogenic agent and banned under PFA Rules. Recently in Bangladesh peoples are consuming toxic fruits and vegetables, which are ripened by a hazardous chemical, calcium carbide. This poses great health risks to consumers. A significant number of pregnant women consume fruit ripened with carbide, the children born with abnormalities. Moreover, the widespread use of formalin, in preservation of fruit and vegetables are posing a threat to public health. The chemical used as a solution in water keeps makes fruits like mangoes attractive. Use of non-edible coloring materials and toxic coating materials in fruits for extending shelf life and attractiveness also cause serious health hazardous in Bangladesh. This paper is mainly focuses on the use of hazardous chemicals, coloring substances in the fruits and vegetables and their effects on health hazard have been addressed. Research results conducted by the postgraduate students here in BAU-GPC (the largest fruit repository in Bangladesh) also addressed e.g. how to produce attractive, chemical free safe fruits for the consumers and use of edible coating like Chitosan, Aloe Vera gel, non-chemical ripening processes, non-chemical process of extending shelf life of different fruits has also been suggested.

Keywords: fruits and vegetables, coloring materials, hazardous chemical, indiscriminate use, and health hazard**.**

1. Introduction

There are about 70 types of fruits and 100 vegetables are grown in Bangladesh. The most widely cultivated fruits are mango, jackfruit, black berry, jujube, pineapple, litchi, guava, papaya, coconut, custard apple, wood apple, elephant apple, Indian blackberry, tamarind, cashew nut, pomegranate, Palmyra palm, rose apple, and Indian olive. There are many minor edible fruits too, which are locally available such as Burmease grape (latkan), monkey jack, rattan, river ebony, velvet apple, cowa, wild date palm etc. (1, 2). The major vegetables are brinjal, potato, tomato, cauliflower, cabbage, beans, aroids etc.

There are near about 24 Million household which are involve in fruit production and total land area is 0.45 million hectare, from where about 80% fruits are harvested and rest 20% comes from commercial orchard. The total fruit production per year is 1.8 million ton but we need 43.44 lac ton i.e. deficiency is 25.68 lac ton. In homestead, women are playing vital role in fruit cultivation. Among the produced fruits near about 83% land are occupied by mango, banana, papaya, jujube, jackfruit, litchi, pineapple **(Fig.1**). But the fruit growers are not getting optimum yield due to insufficient knowledge of production techniques, inadequate supply of quality planting materials, post harvest losses etc.

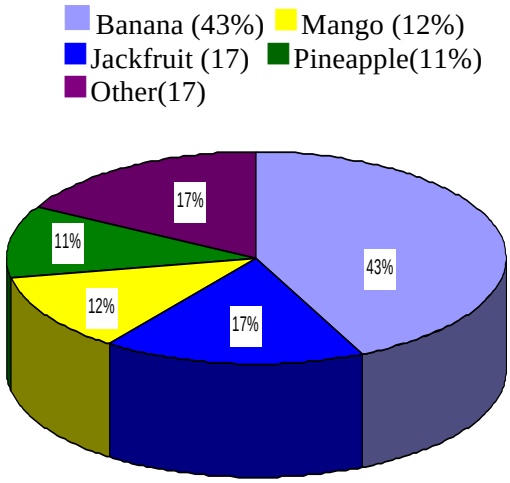

***Fig. 1**: Percentage (%) land cover by different fruits*

We need 115 gram fruit/day/head but we are now consuming about 77 gram/day/head. Hence 70% people are suffering malnutrition especially anemia disease severely found in children and woman of poor and extreme poor household. In Bangladesh major fruits are available (54% of total production) in May, June, July, August and these four months are treated as fruit festival months (Madhu mash) in Bangladesh. The rest 46% is grown in the rest 8 months. A small number of fruits (papaya, sapodilla, coconut and banana) are available throughout the year. Hence it indicates people are not getting nutrient from fruits round the year. To reduce malnutrition, it needs to use homestead space planning for year round fruit production. For this, mango, jujube and papaya were selected for poor and extreme poor household. Mango will give fruits in May-July, Jujube for February-March and papaya for round the year.

Fruit growing has no negative influence on the society and environment; rather it is socially and environmentally accepted. Farmers used frequent number of pesticide and fungicide (20-25 spray/season) spray for mango and litchi production which is harmful for both human body and the environment. Mostly male workers are engaged in fruit sub-sector; but female workers are engaged in homestead fruit production. Similarly in vegetables huge amounts of pesticides are used which is now making big problems.

2. Materials and Methods

2.1. Materials

2.1.1. Harmful Chemicals used in Fruits

- Indiscriminate use of pesticides
- Calcium carbide is reportedly being used in fruit for artificial ripening of fruits. Calcium carbide is a carcinogenic agent and banned under PFA Rules, 1955(USA).
- Formalin is used for extending the shelf life of fruits also causes several health problems.
- Industrial color for coloring the foods, fruits, vegetables etc
- Different non-edible coating materials
- Ether in excess (more than 100pmm)
- Industrial coloring materials also used for coloring the fruits and vegetables to get high market price.

2.1.2. Indiscriminate using of pesticides

Background of Synthetic chemical pesticide use in Bangladesh:

- 1955:Bangladesh entered into era of chemical pesticide with the importation of 3 MT of chemical pesticides.
- 1955-1973: Pesticides were distributed free of cost.
- 1973-1979: Pesticides were sold with half subsidized rate.
- 1979-onward: Subsidies on pesticide were withdrawn and opened to the private sector.
- During 2011-12 about 51,560 ton pesticides have been imported, spending about one hundred fifty thousands US$, where 43.79% is insecticide, 56.07% fungicides and 0.14% miticide (Alam, 2014). In Bangladesh during 1996-97 to 2009-10, pesticide consumption increased 328.4% and per ha pesticide use increased 598.8%. In 2010-11 for the first time a decline trend of pesticide use were observed (3397 MT less used than previous year)

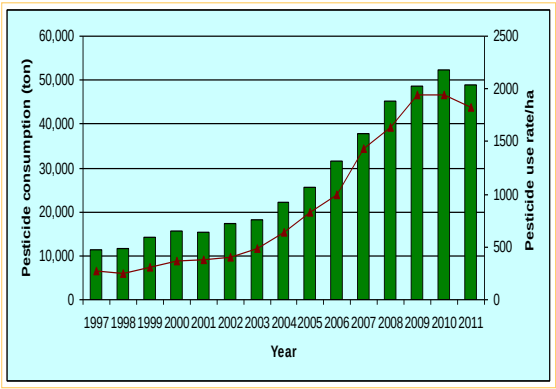

***Fig 2.** Year wise pesticide use pattern in Bangladesh (Source: PPW, DAE, 2012)*

2.1.3. Present Pest Management Scenario in Bangladesh

The worst scenario prevailed in the pest management arenas of the country, which has been improving in the recent past.

Indiscriminate use of synthetic toxic chemical pesticides (misuse and overuse, use of impure and improper, non-registered or sometimes banned pesticides) is a common scenario especially in the high valued crops, viz. some vegetables and fruit crops.

Eliminate the natural enemies of pests.

Upset the ecological balance.

Environmental degradation/ pollution.

Beyond the economics: farmer, practices and identities.

Enter food chain and lead to bioaccumulation and bio magnifications.

Suggestions

Extensive research work especially in the public sector should be undertaken for the development of effective and cheap bio-pesticide based IPM technologies against major insect pests & diseases of coconut.

Emphasis on biological control, host plant resistance should be given for sustainable management.

Extensive promotional works all over the country should be undertaken for the quick dissemination of the developed bio-pesticide based IPM technologies.

Private sector should assist in different aspect to develop the commercial venture of the bio-pesticides or microbial, so that those products can be available at the farm level.

Steps should be taken to give legal permission to the authorities and solve related issues for easy availability of different bio-pesticides.

2.1.4. Indiscriminate use of Calcium Carbide:

Recently in Asian countries peoples are consuming toxic fruits, which are ripened by a hazardous chemical, calcium carbide. This poses great health risks to consumers.

Calcium carbide has cancer-causing properties and is capable of causing neurological disorders. It can result in tingling sensation and peripheral neuropathy. If pregnant women consume fruit ripened with carbide, the children born could develop abnormalities.

"Free radicals from carbide play a major role in the ageing process as well as in the onset of cancer, heart disease, stroke, arthritis and allergies

The use of calcium carbide is not only toxic to consumers; it may also be harmful to those who handle it. It affects the neurological system, resulting in headache, dizziness, mood disturbances, sleepiness, mental confusion and seizures on a short-term basis, while in the long-term it can cause memory loss and cerebral oedema.

The use of carbide gas from calcium carbide to ripen fruits is extremely hazardous to the human body as calcium carbide also contains traces of arsenic and phosphorus. The practice of ripening fruits using carbide is banned in many other countries but it is freely used in many Asian countries.

In natural conditions, fruits get ripening by the action of a ripening hormone in the fruit, which produces ethylene naturally. The use of calcium carbide is dangerous as it produces acetylene gas, which is flammable and explosive even at a low concentration as compared to ethylene.

When calcium carbide is used for artificial ripening, a chemical reaction takes place because of the moisture content in the fruit. Heat and acetylene gas are produced and the ripening process is hastened. However, fruits ripened with calcium carbide are soft and have good peel/skin color but poor in flavor. They also have a shorter shelf-life. An artificially ripened banana would present a yellow outer skin, but the flesh inside may not be ripe and remains green and raw. During natural ripening, a wide spectrum of biochemical changes takes place such chlorophyll degradation, biosynthesis of carotenoids (antioxidants, immune system booster, anti-cancer agent), anthocyanins (powerful antioxidants), essential oils, and flavor and aroma components. But all these healthy chemical changes, which enhance the quality of the fruit, are lost with artificial ripening. Besides being toxic, artificially ripened fruits are less nutritious.

Fig.3*. Health hazard: Workers at a fruit market using calcium carbide to ripen raw mangoes*

In USA, Rule 44-AA of the PFA Prevention of food Adulteration Rules, 1955 prohibits the use of carbide gas for ripening of fruits.

"Rule 44-AA Prohibition of use of carbide gas in ripening of fruits: No person shall sell or offer of expose for sale of have in his premises for the purpose of sale under any description fruits which have been artificially ripened by use of acetylene gas, commonly known as carbide gas."

2.2. Methods

2.2.1. Indiscriminate using of Formalin

Formalin is a colorless strong-smelling chemical substance usually used in industry of textiles, plastics, papers, paint, construction, and well known to preserve human corpse. It is derived from formaldehyde gas dissolved in water. The widespread use of formalin, in preservation of fish, fruit and other food items is posing a threat to public health. The chemical used as a solution in water keeps fish fresh and makes fruits like mangoes attractive. This chemical, usually used to stop dead bodies from rotting, is now being used to preserve edible items.

Exposure from its gas or vapor can cause irritation to the eyes, nose and respiratory tract, causing sneezing, sore throat, larynx constriction, bronchitis and pneumonia. Multiple exposures can lead to asthma. It can also affect the skin, causing dermatitis or allergic reaction. Serious inhalation or ingestion can cause severe pain with inflammation ulceration and necrosis of the mucous membranes, which line almost every internal organ. This may show as symptoms of nausea, vomiting blood, diarrhea with bloody stool, blood from the urine, acidosis, vertigo, and circulation failure, then death. 30mL is suggested the lethal dose of formalin. The limit allowed in air that is still safe for human is less than 2 ppm.

2.2.2. Use of Ethrel /Ethylene /Ethephon

Excess ethrel are used to artificially ripen fruits. AS per PFA up to 100 ppm ethylene is permissible, but condition that its must be applied on physiologically mature fruits.

The other popular method is to ripen fruits specially bananas through heating in a closed environment lead to poor quality

Ministry of Agriculture (USA, India, Canada and others) has clarified that the fruits are exposed to ethylene gas (fruit ripening plant hormone) in low concentration of 10-100 ppm exogenously to trigger their ripening. It is considered safe in the concentration varying from 0.001-0.01% depending upon the crop, variety and maturity . There is no specific provision in PFA for ripening agents.

2.2.3. Coloring materials

Dyes: Eating foods containing industrial dyes and colors causes violent allergic reactions, respiratory problems, asthma, liver disorders and kidney dysfunction and bone marrow disorders. Nowadays, coal tar dyes are being used in sweetmeats.

Asthma caused by toxic dyes used in most of the fruits

The Canadian Food Inspection Agency (CFIA) is now warning the public not to consume the Heritage brand Palm Oil that contain a non-permitted color. This cause cancer in laboratory animals and also be significant for human health specially mental disorder, headache, allergy etc

2.2.4. Coating materials

The coating of foods especially fruits is a very old practice. Different fruits have been coated for many years and the practice will continue in the foreseeable future. Coating of fruits is carried out by applying a thin layer of wax on the surface. The coating process can be carried out by dipping, brushing or spraying with wax. This coating is normally referred to as edible coating. An edible coating is a thin layer that is applied on the surface of a fruit or vegetables, which is consumed together with the fruit. Edible coatings are considered to be safe for human consumption. Therefore, these coatings are expected to be consumed together with the fruits.

As per rule 48-E of the PFA Rules. 1955 fresh fruits and vegetables shall be free from rotting and also from coating of waxes, mineral oils and colors.

However, there is provision for coating fruits with food additive viz. bee wax (white/yellow) carnauba wax or shellac wax as glazing agent is accordance with the Good Manufacturing Practice for use of food additive under proper label declaration as defined in sub-rule (ZZZ) (24) of Rule 42.

Recently we are using Chitosan and Aloe vera gel, which are edible coating materials and safe from health hazards (4).

2.2.5. Ways to minimize likely-hood of buying waxed fruits:

The natural waxes applied on fruits are not known to have any negative effect on health. However, for some personal reasons you may feel it is good to eat un-waxed fruits. The simple ways to reduce the chances of buying waxed fruits.

- Grow your own (and after harvesting do not wax them).

- Buy fruits from markets in locality where that fruits are grown. There are high chances that the farmers have not waxed their fruits after harvesting.
- Buy the dull fruits. However, this is not a guarantee that they are not waxed.
- Check the label and buy un-waxed fruits.

2.2.6. Other contaminants that their source is in Fruit and vegetables and ill health effects?

Crop contaminants (aflatoxins, patulin, ochratoxin, etc.) naturally occurring toxic substances and heavy metals are the major contaminants found in fruit and vegetables. Heavy metals are present in the irrigation water and other manures. Infested seeds, irrigation water and soil act as the source of the fungal toxins. Pesticides can leave adverse effects on the nervous system. Some harmful pesticides can cause several hazardous diseases like cancer, liver, kidney, and lung damage. Certain pesticides can also cause loss of weight and appetite, irritability, insomnia, behavioral disorder and dermatological problems. The pesticide residue found in fruit and vegetables include residues of both banned (Aldrin, Chlordane, Endrin, Heptachlor, Ethyl Parathion, etc.) and restricted pesticides for use in India/Bangladesh (DDT, Endosulfan, etc.).

Heavy metals also cause adverse effect in human metabolic system, skin diseases, heart problems, etc.

2.2.7. The residue limits of different chemicals

Bee wax (white and yellow) or carnauba wax or shellac wax are permitted to be used in accordance with the Good Manufacturing Practice for use of food additives.

- Since use of carbide gas is prohibited in ripening of fruits under PFA, no tolerance limit for its residue is permitted.
- No tolerance limit for colour and mineral oil on fruits and vegetables has been allowed.
- The Maximum Residual Limit (MRL) of pesticide residues are given under PFA Rules, 1955 (Rule 65).
- The presence of heavy metal in the food item (fruit and vegetables) shall not exceed the value given under PFA Rules, 1955 (Rule 57).
- The presence of crop contaminants and naturally occurring toxic substances in fruit and vegetables shall not exceed the maximum limit prescribed under PFA Rules, 1955 (Rule 57A and 57B)

3. Results and Discussion

Select fruits and vegetable without spots or necrosis (lesions) and any abnormality.

Wash fruits and vegetables thoroughly with running potable water (preferably) before eating and cooking.

Purchase fruits and vegetables from known dealers.

- Peeling of fruits before consumption and vegetables before cooking will reduce exposure to pesticide.
- Do not buy and consume cut fruits from open market.
- Throw away fruits and vegetables infected by mould/fungus.
- To minimize the hazards of pesticide residues, discard the outer leaves of leafy vegetables such as lettuce and cabbage.
- Do not wash fruits and vegetables with detergents, as they may get absorbed inside.
- Ensure the quality of fruits and vegetables by sending them to voluntary testing laboratories.
- Wash your hands with soap and potable water, use clean utensil and clean cutting board with stainless steel knives
- Do not choose fruits that are attractive on the outside as they may not be good for health. Fruits that have a uniform color, for example, a bunch of bananas having a uniform color, are more likely to have been artificially ripened.
- Wash the fruits thoroughly before consuming. Keep them under running water for a few minutes, so that the chemicals are washed away.
- Do not buy fruits sold during their off season, as they are more likely to be artificially ripened.
- While eating mangoes and papayas, always remove the peel before cutting fruits into pieces.

3.1. How to identify chemically treated artificially ripened Mangoes?

Artificially ripened mangoes pose a major health risk. Most of the mangoes, which come in the month of March or first weeks of April, are artificially ripened using harmful chemical treatment like using calcium carbide. Greedy traders and fruit vendors want to make quick money by bringing in artificially ripened mangoes before the naturally ripened mangoes enter the market. Since natural ripening process takes time, the artificially ripened mangoes enter the market quickly much before and the prices will be high since demand is more, people are eager to taste the season’s mangoes and hence prices would be more too. The question is how to identify such artificially ripened mangoes in the

market, which are ripened using harmful chemicals like carbides?

These mangoes are plucked before reaching the physiological maturity and are not allowed to ripen naturally. Mangoes and other fruits usually produce ethylene gas, which fastens ripening. But these prematurely plucked mangoes can't produce ethylene, so vendors use calcium carbide to ripen them, which makes the mangoes look ripe within 12 hours. Calcium carbide is allowed to react with moisture that produces acetylene gas that causes the artificial ripening of the mango fruits. Acetylene is dangerous to the neurological system.

Such chemically ripened mangoes are a major health risk. Consumers who eat artificially ripened mangoes are can develop mouth ulcers, gastric problems and skin rashes. It can also cause diarrhea and in the longer run can lead to renal problems, infertility, and also cancer. They can also result in headaches, sleep disorders, mood disturbances, memory loss and even seizures. Besides this, the artificially ripened mangoes will not have the benefits of Vitamin A available in naturally ripened mangoes. The best way to avoid artificially ripened mangoes is not to buy mangoes till the end of April which is when the actual mango season kicks in. If there are mangoes in the month of March or first weeks of April then there are good chances of they being artificially ripened fruits. An artificially ripened mango will not smell right unlike a naturally ripened mango, which will have a good aroma! An artificially ripened mango will have a yellow outer skin like a naturally ripened mango but the tissue/flesh inside will not be ripe. An artificially ripened mango will be dry and less juicy compared to a naturally ripened mango that would have sucked enough water in the tree.

If there are green patches here and there no the yellow skin then it is most probably artificially ripened.

In artificially ripened mangoes, the tip of the fruit (that end which is attached to the tree) would still be raw, greenish in color, very hard and no juice oozes from the tip. This can be noticed just by sheer look at the tip. However, in a naturally ripened fruit, the tip will be golden yellow in color, soft and juicy when you touch it and show signs of ripening. A close observation will do the job for you.

Food safety and sanitation are considered to be a key issue to ensure overall food security in Bangladesh (5). Food is the major source of human exposure to pathogenic agents, both chemical and biological (viruses, parasites, bacteria), from which no individual is spared. The importance of food safety stems from: (i) food being the primary mode of transmission of infectious disease; (ii) the intricate linkage with development- governs individual and community health, national productivity, and promotes export potential & thus earn foreign exchange; (iii) emerged as prominent sources of conflict in international agricultural trade (7).

4. Conclusion

4.1. Regulation of Food Law: (5,6,7,8,9)

Implementation of Prevention of Food Adulteration Act and Rules rests with State/U.T. Governments. The Commissioner Food Safety/Food (Health) Authority of States/U.Ts. have been advised to keep strict vigil to check the use of carbide gas and other hazardous chemicals for ripening of fruits and coloring of fruits and vegetables and to take legal action for violation of the provisions of the Act/Rule.

4.2. Some recommendations for Action Plan for food safety

Review of laws/infrastructure/coordinating mechanism and provide technical assistance to update those towards regional harmonization.

- Review of standards and certification systems in purview of international requirements.
- Review of research and study program and help conducting research and study projects.
- Technical assistance in 10 years' training/ awareness building program.
- Assist in developing risk analysis infrastructure and making risk limits for adulterants/ contaminants.
- Provide support for publishing a regional food safety bulletin containing news and views on food safety data /events /information /development.
- Assist in establishing food safety cell or commission or council at the SAARC secretariat.
- All of the Asian countries should prioritize their efforts in establishing and evaluating priorities in food borne disease prevention & control.

Establish a Regional epidemiological network among the SAARC countries on all possible ways to combat FB-disease outbreaks, particularly the possible risks of being contaminated with a used range of FB-diseases in all countries of SAARC.

Chalk out long term & sustainable resources, means & ways to fight back FB-illnesses from respective countries.

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Designed composter device used for organic waste treatment by aerobic method with volume of 90 liters

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Abstract:

Day by day, huge amount of organic wastes, (such as vegetables, fruits and food wastes) have been produced from both domestic kitchens and canteen. To reduce pollution emissions and waste landfill, the composter equipment with household scale was designed to recycle these wastes into humus fertilizer (the compost)

Based on the theory of decomposition process of organic matter, composter was constructed with two compartments corresponding to the thermophile phase and Cool-down phase. Analytical results of compost samples obtained after 4 weeks showed that they had reached the basic norms of organic fertilizers. Additionally, the compost also gives positive effects and stimulation on crop growth [2]

Keywords: Composter, composting, organic waste treatments.

1. Introduction

Everyday, humans waste thousands of tons of garbage with biodegradable organic matters such as vegetables, tubers, fruits and leftover rice. These, if not handled properly or not processed will give odors causing serious environmental pollution. On the other hand, if properly handled, they could be a potential source of organic fertilizer used for gardening and crops in agricultural sectors. They could have the potential to replace inorganic fertilizers.

The aim of the present investigation was to design a composter that will allow producing compost from organic wastes in order to reduce wastage of garbage as well as producing fertilizer to be used for agriculture purposes.

2. Materials and Methods

2.1. Specifications of the composter device

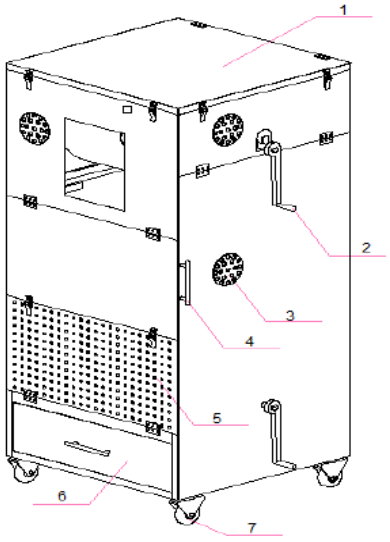

Fig.1. *General design of composter device.*

Descriptions: 1. covers equipment; 2. Winch; 3. Vents; 4. Handles; 5. Vents; 6. drip tray; 7. wheel

Table 2. *Specifications of composter device*

Numerical order	Size	Unit	Assizes
1	Height	cm	164
2	Length	cm	70
3	Width	cm	70
4	Weight	kg	78
5	Above can	lít	87
6	Bellowed can	lít	264
7	Spec. of front door	cm^2	70 x 52cm
8	Spec. of Side door	cm^2	70 x 50m
9	Spec. of Inlet door	cm^2	54 x 25cm
10	Spec. of tool storage area	cm^2	70 x 37cm
11	Drawer	cm^3	64x60x19cm
12	Materials	-	Inox

2.2. Experiment process

Materials for composting include kitchen wastes, weeds and plant debris

Soft materials: spinach, gourd, and mustard; Hard materials: skin of luffa, bark of gourd, broccoli, bark of chayote, ...

Initially, the average size of samples used are 7-22 cm, after processed, it changes into size of 0.2-3 cm

Table 2. *The temperature changes in the decomposition*

Material	Component (70% vegetables + 30% compost)		Day of May, 2012	T (^{0}C)
Spinach; gourd; mustard; bark of melon, gourd, chayote, broccoli and chayote Size 5 - 15cm.	Vegetables	Compost	19	30.0
	14 kg: sizes of 0.5-1cm	6 kg	20	35.0
			21	39.25
			22	39.0
			23	39.5
			24	37.5
			25	30.0

3. Results and Discussion

Initial After 5 -7 days Produced compost

Fig.2. *Compost producing.*

Table 3. *The results of sample analysis compost*

No	Items	Analysis Method	Analysis	Standards (38/2002/ QD-BNN)	Note
1	Humidity	10TCN 302-97	73%	< 25%	Over

No	Items	Analysis Method	Analysis	Standards (38/2002/ QD-BNN)	Note
2	pH	pH meter	6 – 6.5	5.0 – 7.0	Pass
3	Total Nitrogen	TCVN 8557-2010	3.38 g/kg	> 2.5%	Pass
4	Microbial density useful for plant	TCVN 4884:2005 (ISO 4833:2003)	4.2×106	$>10^6$ (CFU/g)	Pass
5	The total content of Carbon	10TCN 366-2004	7.74%	>13%	Under
6	The total content of Potassium	TCVN 8562-2010	2.9 g/kg	>2.5%	Pass
7	*E.coli*	TCVN 7924-2; 2008 ISO 16649-2;2001	-	LOD <10 CFU/g	Pass
8	Pb	TCVN 6496-2009	2.80 mg/kg	≤ 300 mg/kg	Pass
9	Cd	TCVN 6496-2009	0.019 mg/kg	≤ 2.5 mg/kg	Pass
10	Hg	TCVN 6496-2009	0.037 mg/kg	≤ 2.0 mg/kg	Pass
11	The decomposed	reached	reached	reached	Pass
12	Particle's diameter	reached	reached	4 – 5 mm	Pass

Tables 4. *Results of experiments of applying composter to bina*

Day	15.07.2012 (1st day)	29.07.2012 (14th day)	17.08.2012 (33rd day)	22.08.2012 (38th day)
	Drilling	Planted in pots (height)	Measure the height	Measure the height and weight (HxW)
1	Formula 1: 3.5 kg (0.7 compost + 2.8 kg soil)	Plant 1: 10 cm Plant 2: 10 cm	Plant 1: 23 cm Plant 2: 31 cm	Plant 2: 67 cm x 30.75g
2	Formula 2: 3.5 kg (0 compost + 3.5 kg soil)	Plant 1: 10 cm Plant 2: 10 cm	Plant 1: 16 cm Plant 2: 19 cm	Plant 1: 25 cm x 25.12g Plant 2: 28 cm x35.74g

The physical and chemical analysis showed that the criteria of the compost product such as pH, density of useful microorganisms, *E. coli*, Pb, Cd, Hg, ratio of decomposed ripening, particle diameter all reach the common standards for organic fertilizer under Circular No. 36/2010 / TT-BNN. Some others have not been achieved include total nitrogen and potassium in the compost product. It is the result of the input materials, which are mainly garbage, poor of nitrogen and potassium.

In addition to physical and chemical analysis, the compost products were also tested and applied to *Basella alba* for the effects of compost to plant growth and development. The results showed that plants fertilized with compost reach much better growth than the ones without supplemented compost.

4. Conclusion

The composter device is useful for households and cantin to reduce excess pollutants into the environment and society as well as actively and effectively produce compost from household organic wastes; easy to operate, has reasonable price and create quality compost positively replaced for inorganic fertilizers.

References

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[2]. Phuoc Nguyen Van (2011), Management and disposal of solid waste, Publisher National University of Ho Chi Minh Ciy