Proceedings of the 4th AFSA Conference 2018
on
FOOD SAFETY AND FOOD SECURITY

HELD AT ANGKOR PARADISE HOTEL SIEM REAP, CAMBODIA
ON AUGUST 10-12, 2018

Borarin Buntong
Antonio Acedo Jr.
Md. Latiful Bari
Ryohei Kada
Takashi Uemura

ASIAN FOOD SAFETY AND SECURITY ASSOCIATION

Proceedings of the 4th AFSA Conference 2018
on
FOOD SAFETY AND FOOD SECURITY

HELD AT ANGKOR PARADISE HOTEL SIEM REAP, CAMBODIA
ON AUGUST 10-12, 2018

Borarin Buntong
Antonio Acedo Jr.
Md. Latiful Bari
Ryohei Kada
Takashi Uemura

ASIAN FOOD SAFETY AND SECURITY ASSOCIATION

PROCEEDING EDITORS

Borarin Buntong, Ph.D.
Chairman, Conference Organizing Committee
Division of Research and Extension,
Royal University of Agriculture,
Phnom Penh, Cambodia;
E-mail: borarin@yahoo.com

Antonio Acedo Jr., Ph.D.
Secretary, Conference Organizing Committee
Mekong Institute (MI), Thailand
E-mail: junacedo@yahoo.com

Md. Latiful Bari, Ph.D.
Member, Conference Organizing Committee
Center for Advanced Research in Sciences
University of Dhaka, Dhaka-1000
Bangladesh
E- mail: latiful@du.ac.bd

Professor Ryohei Kada, Ph.D.
Shijonawate Gakuen University
Daitoh, Osaka 574-0011
E-mail: kada0113@gmail.com

Takashi Uemura, Ph.D. (AFSA President)
Professor Emeritus
Osaka Prefecture University
Kadoma, Osaka 571-0075 JAPAN
E-mail: takashi_uemura@msn.com

ISSN No: 2306-2150

Published by Asian Food Safety and Security Association (AFSA)
Email: afsa.secretariat@gmail.com

Price: BDT 1500, US$ 30.00 or JPY 6000 + E

Printed and Published in 2018 at Shijyonawate Gakuen University, Osaka, Japan

Asian Food Safety and Security Association

Printed at: osaka@live.com
Osaka, Japan

PREFACE

The passion 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 AFSSA Conference was held in Osaka on September, 2012 hosted by Osaka Prefecture University, JAPAN. The 2nd AFSA conference was held on August 15–18, 2014 at Dong Nai University of Technology, Bien Hoa City, Vietnam. The 3rd AFSA conferences which were held on September 15-17, 2016 at KIIT University, Bhubaneswar, India.This is the 4th AFSA conference which was held on August 10-12, 2018 at Angkor Paradise Hotel, Siem Reap, Cambodia.

The 4th AFSA Conference was jointly organized by AFSA, Royal University of Agriculture, Cambodia, Mekong Institute, Thailand. Sustaining of our passion, by holding the conference bi-annually, has been supported by private and public institutes/organizations of Asia Pacific Institute of Food Professionals (APIFP), International Committee on Food Microbiology and Hygiene (ICFMH), USAID, CIAT Asia Regional Office, Hanoi, Vietnam, Global Knowledge Center on Crop Biotechnology, ISAAA SEAsiaCenter, Los Banos, Philippines Horticulture Innovation Lab and Postharvest Technology Center, UC Davis, USA, and CESAIN, RUA, Cambodia, as well as by students, volunteers and financial supporters, and the Angkor Paradise Hotel, Siem Reap, Cambodia was the host for the 4th conference. Many distinguished guests have strongly encouraged us. We express our sincere thanks to all those hosts and supporters. 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.

This Regional Conference is qualitatively improving as an international meeting; researchers from 18 different countries joined in this 4th conference, where 250 higher levels of research works was presented and hot discussions were made in achieving food safety in this area.

This proceeding contains selected papers submitted by the presenters of different countries based on the presentation made in the conferences. All the submitted paper has been reviewed by the editorial board members and only few manuscripts were selected and recommended by the board members. The views expressed in the manuscripts are those of the authors, and the editorial members take no responsibilities for the content or comments, nor the views of Asian Food Safety and Security Association. However, it is believed that the book will act as a good source for relevant information in relation to food safety and food security.

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

Borarin Buntong,
Antonio Acedo Jr
Md. Latiful Bari
Ryohei Kada
Takashi Uemura

1. QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *SALMONELLA* SPP. LIGNIERES IN STREET-VENDED GRILLED CHICKEN INTESTINES

Abigail T. Atienza[1*], Ida F. Dalmacio[1], Nacita B. Lantican[1], Lotis E. Mopera[2]
[1]Institute of Biological Science, College of Arts and Sciences, University of the Philippines Los BañosCollege, Laguna, Philippines, 4031
[2]Food Science Cluster, College of Agriculture and Food Science, University of the Philippines Los Baños College, Laguna, Philippines, 4031
Corresponding author's Email: atatienza@up.edu.ph

Abstract

With the boost in consumption of street foods, concerns regarding their safety also heightened since these foods are prone to microbial contamination and therefore, can pose as health hazards. In Los Baños, Laguna, the prevalence study performed revealed that 15% of grilled chicken intestines and 9% of dips sold in the area were contaminated with *Salmonella* spp. A quantitative microbial risk assessment model (QMRA) with inputs from in-house experiments, vendors' and consumers' surveys, and published literature was simulated using Monte Carlo analysis to further estimate the probability of infection after consuming *Salmonella*-contaminated grilled chicken intestines. A mean probability of infection of 0.006113 per serving of grilled chicken intestines was calculated with ~137,000 annual predicted infection cases. Sensitivity analysis of the QMRA model identified three most correlated factors that contribute to the estimated risk of infection, i.e. concentration of *Salmonella* in grilled chicken intestines, consumption frequency, and the number of sticks consumed per eating session. Prevalence study of pathogen on the grilled product and the quantified probability of infection further concluded that *Salmonella* is indeed a significant hazard of concern. Integration of HACCP principles in the production based on the result of the risk assessment was also done to improve the process towards safety. Critical control points identified were boiling of raw material and stewing of boiled offal. Critical limits for the internal temperature and cooking time were set at ≥70ºC for 2 minutes to conform with zero infection estimate per annum.

Keywords: *Salmonella*, street foods, ready-to-eat foods, chicken intestines

1. Introduction

Around 2.5 billion people around the globe consume street foods each day (FAO, 2007). Accessibility and availability are considered as the major determinants of its popularity and continued existence (Winarno and Alain, 2015; Barro *et al*., 2007; Rane, 2011; Fellows and Hilmi, 2012). This attractiveness of street foods is brought about by the burst of population and rapid urbanization of the cities specifically in developing countries (Winarmo and Alain, 2015; Fellows and Hilmi, 2012). The street food trade not only offers inexpensive option for the consumers but also convenience for individuals who have no time to cook (Fernandez, 2002; Fellows and Hilmi, 2012). Street foods also provide opportunity of employment for the locals particularly for those who have limited education and skills (FAO, 2004). The socio-economic importance of street foods had paved the way for the evolution of the trade into a large and complex food sector where many consumers and small-scale entrepreneurs depend in a daily basis. Although street foods have emerged as a vital component of the global food chain, they are also deemed as public health risks. Hazards and violations of good practices can occur easily at every stage of street food production (Barro *et al.*, 2007). The consequence of serious food poisoning particularly by microbial contamination is mainly attributed to unsanitary environmental conditions, poor hygiene practices of food handlers and improper processing and storage before retail of street foods (Rane, 2011). These culpable practices facilitate introduction and proliferation of food-borne

pathogens to street foods that eventually lead to infection and intoxication after consumption. Typhoid fever, salmonellosis, cholera and other gastro-enteric diseases were reported as illnesses commonly associated with street foods (Abdussalam and Kaferstein, 1993).

Given the high probability of food borne illnesses upon consumption of street-vended foods, risk mitigation measures have been proposed to improve their safety (WHO, 1996). One of the measures recommended is the implementation of Hazard Analysis Critical Control Points (HACCP), a prevention-based system which identifies and monitors specific food safety hazards (WHO, 1996; Azanza and Gedaria, 1998). While HACCP significantly improves safety in any given food system, there are several areas where its application is limited due to reliance on qualitative considerations of hazards (Buchanan and Whiting, 1998; Mayes, 1998). More importantly, this program does not directly relate to public health goals (Buchanan and Whiting, 1998). By integration of quantitative microbial risk assessment (QMRA) elements on the HACCP system, these limitations can be overcome. QMRA gives a quantitative estimate of public health impact of a given microbial hazard and can be employed to identify processing, storage, and retail practices that can encourage pathogen introduction and proliferation through dynamic modeling (Buchanan and Whiting, 1998). In addition, ''what if'' scenarios can be done in QMRA to model instances and conditions that may create or reduce chances for microbial contamination to occur (Heidinger*et al*., 2009). As soon as these practices and scenarios are known, a more efficient HACCP program can be developed.

In the Philippines, street foods have become an essential part of the lifestyle of the Filipinos (Fernandez, 2002). Economic and cultural factors play key roles in the persistence of street foods in the country (Fernandez, 2002). Street food hawkers are seen everywhere with a variety of delicacies to offer. Among these local street delicacies, grilled animal by-products are the frequent offerings. One of the most sought street food offal is grilled chicken intestines locally termed as *isaw*. However, the risk associated with the consumption of this grilled product is quite high due to substantial density of pathogenic microorganisms in raw chicken intestines. Among other food-borne pathogens, contamination of grilled chicken intestines with *Salmonella* is considered a critical risk. It has been shown that intestines of healthy chickens naturally harbour and can be colonized by *Salmonella* in large numbers (Firildak *et al*., 2015). The organism is also repeatedly implicated in outbreaks concerning poultry and poultry products causing salmonellosis and typhoid fever infection worldwide (McEntire *et al.*, 2014). This study estimated the risk of consumers in Los Baños, Laguna of having *Salmonella*-related infection after consumption of street-vended grilled chicken intestines. With the results of the completed risk assessment as reference, HACCP principles were also implemented to identify strategies and measures to reduce the risk of *Salmonella* contamination in grilled chicken intestines.

2. Materials and methods

Bacterial cultures growth conditions

Stock cultures of all reference strains and *Salmonella* isolates were maintained in nutrient agar (NA) slants overlaid with mineral oil and in glycerol stocks stored in a biofreezer. Working cultures were prepared by reviving stock cultures in NA followed by incubation at 37 °C for 18-24 hours. Reference strains of *Salmonella* TyphimuriumATCC® 14028, *Escherichia coli* ATCC® 25922 and *Staphylococcus aureus* ATCC® 6533 served as quality control cultures for the enumeration and identification tests performed.

Prevalence of Salmonella in street-vended grilled chicken intestines and dips

Twenty (20) grilled chicken intestines and forty-four (44) sauces and vinegar dips were purchased from 20 randomly selected street-vending stalls in Los Baños, Laguna, Philippines from June 2015 to October 2016. Approximately 100g of the samples were collected in packaging provided by vendors at the start and end of their vending operations. Collected samples were placed in a cooler and immediately transported to the laboratory for processing. Twenty five (25) grams of each of the samples were placed in a sterile

sample bag and mixed with 225 ml of lactose broth (LB). Grilled chicken intestines were further homogenized using a mechanical stomacher. All samples in LB were held at 37 ºC for 24 hours for enrichment purposes. From the enriched samples, 1 mL and 0.1 mL aliquots were transferred to tetrathionate broth (TB) and Rappaport-Vassiliadis broth (RVB) and were incubated at 37 ºC for TB and 42 ºC RVB for 24 hours. A loopful from each of the enrichment media were streaked on xylose lysine deoxycholate agar (XLDA), bismuth sulfite agar (BSA), brilliant green agar (BGA) and MacConkey agar (MCA). All agar plates were further incubated at 37 ºC for 24 hours. Colonies showing typical *Salmonella* characteristics in each selective medium were picked and purified. After purification, isolates were stab-streaked in triple sugar iron agar (TSIA) and lysine iron agar (LIA) tubes. Color reaction, gas and H_2S production were noted after 24 hours of incubation at 37º C. Cellular morphology and Gram reaction of the presumed *Salmonella* isolates from TSIA and LIA tubes were also observed by viewing under the microscope after Gram staining (USFDA-BAM 2015). Identity of presumed isolates was confirmed by molecular analysis of isolates using *inv*A (Chiu *et al.,*1996) and *fim*Y (Yeh *et al.,* 2002) PCR-based *Salmonella* assays

QMRA model

A probabilistic risk assessment model was developed to estimate the risk of consumers of Los Baños, Laguna of contracting a *Salmonella*-related infection after eating street-vended grilled chicken intestines. This model considers steps from purchase of raw materials from sources to consumption of the grilled product by the target population. Scope and inputs for the QMRA model were decided based on the generalized production to consumption pathway for street-vended grilled chicken intestines which was created from the interviews and actual observations of the preparation and vending activities of grilled chicken intestines vendors with their permission.

Risk simulation, risk probability and sensitivity analysis

Risk simulation, risk probability and sensitivity analyses were executed using MS Excel 2007 with @Risk software Student Version 7.5 (Palisade, Inc., Ithaca, NY, USA). The QMRA model was implemented using Monte Carlo Simulation with a Latin Hypercube sampling scheme. Simulation or recalculation of the model was set at 100,000 iterations.

Scenario exploration

Scenario exploration was performed with the purpose of reducing the probability of *Salmonella* infection which will consequently lessen the predicted number of infection cases associated with grilled chicken intestines' consumption. This was done by altering the values of selected model variables which are determined by sensitivity analysis as the major determinants of risk of infection. In each simulation of mock scenarios, all other QMRA model inputs except the modified variable/s were set in the baseline scenario.

Hazard analysis, critical control points and critical limits determination

The conclusion whether *Salmonella* is a significant hazard or not was drawn from the result of the prevalence study performed on street-vended grilled chicken intestines and estimated probability of infection. For the critical control points (CCP) identification, the sensitivity analysis by Spearman correlation ranking was used to determine the steps of the production process which have the most significant effect on the concentration of *Salmonella* in the final grilled product. Lastly, critical limits for the identified CCPs were determined based on the resulting scenario that gave zero infection estimate per year to conform to the assigned zero tolerance standard for *Salmonella* in RTE (Ready-to-eat) foods.

3. Results and discussion

Prevalence of Salmonella in street-vended grilled chicken intestines and dips

Because of the undesirable conditions under which street vendors operate, there is a common assumption that contamination, either physical or biological in nature, is inevitable in street food

products (Winarno and Alain, 2015). To confirm if *Salmonella* is present and a hazard of concern in street-vended grilled chicken intestines sold in Los Baños, Laguna, the prevalence of the organism in the given product was determined. Grilled chicken intestines, purchased from ambulant and stationary grilling stalls across the town, were evaluated using the protocol intended to detect and identify *Salmonella*. Out of 20 samples tested, three (3) grilled chicken intestines (15%) were positive for the organism.Aside from grilled offal, sauces and vinegar concoctions used as dips were also examined for the presence of the concerned pathogen. It was found that *Salmonella* can thrive in sweet and spicy sauces but not in the vinegar dips. Two (2) sweet sauces and two (2) spicy sauces tested contained the pathogen, and this positive portion constituted 9% of the total dip samples.

QMRA model

The reported *Salmonella* contamination in street-vended grilled chicken intestinesand dips in the prevalence study conducted implies a health risk to the public particularly for the consumers residing in Los Baños, Laguna. Microbiological quality standards for Ready-to Eat (RTE) foods including grilled chicken intestines (NSW/FA, 2009) indicate that if *Salmonella* is detected in 25g sample, the given RTE food product should be considered as potentially hazardous. With the given hazardous category of the said street food, a QMRA model was developed to assess the risk of *Salmonella* infection associated with street-vended grilled chicken intestines. Table 1 summarizes the description and probability distribution of variables included in the QMRA model.The initial concentration and prevalence of the organism in the main raw material was modelled as Exponential and Beta distribution with parameters α=11 and β=1 based on the result of the prevalence study on raw chicken intestines (Atienza &Dalmacio, Unpublished article B). The Beta of positive samples was based on the total number of samples tested. A binomial distribution was also included in the QMRA to represent whether the chicken intestines used as raw material was contaminated with *Salmonella* or not. The same type of probability distribution was used to model the prevalence and the probability of the presence of the organism in the dips.For the thermal processing inputs, temperature, cooking time and thermal resistance of the organism were the variables considered. It was assumed that 55ºC, 62.5ºC and 70ºC were the minimum, most likely and maximum internal temperatures when cooking chicken intestines. These values were then used to model the inactivation temperature using PERT distribution. The PERT (Program Evaluation and Review Technique) distribution is a special case of BetaGeneral distribution which specifies minimum, most likely and maximum values for the identified variable. The cooking time was also modelled using PERT distribution with 2, 3.5 and 5 minutes entered for the minimum, maximum and most likely values. Since the data obtained from the vendors were in ranges, the median of 2-5 minutes which is 3.5 was assumed to be the most likely value. Thermal resistance of *Salmonella* was described using the D-value model (Atienza &Dalmacio, Unpublished article B) derived from inactivation experiments in pre-cooked chicken intestines. The number of log reductions achieved in the thermal processing steps was determined by dividing the cooking time by the D-value. The final *Salmonella* concentration after each cooking step was calculated by subtracting the number of log reductions obtained from the concentration of the pathogen before the cooking process. On the other hand, time, temperature and growth rate were the variables considered to describe the growth of the pathogen during storage and retail. Duration and temperature conditions at these stages were observed and noted while doing the production to consumption pathway. Storage time and vending operation were represented according to the distribution which incurred the lowest value as ranked by the Akaike Information Criterion (AIC) after fitting the storage and vending hours data through the distribution fitting function of @Risk software version 7.5 (Palisade, Inc., Ithaca, NY, USA). It was observed that prior to retail, pre-cooked chicken intestines were either stored at refrigerated temperature or at room temperature. Ambient storage temperature was represented as PERT distribution with 25, 27.9 and 38.5 ºC minimum, most likely and maximum temperatures for storage. These inputs were derived from temperature observations in Los Baños, Laguna

for the year 2016 collected by the DOST-PAGASA Weather Station. Although the lowest temperature observed was less than 25ºC, it was assumed that the said temperature is the minimum since the lowest temperature recorded is usually observed early morning, and there is a high chance that it will not be observed during vending operation which commonly starts mid-afternoon and ends before midnight. For the low temperature storage, a certain value of 4ºC was defined and it was assumed that temperature variation was absent when the pre-cooked product was stored in a refrigerator. This was done because the only available growth rate of *Salmonella* in pre-cooked chicken at low temperature range is at 4ºC. Data on the temperature variability during cold storage was also not available. With this assumption, growth rate for the low temperature storage was modelled by a normal distribution with standard error of ± 0.0004045 (Atienza &Dalmacio, Unpublished article A). On the other hand, the growth rate utilized for the ambient storage was described by the square root growth model generated using maximum growth rates of the chosen *Salmonella* isolate grown in pre-cooked chicken intestines at 25ºC to 40ºC (Atienza & Dalmacio, Unpublished article A). The log of growth during vendors' and consumers' storage and retail is calculated by multiplying the growth rate by number of hours of storage and retail. *Salmonella* concentration at the end of each stage is computed as the cumulative log from the preceding stage. Although a lag period was observed on the growth of *Salmonella* at 25ºC and 40ºC, insufficient data points are available to model the lag phase of the organism, hence it was assumed that lag phase does not occur at the said temperatures leaning to fail-safe scenario. Growth level probabilities for each condition was computed separately to account for the two storage conditions observed and then recombined as discrete distribution with input values of estimated probability of growth for each condition and the percentage of vendors who practice low temperature and ambient storage. The same goes for the estimation of the final concentration of *Salmonella* before consumption. Based on the consumer's survey conducted, it was shown that a portion of consumers do not immediately eat the product after grilling but take it away for later consumption. Hence, the growth of the pathogen at the point of consumption was represented by a discrete distribution considering the proportion of those who immediately consume the product after grilling and those who take away the product for later consumption. Serving size was determined by the weight of grilled chicken intestines per stick and the surveyed number of sticks consumed per eating session which were represented by Laplace and exponential distribution as suggested by the calculated AIC after distribution fitting of incurred data. The dose-response relationship model relating the concentration of *Salmonella* ingested after eating grilled chicken intestines to the probability of infection was derived from the risk assessment of *Salmonella* performed by FAO/WHO (2002) in eggs and broiler chickens. This model follows a Beta-Poisson distribution, where it is assumed that the cells of the pathogen are Poisson distributed and each cell has a probability of causing infection defined by a Beta distribution (α, β).

Survey on grilled chicken intestines consumption

A survey from July 2016 to March 2017 was conducted to determine the consumption pattern among residents of Los Baños, Laguna for street-vended grilled chicken intestines. The survey was designed to determine the amount of grilled chicken intestines ingested and the eating habits of the target population and consequently measure the dose or the extent of exposure of the locals to Salmonella upon eating the concerned street food product. Three hundred eight (308) residents were asked about their consumption frequency and amount of grilled chicken intestines (number of sticks) consumed per seating. Most of the respondents were teenagers and adults belonging to the 13-19 and 20-49 age groups. Gender of the sample population was almost evenly divided between male and female. Fifty one (51%) percent of the total respondents were male while 49% were female. As for the income category, interviewed residents were also almost equally distributed to four designated income brackets. The respondents were then asked using the prepared questionnaire if they eat street-vended grilled chicken intestines. Eighty-one (81%) percent (n=248) of the total respondents answered they do eat the said street food product. Among these consuming individuals, 91% (n=225) eat the

product with dips provided by the vendor while 14% (n= 34) take them forlater consumption. With regards to eating frequency, an average of 5.41 times per month was computed for the whole sample population. Minimum and maximum consumption frequency recorded per month was 1 and 90 times respectively.

Table 1: QMRA model inputs included in the simulation

VARIABLE	DESCRIPTION	DISTRIBUTION
Prevalence of *Salmonella* in raw chicken intestines	Proportion of raw chicken intestines samples tested positive with *Salmonella*	Beta (α=11, β=1)
Probability of contaminated raw chicken intestines	Probability that *Salmonella* is present in the raw chicken intestines utilized in the production	Binomial (1, Beta (α=11, β=1))
Concentration of *Salmonella* in raw chicken intestines	Level of *Salmonella* in raw chicken intestines	Exponential(0.2864,Shift(0.09136))
Percent reduction after washing	Reduction in *Salmonella* concentration after removal of intestinal contents and washing	Triangular (-0.1428,1,1)
First thermal processing	Reduction in *Salmonella* concentration during boiling of raw chicken intestines	Time: PERT (2,3.5,5) Temperature: PERT (55,62.5,70) $\mathrm{D-value} = 10^{(-0.185\mathrm{T}+11.87)}$
Second thermal processing	Reduction in *Salmonella* concentration during stewing of boiled chicken intestines	Time :PERT(2,3.5,5) Temperature:PERT (55,62.5,70) $\mathrm{D-value} = 10^{(-0.185\mathrm{T}+11.87)}$
Growth during vendors' storage	Growth of *Salmonella* inpre-cooked chicken intestine in ambient and low temperature storage before retail	Time: Logistic (5.6151,0.77387) Temperature (Ambient): PERT (25,27.9,38.5) Temperature (4ºC): 4. Fixed value Growth rate Ambient: $\mu_{max} = (3.044\mathrm{E}-5+55.8207)^2$ 4ºC: $\mu_{max} = 0.000044 \pm 0.0004045$ Growth (Both storage temp): Discrete
Growth during retail	Growth of *Salmonella* in pre-cooked chicken intestines during retail	Time: Uniform (4.7368,10.2632) Temperature: PERT (25,27.9,38.5) $\mu_{max} = (3.044\mathrm{E}-5+55.8207)^2$
Third thermal processing	Reduction in *Salmonella* concentration during grilling of pre-cooked chicken intestines	Time: PERT(2,3.5,5) Temperature: PERT (55,62.5,70) $\mathrm{D-value} = 10^{(-0.185\mathrm{T}+11.87)}$
Prevalence of *Salmonella* in dips	Proportion of sauces and vinegar dip samples positive with *Salmonella*	Beta (α=3, β=33)

Growth during consumer's storage time	Growth of *Salmonella* in grilled chicken intestine after purchase	Time: Exponential Temperature: PERT Growth rate: $\mu_{max} = (3.044E-5+55.8207)^2$
Concentration of *Salmonella* in grilled chicken intestines upon consumption	Concentration of the pathogen upon intake of the product	Discrete (Concentration at the point of sale, Concentration after consumer's storage: % Immediately consumed, % Take out)
Serving size	Amount ingested per eating session	Weight per stick: Laplace (12.83,4.7899) Number of sticks per eating session: Exponential (2.5357, Shift (-0.0082328)
Number of exposure events per year	Consumption frequency per year	Inverse gaussian (56.828,19.655, Shift (-4.5029))
Probability of infection	*Salmonella* dose-response relationship model relating dose/amount ingested to probability of infection	Beta-Poisson Probability of infection = $(1-(1+c/\beta)^{-\alpha}$ α=0.1324, β=51.45 FAO/WHO, 2002

The number of *isaw* sticks consumed was also asked to determine the amount of grilled chicken intestines ingested per eating session. The minimum, maximum and average numbers of sticks consumed per month within the sample population were 1, 15 and 3.15, respectively.

Simulation of risk assessment model inputs and sensitivity analysis

To complete the risk assessment, risk characterization step was done to integrate all the data gathered to estimate the risk of *Salmonella* infection within the target population. Table 12 contains the generated outputs in the baseline scenario after simulation of the developed QMRA model. The baseline scenario represents the current knowledge on the production and consumption of the concerned product based on the information gathered and considered in this study. The model predicts an average *Salmonella* concentration of 11.12 MPN per serving of grilled chicken intestines. The large difference of the minimum and maximum values achieved implies a wide spread of predicted pathogen level upon consumption of the food product which can be attributed to the large variability of uncertain inputs of the model. Specifically, wide ranges of values were observed and considered on the holding time (2-8 hours) and temperature (4-38.5ºC) during preparation and retail operations. Amount in grams per stick of grilled chicken intestines also sold by vendors also varies considerably which tantamount to increase in variability per serving of the product (7.2 -35.42g).

Table 2: QMRA model outputs for the baseline scenario

MODEL OUTPUT	MEAN	MINIMUM	MAXIMUM
Concentration of *Salmonella* (MPN) /serving	11.12	-1,643	29,144
Probability of infection/serving	0.006113	-0.3056	0.5681
Probability of infection/serving/year	1.220	-23.65	9821
Predicted number of infection cases/year	137,191	-2.661E6	1.105E9

The relative frequency distribution of the probability of infection per serving after the estimated *Salmonella* concentration was incorporated in the dose-response model was illustrated in Figure 10. It was shown that more than 90% of the iterations fall from -0 to 0.022 range. On the average, a probability of infection of 0.006113 per serving of grilled chicken intestines was estimated. Whiting and Buchanan (1997) on their discussion on dose-response relationship stated that if a food process results to a probability of infection of 10^{-1} or 10^{-2} after a single consumption, the final product of the process can be deemed as grossly unsafe. From this, it can be concluded that the process for grilled chicken intestines is within the safe zone if the mean probability of infection per serving will be used as the basis. However, if the worst-case scenario or the maximum probability of infection achieved per serving will be considered, this conclusion will be reverted and would classify street-vended grilled chicken intestines as potentially hazardous. Simulation outputs also showed that annually on the average, there is an approximately 25% chance that a single consumer who eats street-vended chicken intestines might get infected with *Salmonella*. Multiplying the probability of infection per serving with the distribution of consumption frequency obtained from the consumers' survey will give the predicted annual risk of *Salmonella* infection in Los Baños.

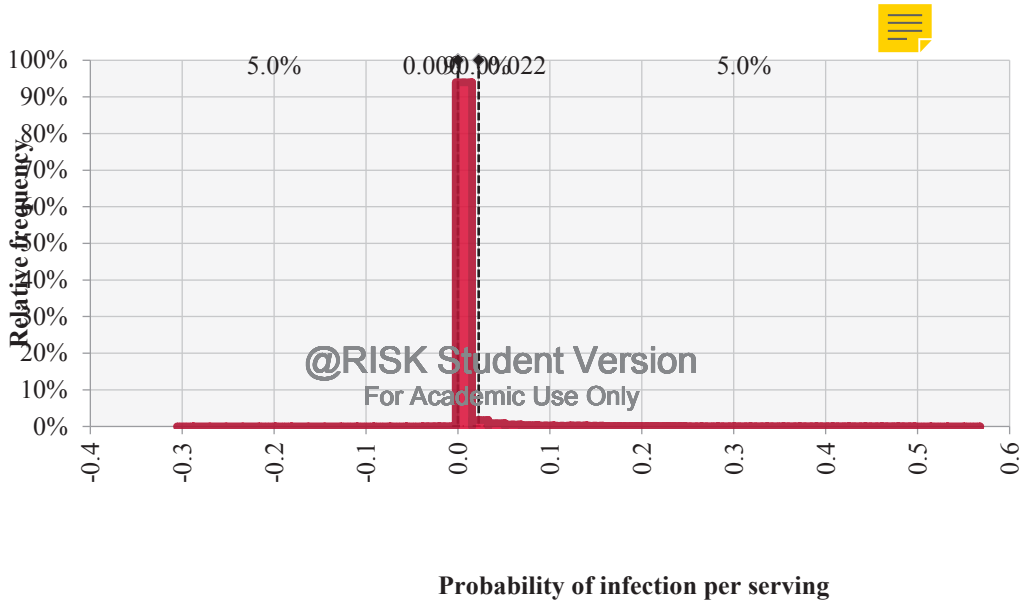


Figure 1: Relative frequency distribution of probability of *Salmonella* infection predicted per serving of grilled chicken intestines within Los Baños, Laguna.

From the estimated probability of infection per serving per year, the predicted number of infection cases per year was determined by multiplying it by the proportion of those who eat street-vended grilled chicken intestines in Los Baños. The total population of the town accounted for the year 2015 was 112, 243 based on the consolidated Barangay Management Information System recorded in 2016 and from the consumption survey performed, it was shown that 81% of the population consumes street-vended chicken intestines. Utilizing this percentage of consuming population, the calculated number of *Salmonella* infection cases ranges from –2,660,628 to 1,104,843,000 with an average of 137,191 cases per year. Although these numbers are relatively high, it should be given emphasis that the model did not aim to estimate the probability of illness (i.e. any derangement in the whole body function or any of its parts) which involves a series of complex events which relies on the interaction of *Salmonella*, the host and food determinants among others (Sant'Ana*et al*., 2014) but predicted the probability of infection (i.e. invasion of the pathogen in the host's body). Thus, it should be kept in mind that not all infection cases foreseen will result to a disease. Determination of the probability of disease requires information on the proportion of healthy and susceptible individuals within the target population and severity of manifestation after *Salmonella* infection. Unfortunately, data for these within the premise of the risk assessment performed are currently not available.

Sensitivity analysis on uncertain model inputs was also performed to determine which of the inputs have the greatest impact on count predictions. In this study, the sensitivity analysis was done by calculating the Spearman correlation coefficient for each uncertain parameter and ranked them in accordance with the magnitude of their effect or correlation with probability of infection. The magnitude of association of the model inputs with the probability of infection was used to determine the critical parameter that must be monitored and consequently to develop possible mitigation strategies to reduce the number of infection cases due to consumption of *Salmonella*-contaminated grilled chicken intestines. A positive correlation coefficient represented an increase in estimated risk of infection with increase in the associated uncertain model variable, whereas a negative correlation coefficient means a decrease in probability of infection. As illustrated in Figure 11, the final concentration of *Salmonella* upon consumption has the most impact on the annual probability of infection followed by consumption frequency and number of sticks eaten per seating. The result suggests that possible interventions to reduce probability of infection can be on provision of sufficient control in the production and retail processes to eliminate *Salmonella* or significantly decrease

pathogen's concentration (if cannot be totally eliminated) and by information dissemination to increase consumer's awareness on the possible risk associated with consumption of grilled chicken intestines and consequently reduce the frequency and amount consumed by the target population. To closely examine the factors affecting *Salmonella* concentration upon consumption, a separate sensitivity analysis was done for the said model output. Correlation coefficient ranking of the uncertain inputs indicates that temperature during thermal treatments has the greatest influence on the final concentration of the pathogen (Figure 12). This is followed by *Salmonella* growth during storage prior to retail which obtained a correlation coefficient of 0.11. This factor was mainly affected by the holding time and temperature of the product before vending. Percent reduction after raw material washing, cooking time, time and temperature during retail and initial pathogen concentration in raw chicken intestines also have marginal correlation with the final concentration of the *Salmonella* in the final grilled product.

Scenario exploration

Different scenarios were explored with the purpose of reducing the probability of infection and subsequently mitigating the predicted number of infection cases per year incurred in the baseline scenario. Focusing on the result of sensitivity analyses, model inputs which are shown to be most correlated with the probability of infection and final pathogen concentration were assigned with certain values, simulated with other inputs of the baseline model and finally evaluated for decrease on the predicted number of *Salmonella* infection cases.

Table 13 summarizes the range of predicted number of Salmonella infection of 16 generated what-if-scenarios. The baseline scenario was also included as Scenario 1 for comparison purposes. Scenarios 2 to 4 have fixed temperature values for first two thermal treatments in the food while Scenarios 5 to 7 have pre-determined cooking time values for the first and second cooking steps. The final thermal treatment of the process which is grilling is not considered for time and temperature fixed values since in actual cases, it would be very difficult to monitor the temperature of the product during grilling and the cooking time for this step is usually not controlled by the producers but decided by the consumer.Among these scenarios, set ups that have fixed temperatures for the first and second cooking steps had the most substantial reduction and increase in predicted number of infection (Scenarios 2-4). A six-fold increase in the mean number of *Salmonella* infection cases was observed when the internal temperature of food for the first two cooking steps were set to 60 ºC. When the internal temperature setting was changed to 65ºC the mean number predicted was ~ 1 infection case per year. If the setting was further elevated to 70ºC, result will be calculated as 0. This shows the tremendous effect of variation in cooking temperature in the predicted number of *Salmonella* infection cases. Standardizing the cooking time for 2 and 3 minutes would increase the predicted infection cases by 218% and 7%, respectively. Meanwhile, cooking the product for 5 minutes would mean an average of 58% reduction in infection cases.

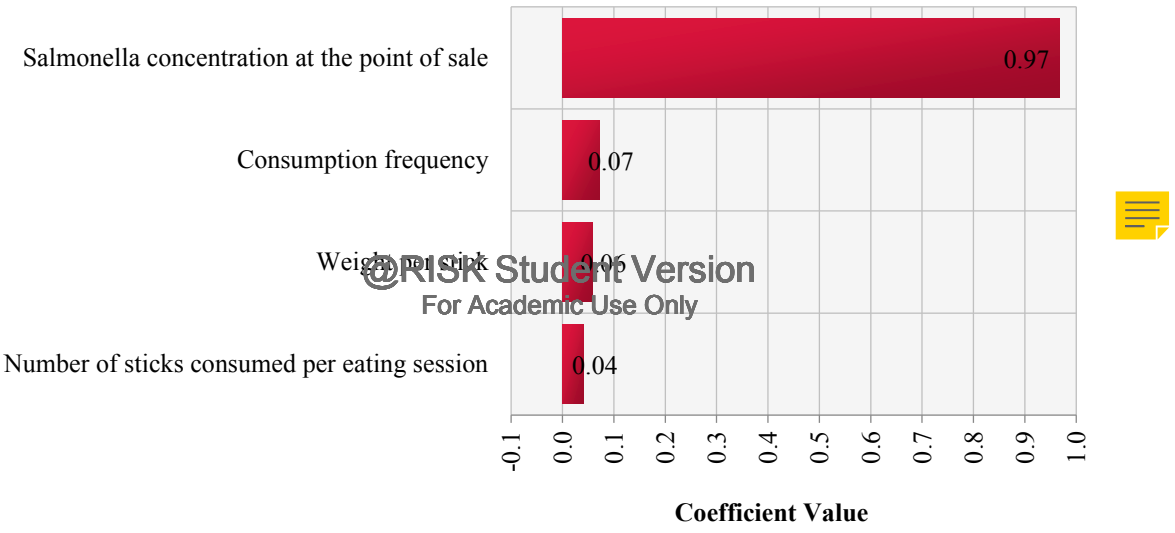


Figure 2: Sensitivity analysis on factors affecting the annual probability of *Salmonella* infection related to consumption of street-vended grilled chicken intestines.

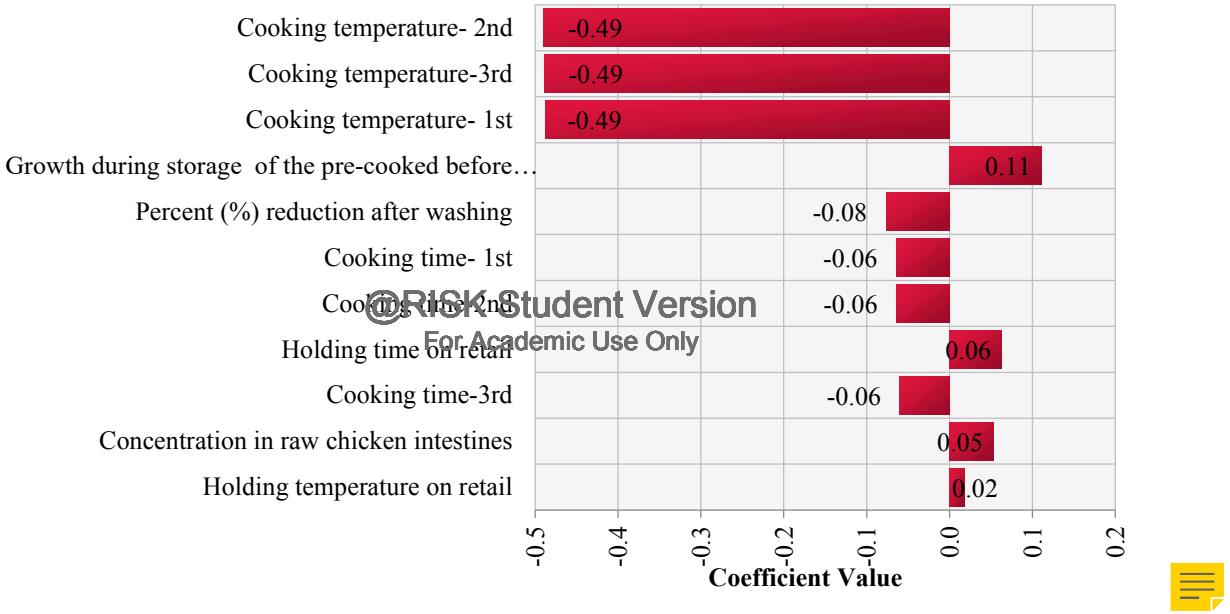


Figure 3: Sensitivity analysis on factors affecting the final concentration of *Salmonella* in grilled chicken intestines.

Hazard analysis, critical control points and critical limits determination

Hazard analysis based on the results of the risk assessment performed is somehow straightforward. The estimated probability of infection per serving of the product and the relatively high predicted number of infection cases suggest that there is indeed a high chance to get infected and consequently suffer from disease caused by *Salmonella* after consuming street-vended grilled chicken intestines. The measured risk of infection was supported by the presence of the pathogen in the performed prevalence study on the product and its dips at the point of sale conducted in the hazard identification step. Also, the result of the prevalence study in raw chicken intestines sold within the area of study reinforces the conclusion of the hazard analysis. With the given qualitative and quantitative evidences, it is now certain that *Salmonella* is a hazard in street-vended grilled chicken intestines. Next, the HACCP principle that was implemented was the identification of critical control points (CCP) in the production and retail process. Control points within the pathway were selected through uncertain models inputs which were shown to have the most impact based on the result of the sensitivity analysis done for the final concentration of *Salmonella* in the grilled product (Figure 2) of US FDA (2017) on their published HACCP guideline, control points identified are washing of raw chicken intestines, storage of pre-cooked product before and during retail and the three cooking steps identified in the food pathway. Other decision trees in the literature would not classify earlier control steps as contender for CCP if there is/are later treatment/s that could control *Salmonella* but the decision tree adapted in this study included all the steps that would minimize the likelihood of the given hazard.

This is the reason why the washing step of raw chicken intestines and the first thermal treatment were regarded as candidate CCP, even if there were succeeding control points in the process. However, not all control points identified above were regarded as CCPs. The ease of monitoring and acceptability of control measures to the vendors should also be considered in deciding on the final list of CCPs. Given the financial capacity of the street food vendors, regular monitoring of Salmonella concentration in raw chicken intestines after removal of intestinal contents and washing with water could be a tedious and expensive task since they will be obliged to send off samples and pay for the testing. Time and temperature control during storage of pre-cooked chicken intestines prior to and during retail can also be demanding for most of the vendors. Each of them had his/her own pace for product preparation and vending which is usually based on the volume of the grilled products they produce. Thus, standard holding and retail time will not be easy to impose. Also, many of these hawkers do not own a refrigerator to hold the product at low temperature. Provision of cold storage for the pre-cooked product would mean additional cost. Monitoring of time and temperature of the first two cooking steps control points are much easier to do given that a thermal probe is available to check the internal temperature of the food while cooking. The cost of a thermal probe would be less expensive as compared to cooling equipment. Scenario exploration had also shown that control of the said conditions of thermal processing can be sufficient enough to eliminate the pathogen using the inputs in the QMRA model. Thus, time and temperature for the said thermal treatments were assigned to be the process CCPs. Critical limits for the time and temperature for the thermal treatments regarded as CCPs were determined utilizing the result of scenario exploration. Conditions for cooking raw and pre-cooked chicken intestines that showed the acceptable risk were chosen to be the critical limits for the CCPs. In this study, acceptable risk is defined as the tolerable predicted number of Salmonella infection cases related to street-vended chicken intestines consumption. Acceptable risk can be based from a long list of standpoints and different risk criteria may be appropriate for different kinds of microbial agents depending on the severity of disease and potential sequelae of each agent (WHO, 2001). Making judgement based on the maximum predicted number of infection cases demonstrated in scenario exploration, critical limits for cooking temperature (internal temperature of the product) must be >70ºC to conform with zero infection case per year. This zerotolerance performance criterion was used as the basis for selecting critical limits instead of the average number of cases because the upper limit of the prediction range equates to the worst case that could take place for all the uncertain exposure assessment model inputs. Meanwhile, the limit for cooking time was set to 2 minutes. The minimum cooking time duration entered in the model was chosen since it was noted earlier that prolonged cooking was avoided by the producers during the product preparation. Nevertheless, the predicted number of cases would still be within the limit since the worst-case scenario (least cooking duration) had already been considered in the selecting critical limits for cooking temperature.

4. Conclusion

Overall, it was shown that street-vended grilled chicken intestines in Los Baños, Laguna pose health risk to consumers in the area due to probable Salmonella contamination. Infection upon consuming the street food product is apparent, thus intervention must be done to minimize this risk. QMRA was demonstrated to be an efficient food safety tool to describe the ecology of the pathogen along the food pathway and estimate the magnitude of the given hazard at the point of consumption. Lastly, the results from the QMRA can also be very valuable when devising a HACCP plan especially on evaluation of the severity of hazard and on developing strategies to lessen the occurrence of the hazard and consequently reduce public health risk.

Table 3: Scenario exploration on the effect of change in model inputs values on the estimated number of *Salmonella* infection cases per year due to consumption of grilled chicken intestines

SCENARIO	DESCRIPTION	MEAN NUMBER OF INFECTION CASES PER YEAR	MINIMUM	MAXIMUM
1	Baseline Scenario	137,191	-2.660E6	1.104E9
2	First and second cooking temperature fixed at 60 ºC*	950,919	-3.932E7	1.913E10
3	First and second cooking temperature fixed at 65 ºC*	1.281	-59.71	112,828
4	First and second cooking temperature fixed at 70 ºC*	0	0	0
5	First and second cooking time fixed for 2 minutes	436,401	-7.123E7	1.671E10
6	First and second cooking time fixed for 3 minutes	147,359	-1.921E6	1.236E9
7	First and second cooking time fixed for 5 minutes	58,010	-4.464E7	9.012E8

* Refers to the internal temperature of the food product

5. Acknowledgement

The authors would like to thank the Office of the Vice Chancellor for Research and Extension of the University of the Philippines Los Bañosfor providing funds for the conduct of the study through Professor Emeritus Grant. the Common Services Laboratory, Food and Drug Administration Philippines for providing reference bacterial strains used as quality control cultures.

References

Abdussalam, M., Kaferstein, F.K. (1993). Safety of Street Foods. World Health Forum 14:191-194.

Atienza, A.T., Dalmacio, I. F. (2016). Modelling Growth and Thermal Inactivation of Salmonella spp. in Pre-cooked Chicken Intestines. (Unpublished article A).

Atienza, A.T., Dalmacio, I. F. (2016). Exposure Assessment of Salmonella spp. in Street vended Grilled Chicken Intestines. (Unpublished article B).

Azanza, M.P.V, Gedaria, A. I. (1998). Microbial Hazards of Street-Vended Grilled Chicken Intestine. Science Diliman 10 (2): 1-11.

Buchanan, R. L., & Whiting, R. C. (1998). Risk assessment: a means for linking HACCPplans and public health. Journal of food protection, 61(11), 1531-1534.

Chiu, C. H., & Ou, J. T. (1996). Rapid identification of Salmonella serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture multiplex PCR combination assay. Journal of Clinical Microbiology, 34(10), 2619-2622.

Fernandez, D. G. (2013). Balut to barbecue: Philippine streetfood. Budhi: A Journal of Ideas and Culture,5(3 6.1), 329-340.

Firildak, G., Asan, A., & Goren, E. (2015). Chicken carcasses bacterial concentration at poultry slaughtering facilities. Asian J. Biol. Sci, 8, 16-29.

Fellows, P., Hilmi, M. (2012). Selling Street and Snack Foods. Diversification Booklet Number 18 Rural Infrastructure and Agro-Industries Division of FAO. Available at: http://www.fao.org/docrep/015/i244e/i2474e00.pdf. Accessed 15 June 2015.

Food and Agriculture Organization/World Health Organization (2002). Risk assessment of Salmonella in Egg and Broiler Chickens. Available at: http://www.fao.org/3/a-y4392e.pdf. Accessed 15 April. 2017.

Food and Agriculture Organization/World Health Organization (2004). Risk assessment of *Listeria monocytogenes* in Ready-to-Eat Foods. Available at: http://www.fao.org/3/ay5394e.pdf. Accessed 12 April. 2017.

Food and Agriculture Organization. (2007). Spotlight: School Children, Street Food and Micronutrient Deficiencies in Tanzania. Web :http://www.fao.org/AG/magane/0702sp1.htm

Heidinger, J. C., Winter, C. K., & Cullor, J. S. (2009). Quantitative mcrobial risk assessment for Stahylococcus aureus and Staphylococcus enterotoxin A in raw milk. *Journal of Food protection*, *72*(8), 1641-1653.

Mayes, T. (1998). Risk analysis in HACCP: burden or benefit?. *Food control*, *9*(2-3), 171-176.

McEntire, J., Acheson, D., Siemens, A., Eilert, S., & Robach, M. (2014). The public health value of reducing salmonella levels in raw meat and poultry. *Food Protection Trends*, *34*(6), 386-392.

New South Wales Food Authority. (2009). Microbiological quality guide for ready-to-eat foods: A guide to interpreting microbiological results

Nicolas, B., Razack, B. A., Yollande, I., Aly, S., Tidiane, O. C. A., Philippe, N. A., & Sababénédjo, T. A. (2007). Street-vended foods improvement:

Contamination mechanisms and application of Food Safety Objective Strategy: Critical review. *Pakistan Journal of Nutrition*, *6*(1), 1-10.

Rane, S. (2011). Street vended food in developing world: hazard analyses. *Indian journal of microbiology*,

51(1), 100-106.

Sant'Ana, A. S., Franco, B. D., & Schaffner, D. W. (2014). Risk of infection with Salmonella and Listeria monocytogenes due to consumption of ready-to-eat leafy vegetables in Brazil. Food Control, 42, 1-8.

US Food and Drug Administration.(2015). BAM Online (Bacteriological Analytical Manual Online) Available at: http://www.fda.gov/food/scienceresarch/laboratorymethods/bacteriologcalanalyticmanualbam/default.htm. Accesse March 2015.

US Food and Drug Administration. (2017). HACCP Principles & Application Guidelines. Available at:https://www.fda.gov/downloads/Fod/GuidaneRegulation/UCM25244 pdf. Accessed 12 April 2017

Whiting, R. C., & Buchanan, R. L. (1997). Development of a quantitative risk assessment model for Salmonella enteritidis in pasteurized liquid eggs International Journal of Food Microbiology, 36(2-3), 111-125.

Winarno, F.G., Alain, A. (2015). Street foods in developing countries: lessons from Asia. Agriculture and Consumer Protection. Food and Agriculture Organization Corporate Document Repository. Available at: http://www.fao.org/docrep/u3550t/3550t08.htm. Accessed 18 June 2015.

World Health Organization. (1996). Essential Safety Requirements for Street-Vended Foods. Food Safety Unit Division of Food and Nutrition.

Yeh, K. S., Chen, T. H., Liao, C. W., Chang, C. S., & Lo, H. C. (2002). PCR amplification of the Salmonella typhimurium fimY gene sequence to detect the Salmonella species. International journal of food microbiology, 78(3), 227-234.

2. ACCEPTABILITY AND MARKETABILITY OF GINGER TURMERIC TEA GRANULES

Rosemarie R. Jadie[1], Maria Corazon R. Naz, RND, MSN[2]
[1]Bicol University Gubat Campus
[2]Bicol University Extension Management Division, Legazpi City
Corresponding author's Email: rosejadie@yahoo.com.ph

Abstract

The community farmers in Sorsogon City Philippines are engaged in farming ginger and turmeric. Year after year, they find it hard to sell some of their products that results to spoilage. On the other hand, the consumers of today are clamoring for a herbal tea that could help them find relief of some of their illnesses. Along these premises the researcher stands on the cause of serving the needs of the farmers and the consumers. This study aimed to develop ginger-turmeric tea formulations as a product for consumption. More specifically, it sought to 1) utilize ginger turmeric and herbs in the formulation and production of tea; 2) determine the most acceptable tea formulation based on the sensory characteristics such as color, flavor strength, and the specific taste of the product; 3) determine the acceptable ratio of water and tea granules; 4) determine the nutritional value and toxicity of the tea formulations. The study made use of descriptive-evaluative research using the hedonic scale instrument, experimental study on the formulation of tea, focused-group discussions, interviews, product development, product sampling for tasting or sensory evaluation and laboratory testing. *Results and Discussion:* The salient findings of the study revealed that: 1.Ginger and turmeric roots can be combined with herbs. Five tea formulations were developed: a) GT 16 in1 (Ginger *Zingiber Officinale*, Turmeric Yellow *Curcuma Longa)*, Turmeric white *Curcuma zedoaria*, pandan *Pandanusamaryllifolius*, rosemary *Rosmarinusofficinalis*, anise *Pimpinellaanisum*, sambong *Blumeabalsamifera Linn.*, alagaw *PremnaOdorata Blanc*, banaba *Lagerstroemia Speciosa Linn.*, guyabano A*nnonamuricata*, lagundi *Vitex Negundo*, oregano *Origanum Vulgare*, herbabuena *Mentha Cordifolia Opiz*, malunggay *Moringa Oleifera*, lemon grass *Cymbopogon Citratus* and cinnamon *Cinnamomumzeylanicum*), b) GT Rosemary, c) GT Oregano, d) GT Pandan and e) GT Gubayano. 2) GT 16-in-1 was extremely acceptable to young and older consumers in terms of color, taste and flavor strength at mean score of 8.42, followed by other formulations. A ratio of 200 ml hot/cold water to one tablespoon GT 16-in-1 was most favored at 7.7 mean score, 3) Per serving, the product has low calorie, low fat with 6 to 7 % carbohydrate adequacy because of sugar, contains 2% of recommended protein and magnesium, and negligible amounts of other minerals. In conclusion, the ginger, turmeric and herbs can be utilized to produce tea granule acceptable for its sensory attributes, right proportion of serving, nutritious and healthy, non-toxic and is very suitable to human consumption.

Keywords: Utilization, Ginger Turmeric Herbs, Tea granules, production, consumption

1. Introduction

Globally, individuals are mindful of their well-being. Hence, the search for healthy food supplement is a trend such that variety of products is introduced in the market. Ginger and Turmeric is one among the claimed super foods of the century (www.greenmedinfo.com**).**In fact, there are lots of similar products that are available in the market. These two herbs are abundant in the Philippines particularly in the Province of Sorsogon, Philippines, and have remarkable health benefits, which motivated the researcher to formulate the product and conduct this study.

2. Objectives

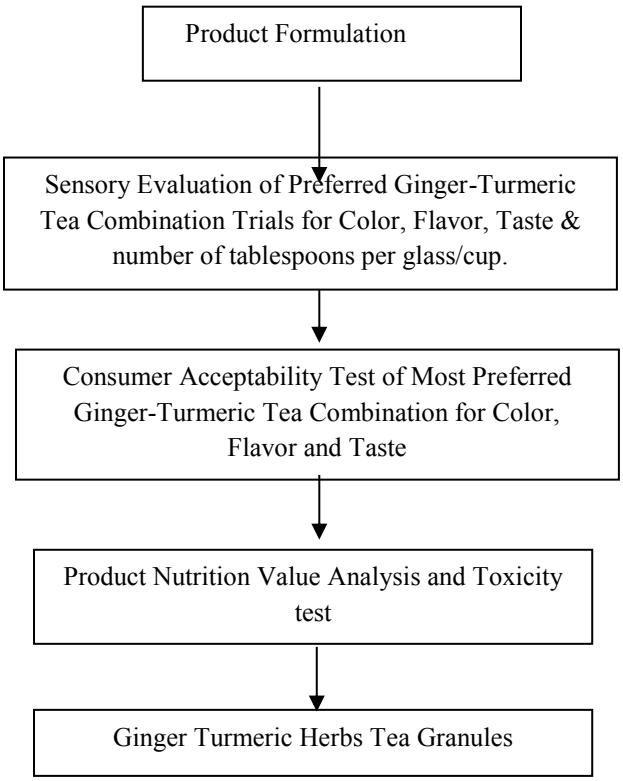
Specifically, the study sought to: 1) utilize ginger and turmeric in the formulation and production of tea granules 2) determine the health qualities of the herbs 3) determine the most acceptable tea formulation based on the

sensory characteristics such as color, flavor strength, taste; 4) determine the acceptable ratio of water and tea granules. 5) determine the nutritional value and toxicity of the tea formulations.

3. Materials and methods

The study made use of descriptive-evaluative research using the hedonic scale instrument, focused-group discussions, interviews, product development, product sampling for tasting or sensory evaluation and laboratory testing.

Scientific Basis/Theoretical/Conceptual Framework:



Product process formulation/production

The product formulation include the following steps: (1) Selection of good quality ginger and turmeric;(2) weighing the ginger turmeric on the desired formulation; (3) extracting the juice; (4) combining the juice withsugar; (5) cooking the combined ingredients for 1 one hour or until sugar appearance; (6) set aside and let it cool; (7) determination of the acceptable taste, color and flavor and the right amount of tea in table spoon in one cup of 200 ml water ; (8) determination of the nutritional value and toxicity of the product and productionof the tea granules.

4. **Results and discussion**

The study was conducted at Gubat Campus, Sorsogon City for a period of three (3) months. The result of the study yielded the following findings:

a. The Ginger and Turmeric can be produced into tea granules and it can be combined with herbs to further improve the product. The researcher was able to formulate five variants namely: a) Ginger Turmeric 16 in1 (Ginger, Turmeric Yellow, Turmeric white, pandan, rosemary, anise, sambong, alagaw, banaba, guyabano, lagundi, oregano, herbabuena, malungay, lemon grass and cinnamon); b) Ginger Turmeric Rosemary; c) Ginger Turmeric Oregano; d) Ginger Turmeric Pandan and e) Ginger Turmeric GT Guyabano.

b. Almost all the herbs used in the formulation have distinct health properties that is beneficial to human consumption. Among its common properties are anti-inflammatory, anti-cancer, diuretic, anti-oxidant, anti-bacterial, pain reliever and others. Below are the more detailed properties of the herbs contained in the 16 in 1.

c.

Table 2: Describes the properties of the herbs

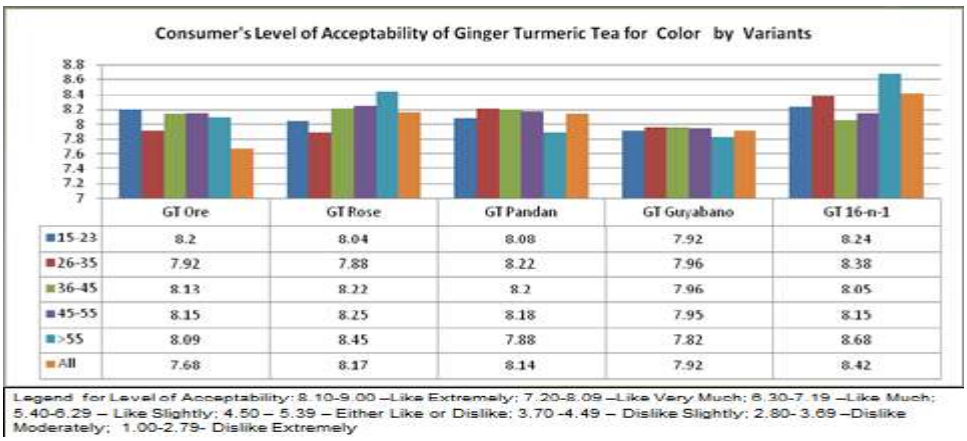
Herbs	Properties
Ginger (*Zingiber Officinale)*	Loaded with nutrients, bioactive gingerol compounds that have powerful benefits such as anti-inflammatory and antioxidant properties, it can treat nausea, muscle pain and soreness. may drastically lower blood sugars and improve heart disease risk factors, treat indigestion, reduce menstrual pain, may lower cholesterol level, may help prevent cancer, may improve brain function and protect against alzheimer's disease, can help fight infections.
Yellow Turmeric (*Curcuma Longa)*	Has a powerful anti-inflammatory and antioxidant properties**,** can improved brain function and a lower risk of brain and heart disease and cancer, in treating Alzheimer's disease, arthritis is a common disorder characterized by joint inflammation, and may help delay aging and fight age-related chronic diseases.
White Turmeric or Curcuma Mango (*Curcuma zedoaria*) 	Has the ability to purify the blood, can inhibit cancer cell growth, useful as antipyretics, treat pain shortness of breath (asthma), treat inflammatory disease of the respiratory tract (bronchitis), useful as an antitoxin (antidote), can help reduce abdominal fat, useful as a laxative (laxative) and antioxidant, treats fever, colds and bloating, treats pain, skins ailments wounds and anti-inflammation , treats respiratory disorders, acts as aphrodisiac, treat urinary tract infections, regulates menstruation, normalize body temperature, improve digestions, prevent stress ulceration in gut and intestines, treats cough, vomiting , colic and dyspepsia, anti-venom. 1.White Turmeric can inhibit cancer cell growth. 2. White Turmeric can narrow female organs. 3. White Turmeric may help treat itching of the female. 4. White Turmeric can strengthen lust. 5. White Turmeric is useful as antipyretics (fever). 6. White turmeric to treat pain shortness of breath (asthma). 7. White turmeric to treat inflammatory disease of the respiratory tract (bronchitis). 8. White Turmeric may be useful as an antitoxin (antidote). 9. White Turmeric can help reduce abdominal fat. 10. White Turmeric may increase appetite. 11. White Turmeric may be useful as a laxative (laxative). 12. White Turmeric can be used as an antioxidant. 13. White Turmeric beneficial to treat fever, colds, bloating, etc.
Rosemary (*Rosmarinus officinalis)*	Has the ability to boost memory, improve mood and relieves stress, reduce inflammation, relieve pain, protect the immune system, stimulate circulation, detoxify the body, protect the body from bacterial infections, prevent premature aging and a breath freshener
Star Anise (*Pimpinella anisum*)	Remedy for asthma, bronchitis cough as well as digestive disorders such as flatulence, bloating, stomach pain, nausea and indigestion, **Can Significantly Reduce Your Risk Of Various Cancers,**
Banaba (*Lagerstroemia Speciosa Linn.*)	Contain a compound called corosolic acid for glucose lowering effect, helps the body break down sugars and carbohydrates and send them out to the body as energy through a process known as glycolysis, stimulates the release of cancer-cell-killing enzymes, shown effective against cervical cancer cells, leukemia, breast and liver cancer cells.
Alagaw (*Premna Odorata Blanc)*	Loosens phlegm and relieve cough**s,** tuberculosis and headaches,

	other properties are carminative, parasiticide, sudorific, and pectoral**,** fever and colds, cough and bronchitis, fever blister**s** of the lips and stomachaches, for flatulence (gas pains) in adults and have shown antimicrobial, cardiotonic, anticoagulant, hepatoprotective, antitubercular, antitumor
Malunggay (*Moringa Oleifera)* 	Normalize blood sugar level (Diabetes) and effective in combating malnutrition's., also effective as anti-infectious, antibacterial and antifungal**,** anticancer (Phytochemicals), rich in potassium, calcium, phosphorus, iron, vitamin A and D, Essential amino acids as well as known as antioxidants such as β-carotene, vitamin C and flavonoids, anti-inflammatory**,** strengthen the immune aystem, helps to restore skin condition**,** relieves headache**,** strengthen the eye muscles**,** prevents intestinal worms (Anthelmintics)**,i**Increase semen count**,** relax and promote good-night sleep**, t**reat common cold, fever and asthma, heals ulcers**,** clean dirty or polluted water**,** treat rheumatism**,** hypocholestemia or high cholesterol**.**Malunggay leaves contain calcium, potassium, protein, iron and vitamin A, B, and C in which they contribute and balance the cholesterol level in the body.
Herbabuena(*Mentha Cordifolia Opiz* 	Carminative, stimulant, aromatic, emmenagogue.Crushed or bruised leaves for insect bites.Decoction and infusion of leaves and stems used for fever, stomach aches, dysmenorrhea, and diuresis.Pounded leaves for insect bites, fevers, toothaches, headaches. Effective for **dizziness, headaches, toothachesflatulence, aough, Arthritis:**
Lemon Grass (*Cymbopogon Citratus)* 	Good source of **vitamins A B and C, folate, folic acid, magnesium, zinc, copper,iron, potassium, phosphorus, calcium and manganese.** It has many beneficial medicinal properties including analgesic, anti-inflammatory, antidepressant, antipyretic, antiseptic, antibacterial, antifungal, astringent, carminative, diuretic, febrifuge, galactogogue, insecticidal, sedative, and anti-cancer properties, helps digestion. helps treat digestive health problems like indigestion, constipation, heartburn, diarrhea, bloating, flatulence, stomach spasms, vomiting and cramps, has antimicrobial properties that help relieve gastroenteritis, controls cholesterol levels, remove toxins, uric acid and bad cholesterol, heals colds and flu, can relieve pain in muscles and joints, as well as headaches resulting from a cold or the flu, fights Cancer and reduces Arthritis Pain
Lagundi (*Vitex Negundo*)-	Relieves digestive troubles, tonic for liver, treats respiratory complaints, combat rheumatism, heals skin disease and analgesic for pain relief.
Oregano (*Origanum Vulgare)*	Contains vitamins A, C, E, and K, as well as fiber, folate, iron, magnesium, vitamin B6, calcium, and potassium. Additionally, oregano, contains potent phytochemicals, an **antioxidants for immune system support, antifungal, antibacterial (**Carvacol and thymol, two phytochemicals in oregano, are powerful antimicrobials) , a**nti-inflammatory properties** (Oregano contains beta-caryophyllin (E-BCP), a substance that inhibits inflammation and may also be beneficial for conditions including osteoporosis and arteriosclerosis, as well as metabolic syndrome), **useful for upper respiratory infections, cancer-fighting effects**A phytochemical in oregano, carnosol, has also been "evaluated for anti-cancer property in prostate, breast, skin, leukemia, and colon cancer with promising results."
Pandan (*Pandanusamaryllifolius*)	Relieves headache and arthritis, treats ear pains,eases chest pains, reduces fever, as a laxative for children, strengthen gums and reduces stomach spasms, helps in speeding in the recuperation of women , who have just given birth and are still weak, **l**ower high blood pressure**,** overcoming weak nerves**,** rheumatism or anti-rheumatic and stiff neck**,** boost appetite**,** nourish and boost your health**,** pain relief**,**

	reduce cramps**, a**nti-carcinogenic properties, relieves anxiety and stress**,** gum pain**,** after child birth relaxing energy, p**andan leaf teacanbe given to them as a relaxants,** reduce fevers, detoxing agent, mild laxative for children anti-hyperglycemic, **lowers their sugar blood,**treat muscle pain, treat men's problem**premature ejaculation or impotence.**
Sambong **(***Blumeabalsamifera Linn.)* 	**Diuretic,lowers blood pressure and lowers levels of cholesterol,** used for treating kidney stones and as a cure for hypertension**,** considered as anti-diarrheal and anti-spasmodic, cures commoncold and helps in the treatment of urinary tract infections**,** delays renal failure, dissolves kidney stones, acts as expectorant to get rid of mucus and phlegm.It **provides relief from stomach ailments, analgesic properties, as antioxidants.**
 Guyabano (*annonamuricata)*	Has Vitamin C, Riboflavin, Phosphorous, Thiamine, Calcium, Carbohydrates. It is anti-cancer, antibacterial, antiviruses, antiparasiticboost Immune system, boost the mood, boost energy, cures back pain, rheumatism, prevents migraine, urinary tract infection, constipation, anemia, leg cramps, lowers cholesterol levels, calm nerves, strengthen bones, hypertension remedy and diabetes treatment.
 Cinamon (*Cinnamomumzeylanicum)*	Antibacterial, anti-oxidant, antimicrobial, anti-inflammatory, antifungal, fights diabetes, fights free radicals, lowers bad cholesterol, provides bone , helps treat ADHD, Parkinson's disease, treats symptoms of Alzheimer Disease, colds, fights against salmonella, combats premenstrual syndrome, fights against heart disease, helps fights cancer, fights arthritis,

d. The GT 16-in-1 was extremely acceptable to young and older consumers in terms of color, taste and flavor strength at mean score of 8.42, followed by other formulations. A ratio of 200 ml hot/cold water to one tablespoon GT 16-in-1 was most favored at 7.7 mean score.

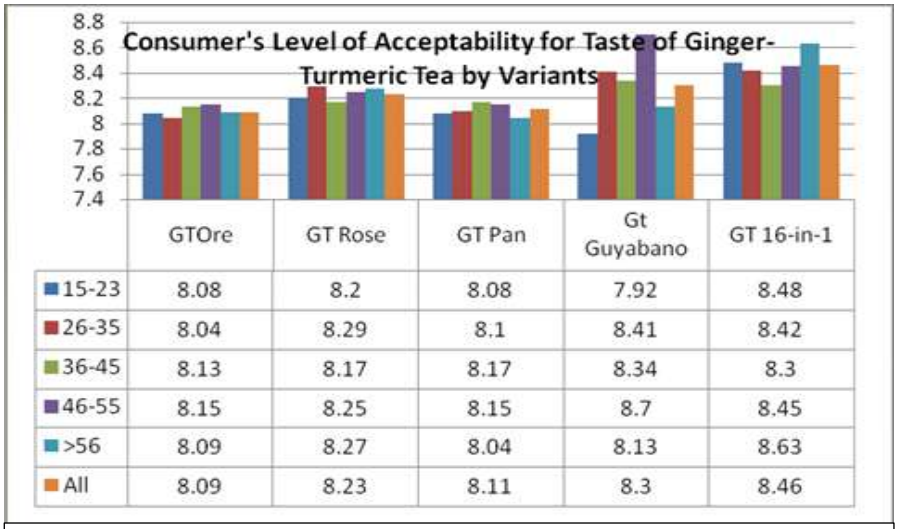
Figure 1 and Table 1. Describes the consumers level of acceptability of Ginger Turmeric Tea for color by variants. As reflected GT 16 in 1 is the most acceptable product formulation specially for those ages 56 and above because they found it more favorable due to the health benefits of the herb combinations. Although as presented almost all the colors of the five variants are relatedly closed to each other but, we have to see that "beauty is no quality in things themselves: It exists merely in the mind which contemplates them; and each mind perceives a different beauty. One person may even perceive deformity, where another is sensible of beauty; and every individual ought to acquiesce in his own sentiment, without pretending to regulate those of others. (Hume 1757, 136)



	GT Ore	GT Rose	GT Pandan	GT Guyabano	GT 16-n-1
15-23	8.2	8.04	8.08	7.92	8.24
26-35	7.92	7.88	8.22	7.96	8.38
36-45	8.13	8.22	8.2	7.96	8.05
45-55	8.15	8.25	8.18	7.95	8.15
>55	8.09	8.45	7.88	7.82	8.68
All	7.68	8.17	8.14	7.92	8.42

Legend for Level of Acceptability: 8.10-9.00 –Like Extremely; 7.20-8.09 –Like Very Much; 6.30-7.19 –Like Much; 5.40-6.29 – Like Slightly; 4.50 – 5.39 – Either Like or Dislike; 3.70 -4.49 – Dislike Slightly; 2.80- 3.69 –Dislike Moderately; 1.00-2.79- Dislike Extremely

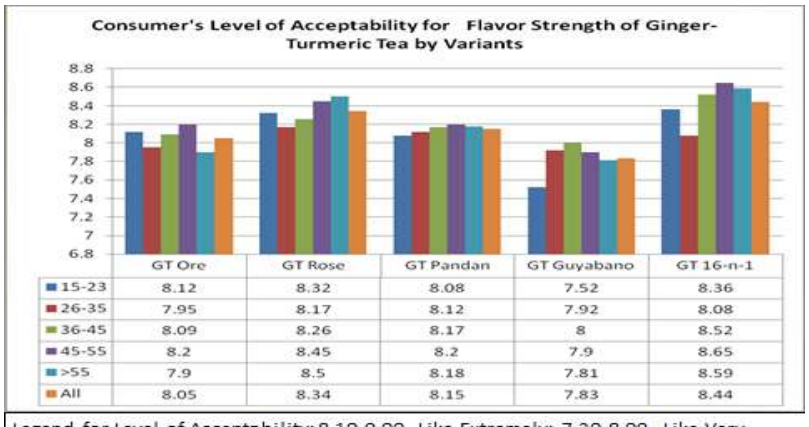
Figure 2 and **Table 2** Describes the consumers' level of acceptability of Ginger Turmeric Tea for taste by variants. As reflected GT 16 in 1 is the most acceptable product formulation specially for those ages 46-55 and above because they found the taste more attractive as a result of the combined taste of the herbs. Richard Bowen in his article "Physiology of Taste" discussed that among humans, there is substantial difference in taste sensitivity. Roughly one in four people is a "supertaster" that is several times more sensitive to bitter and other tastes than those that taste poorly. Such differences are heritable and reflect differences in the number of fungiform papillae and hence taste buds on the tongue.



	GTOre	GT Rose	GT Pan	Gt Guyabano	GT 16-in-1
15-23	8.08	8.2	8.08	7.92	8.48
26-35	8.04	8.29	8.1	8.41	8.42
36-45	8.13	8.17	8.17	8.34	8.3
46-55	8.15	8.25	8.15	8.7	8.45
>56	8.09	8.27	8.04	8.13	8.63
All	8.09	8.23	8.11	8.3	8.46

Legend for Level of Acceptability: 8.10-9.00 –Like Extremely; 7.20-8.09 –Like Very Much; 6.30-7.19 –Like Much; 5.40-6.29 – Like Slightly; 4.50 – 5.39 – Either Like or Dislike; 3.70 -4.49 – Dislike Slightly; 2.80- 3.69 –Dislike Moderately; 1.00-2.79- Dislike

Figure 3 Table 3 Describes the consumers level of acceptability of Ginger Turmeric Tea for flavor strength by variants. As reflected GT 16 in 1 is the most acceptable product formulation specially for those ages 56 and above because they found it more favorable due to the health benefits of the herb combinations. In detail GT Rosemary has a slightly minty, sage-like, peppery, balsamic taste and woody aftertaste, GT Oregano, has an aromatic, warm, and slightly bitter taste, GT Pandan has very fragrant and aromatic naturally sweet taste and soft aroma, while GT Guyabanotaste is a little bit sour while GT 16 in 1 has the combination of taste of all the and the benefits of all the herbs.



	GT Ore	GT Rose	GT Pandan	GT Guyabano	GT 16-n-1
15-23	8.12	8.32	8.08	7.52	8.36
26-35	7.95	8.17	8.12	7.92	8.08
36-45	8.09	8.26	8.17	8	8.52
45-55	8.2	8.45	8.2	7.9	8.65
>55	7.9	8.5	8.18	7.81	8.59
All	8.05	8.34	8.15	7.83	8.44

Legend for Level of Acceptability: 8.10-9.00 –Like Extremely; 7.20-8.09 –Like Very Much; 6.30-7.19 –Like Much; 5.40-6.29 – Like Slightly; 4.50 – 5.39 – Either Like or Dislike; 3.70 -4.49 – Dislike Slightly; 2.80- 3.69 –Dislike Moderately; 1.00-2.79- Dislike Extremely

Figure 4 and **Table 4** Describes the consumers' level of preference on ratio of glass of water to tablespoon formulation of GT by variants. As reflected the consumers prefers to the 1 glass of 200 ml water to 1-2 tablespoon GT tea weight a score of 7.64 - 7.7.

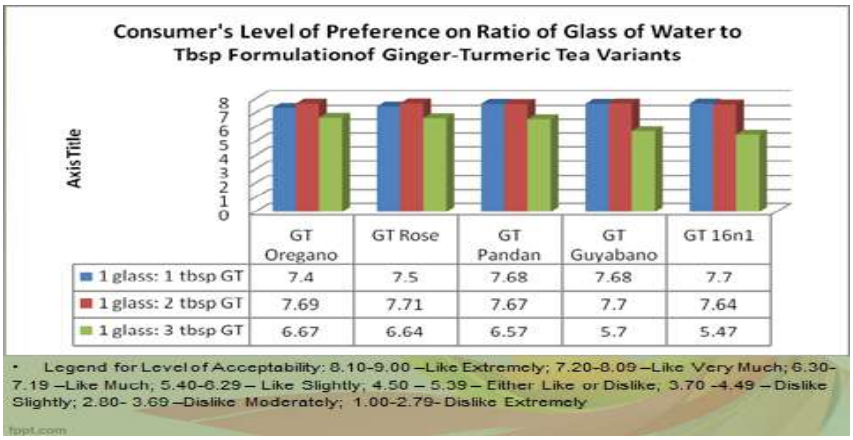


Table 5 describes the nutritional value of the 16 in 1 Ginger Turmeric Tea Formulation. Per serving, the product has low calorie, low fat with 6 to 7 % carbohydrate adequacy because of sugar, contains 2% of recommended protein and magnesium, and negligible amounts of other minerals. It is non-toxic to mice and is very suitable to human consumption because of health benefits. As revealed, through the above cited minerals and vitamins, the product may aid in maintaining normal nerve and muscle function, growth, maintenance, and repair of cells and tissues, supports a healthy immune system, keeps the heart beat steady, and helps bones remain strong. It also helps regulate blood glucose levels, blood sugar regulation, and aid in the production of energy and protein, as well as healing wounds.

Table 5 . Nutritional Composition of Two Tablespoon Formulation of Ginger-Turmeric Tea with G-in 6 Variant

Nutrients	Amount per 900 g	Amount per 30 g				
			16-18 yo	19-29 yo	16-18 yo	19-29 yo
Energy,kcal	394	13.13	3010	2530	0.43	0.52
Fat, g	9.54	0.32	1000	155	0.32	0.2
Carbohydrate,g	879.3	29.3	489	411	6	7
Protain, g	39.96	1.33	73	71	2	2
Magnesium,mg	165	5.5	265	240	2	2
Phosphorus,mg	207	6.9	1250	700	0.55	0.98
Manganese,mg	18.7	0.62	-	-	-	-
Ash, g	0.4	1.2	-	-	-	-
Moisture,g	1.31	8.93	-	-	-	-

On Toxicity, using the GT 16 in 1 formulation, toxicity test was conducted by the Regional Center for Food and Quality Assurance of Bicol University, by studying ten mice. Five of which were subjective to drink the one tablespoon ginger turmeric to 1 glass ratio of ginger turmeric tea at 200ml per dose. The other five was not subjected to the test. After 14 days of feeding the controlled mice, it was found out that there were no signs of toxicity and effects on mortality among the animals. The controlled group of 5 mice is still as strong as the 5 mice which did not drink the formulation. 5) On Marketability, the preferred package was 100 g in cellophane pack (56%) that can be bought from direct dealers, friends (46 %) and other outlets, and is very marketable with return of investment (ROI) of 138.45 % as reflected in Table 6, Table 7 and Table 8.

Table 6 Preferred packaging of the product

Packaging	Frequency	%
100 grams Cellophane	28	56%
20 grams Cellophane	3	6%
250 grams Cellophane	5	10%
500 grams Cellophane	5	10%
45 Grams Cellophane	3	6%
100 grams bottled	3	6%
200 grams bottled	3	6%
total	50	100%

Table 7 Preferred type of Seller

Type of seller	Frequency	%
Friends/direct seller	23	46%
Sari-sari store	18	36%
Grocery	8	16%
Mobile vending	1	2%
Total	50	100%

Table 8 Product sales and income

Income	Amount	Amount
Sales		
1,440 packs of 100 grams x P 45.00		P 64, 800.00
Expenses:		
72 kilos ginger x P 80	P 5,670.00	
72 kilos turmeric x P 80	5,670.00	
Herbs	1,000.00	
Sugar 144 kilos x P 44	6,336.00	
Fuel/Cooking P 700 x 5	3,500.00	
Labor	5,000.00	
Subtotal	P 27,176.00	P 27,176.00
Net income		P 37,624.00
ROI=	P 37,624 x 100 / 27,176	= 138.45 %

5. Conclusion

Form the stated results, the study forwards the following conclusions: Ginger, Turmeric can be produced into tea granules and it can be combined with herbs to further improve the product; almost all the product formulations were accepted by the customers with GT 16 in 1 as the most acceptable in terms of color, taste flavor; The most accepted drinking ratio is 1 tablespoon to 1 glass/cup 200 ml hot or cold water; The product is non-toxic, nutritious and is very suitable for human consumption.

From the above findings and conclusions, the study recommends the following: the product must be submitted to the Bureau of Patents for patenting; commercialization of the product can be conducted by product pitching of the technology among cooperatives, local government units and business companies; continue to conduct marketing of the product through direct selling and rationing to groceries and department stores; organize farmers who will establish demonstration farms and plantation of ginger and turmeric that would ensure a steady supply of the raw materials and submit the product for clinical test for a more authentic data that the product can heal some illnesses.

6. Acknowledgement

The writer would like to convey her heartfelt gratitude to the following: Bicol University General Administration and Bicol University Gubat Campus Staff who assisted in the whole duration of the study; Bicol University Research and Development Staff for the technical backstopping from proposal preparation until the final polishing of the result; Bicol University Extension Service Center staff who assisted the researcher in the conduct of the product testing; Regional Center for Food and Quality Assurance of Bicol University for the technical assistance the conduct of toxicity test; to DOST for the technical assistance in the conduct of Nutritional Analysis; and most of all to God Almighty who gave us the wisdom and strength throughout conduct of the study. To God be all the Glory!

References

1. Healing Wonders of Philippine Medicinal Plants, A Guide to The Use of PhilippineMedicinal Plants as an Alternative Medicine. http://www.filipinoherbshealingwonders.filipinovegetarianrecipe.coalagaw.htm
2. Hume, David. (1757). "Of the Standard of Taste," *Essays Moral and Political*, London: George Routledge and Sons, 1894. retrieval 2-11-15
3. Nutritional Analysis Report. (2017). Department of Science and Technology, Industrial technology Development Institute Standard Testing Division, Philippines.
4. Paul, H. (2014). 13 Health Benefits of Sambong https://www.linkedin.copulse/20141031154644-276727113-health-benefits-of sambong
5. Toxicity Test Report. (2017). Bicol University Regional Center for Food and Quality Assurance, Philippines. Yellow Turmeric Benefits that Rival Medications, https://draxe.com/tumericbenefits/1-32017 Patents
6. Jadie, R. R. (2016). Process of Producing Te from Turmeric and Ginger, Philippine Patent Utility Model, 2/2015/000671

3. SELECTION OF BEST DRYING METHOD TO SECURE CONTINUOUS SUPPLY OF EDIBLE GRADE MUCILAGINOUS MATERIALS FOR FOOD INDUSTRY

Kasunmala I.G.G., Navarathne S. B. and Wickramasinghe I
Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
Corresponding author's Email: kasunmala@sci.sjp.ac.lk

Abstract

Continuous supply of raw materials is a most common problem in food industry. *Neolitsea cassia, Cinnamomumverum, Terminaliaarjuna* leaves, and *Dilleniaretusa*fruits are some of the common materials used for extraction of mucilage in Sri Lankan food industry. Mucilaginous materials extracted from these materials were used as textural improvers, thickeners, stabilizers etc. Due to the seasonality and geographical factors, the availability of those raw materials year around is challenging issue in food security. Hence preservation of materials is an important task. Therein, the aforementioned raw materials were subjected to four different drying methods namely solar drying, mechanical drying, dehumidified air drying and shade drying. Mucilaginous materials were extracted manually in 1% citric acid solution and filtered. Viscosity and the drymatter content were measured for each drying method. They were compared with mucilaginous material extracted fresh raw materials. Results revealed that, mucilaginous material extracted from *Neolitsea cassia* leaves showed the highest yield and the viscosity both fresh and dried formes (fresh: 2.68± 0.15%, 3.9cP, dried: 2.63± 0.15%, 3.75cP) and there was a significant different ($P \leq 0.05$) between dehumidified air drying over other drying methods regarding the requiredproperties. Dehumidified drying exhibited the highest viscosity and yield which was nearly about fresh leaves and fruits, 98.1% and 95.4% respectively. Shelf life study was also showed significant retention of viscosity over six months. Hence dehumidified air drying was the best drying method in drying the raw materials to secure the availability of raw materials throughout the year.

Keywords: Dehumidified drying, *Neolitsea cassia, Cinnamomum verum, Terminalia arjuna*l eaves, *Dillenia retusa* fruits, mucilaginous materials, food security.

1. Introduction

"Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" [13]. Continuous supplying of raw materials year around is challenging issue in food industry.This is basically due to seasonal variations and geographical factors which negatively affect for the production of raw materials. To overcome this issue preservation of raw materials is an important task in food industry.

Applying drying methods is most common way to preserve raw materials. There are several drying methods used in food industry. But the most common and the economic ways of drying raw materials are sun drying, hot air oven drying, dehumidified air drying and shade drying. Those methods can apply without much technical knowledge, so those methods can easily be introduced to the local famers.

Gum and mucilage materials are naturally originated polymers, which extensively make use of conventional as well as novel food preparations [5]. This paper reports about most common mucilaginous materials used in Sri Lanka.Mucilage's are generally normal products of metabolism, formed within the cell (intracellular formation) and/or are produced without injury to the plant [7]. *Neolitsea cassia, Cinnamomum verum* and *Terminalia arjuna* leaves, and *Dillenia retusa* fruits are the most commonly available mucilaginous sources which are used in traditional food preparations as a textural improvers and thickening agents [4]. But most of them are under utilized in food industry due to insufficient knowledge about

these mucilaginous materials and lack of continuous supply of raw materials.

The aim of the present study was to select a prsavationmethord for raw materials to secure continuous supply of edible grade mucilaginous materials for food industry. This research manuscript describes the effect of four drying methods on drying four raw materials of mucilaginous materials with a view to select the best drying method and shelf life of the raw materials.

2. Materials and methods

Fresh matured *Neolitsea cassia, Cinnamomum verum* and *Terminalia arjuna* leaves, and *Dillenia retusa* fruits were collected from trees from Galle, Sri Lanka. Lying between 80° 13' 15.5208" East longitudes and 6° 3' 12.6684" North latitudes, Galle district is situated on the southern coast of the Sri Lanka. They were washed and air dried for the subsequent use of the study.

Dehydration of raw materials

*Neolitsea cassia, Cinnamomum verum*and *Terminalia arjuna* leaves, and *Dillenia retusa* fruits were subjected to four different drying methods with a view to select best drying method for the raw materials. Leaves were used as it is and fruits were cut into slices to reduce the case hardening effect.

a. Sun drying – Leaves and fruits were arranged as thin layer on drying trays and kept under direct sunlight for 12 hr.
b. Hot air oven drying – Leaves and fruits weredried in hot air oven (Leader-GPME350SSVISS080, UK) at 55 °C for 4 hr.
c. Dehumidified airdrying - Leaves and fruits weredried in a dehumidified air dryer at 4 °C for two weeks.
d. Shade drying - Leaves and fruits were arranged as thin layer on a trays and kept under shade condition (room temperature and normal atmospheric humidity) without contact with direct sunlight for one week.

Extraction of mucilaginous materials from Neolitsea cassia leaves

One hundred grams of leaves were hot water blanched for 15 min and thereafter blanched, leaves were washed with cold distilled water and gently crushed manually with 1% citric acid solution with 1:8 leaves: water ratio for 15 minutes. Extracts were filtered through six layers of muslin cloths. The filtrate was again filtered through a granular activated carbon filter (Haycarb PLC -RPMC 1003) with a filtration rate of 1 ml/min and the filtration was centrifuged (Hermile-Z206A, Germany) at 3500 rpm for 20 min and mucilaginous material was precipitated separately three times with the volumes of 95% ethanol. And it was dried in dehumidified air drying at 45 °C for 8 hours. The dried mucilage was collected, ground and sieved (Mesh 18, Microsil sifter, India). The ground mucilage product was separately stored in an airtight container at ambient conditions.

Determination of the Response Variables

Two responsevariables were used to analyze the best dehydration method, mucilaginous yield and viscosity. The yield was calculated as the ratio of dry weightsof the powder obtained after lyophilization to the initialpowder weight and expressed as g/kg. The apparent viscosityof the hydrated samples (2.5% w/w) was measured at constantconditions (temperature 25°C, pH 7, and shear rate 100 rpm)using DV-II+ programmable viscometer (Brookfield Engineering Instruments, USA) fitted with LV-1 spindle.

Shelflife analysisNeolitsea cassia leaves

Neolitsea cassia leaves were driedusing different drying methodswere subjected to shelf life analysis to determine maximum period of time the dried leaves can be kept without any interruption to the process variables.

Dried *Neolitsea cassia* leaves obtained from above drying methods were kept in an airtight container for six months at ambient room temperature (Temperature and RH). The apparent viscosityof the hydrated samples (2.5% w/w) was measured once a month at constant conditions.

Statistical analysis

Data were analyzed with Statistical Package minitab version 17.0 using oneway analyses of variance. Significance was defined at $P<0.05$ by

using Tukey's test. Percentage retention of yeid and viscosity was calculated with minitab version 17.0.

3. Results and discussion

*Neolitsea cassia, Cinnamomum verum*and *Terminalia arjuna* leaves, and *Dillenia retusa* fruits are some of the common sources that are used in extracting of mucilage materials which usually use as textural improvers, thickeners, stabilizers etc. Seasonal and geographical variation of these materials reduce the continuous supply and that makes hard to use them as raw material in food industry. Hence preservation of raw materials is the only option with a view to ensure continuous supply of these braw materials.

Four drying methods were used to dehydrate the selected raw materials and results pertaining to the magnitude of mucilage materialare given in Table 1 and 2.

Table 1: Yield of selected mucilaginous materials from different drying methods

Raw material	Yield of fresh raw materials	Yield after dehydration			
		Sun drying	Hot air oven drying	Dehumidified airdrying	Shade drying
Neolitsea cassia leaves	2.86±0.02^{a}	1.03±0.02^{b}	0.83±0.02^{c}	2.85±0.03^{a}	1.54±0.02^{d}
*Cinnamomumverum*leaves	1.10±0.02^{a}	0.46±0.02^{b}	0.27±0.01^{c}	1.07±0.01^{a}	0.86±0.01^{d}
*Terminaliaarjuna*leaves	1.85±0.01^{a}	0.85±0.03^{b}	0.58±0.04^{c}	1.83±0.02^{a}	1.12±0.03^{d}
*Dilleniaretusa*fruits	9.16±0.02^{a}	6.62±0.03^{b}	5.14±0.03^{c}	9.11±0.02^{a}	8.98±0.02^{d}

Each point corresponds to the average value ± standard deviation of six independent determinations. Different letters for each column indicate significant differences ($P< 0.05$).

Table 2: Viscosity of selected mucilaginous materials from different drying methods

Raw material	Viscosity of fresh raw materials/ Cp	Viscosity after dehydration/ Cp			
		Sun drying	Hot air oven drying	Dehumidified airdrying	Shade drying
Neolitsea cassia leaves	3.85±0.05^{a}	2.38±0.05^{b}	1.63±0.05^{c}	3.75±0.05^{a}	2.86±0.05^{d}
*Cinnamomumverum*leaves	1.83±0.05^{a}	0.93±0.05^{b}	0.55±0.10^{c}	1.75±0.06^{a}	1.50±0.00^{d}
*Terminaliaarjuna*leaves	2.63±0.05^{a}	1.13±0.05^{b}	0.78±0.05^{c}	2.50±0.08^{a}	1.89±0.05^{d}
*Dilleniaretusa*fruits	4.63±0.13^{a}	3.43±0.05^{b}	2.95±0.08^{c}	4.43±0.10^{a}	3.95±0.18^{d}

Each point corresponds to the average value ± standard deviation of six independent determinations. Different letters for each column indicate significant differences ($P< 0.05$).

Mucilaginous yield

Yield of four mucilaginous materials with four drying methods were tabulated in Table 1. Drying of raw materials significantly affect ($P<0.05$) the mucilaginous yield except in dehumiditified drying compared with fresh raw materials. Dehumiditified drying had a little effect on mucilaginous yield compered to other drying methods and it yielded the highest mucilage out put. Hot air oven dry showed the lowest yeid. Shade drying showed the second best mehod and sun drying was the least method compering the yield of mucilaginous material. All four mucilaginous materials exhibit same patern of mucilaginous yield for each drying method.

Viscosity variation

Viscosity variation of four mucilaginous materials with four drying methods shown in Table 2. Drying of raw materials decresed the viscosity compared to fresh mucilaginous materals. Viscosity was significantly ($P< 0.05$) decresed in the manner of hot air oven dring, sun drying and shade drying but a little reduction of it shown by the dehumiditified drying.Hot air oven dring gave the lowest viscosity whereas dehumiditified drying exhibit highest viscosity. All four raw materials

revealed same pattern of viscosity reduction to each drying method and those variations were compatible with the yield variation of the mucilaginous materials.

Percentage retention of yield and viscosity of mucilaginous materials after drying the raw materials with four drying methods aregiven in Table 3.

Percentage retention of yield and viscosity

Table 3: Percentage retention of yield and viscosity of dried mucilaginous materials

Raw material	Yield retention after dehydration				Viscosity retention after dehydration			
	SD	AOHD	DAD	ShD	SD	AOHD	DAD	ShD
Neolitsea cassia leaves	36.01%	29.02%	99.65%	53.84%	61.82%	42.34%	97.40%	74.29%
*Cinnamomumverum*leaves	41.82%	24.55%	97.27%	78.18%	50.82%	30.05%	95.63%	81.97%
*Terminaliaarjuna*leaves	45.95%	31.35%	98.92%	60.54%	42.97%	29.66%	95.05%	71.86%
*Dilleniaretusa*fruits	72.27%	56.11%	99.45%	98.03%	74.08%	63.71%	95.68%	85.31%

SD: Sun drying, AOHD: Hot air oven drying, DAD: Dehumidified airdrying, ShD: Shade drying.

When considering the percentage retention of yield and viscosity, both yield and viscosity showed same pattern. Highest yield and viscosity retention exhibit in dehiumiditified dryng method (over 97% retention on yield and over 95% retention of viscosity). Significant reduction was observed in hot air oven drying which was less than 56% for yield and less than 63% for hot air oven drying. All the four mucilaginous materials revealed same pattern of percentage retention of yield and viscosity.

According to the previous studies, organoleptic properties, extractability and physiochemical properties of *Neolitsea cassia*showed best quality among the selected four mucilaginous materials [4]. Hence, shelf life analysis was carried out based on *Neolitsea cassia*leaves with four different drying mehods.

Shelf life analysis

Viscosity variations of selected raw materials over six month of period aregraphically represented in Figure 1.

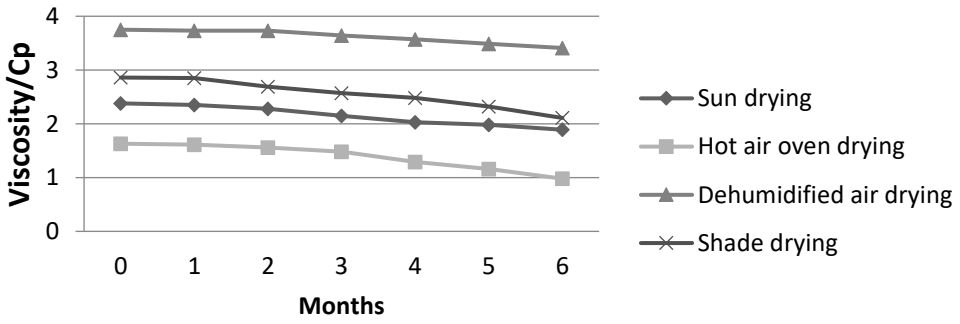


Figure 1: Shelf life analysis of *Neolitsea cassia*

According to the figure 1, dehumiditified drying showed a significant retention of viscosity over six months of period. Shade drying was the second-best method but there was a significant ($P< 0.05$) decrese in viscosity by this method. Hot air oven drying exhibit the least quality of mucilaginous material when comparing those drying methods, sun drying and shade drying can be done without any hiderence but diuranal factores may affect the drying process. Hot air oven drying and dehumiditified drying requires equipment but economical, can prafom without interruption of environment conditions, and drying can be practiced as most hygienical way of drying of raw materials. Dehumiditified drying is bit time consuming but gave a good quality product compared to hot air oven drying. It is mainly due to that drying was occurred at very low temperature.

4. Conclusion

Due to the seasonality and geographical factors, the availability of raw materials for edible grade mucilaginous materials year around is challenging issue in food security. To overcome this burden preservation methods were developed. Dehumidified drying exhibited the highest viscosity and yield which was nearly about fresh leaves and fruits. Furthermore, it showed significant retention of viscosity over six months. Hence dehumidified air drying was the best drying method in drying the raw materials to secure the availability of raw materials throughout the year.

5. Acknowledgement

The authors thank the University of Sri Jayewardenepura, Sri Lanka for financial assistance (Grant No:ASP/01/RE/SCI/2016/19).

References

1. Aravindh, M. A., & Sreekumar, A. (2015). Solar drying—a sustainable way of food processing. In *Energy sustainability through green energy* (pp. 27-46). Springer, New Delhi.
2. Esper, A., & Mühlbauer, W. (1998). Solar drying-an effective means of food preservation. *Renewable Energy*, *15*(1-4), 95-100.
3. Farkas, J. (2007). Physical methods of food preservation. In *Food Microbiology: Fundamentals and Frontiers, Third Edition* (pp. 685-712). *American Society of Microbiology*.
4. Jani, G. K., Shah, D. P., Prajapati, V. D., & Jain, V. C. (2009). Gums and mucilages: versatile excipients for pharmaceutical formulations. *Asian J Pharm Sci*, *4*(5), 309-323.
5. Kasunmala, I. G. G., Navarathne, S. B., & Wickramasinghe, I. (2017). Extraction and characterization of mucilaginous material from DawulKurundu Leaves (Neolitsea involucrate) and Godapara Fruits (Dillenia retusa). *Extraction*, *2*(2).
6. Kumar, Y., Tiwari, S., & Belorkar, S. A. (2015). Drying: an excellent method for food preservation. *International Journal of Engineering Studies and Technical Approach*, *1*(8), 1-17.
7. Maru, S. G., Prakash, S. B., Dharmesh, S. B., Agham, S. A., & Aghera, N. J. (2012). Natural polymer: gums and mucilage as good pharmaceutical excipients. *Tech Med*, *1*, 64-74.
8. Nep, E. I., & Conway, B. R. (2011). Physicochemical characterization of grewia polysaccharide gum: Effect of drying method. *CarbohydratePolymers*, *84*(1), 446 453.
9. Ratti, C. (2001). Hot air and freeze-drying of high-value foods: a review. *Journal of food engineering*, *49*(4), 311-319.
10. Sagar, V. R., & Kumar, P. S. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. *Journal of food science and technology*, *47*(1), 15-26.
11. Song, X., Zhu, T., Zhuang, D., Wang, C., & Yao, B. (2015). Cooling and Dehumidification Capacity Chart of Surface Air Cooler in Air Conditioning. *Procedia Engineering*, *121*, 2014-2020.
12. World Food Summit. (1996). Rome Declaration on World Food Security.

4. INVESTIGATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN MILK AND DAIRY PRODUCT

Chatartorn Cha-aim[1], Wiparat Nisapai[2], Pongsert Sriprom[1*]
[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[2]Faculty of Engineering, Chaiyaphum Rajabhat University, Nafai, Mueang Chaiyaphum, Thailand
Corresponding author's Email: pongsert.sr@kmitl.ac.th

Abstract

The presence of organochlorine pesticides (OCPs) in milk and dairy products can conduct to human exposure. This study was to investigate the presence of organochlorine pesticide in fresh milk and UHT milk. The samples were prepared by AOAC and QuEChERS modification method. Gas chromatography with mass spectrometer (GC-MS) was used to detect organochlorine pesticide and organochlorine derivative compounds in fresh milk, and UHT milk. The results showed that AOAC modification method can detect organochlorine derivative be similar to QuEChERS modification method. The organochlorine pesticide residues were found at least one type in all milk sample, especially Hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH) and heptachlor epoxide were presented in all sample both fresh milk and UHT milk. Organochlorine pesticides are widely used in Thailand over the last three decades and accumulated in cattle feed as the main source of contamination. Furthermore, there will be a study in quantitative and risk assessment in health effect.

Keywords: Cattle, GC-MS, pesticide contamination

1. Introduction

The agricultural is a major sector in Thailand. Pesticides have been extensively used for increasing productivity by protecting crops from insects and other pests. Especially organochlorine pesticides are beneficial compound in many applications their properties are lipophilic, high persistence, hardly metabolite and significant toxicity to plants, animals and humans. In Thailand, the ministry of industry had noticed the organochlorine compounds list which had been banned. For example, endrin in 1982, DDT in 1983, dieldrin, aldrin, heptachlor in 1988, chlordane in 2000, beta-HCH in 2001 and endosulfan in 2004 (The ministry of industry, 2013).

The feed was including pasture grasses, silage crop, cereal grain, sugar beet, etc. were contaminated by organochlorine pesticides from agricultural, so when it used for animal feed organochlorine pesticides will accumulate in animal tissue, in particular milk that high-fat content. Tsiplakou E. et al (2010) studied on pesticides residue in feedstuff in Greece, the sample was alfalfa hay and the concentrated feed consisted of maize grain, barley grain, soybean meal, wheat middlings, mineral and vitamin premix and they founded Σendosulfan 0.1 and 5.36 mg/kg respectively. Rattanawat C. et al (2015) studies on organochlorine pesticide levels in food web in rice paddies of Bueng Boraphet wetland, Nakhon Sawan province, central Thailand founded aldrin, α-HCH, β-HCH, γ-HCH and heptachlor epoxide in rice grain and rice leaf. The result confirmed that the animal feed is the main source of pesticides contamination of dairy product.

Milk is a perfectly natural food for infants and children with fat content about 3-4% that an important source of organochlorine accumulation. There are many reports show the occurrence of organochlorine pesticide residues in milk. A study of Agata W. et al (2013) in raw cow's milk in Poland showed that high level of ΣDDT, but less than the acceptable daily intake (ADI). 80% of milk samples marketed in Iran found at least one organochlorine pesticide residue while lindane (0.021 mg/kg) and DDT (0.125 mg/kg) are exceeded the FAO/WHO standard limits (0.004 and 0.05 mg/kg) (Ashnagar A. et al, 2009). The occurrence of residues in raw, pasteurized and UHT milk in Brazil was reported by Heck M.C. et al (2007), HCH and pp'-DDE were found in all samples and pp'-DDE, op'-DDD, lindane were found at

high concentration, however no EDI exceeded the corresponding ADI value.

Thai government established the school milk supplementary project in 1992, to encourage nutritional in children and to support the dairy industry in Thailand by using raw milk from domestic farmers. The proportion of raw milk entering the school milk supplements project accounts for 40 percent of all raw milk produced in Thailand. Every child in kindergarten to primary school drink school milk. Nevertheless, the researcher in Thailand study a few in milk contamination, In 1983 Malinee L. et al studied on residues in milk samples collected from Nong-Po, Ratchaburi province and from the Department of Animal Science, Kasetsart University, Kam pangsan campus the result showed that heptachlor, heptachlor epoxide, aldrin, dieldrin and pp'-DDT were found in milk from Nong-Po, Ratchaburi, but no detected pp'-DDT in milk from Kam pangsan campus. Shinsuke T. et al (1991) founded a high level of ΣHCH as 69 ng/g fat weight basis in butter but did not exceed the maximum allowable residue limit in foodstuffs by the ministry of public health. However, it no presently reports in milk contamination.

Finally, the aim of this study was to compare difference extraction method and to investigate the contamination of OCPs residues in difference milk type supply from school milk supplementary project.

2. Materials and Methods

Reagents and standards

All the chemicals used were of analytical grade or higher. Acetic acid and sodium chloride were purchased from Carlo Erba reagents S.A.S. (France). Acetonitrile, Diethyl ether, Methanol, Petroleum ether were purchased from RCI Labscan (Thailand). Anhydrous sodium sulfate was purchased from Sigma Aldrich (Germany). Florisil, magnesium sulfate, sodium acetate were purchased from Merck (Germany). Hexane was purchased from Honeywell (USA). Potassium oxalate was purchased from BDH laboratory supply (USA). The DI water was purified with ELGA (England). Standard organochlorine pesticides (Aldrin, Cis-Chlordane, Trans-Chlordane, Oxy-Chlordane, Dieldrin, p,p'-DDD, p,p'-DDE, o,p'-DDT, Endrin, p,p'-DDT, HCB, α-HCH, β-HCH, α-HCH, Heptachlor,Heptachlor Epoxide were purchased from Restek (USA).

Sample collections

Fresh milk was collected from dairy cooperative organization in the school milk supplementary project by packaged in polyethylene bags and the samples were transported in cooling boxes containing ice packs to the laboratory. Then the sample were immediately stored frozen at -20°C until analysis. UHT milk of school milk supplementary products was collected from pre-elementary school and storage at room temperature.

Extraction and clean up

The extraction method was compared by five different methods according to table 1. The sample was spiked with 0.2 ppm of the solvent mixtures, including Aldrin, Cis-Chlordane, Trans-Chlordane, Oxy-Chlordane, Dieldrin, p,p'-DDD, p,p'-DDE, o,p'-DDT, Endrin, p,p'-DDT, HCB, α-HCH, β-HCH, α-HCH, Heptachlor andHeptachlor Epoxide. The extracted was analyzed by GC-MS. The GC analyses were carried out on a Thermo Trace 1300 seriesgas chromatography equipped with an ISQ mass spectrometer and Triplus RSH autosampler, a capillary column (stx®-CL Pesticides: 30 m x 0.32 mm i.d., 0.5 µm df). The column oven temperature was programmed as front inlet temperature at 250°C, oven temperature as initial time 1 min, Ramp 1 temperature 35°C, Final temperature 190°C, Ramp 2 temperature 155°C, Final temperature 250°C, Final time 3 min, flow rate at 1.2 ml/min, MS transfer line at 250°C, ion source temperature at 230°C and electron energy at 70 eV.

Table 1: Method of organochlorine pesticide extraction and clean up.

Method	Reference	Principle
A	AOAC 970.52	AOAC

B	Heck M. et al. (2007)	
C	Ashnagar A. et al (2009)	
D	In-Seek J. et al (2012)	QuEChERS
E	Xianyu C. et al. (2014)	

3. Results and discussion

Comparison of the extraction method

Nowadays, have many methods to determine OCPs residues in milk and dairy product. The standard method is AOAC 970.52 and other modification method use liquid-liquid extraction techniques and requires a large amount solvent. The new technique as QuEChERS (quick, easy, cheap, effective, rugged and safe) was developed to use in high-fat food matrix, so this study compares five extraction methods as shown in Table 2.

Table 2: Methodologies for organochlorine pesticide determination

Method	Extraction procedure	Clean up Procedure	Condition
A	Addition of potassium oxalate followed by methanol, ether and petroleum ether	Florisil	Use K-D concentration for collect eluting solvent in clean up method
B	Petroleum ether	Florisil	Extract only fat by centrifugation
C	Addition of aqueous potassium oxalate solution followed by methanol, diethyl ether and hexane	Petroleum ether : acetonitrile	Lipid-lipid partitioning by NaCl solution
D	Acetonitrile in 1% acetic acid	PSA and C18	Clean up by freezing centrifugation
E	Acetonitrile and hexane	PSA	Use sonicator in extraction step

The comparison of extraction and clean-up method for the16 pesticides was determined by comparing the peak area of post-extraction spike sample with the corresponding area in blank sample. In AOAC principle method C can detect most pesticide at 11 of 16 pesticides follow by method A and B at 9 and 6 pesticides respectively while QuEChERS principle method D is more detected than method E at 11 and 1 pesticide detection, the detected pesticides in each different method was presented in Table 3.

Method C use many types of solvent to extract organochlorine pesticide for reducing interfere and method D use acetic acid as a preservative to ensure the stability of the pesticides and improve clean up efficiency by freezing centrifugation. Actually, both principles are closely detected. However, the pesticide detection depends on GC condition and limit of detection so, the optimization method should confirm by recovery study.

Table 3: Pesticide residue found in milk by difference method

No.	Pesticide	AOAC 970.52	Heck M. et al. (2007)	Ashnagar A. et al. (2009)	In-Seek J. et al. (2012)	Xianyu C. et al. (2014)

1	HCB	-	✓	✓	✓	-
2	α-HCH	-	✓	✓	✓	-
3	γ-HCH	-	-	✓	✓	-
4	β-HCH	✓	-	-	✓	-
5	Heptachlor	-	-	✓	✓	-
6	Aldrin	-	✓	-	✓	-
7	Oxy-Chlordane	✓	-	-	-	-
8	Hept.-Epoxide	-	✓	✓	-	-
9	Trans-Chlordane	✓	-	✓	-	-
10	Cis-Chlordane	✓	-	-	✓	✓
11	p,p'-DDE	-	✓	-	✓	-
12	Dieldrin	✓	-	✓	✓	-
13	o,p'-DDT	✓	-	✓	-	-
14	Endrin	✓	-	✓	✓	-
15	p,p'-DDD	✓	✓	✓	-	-
16	p,p'-DDT	✓	-	✓	✓	-

✓: Detect, - : Not Detect.

Organochlorine pesticide residues in milk sample

Seven samples of fresh samples of school milk supplementary products were determined the organochlorine pesticide residues using GC-MS. The results show that the residues were detected at least one type in all milk sample. HCB, β-HCH, Heptachlor, Aldrin, Oxy-Chlordane, Hept. -Epoxide, p,p'-DDE, Dieldrin and p,p'-DDT were detected in all sample. o,p'-DDT and Endrin were detected in six samples. α-HCH and γ-HCHwere detected in five samples. Trans-Chlordane, Cis-Chlordane and p,p'-DDTwere detected in four samples as shown in Figure 1.

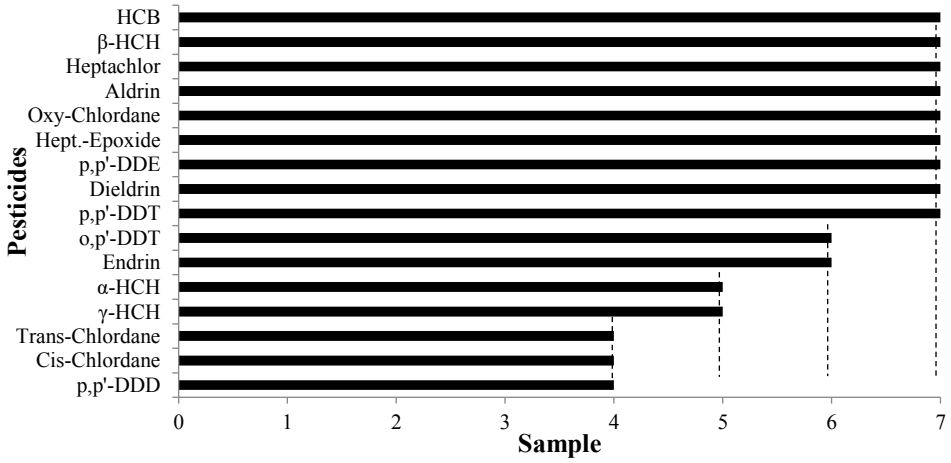


Figure 1: Summary of pesticide detections inFresh Milk (n =7)

Sixteen samples of UHT milk of school milk supplementary products were determined. The results were detected HCB, β-HCH, Hept.-Epoxide, p,p'-DDD all UHT sample. Heptachlor and Trans-Chlordanewere detected in fifteen samples. α-HCH, Aldrin, Cis-Chlordane and p,p'-DDE were detected in fourteen samples. γ-HCH, Dieldrin and o,p'-DDT were detected in thirteen samples. Oxy-Chlordane was detected in twelve samples. Endrin and p,p'-DDTwere detected in ten samples as shown in Figure 2.

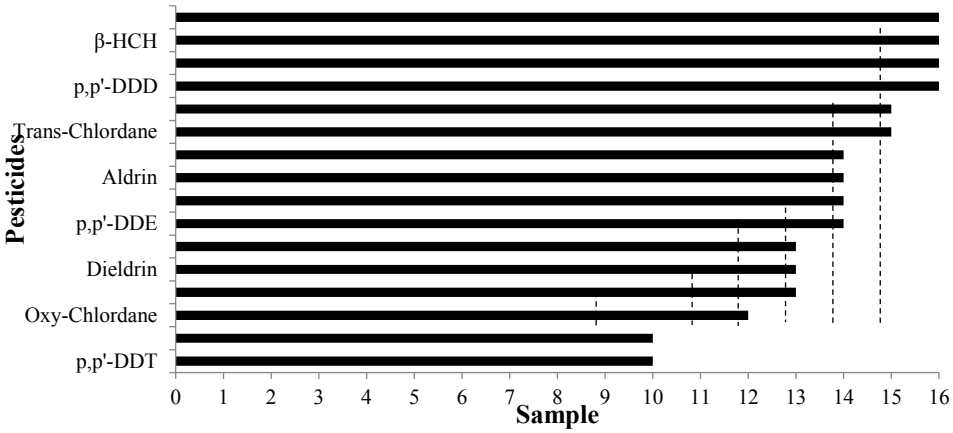


Figure 2: Summary of pesticide detections inUHT Milk (n = 16)

The result indicated that milk sample was contaminated by organochlorine pesticide from environmental contamination. Hexachloro benzene (HCB), Beta-hexa chloro cyclohexane (β-HCH) and heptachlor epoxide were presented in all sample both fresh milk and UHT milk. HCB residues may be caused by it is a byproduct during the manufacture of other chemicals mainly solvents and pesticide. β-HCH is the most persistent isomer, it is the predominant isomer in soils and animal tissues because its configuration favors storage in biological media and affords it greater resistance to hydrolysis and enzymatic degradation. As same as heptachlor epoxide is a manufactured chemical and can occur form break down of heptachlor by bacteria and animals. it can stay in the soil and water for many years and plants can take up from the soil, so it can build up in the tissues of a cattle (ATSDR, 2005). Moreover, the detection limit must be studied and compared with the maximum residue limit level by regulatory for food safety assessment.

4. Conclusion

This study was to compare difference extraction methods and to investigate the contamination of organochlorine pesticides residues in different milk type supply from school milk supplementary project. The study shown five difference methods get difference result, but AOAC and QuEChERS principle detect the same amount of pesticide residue about 11 types. The presence of organochlorine pesticide residues in all milk samples have been banned for many decades but these chemical substances have been found in the environment. Furthermore, there will be a study in quantitative and risk assessment in health effect.

References

1. AOAC international. (2016). Official Methods of Analysis. 20th ed. AOAC 970.52 Chapter 10, AOAO international, Maryland, 1-10.
2. Ashnagar, A., Naseri, N. G., & Farmad, M.C. (2009). Determination of organo-chlorine pesticide residues in cow's milk marketed in Ahwaz city of Iran. International Journal of PharmTech Research, 1(2), 247-251.
3. Chaiyarat, R., Sookjam, C., Eiam-Ampai, K., & Damrongphol, P. (2015). Organo-chlorine pesticide Levels in the food web in rice paddies of Bueng Boraphet wetland, Thailand. Environmental monitoring and assessment, 187(5), 230.

4. Chen, X., Panuwet, P., Hunter, R. E., Riederer, A. M., Bernoudy, G. C., Barr, D. B., & Ryan, P. B. (2014). Method for the quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry. Journal of Chromatography B, 970, 121-130.
5. Heck, M. C., dos Santos, J. S., Junior, S. B., Costabeber, I., & Emanuelli, T. (2007). Estimation of children exposure to organo-chlorine compounds through milk in Rio Grande do Sul, Brazil. Food Chemistry, 102(1), 288-294.
6. Jeong, I. S., Kwak, B. M., Ahn, J. H., Jeong, S. H. (2012). Determination of pesticide residues in milk using a QuEChERS-based method developed by response surface methodology. Food chemistry, 133(2), 473-481.
7. Martins, J. G., Chávez, A. A., Waliszewski, S. M., Cruz, A. C., & Fabila, M. M. G. (2013). Extraction and clean up methods for organochlorine pesticides determination in milk. Chemosphere, 92(3), 233-246.
8. Tanabe, S., Kannan, K., Tabucanon, M. S., Siriwong, C., Ambe, Y., & Tatsukawa, R. (1991). Organochlorine pesticide and polychlorinated biphenyl residues in foodstuffs from Bangkok, Thailand. Environmental pollution, 72(3), 191-203.
9. The Agency for Toxic Substances a Disease Registry. (2005). Fact sheet of Heptachlor and heptachlor epoxide. Atlanta. The ministry of industry (2013). The notification of the ministry of industry of hazardous substances list.
10. Tsiplakou, E., Anagnostopoulos, C. J., Liapis, K., Haroutounian, S. A., & Zervas, G. (2010). Pesticides residues in milks and feedstuff of farm animals drawn from Greece. Chemosphere, 80(5), 504-512.
11. Witczak, A., Pohoryło, A., & Mituniewicz Małek, A. (2016). Assessment of health risk from organochlorine xenobiotics in goat milk for consumers in Poland. Chemosphere, 148, 395-402.

5. MICROBIOLOGICAL QUALITY OF RAW MATERIALS IN PRODUCTION OF SHRIMP CHILI PASTE

Vanida Kongkachod[1], Adisorn Swetwiwathana[2], Kittichai Baniong[2], and Aphacha Jindaprasert[2*]
[1]Master course student of Department of Agro-Industry, KingMongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand
[2]Department of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand
Corresponding author's E-mail: aphacha.ji@kmitl.ac.th

Abstract

The study was to assess the microbiological quality 27 sample of raw materials in production of shrimp chili paste (Nam Prik Kapi) from restaurant. The samples were tested for total plate count (TPC) and 4 pathogenic bacteria consisted of *Bacillus cereus, Clostridium perfringens, Salmonella* spp. and *Staphylococcus aureus* of raw materials such as shrimp paste (kapi), chili, garlic, lime juice, palm sugar, eggplant, water that used in producing ready-made shrimp chili paste. The study revealed that TPC were found in 27 samples range of 39 – 9.0 x 10^5cfu/g.*B. cereus* cells and spore were found in shrimp paste, shrimp chili paste as kept in a refrigerator for 3 days at 50, 43, 26 cfu/g and 23, 60, 10 spore/ g, respectively. All raw materials were not detected *C. perfringens, Salmonella* spp. and *S. aureus*. The pH values of shrimp paste and shrimp chili paste were in the range of 4 . 40 – 4. 72. *B. cereus,* isolated in shrimp chili paste as kept in a refrigerator for 3 days, were confirmed biochemical characteristics test and identified using 16S rRNA sequence.

Keywords: *Bacillus cereus,* shrimp paste (kapi), shrimp chili paste, safety

1. Introduction

Shrimp chilli paste) Nam Prik Kapi(, is a popular food items in Thailand, is usually consumed with food or vegetables and about 98% of Thai population consumed chilli paste (Department of Health, Ministry of Public Health, 2010) and more than 64. 1% of the respondents replied that chilli paste as an indispensable family meal (Thai Health Promotion Foundation, 2010).The shrimp chilli paste prepared by mixing shrimp paste, chilli & garlic, lime juice, palm sugar and water together in a milling machine until the mixture becomes smooth. Before serving, eggplants are sprinkled on the top. The Academic Centre of Thai Health Promotion Foundation (2010) found ready to eat chilli paste including shrimp chilli paste, fermented fish spicy dip, pimp chilli paste, red hot chilli dip and hill dipping sauce was contaminated with yeast & mold, colifroms, *Escherichia coli*, *B. cereus* and *C. perfringens.* The main reasons for microbial contamination in chilli pastes were the ingredients used including cooked fish or fermented shrimp containing *B. cereus,* a spore forming pathogenic bacteria capable of producing toxin. (Academic centre Thai Health Promotion Foundation, 2010). *B. cereus is* a gram-positive bacterium and widely distributed in soil and vegetation and thus can contaminate raw or processed foods. Pathogenic *B. cereus* strains can cause a range of infections in humans, as well as food poisoning of an emetic (intoxication) or diarrheal type infection (Berthold pluta A et al., 2015). In this study we evaluated the microbiological quality of laboratory prepared shrimp chili paste and compared the quality of restaurant prepared shrimp chili paste. Total bacterial load and the presence of *B. cereus, C. perfringens, Salmonella* spp. and *S.aureus* were investigated. into sterile plastic bag in a refrigerator at 4°C for 3 days.

2. Materials and methods

Shrimp chili paste preparation

In the laboratory, 100 g of shrimp chili paste were prepared and the ingredients shrimp paste 50.0 g, lime juice 20.0 g, palm sugar 50.0 g, chili 8.0 g, peeled garlic 2.0 g and water 9.0 g, were ground using a milling machine for 10 minutes and finally 2.0 g eggplants were added. This ready-to-eat shrimp chili paste was kept

Sample collection

A total 27 samples were collected from November, 2017 to January, 2018. The raw ingredients including 3 shrimp paste samples, 3 chill samples, 3 garlic samples, 3 lime juice samples, 3 palm sugar samples, 3 eggplant samples and 3 tap water samples that used as materials for shrimp chili paste production, and 3 ready-made shrimp chili paste samples and 3

shrimp chili paste samples kept in a refrigerator for 3 days were also collected. All the samples were collected in sterile plastic bag and kept in foam box containing ice to transport to the laboratory within 2 hours and the microbiological and chemical analysis was done for within 24 h.

Microbiological analysis

Twenty five grams of each samples were aseptically homogenized with 225 ml sterile peptone solution)0.1% w/v(in separate sterile plastic bag for 2 min in a stomacher.Serial dilutions)1:10(of each homogenized sample were made in the same diluents, and 1.0 ml of diluted and undiluted samples pour plated on to Plate Count Ager)PCA(and incubated at 37^0C for 48 h before being counted the total bacterial load. *B.cereus* counts were determined by Manitol egg yolk poly- myxin)MYP(agar and incubated at 30ºC for 18 - 24 h. For detection of spores, peptone water was added to the samples and heated in a water bath at 80ºC for 10 minutes before being plated on to MYP agar and incubated at 30^0C for 18 - 24 h. The typical pink colonies on MYP agar were picked and confirmed by streaking onto trypticase soy-sheep blood agar and incubated according to BAM, 2001, procedure. The presumptive colonies were isolated and were confirmed using 16S rRNA sequence and phylogenetic analysis as described by Gibthai Co. Ltd., Thailand. *S.aureus* was determined using selective 3M Petrifilm Staph strip and incubated at 35^0C for 24 h according to AOAC method. The typical red–violet colonies were counted as *S. aureus*)3M Food Safety, 2000(. The *C. perfringens* were determined by inoculating 1 ml of diluted or undiluted samples in Cook Meat medium)CM(broth and incubated at 35^0C for 24 h)BAM, 1992(. Detection of *Salmonella* was done according to the ISO 6579/2002 method. Briefly, twenty five grams of samples were aseptically homogenized with 225 ml of pre-enrichment broth)0.1% w/v buffered peptone water(and incubated at 37^0C for 24 h. The typical colonies were counted and the classification of *Salmonella* serotype was done by slide agglutination test method and serotyping with antisera purchased from S & A Laboratory Ltd., Thailand.

Chemical analysis

The pH was measured in triplicate with a pH meter)Mettler Toledo, China(. The shrimp paste, lime juice, ready-made shrimp chilli paste and shrimp chilli paste kept as such in a refrigerator for 3 days samples was determined according to method by Thai Industrial Standards)TIS 1080–1992 Kapi) and the results were expressed as mean of three reading.

3. Results and discussion

The microbiological quality of raw ingredients and laboratory prepared shrimp chili paste samples kept as such in a refrigerator for 3 days was shown in Table 1. The results revealed that total bacterial load ranged from 3.9 x 10^1 to 9.0 x 10^5 cfu/g, was within the acceptable limit as mentioned in International Commission on Microbiological Specifications for Foods (ICMSF, 2002). In addition, *B. cereus* cells and spore were observed in shrimp paste, shrimp chili paste and shrimp chili paste kept as such in a refrigerator for 3 days at 50, 43, 26 cfu/g and 23, 60, 10 spore/g, respectively. However, *C. perfringens, Salmonella* spp. and *S.aureus* were not detected in any of the samples analyzed. Wattanahongsiri (2016) reported higher bacterial load in 24 shrimp paste samples from closed container, open container sold in fresh markets and in hospitals' kitchen, respectively. In addition, *B. cereus* was detected in 22 samples, and *C. perfringens* was found in 3 samples, Furthermore, Phromcharoen et al. (2003) reported higher bacterial load ranging from 3.0 x 10^3 -3.0 x 10^7 cfu/g were detected in 62 Thai chili pastes samples and lower level < 10^5 cfu/g of *B. cereus* was found in Thai chilli pastes samples, and this reduction might be caused by controlling the raw materials quality.

Table 1: Microbiological quality of raw ingredients and laboratory prepared shrimp chilli paste samples kept as such in a refrigerator for 3 days *

Sample	No. of sample	Total plate count)cfu/g(	*B. cereus cell*)cfu/g(	*B. cereus spore*)*spores*/g(	*C. perfringens (cfu/g)*	*Salmonella*)*cfu*/25 *g*(	*S. aureus*)cfu/g(
Chilli	3	9.0 x 10^5	N/D	N/D	N/D	N/D	N/D

Garlic	3	3.6×10^3	N/D	N/D	N/D	N/D	N/D
Lime juice	3	N/D	N/D	N/D	N/D	N/D	N/D
Palm sugar	3	3.9×10^1	N/D	N/D	N/D	N/D	N/D
Eggplant	3	1.5×10^6	N/D	N/D	N/D	N/D	N/D
Water	3	3.1×10^2	N/A	N/A	N/A	N/A	N/A
Shrimp paste	3	1.2×10^4	50	60	N/D	N/D	N/D
Shrimp chilli paste	3	6.6×10^4	43	23	N/D	N/D	N/D
Shrimp chilli paste as kept in a refrigerator for 3 days	3	8.7×10^4	26	10	N/D	N/D	N/D

N/D = Not detected; N/A = Not analyzed
*A total samples during the November, 2017 to January, 2018

B. cereus from the shrimp paste, shrimp chilli paste and shrimp chilli paste kept in a refrigerator for 3 days were identified biochemical characteristics test) Table 2). *B. cereus* is a genus of Gram-positive, rod-shaped and motile bacteria. It produces β-hemolysis on the sheep blood agar, produces acid from glucose anaerobically, produce acetyl methyl carbinol (Voges Proskauer (VP)-positive); and decomposes L-tyrosine (Tyrosine-positive). However, it was negative for rhizoid growth and does not produce protein toxin crystals. Selected *B. cereus* isolates in shrimp chilli paste as kept in a refrigerator for 3 days)Sample name: BS-1) was confirmed using 16S rRNA sequence and phylogenetic tree analysis (Figure 1). The results showed that bacterial isolate (BS-1) was *B. cereus.*

Table 2: Identification of *B.cereus* detected from the Shrimp paste, Shrimp chilli paste and shrimp chilli paste as kept in a refrigerator for 3 days at the Department of Health, Ministry of Public Health

Sample	NA	Gram stain	Motility	VP	Glucose	Hemolyse	Rhizoid growth	Tyrosine	Toxin crystal	Growth 43^0C	B. cereus
Shrimp paste	+	+	+	+	+	++	-	+	-	+	+
Shrimp chilli paste	+	+	+	+	+	++	-	+	-	+	+
Shrimp chilli paste kept in a refrigerator for 3 days	+	+	+	+	+	++	-	+	-	+	+

NA= Nutrient growth; VP=Voges Proskauer; + = Positive ; - = Negative

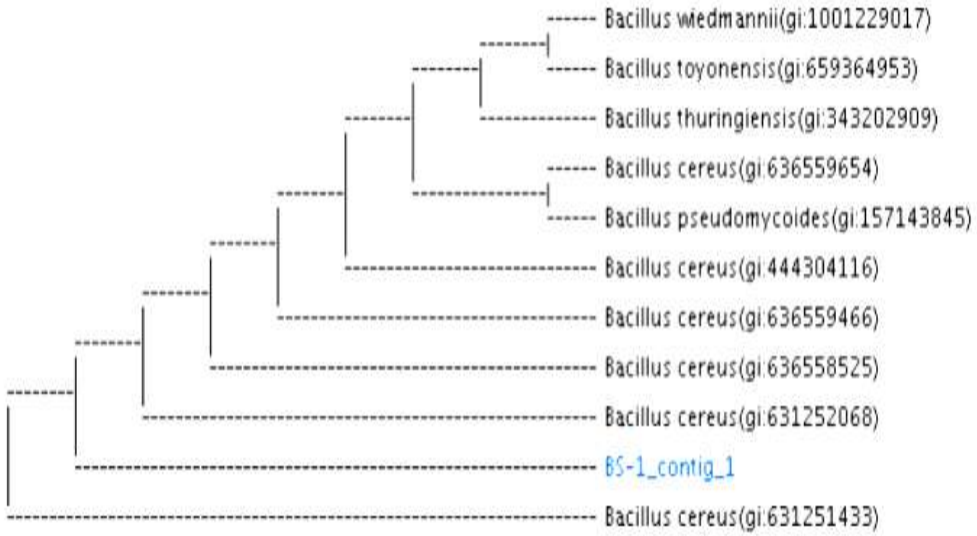


Figure 1: Comparative phylogenetic analysis of the *B. cereus* isolates (sample name: BS-1) from GenBank.

Table 3 showed the pH values of lime juice was recorded as 2.37 and shrimp paste as 7.06, which was within acceptable level since Thai Industrial standards (TIS 1080 - 1992) is in the ranges of 6.5 – 7.8, shrimp chili paste and shrimp chili paste kept in a refrigerator for 3 days were ranged from 4.40 – 4.72. From Table 1 and Table 3, the level of *B. cereus* vegetative cells and spore trended to decrease, when pH of shrimp chili paste decreased while kept in a refrigerator. These findings are in agreement with the findings of Phrom-charoen et al. (2003), who detected *B. cereus* strains in Thai chill pastes and pH values of the chili paste contains sour ingredients were in the range of 4.21 - 5.23, respectively.

Table 3: The pH values of lime juice, shrimp paste, shrimp chilli paste and shrimp chilli paste kept in a refrigerator for 3 days

Sample	No. of sample	pH
Lime juice	3	2.37 ± 0.01
Shrimp paste	3	7.06 ± 0.37
Shrimp chilli paste	3	4.72 ± 0.11
Shrimp chilli paste as kept in a refrigerator for 3 days	3	4.40 ± 0.12

4. Conclusions

In summary, *B. cereus* cells and spore were found in shrimp paste, shrimp chilli paste kept in a refrigerator for 3 days. The main reasons of the shrimp chilli paste contamination might be due to the non-properly cooked ingredients. Thus, proper cooking temperature and holding time is important to control *B. cereus* in shrimp chilli paste production. These measures will improve the safety quality and shelf life of the popular shrimp chilli paste food in Thailand.

5. Acknowledgements

The authors would like to thanks Faculty of Agro-Industry, King Mongkut's Institute of Technology, Ladkrabang Bangkok, Thailand for financial support to accomplish this research work.

6. References

1. Academic Centre Thai Health Promotion Foundation.)2010(. Chili menu miracle. Thai Health Promotion Foundation. Ministry of Public Health.
2. Berthold-Pluta, A., Pluta, A., & Garbowska, M. (2015). The effect of selected factors on the survival of *Bacillus cereus* in the human gastro intestinal tract. Microbial pathogenesis, 82, 7-14. Available at: FoodScienceResearch/LaboratoryMethods/cm063346.htm. (2017).
3. ISO 6579: 2002 Microbiology of food and animal feeling stuffs –Horizontal method for the detection of Salmonella spp. Available at https://www.salmonella360.com/c3/assets/fullsize/955. April 1, 2017.
4. ICMSF (International Commission on Microbiological Specifications for Foods). 2002. Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. Elsevier Inc Ltd.
5. Phromchroen, R., Tuitemwong, P., Tuitemwong, K., and Suthirawut, S. (2003). Effects of intrinsic factors on toxin production of *B. cereus* in Thai chilli pastes. Proceedings of 41th Kasetsart University Annual Conference Science, Natural Resources and Environ- mental Economics. : 221-229.
6. Thai Health Promotion Foundation. (2010). According to the study on consumption behaviour and food safety of chilli paste by the Department of Health, Ministry of Public Health. Nonthaburi. Bangkok, Thailand
7. Thai Industrial standards Institute)B.E.2532(.Thai Industrial standards TIS 1080 – 1992 Kapi.Thai Industrial standards Institute.Ministry of Industry, Nonthaburi Bangkok, Thailand
8. Wattanahongsiri, W. (2016). Effect of heat on *Bacillus cereus* survival during shrimp paste fried rice preparation in a hospital kitchen. Master of Science in Food Service and Catering Technology King Mongkut's Institute of Technology Ladkrabang. Bangkok.
9. U. S. Food and Drug Administration.)2001(. BAM: Aerobic plate count. Available at: http://www.fda.gov/Food/
10. U.S, Food and Drug Administration.)2001(. BAM: Bacillus cereus. Available at: http://www.fda.gov/Food/
11. U.S, Food and Drug Administration.)1992). BAM: Clostridium perfringens. Available at https://www.fda.gov/food/foodscienceresearch/laboratorymeods/ucm070878.htm. April 1, 2017.
12. 3M Food Safety. (2000).Staph Express Count Plate and Disk. Available at : http://multimedia.3m.com/mws/media/241279O/petrifilm-staph-expressbrochure pdf.April

6. DO LAND TITLING IMPROVE AGRICULTURAL PRODUCTIVITY AND LAND SALES VALUE?: EVIDENCE FROM RURAL CAMBODIA

Jungwook Ahn, and Taeyoon Kim
Graduate School of International Agricultural Technology, Seoul National University
Corresponding author's Email: ahn0351@snu.ac.kr

Abstract
This paper examines the effects of land titling on agricultural productivity and land sales value in rural Cambodia by using CSES (Cambodia Socio-Economic Survey) parcel-level data conducted in 2014. However, the land titling are likely to have endogenous to both agricultural productivity and land sales value because households with higher productivity and income have better opportunity for obtaining land papers. Therefore, we apply the five mode of land acquisition as an instrument variable to solve biased estimates from OLS (Ordinary Least Squares). Our study also investigates the effects of two types of land titles such as official paperissued by the government (complete title) and application receipts (intergrade title) since parcels with the government paperare likely to be in tenure security that can positively affect productivity compared to parcels with application receipt. The study finds that parcels held with land titling are over 17% more productive and have over 30% higher land sales value than others. However, there is no difference between paper from the government and application receipt.

Keywords: Land titling, agricultural productivity, land sales value, endogeneity, Cambodia

1. Introduction

The development of land property rights has grown with economic growth and poverty reduction in many developing countries where agriculture is the predominant sector. However, the evaluation of property rights and their effect on agricultural productivity have been controversial for a long time.

Farmers with leases or titles improve productivity through fixed investment in Zambia(Smith, 2004). Low-cost land certification implemented in Ethiopia has strong significant impacts on the maintenance of soil conservation structures, investment in trees, and land productivity (Holden et al., 2009). Land certification increases land related investment and rental market participation in Ethiopia (Deininger et al., 2011). Feder (1987) examines that secure land ownership can lead to increase productivity in Thailand with the economic theory that farmers with secure ownership have ability to invest, so that it leads to higher input and output. Plots with land papers in rural Cambodia have higher productivity and land sales values, while the papers are found to have weak effects on access to credit (Markussen, 2008).On the other hand, land title has no significant effect on plot investment in Madagascar, so little effect on land productivity and land sales values (Jacoby and Minten, 2007). Land titling does not affect positive benefits related secure property, such as access to credit in Peru (Kerekes and Williamson, 2010).

In the study, we investigate the effects of land titling on agricultural productivity by using CSES (Cambodia Socio-Economic Survey) parcel-level data conducted in 2014.Besley (1995) suggests that property rights have potentially impacts on increase of agricultural productivity through three main channels. First, land property rights can increase incentives to invest with confidence of the landowner. Farmers without the property rights have little incentive to plant because there is no reasonable assurance that one will have possess the land at the time of harvest. Second, property rights may increase agricultural productivity by helping create a market for land, leading to more efficient allocation. An active land market increase agricultural productivity since land is possessed by those who use the land most productively.Third, property rights can increase productivity by easing access to credit. Land rights enables farmers to assure the land as collateral and thus to have access to credit that can invest for their farm land.However, it is

difficult to test these hypothesis of the three channels directly because CSES 2014 does not provide all these information.

Therefore, we attempt to examine the effects of land titling on land sales value as well as agricultural productivity. This is becauseland sales value is an alternative measure of productivity since the value of a parcel is the discounted sum of future income flows the parcel is likely to generate (Markussen, 2008).

In Cambodia, private property rights to land were officially reintroduced in 1989.Many households submitted applications for formal land titles to get formal land by government and more than four million applications have been submitted. However, only a small fraction of land householdshave actually got formal land titles being issued by the government due to the limited administrative capacity of the govern - ments. Therefore, many land households still have the application receipts instead of the official papers. However, many people considered application receipt to be titles and the receipts was useful in terms of an insurance in case of land grabbing, land sale, and inheritance of property because the 1992 Land Law allowed application receipts to be used for land ownership (Sovannarith et al., 2001). However, the rule was changed in the 2001 Land Law. As a result, the receipts are no longer allowed to be used for land ownership by the law. Accordingly, we assume that parcels with the government paper are likely to be in tenure security that can positively affect productivity compared to parcels with application receipt. Therefore, our study also analyzes the impacts of different two types of land paperssuch as the government official land paper and application receipt.

Section 2 describes the history of land property rights in Cambodia. Section 3 presents the CSES data set and provides descriptive statistics. Section 4 shows model and estimation strategies. Section 5 investigates the effect of land titling on agricultural productivity and land sales value. Section 6 concludes.

History of Land Property Rights in Cambodia

In pre-colonial times, the sovereign formally owned all land, but land actually was freely occupied since population density was low. During French colonization (1863-1953), modern system of property rights and the concept of fully private property in land was first introduced by Civil Code in 1920. However, only 10% of landowners (especially the rice growing plains) received land ownership titles because of lack of bureaucratic capacity. After independence in 1953 and up to the Khmer Rouge takeover in 1975, the colonial property rights system was continued. For the Khmer Rouge reign (1975-1993), all private properties in land were abolished and all records of ownership were destroyed. After the fall of the Khmer Rouge, land continued to be used by solidarity groups and owned by the state. Families were allowed small plots, but there was no effort to restore private ownership.

The Land Laws were enacted in 1992, and it encouraged landholders to submit application for formal land titles, but only small amount of these applications have actually resulted in certificates being issued due to the limited administrative capacity of the governments. Furthermore, when households apply for a land title, they need to pay substantial costs in terms of informal fees. The official fee of registration is 3-4$ while the actual fee is sometimes as much as 300-400$ (Markussen, 2008). The Land Laws of 2001 with the goals for the provision of greater tenure security to average Cambodians created LMAP (Land Management Administration Project). LMAP aimed to facilitate a comprehensive reform of land management policies in Cambodia, and one of its purpose is a systematic land titling, issuing 1 million title in 11 provinces during 2003-2007. As of 2010, these processes had collected data on more than 2 million parcels, and had issued around 1.5 million title certificates (Trzcinski and Upham, 2014).

Data and Descriptive Statistics

The research used the national representative CSES (Cambodia Socio-Economic Survey) parcel-level data conducted from January 2014 until December 2014. The survey was conducted by NIS (Cambodia National Institute of Statistics) and supported by SIDA (Swedish International Development Cooperation Agency). The survey data includes around 12,000 households surveyed in 24 provinces and

more than 15,000 parcels information. Table 1 shows descriptive statistics. Table 1 only includes parcels where crops are cultivated in rural households. Only parcels owned by the households are included and rented parcels are excluded.

The first line of Table 1 shows the percentage of parcels with land titling. Among the total parcel cultivating crops, 64% have land titling. As with the view that land papers have a positive impact on agricultural productivity and land sales value, parcels with titling have higher value of output, higher value of land, and are more likely to be irrigated compared to parcels without titling. Parcels with titling are also more likely to acquire land from the states, while parcels without tiling are more likely to acquire land from inheritance or given by relatives. Unlike our expectation, parcels with government paperhave lower value of output and lower value of land compared to parcels with application receipt, but the rate of irrigation is high. However, we need detailed econometric analyses to verify the casual relations.

Estimation Strategies

In this study, Cobb-Douglas form is used to represent the relationship between agricultural productivity and land titling. The model begins as the equation (1):

$$\ln\left(\frac{pY_{vhi}}{L_{vhi}}\right) = \alpha_0 + \alpha_1 T_{vhi} + \sum_{k=2}^{n} \alpha_k \boldsymbol{X}_{vhi} + \gamma_v + \varepsilon_{vhi}, \qquad (1)$$

Table 1: Descriptive statistics

Variable	All parcels	N	Parcels with titling	N	Parcels without titling	N	Parcels with states paper	N	Parcels with receipt	N
Parcels with land titling	0.64	10,362								
Type of land paper										
Paperfrom the government	0.79	6,631								
Application receipt	0.20	6,631								
Cultivated area size, m^2	8,781	10,349	9,013	6,661	8,361	3,688	9,433	5,283	7,541	1,340
Value of output, *riel* /m^2	644.5	10,872	806.5	6,655	351.8	3,684	795.8	5,277	860.8	1,340
Value of land, *riel* /m^2	3,152	10,897	3,466	6,669	2,585	3,693	3,208	5,291	4,379	1,340
Irrigated in at least one season	0.52	10,897	0.57	6,669	0.42	3,693	0.58	5,291	0.52	1,340
Land type										
Wet season land	0.67	10,880	0.66	6,661	0.69	3,684	0.66	5,283	0.67	1,340
Dry season land	0.08	10,880	0.07	6,661	0.09	3,684	0.07	5,283	0.09	1,340
Wet and dry season land	0.07	10,880	0.08	6,661	0.06	3,684	0.09	5,283	0.06	1,340
Kitchen garden land	0.10	10,880	0.10	6,661	0.09	3,684	0.10	5,283	0.10	1,340
Permanent crops land	0.05	10,880	0.06	6,661	0.05	3,684	0.06	5,283	0.05	1,340
Other types of land	0.00	10,897	0.00	6,669	0.00	3,684	0.00	5,281	0.00	1,340
Mode of land acquisition										
Given by the state	0.33	10,897	0.36	6,669	0.27	3,683	0.36	5,291	0.37	1,340
Inheritance or given by relatives	0.46	10,897	0.41	6,669	0.54	3,683	0.41	5,291	0.41	1,340
Bought	0.13	10,897	0.16	6,669	0.07	3,683	0.16	5,291	0.14	1,340
Cleared or occupied for free	0.06	10,897	0.05	6,669	0.09	3,683	0.04	5,291	0.05	1,340
Other modes of acquisition	0.00	10,897	0.00	6,669	0.00	3,683	0.00	5,291	0.00	1,340

Note: 1) Only parcels cultivating crops are included in rural households.
2) Only parcels owned by the households are included.
3) Rented parcels are excluded.

Where p is sale price of crops, Y_{vhi} is crops output on parcel i in household h in village v, L_{vhi} is cultivated area, $\frac{pY_{vhi}}{L_{vhi}}$ means sales of output per cultivated area, T_{vhi} is parcels held with land titling (dummy variable), α_k is coefficients to be estimated, $\boldsymbol{X}_{vhi}$ is a vector of explanatory variables that are exogenous such as household lasize, total inputs cost, irrigation, land type, and household head characteristics, γ_v is village fixed effect, and ε_{vhi} is error term.Household fixed effects can be applied to remove all impacts generated by differences between households. However, in the data, most households have only a few parcels (often only one) so that we do not apply household fixed effects.In addition, village fixed effects can control for village level characteristics, such as agro-climatic conditions, soil quality, market conditions, and infrastructure. However, the

land titling variable (T_{vhi})is likely to have endogenous to agricultural productivity. Parcels with land papers have potentially effect on agricultural productivity at the same time as households with higher productivity and income have better opportunity for obtaining land paper. This means OLS (Ordinary Least Squares) estimation may suffer from simultaneous causality bias. In order to overcome this simultaneous causality bias, we apply an instrumental variables estimator, using 2SLS (Two Stage Least Squares). The first stage is as following equation (2):

$$T_{vhi} = \beta_0 + \beta_1 \sum_{k=1}^{5} Z_{kvhi} + \sum_{k=2}^{n} \beta_k \boldsymbol{X}_{vhi} + \sigma_{vhi}, \qquad (2)$$

where Z_{vhi} is the five mode of land acquisition used as instrument variablesuch as given by the states, inheritance, bought, free occupied, and other modes of acquisition. For an instrument variable to be valid, it must satisfy two conditions: The instrument variable (Z) should explain the variance of T_{vhi} and be independent of the variance of $\ln\left(\frac{pY_{vhi}}{L_{vhi}}\right)$ in the equation (1). Following Besley (1995), Brasselle et al. (2002), and Markussen (2008), we use the mode of land acquisition instruments for property rights in this study since the mode of land acquisition is correlated with property rights and uncorrelated with the productivity. For example, if a parcel is given by the state, there is a chance that obtaining an official document was part of the process of acquiring the parcel. There is also no reason why choices related to agricultural productivity should be linked to the mode of land acquisition.

When dealing with endogenous dummy variables, we must be careful not to fall into "forbidden regression"[1] that a phrase that means replacing a nonlinear function of an endogenous variable with the same nonlinear function of fitted values from a first-stage estimation (Wooldridge, 2010).Therefore, Angrist et al. (2013) suggest that plug in the fitted values of D_{vhi} derived from equation (2) into the equation (1):

Through these 2SLS, we can avoidforbidden regression from dummy endogenous variable and solve the potential simultaneous causality bias between land titling and agricultural productivity.

[1] A forbidden regression occurs when researchers apply 2SLS reasoning directly to nonlinear models. A common scenario is a dummy endogenous variable.

2. **Results and discussion**

Land titling and agricultural productivity

Table 2 shows the results of estimating the model for the sales of crops production per cultivated area (m^2). The first and second columns show the results of estimating the equation (1) and (3) by OLS and 2SLS. The impact of land titling is positive in both models, but only significant (at the level of 10%) in the 2SLS model where the coefficient is also much higher.Parcels held with land titling are over 17% more productive in the 2SLS.

In this model, the Durbin-Wu-Hausman tests fail to reject the hypothesis of instrument exogeneity. In the testing for weak instruments, the F-test for joint significance of instruments in the first stage regression should be at least 10. The first stage F-statistics are much higher than 10 in the models presented here. Therefore, the instruments are not weak.

Many exogenous variables such as season, land, inputs cost, land type, and household head characteristics have a significant effect on the output. This shows similar results in OLS and 2SLS. In particular, wet season has negative effect on the output compared to dry season. In the land type, other types of lands have a positive effect on the output compared to wet season land. In this data, the crops include rice, many types of vegetables, fruits, and cash crops, but, rice accounts for around 80% of the total crops. Therefore, it is assumed that the characteristic of rice, which is influenced by the season, is greatly reflected in this result. Nesbitt (1997) claims that erratic rainfall during wet season induces substantially lower paddy productivity because it causes insufficient or excessive rains during some important period for rice. Maclean et al. (2013) suggest that paddy productivity of wet season is much lower than that of the dry season, mainly due to low

use of higher yield seeds and worse water management in Cambodia.

$$\ln\left(\frac{pY_{vhi}}{L_{vhi}}\right) = \alpha_0 + \alpha_1 \widehat{T_{vhi}} + \sum_{k=2}^{n} \alpha_k \boldsymbol{X}_{vhi} + \gamma_v + \varepsilon_{vhi}, \quad (3)$$

As mentioned above, the land titling needs to be divided into government paper and application receipts. This is because the 2001 Land Law was changed not to allow application receipt to be used for land ownership. The third and fourth columns show the estimating results of the impacts of government paper by OLS and 2SLS. The effect of government paper is positive compared to receipt, but not significant in both models. It can be said that there is no difference between government paper and application receipt in the model of value of output.

Land titling and land sales value

As mentioned in Section 1, land sales value is an alternative measure of agricultural productivity, so the hypothesis of an effect of land titling on agricultural productivity can be tested by analyzing whether land titling have a significant effect on land sales value. We use land sales value as the dependent variable. Information on values of actual sales would probably be more reliable, but CSES survey data does not include land values of actual sales. There are various dependent variables such as parcel size, irrigation, land type, and household head characteristics. Village fixed effects are included to handle with geographical differences in market conditions, soil quality, and infrastructure.There is also potential endogeneity between land titling and land sales value. Therefore, we use the five mode of land acquisition as an instrument variable.

Table 3 represents the results of estimating the model for land sales value per parcel (m^2). The results are similar to the estimated coefficients in the model for agricultural productivity.In the first and second columns, the effects of land titling is positive and significant in the both OLS and 2SLS model. The coefficient of OLS is 0.126 and the thing of 2SLS is 0.307. Therefore, parcels held with land titling have over 30% higher land sales value than others in the 2SLS model.These results strength the argument for a positive effect of land titling on agricultural productivity. The instrument exogeneity is rejected at the 10% level, while the instruments remain highly relevant.

However, the effect of government paper is positive compared to receipt, but not significant in the third (OLS) and fourth (2SLS) columns. It can be said that there is also no difference between government paper and application receipt in the model of land sales value.

3. Conclusion

The study use CSES 2014 parcel-level data to investigate the effects of land titling on agricultural productivity and land sales value. In addition, we attempt to analyze the effects of government paper compared to application receipt.

However, the land titling variable has potential endogenous issue to agricultural productivity and land sales value. This is becauseparcels with land papers have potentially effect on agricultural productivity at the same time as households with higher productivity and high value of land have better opportunity for obtaining land paper. This means OLS (Ordinary Least Squares) estimation may suffer from simultaneous causality bias. In order to overcome this simultaneous causality bias,we apply the five mode of land acquisition as an instrument variable and use second stage to solve simultaneous causality bias from the endogeneity. However, be careful not to fall into forbidden regression due to the endogenous dummy variable.

The study finds that parcels held with land titling are over 17% more productive and have over 30% higher land sales value than others. However, there is no difference between paper from the government and application receipt in the both model of output value and land sales value. It means that many farmers may still be using application receipts in terms of land sale, trade, and property inheritance in rural area. The Cambodian government has endeavored to grant land certification of land ownership for many rural farmers who have suffered from insecure land ownership.

However, many farmers still have the application receipt and may use it for land ownership in rural Cambodia even though 2001 Land Law prohibiting the use of application

receipts for land ownership has been implemented.This situation can cause land insecurity which provoke potentially the threat to losing their land by external aggression. Recently, land disputes have become a serious and persistent issue in Cambodia.These land conflict occur more frequently in remote areas outside the reach of the government.Therefore, the government should strive to provide land certification to farmers who live in remote farm area to improve their land productivity, income, and welfare.

References

1. Angrist, J. D., Pischke, J.-S., & Pischke, J.-S. (2013). Mostly harmless econometrics: an empiricists companion: Cram101 Publishing.
2. Besley, T. (1995). Property-Rights and Investment Incentives - Theory and Evidence from Ghana. Journal of Political Economy, 103(5), 903-937. doi:Doi 10.1086/262008
3. Brasselle, A.-S., Gaspart, F., & Platteau, J.-P. (2002). Land tenure security and investment incentives: puzzling evidence from Burkina Faso. Journal of Development Economics, 67(2), 373-418.
4. Deininger, K., Ali, D. A., & Alemu, T. (2011). Impacts of land certification on tenure security, investment, and land market participation: evidence from Ethiopia. Land Economics, 87(2), 312-334.
5. Feder, G. (1987). Land ownership security and farm productivity: evidence from Thailand. The Journal of Development Studies, 24(1), 16-30.
6. Holden, S. T., Deininger, K., & Ghebru, H. (2009). Impacts of low-cost land certification on investment and productivity. American Journal of Agricultural Economics, 91(2), 359-373.
7. Jacoby, H. G., & Minten, B. (2007). Is land titling in Sub-Saharan Africa cost-effective? Evidence from Madagascar. The World Bank Economic Review, 21(3), 461-485.
8. Kerekes, C. B., & Williamson, C. R. (2010). Propertyless in Peru, even with a government land title. American Journal of Economics and Sociology, 69(3), 1011-1033.
9. Maclean, J., Hardy, B., & Hettel, G. (2013). Rice Almanac: Source book for one of the most important economic activities on earth: IRRI.
10. Markussen, T. (2008). Property Rights, Productivity, and Common Property Resources: Insights from Rural Cambodia1. World Development, 36(11), 2277-2296.
11. Nesbitt, H. J. (1997). Rice production in Cambodia: Int. Rice Res. Inst.
12. Smith, R. E. (2004). Land tenure, fixed investment, and farm productivity: Evidence from Zambia's Southern Province. World Development, 32(10), 1641-1661.
13. Sovannarith, S., Sopheap, R., Utey, U., Rathmony, S., Ballard, B., & Acharya, S. (2001). Social Assessment of Land in Cambodia. A Field Study (Phnom Penh: CDRI, Working Paper 20).
14. Trzcinski, L. M., & Upham, F. K. (2014). Creating law from the ground up: Land law in post-conflict Cambodia. Asian Journal of Law and Society, 1(1), 55-77.
15. Wooldridge, J. M. (2010). Econometric analysis of cross section and panel data: MIT press.

Table 2: Land Titling and Agricultural Productivity

Independent variables	**Dependent variable:Sales of crops production per hectare, riel /m^2 (log)**			
	OLS (1)	**2SLS (2)**	**OLS (3)**	**2SLS (4)**
Parcel with titling (1 = yes, 0 = no)	0.014	0.176*		
	(0.020)	(0.106)		
Parcel with government paper (1 = yes, 0 =receipt)			0.036	0.264
			(0.043)	(0.319)
Season (1 = wet season, 0 = dry season)	-0.239***	-0.239***	-0.212***	-0.213***
	(0.039)	(0.037)	(0.048)	(0.045)
Cultivated area size, m^2 (log)	-0.530***	-0.535***	-0.511***	-0.512***
	(0.017)	(0.017)	(0.022)	(0.021)
Household size (log)	0.061***	0.056***	0.061**	0.063**
	(0.020)	(0.019)	(0.028)	(0.027)
Total inputs cost for cultivation crops, *riel* (log)	0.384***	0.385***	0.378***	0.378***
	(0.017)	(0.016)	(0.021)	(0.020)
Irrigated parcel during at least one season (1 = yes, 0 = no)	0.024	0.020	0.049	0.041
	(0.023)	(0.022)	(0.030)	(0.031)
Land type				
Dry season land (1 = yes, 0 = no)	0.110**	0.111***	0.100	0.104*
	(0.050)	(0.048)	(0.065)	(0.062)
Wet and dry season land (1 = yes, 0 = no)	0.096***	0.090***	0.091*	0.094*
	(0.047)	(0.045)	(0.052)	(0.049)
Kitchen garden (1 = yes, 0 = no)	0.376***	0.377***	0.335***	0.331***
	(0.063)	(0.060)	(0.090)	(0.085)
Permanent crops land (1 = yes, 0 = no)	0.547***	0.548***	0.646***	0.648***
	(0.113)	(0.109)	(0.141)	(0.134)
Other types of land (1 = yes, 0 = no)	0.240	0.215	0.151	0.149
	(0.248)	(0.227)	(0.179)	(0.170)
Household head characteristics				
Household head gender (1 = male, 0 = female)	0.031*	0.033*	0.033	0.030
	(0.018)	(0.018)	(0.023)	(0.023)
Household head age (log)	0.009	-0.001	-0.027	-0.021
	(0.025)	(0.026)	(0.033)	(0.032)
Household head can at least read or write (1= yes, 0 = no)	0.040***	0.034**	0.047**	0.049**
	(0.015)	(0.015)	(0.021)	(0.020)
Constant	4.658***	4.586***	4.674***	4.539***
	(0.279)	(0.275)	(0.308)	(0.330)
Village Fixed Effect	Yes	Yes	Yes	Yes
Observations	9,394	9,394	6,002	6,002
R-squared	0.536	0.532	0.556	0.553
Test of endogeneity (P-value)		0.118		0.467
F-test for joint significance of instruments in 1 stage reg.		58.4		11.2

Note: 1) *** p<0.01, ** p<0.05, * p<0.1 indicate level of significance at 1%, 5%, 10%, respectively.
: 2) Number in parentheses are robust standard error.
: 3) Only parcels growing crops are included in rural households.
: 4) Rented parcels are excluded.
: 5) The omitted category for type of land is the wet season land.
: 6) In the 2SLS regressions, parcel with paper is instrumented with the mode of parcel acquisition.
: 7) Standard errors are adjusted for clustering at the village level.

Table 3: Land Titling and Land Sales Value

Independent variables	**Dependent variable: Sales value of parcel per hectare, riel /m^2 (log)**			
	OLS (1)	**2SLS (2)**	**OLS (3)**	**2SLS (4)**
Parcel held with land titling (1 = yes, 0 = no)	0.126***	0.307***		
	(0.023)	(0.112)		
Parcel with government paper (1 = yes, 0 =receipt)			0.034	-0.363
			(0.041)	(0.333)
Parcel size, m^2 (log)	-	-0.341***	-0.343***	-0.341
	0.337***	(0.010)	(0.013)	(0.013)
	(0.010)			
Irrigated parcel during at least one season (1 = yes, 0 =	0.028	0.025	0.042	0.053
no)	(0.022)	(0.021)	(0.028)	(0.029)
Land type				
Dry season land (1 = yes, 0 = no)	-0.047	-0.045	0.004	-0.004
	(0.041)	(0.039)	(0.058)	(0.056)
Wet and dry season land (1 = yes, 0 = no)	0.122***	0.115***	0.120***	0.112
	(0.036)	(0.035)	(0.044)	(0.042)
Kitchen garden (1 = yes, 0 = no)	0.242***	0.239***	0.244***	0.246
	(0.035)	(0.034)	(0.048)	(0.046)
Permanent crops land (1 = yes, 0 = no)	0.539***	0.537***	0.532***	0.521
	(0.047)	(0.045)	(0.061)	(0.058)
Other types of land (1 = yes, 0 = no)	0.558***	0.534***	0.428**	0.429
	(0.203)	(0.193)	(0.213)	(0.204)
Household head characteristics				
Household head gender (1 = male, 0 = female)	0.016	0.017	0.016	0.021
	(0.020)	(0.019)	(0.025)	(0.024)
Household head age (log)	0.080***	0.066**	0.084**	0.074
	(0.026)	(0.027)	(0.034)	(0.033)
Household head can at least read or write (1= yes, 0 =	0.065***	0.058***	0.065***	0.062
no)	(0.017)	(0.018)	(0.023)	(0.023)
Constant	9.919***	9.843***	10.040***	10.251
	(0.163)	(0.164)	(0.203)	(0.282)
Village Fixed Effect	Yes	Yes	Yes	Yes
Observations	10,345	10,345	6,623	6,632
R-squared	0.631	0.629	0.654	0.648
Test of endogeneity (P-value)		0.095		0.222
F-test for joint significance of instruments in 1 stage reg.		70.3		12.0

Note: 1) *** p<0.01, ** p<0.05, * p<0.1 indicate level of significance at 1%, 5%, 10%, respectively.
: 2) Number in parentheses are robust standard error.
: 3) Only parcels cultivating crops are included in rural households.
: 4) Rented parcels are excluded.
: 5) In the 2SLS regressions, parcel with paper is instrumented with the mode of parcel acquisition.
: 6) Standard errors are adjusted for clustering at the village level.

Table A1: The Results of the First Stages

Independent variables	Dependent variable:	
	Land titling	Government paper
	OLS	OLS
Season (1 = wet season, 0 = dry season)	0.004	0.006
	(0.014)	(0.012)
Cultivated area size, m^2 (log)	0.023***	0.001
	(0.005)	(0.005)
Household size (log)	0.021**	-0.010
	(0.009)	(0.009)
Total inputs cost for cultivation crops, *riel* (log)	-0.009*	-0.002
	(0.005)	(0.004)
Irrigated parcel during at least one season (1 = yes, 0 = no)	0.024**	0.030***
	(0.010)	(0.009)
Land type		
Dry season land (1 = yes, 0 = no)	-0.011	-0.022
	(0.021)	(0.020)
Wet and dry season land (1 = yes, 0 = no)	0.042**	-0.007
	(0.017)	(0.016)
Kitchen garden(backyard) and Chamkar land (1 = yes, 0 = no)	-0.007	0.019
	(0.017)	(0.016)
Permanent crops land (1 = yes, 0 = no)	-0.006	-0.007
	(0.030)	(0.028)
Other types of land (1 = yes, 0 = no)	0.167	0.009
	(0.186)	(0.134)
Household head characteristics		
Household head gender (1 = male, 0 = female)	-0.012	0.015*
	(0.010)	(0.009)
Household head age (log)	0.039***	-0.044***
	(0.014)	(0.013)
Household head can at least read or write (1= yes, 0 = no)	0.034***	-0.010
	(0.008)	(0.008)
Mode of land acquisition		
Given by the states (1 = yes, 0 = no)	0.083	0.065
	(0.079)	(0.073)
Inheritance or gift from relatives (1 = yes, 0 = no)	0.035	0.037
	(0.079)	(0.073)
Bought (1 = yes, 0 = no)	0.238***	0.110
	(0.079)	(0.073)
Free occupied (1 = yes, 0 = no)	0.004	-0.015
	(0.081)	(0.076)
Constant	0.476***	0.586
	(0.169)	(0.139)
Village Fixed Effect	Yes	Yes
Observations	9,394	6,002
R-squared	0.610	0.723

Note: 1) *** p<0.01, ** p<0.05, * p<0.1 indicate level of significance at 1%, 5%, 10%, respectively.
: 2) Number in parentheses are robust standard error.
: 3) Only parcels growing crops are included in rural households.
: 4) Rented parcels are excluded.
: 5) The omitted category for type of land is the wet season land.
: 6) The omitted category for mode of land acquisition is other modes of acquisition.
: 7) Standard errors are adjusted for clustering at the village level.

7. EVALUATION OF THE QUALITY OF POROUS CRUMB STRUCTURE OF LEAVENED PRODUCTS FROM COMPOSITE FLOUR OF CASSAVA, RICE, AND WHEAT

H.A. Rathnayake[1*], S.B. Navaratne[1], C.M. Navaratne[2]
[1]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawilla, Nugegoda, Sri Lanka
[2]Department of Agricultuter Engineering, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka
Corresponding author's Email: heshani@sci.sjp.ac.lk

Abstract

Currently, substitution of wheat flour from composite flour had gained an interest throughout the world due to economic, nutritional, health and safetyaspects. Ability to entrap leavened gas and development of well-developed porous structure are the major factors that have to be considered when developing leavened products from composite flour. The current study was carried out to evaluate the effect of physical and microstructural properties of porous crumb structure developed from different blends of rice, cassava and wheat flour. Thus, four batches of dough samples were prepared according to two-factor factorialdesign and subjected to fermentationfor 90 minutes at 28^{o}C followed by gelatinization by steaming. Thereafter the resulted crumb samples were subjected to determine physical properties and microstructure analysis. Results reviled that, there were no significant different ($P \geq 0.05$) between specific volume, hardness, springiness, gumminess, chewiness and crumb porosity and average pore size except product volume of cassava added sample (50 : 50 of Cassava flour:Wheat flour) and control (100% wheat flour) in comparison with the other samples. There was a significant different ($P \leq 0.05$) between rice flour added samples (50: 50 of RF:WF and 25: 25: 50 RF: CF: WF) and control (100% WF) regarding the aforementioned properties. Additionally, the cassava flour added samples (50:50 of CF:WF and 25: 25: 50 RF: CF: WF) had resulted comparatively more flexible, somewhat gummy and dense cavities.Hence, the current study proved that the application of cassava flour in composite flour for production of rice-relatedleavened products may result better crumb properties.

Keywords: Cassava flour, crumb, rice flour, wheat flour

Introduction

Wheat flour is the most commonly used ingredient in the production of leavened baked products. However, substitution of wheat flour from composite flour had gained the interest in the food industry due to food safety, nutritional and economic reasons. In the case of food safety, people who are suffering from celiac disease (allergy to gluten) (Park et al., 2014) have trouble with consuminggluten-containing foods such as wheat, rye and barley (Hager and Arendt, 2013).Starting with the removal from the oven, leavened baked products tend to undergo deteriorative changes (Hui et al., 2008). This phenomenon is commonly identified as crumb staling where, the increment of crumb firmness is the most commonly identified parameter (Angioloni and Collar, 2009; Skendi et al, 2010; Rathnayake et al, 2018). Hence, development of leavened baked products that have a longer shelf life with improved microbial stability from natural ingredients is also important for food safety and security. Additionally, people become more health conscious and consider the nutritional properties and caloric value of the food they consume. Hence incorporation of natural sources that are rich in nutritional properties has also become important. When considering the economic basis, wheat is not grown in Sri Lanka and the entire requirement is needed to be imported. Around 1208 ('000 MT) of wheat grains had been imported in 2015 (Central Bank of Sri Lanka, 2016) spending around 44,994 million rupees (Department of Census and Statistics, 2016) which is a huge burden for the national coffer for a developing country like Sri Lanka.

Composite flour is a mixture of flours from tubers rich in starch (Ex: Cassava, yam, sweet potato) and/or protein-rich flours (Ex: soy, peanut) and/or cereals (Ex: maize, rice, millet, buckwheat), with or without wheat flour (Seibel, 2011). However, partial substitution of wheat flour by other flour type's may represent considerable technical difficulties due to the lack

in preliminary proteins that is required to form the necessary gluten network for holding leavened gas (Eduardo et al, 2013).

Rice (*Oryza sativa L.*) is the major staple food incertain Asian countries like Sri Lanka that can be considered as an important source of nutrition and energy (Shigeki et al, 2012) which provides 45% calorie and 40%total protein requirement of an average Sri Lankan(Fari et al, 2010).Rice flour had been used as a wheat flour substitute for the development of gluten-free bakery productsin several studies (Fari et al, 2010; López et al, 2004; Heo *et al.*, 2013). Cassava (*Manihotesculenta*), which is also called manioc, tapioca or yuca is one of the most important food crops in the humid tropics (Tonukari, 2004). Certain researches have been carried out by using cassava flour with or without blending other flour types as composite flour for substituting wheat flour (Eduardo et al, 2013; Eriksson et al, 2014; López et al, 2004).

The objective of this study is to evaluate the porous crumb structure of leavened products prepared from Cassava, Rice and Wheat flour blends to study the possibility of incorporating cassava flour for developing a value-added bakery product for food safety and security.

1. Materials and methods

Sample preparation

Rice, Wheat and Cassava flour (160µm) were prepared according to the standard methods. Dough samples were prepared (Moisture content 60%) following straight dough method using each flour types according to thedesign given in Table 1.

Table 1: Experimental design

Sample	Flour ratio
W	Wheat flour = 100g (Control)
RW	Wheat flour: Rice flour: Cassava flour =50g : 50g : 00g
CW	Wheat flour: Rice flour: Cassava flour = 50g : 00g : 50g
RCW	Wheat flour: Rice flour: Cassava flour = 50g : 25g : 25g

Then, approximately 10g of dough samples were measured (KERM ABJ-NM/ABS-N, ABS 220-4N) and subjected forfermentation for 90 minutes at 28-30^{o}C. Thereafter, the fermented dough samples weresubjected to gelatinize for 30 minutes in a household steamer (Philips Electric Steamer HD9104).

Analysis of physical properties and cellular properties

Leavening index (Navaratne, 2007) during dough fermentation, product volume by seed displacement method, (AACC, 2000, Standard 10-05; Zghal et al, 1999, Švec and Hrušková, 2004; Cornejo and Rosell, 2015; Curic et al, 2008) and product specific volume (López et al., 2004; Švec and Hrušková, 2004; Curic et al, 2008) of the crumb samples were determined.

Texture profile Analysis was done as described by Wang *et. al,* (2002) and Angioloni and Collar, (2009) using Texture profile analyzer (CT3, M08-372-F1116) (after 90 minutes from gelatinization time) with slight modifications. There, the crumb samples were sliced using a paper cutter into the size of 20 mm height with 20 mm diameter and the texture analysis was done with the specifications of two compression cycles using the probe with 25 mm diameter (TA11/1000), penetration depth 50%, test speed 1 mm/s, Trigger load 5g, Load cell 4500 g. The results obtained from the study were analyzed by using software TexturePro CT Software TA-CT-PAD-AY.

Crumb porosity (%) and average cell area (cm^2) of scanned images (Cannon Lide-120 with a resolution of 300dpi) of crumb samples were analyzed using Image J software (Curic et al, 2008).

Statistical analysis

All testswere replicated six times and the obtained results were analyzed by one way ANOVA using Minitab 17 statistical software and the graphs were drawn using Microsoft Excel 2013.

3. Results and discussions

Analysis of physical properties

Product volume can be considered as a quantitative measurement of baking per-forrmance (Maktouf et al, 2016) and leavened gas retention capability within the dough mass (He and Hoseney, 1991; Onyango et al, 2009). Apart from that, volume has a direct influence on the firmness of the product (Eduardo et al, 2013). The graphical representation of the results obtained for the product volume is represented in Fig 2. The leavening index is also

an important parameter whichrepresents the quality of the crumb structure and gas retention capability. The obtained results pertaining to the leavening index are showing in Fig 1.

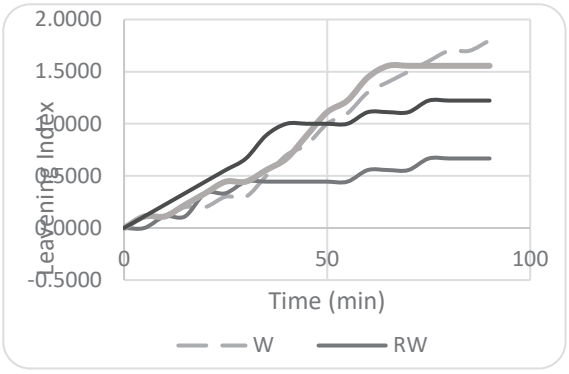


Figure 2: Leavening index

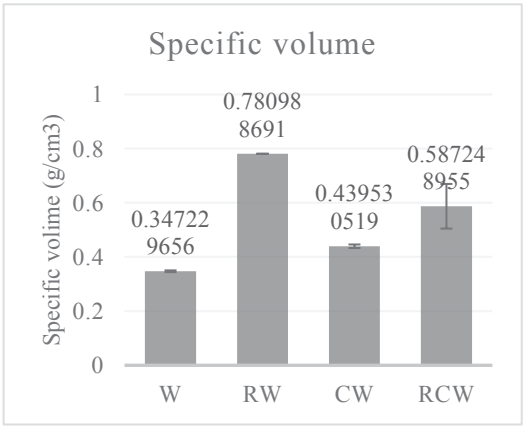


Fig1: Product specific volume.

When considering the results in the Fig 1 and 2, substitution of wheat flour with rice flour (RW) had significant reduction ($P \leq 0.05$) of the leavening index (0.6667 at 80 minutes) as well as product volume (13.2198 cm^3) compared to the leavening index (1.5556 at 80 minutes) and the product volume (22.7497 cm^3) of wheat and Cassava flour (CW) sample. The increment of crumb volume and leavening index in CW compared to RW can be occurredas a result of the viscous nature of cassava flour.

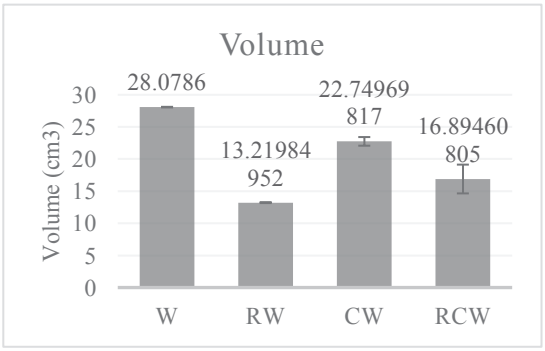


Figure 3: Products Volume

Thus, rheological properties of Cassava flour capable to entrap more leavened gas compared to rice flour. Building ofcertain bondsbetween rice proteins due to the presence of low molecular weight thiols (especially reduced glutathione) which activates proteolytic enzymescan also cause thereduction of the volume of productthat contain rice flour (Fari et al, 2010). Apart from that, literature reviled that, rice flour has few prolamins (2.5-3.5%) and as a result of that, rice flour lacks the formation of a viscoelastic structure (Gujral and Rosell, 2004).

Specific volume is another important visual characteristic for leavened baked products which could strongly influencethe consumer's choice when evaluating product quality (Hager and Arent, 2013) and also specific volume have an effect on crumb hardness and relative elasticity (Scanlon and Zghal, 2001).

Fig 3 represents the sample from cassava-wheat flour blend (CW) had significantly lower ($P \leq 0.05$) specific volume (0.4395 g/cm^3) compared to the sample RW (0.780989 g/cm^3). When considering the sample RCW, product volume (16.89 cm^3) had not significantly increased ($P \geq 0.05$) compared to RW but significantly reduced ($P \leq 0.05$) the specific volume (0.5872 g/cm^3).

Texture profile analysis of the samples

Texture evaluation plays an important role when judging the quality of porous crumb structure of leavened baked food products (Che Pa et al., 2013; Scanlon and Zghal, 2001; Tan et al, 1997). The most commonly considered attributes of leavened baked products include hardness, springiness, adhesives, chewiness, gumminess and cohesiveness (Meterei, et al, 2004; Švec and Hrušková, 2004). Certain parameter resulted from the texture profile analysis pertaining to the four crumb samples (after 90 minutes after gelatinization) are given in Table 2.

Table 2: Texture profile analysis of the products

Sample	Hardness (g)	Springiness (mm)	Gumminess (g)	Chewiness (mJ)
W (Control)	$211.25^{b} \pm 11.03$	$9.3367^{a} \pm 0.1617$	$81.10^{b} \pm 8.910$	$7.06^{b} \pm 0.219$
RW	$691.30^{a} \pm 69.6$	$9.535^{a} \pm 0.490$	$303.60^{a} \pm 51.100$	$30.05^{a} \pm 7.550$
CW	$245.00^{b} \pm 12.58$	$8.840^{a} \pm 0.042$	$101.35^{b} \pm 13.65$	$10.637^{b} \pm 1.908$
RCW	$613.30^{a} \pm 62.5$	$9.633^{a} \pm 0.545$	$310.30^{a} \pm 18.000$	$38.04^{a} \pm 1.509$

^{a,b} ***Values in the same row with different superscripts are significantly different at 0.05 significant level.***

Hardness can be measured from the peak force on the first compression and is defined as the force required for biting bread samples (Meterei, et al, 2004; Švec and Hrušková, 2004; Maktouf et al, 2016). Sample CW has not shown significantlyhigher hardness ($P \geq 0.05$) (223.33 g) compared to the control (sample W) (216.67 g) when considering the other two samples (RW and RCW). Whereas rice flour incorporated sample (RW) had a significantly higher hardness ($P \leq 0.05$) (691.3 g) compared to the control (W). According to the literature, product moisture content, and crumb staling are the most important phenomena responsible for dough hardness. When a baked product gets cooled, crumb staling occurs mainly due tothe retrogradation of amylopectin and moisture migration and redistribution causing the product to harden. Certain additives such as food hydrocolloids/gum, emulsifiers, enzymescan be incorporated to improve the product texture and retard the staling process (Rathnayake et al, 2018).

Springiness is calculated from the distance of the sample recovered after the first compression (Meterei, et al, 2004; Švec and Hrušková, 2004; Maktouf et al, 2016). But there were non significant different ($P \geq 0.05$) in the springiness of the four samples. Gumminess represents the density that persists throughout chewing.

Chewiness may depend on gumminess and springiness that describes how long it takes to chew a food sample to make it consistency suitable for swallowing (Meterei, et al, 2004; Švec and Hrušková, 2004; Maktouf et al, 2016). As mentioned in Table 2, both RW and RCW samples had significantly higher ($P \leq 0.05$) gumminess and chewiness compared to the control sample (W). Whereas, the sample CW had not shown significantly higher ($P \geq 0.05$) gumminess and chewiness compared to the control (W) and those values are significantly lower ($P \leq 0.05$) compared to gumminess and chewiness of the other two samples (RW and RCW). When considering the texture profile analysis results of samples RCW and RW, it proves that the incorporation of cassava flour can increase the crumb springiness, gumminess and chewiness.

Image analysis of porous crumb structure

Currently, image analysis (IA) has been used as a quantitative tool to reliably asses the crumb features (Zghal et al, 1999) where digital scanners had been commonly used to capture high-resolution images of bread crumb in two dimensions (2D) and has been recommended as a fast, convenient, economically feasible and robust method that provides good accuracy by acting independently from external light (Reynold et al, 2011).

Table 3: Results of the image analysis of the samples

Sample	Crumb porosity (%)	Average cellarea (cm^2)
W (Control)	$22.331^{a} \pm 1.794$	$0.0787^{a} \pm 0.001385$
RW	$16.840^{b} \pm 3.610$	$0.006230^{a} \pm 0.001724$
CW	$21.990^{a} \pm 4.360$	$0.0787^{a} \pm 0.1598$
RCW	$18.561^{ab} \pm 1.894$	$0.00614^{a} \pm 0.001525$

^{a,b} ***Values in the same row with different superscripts are significantly different at 0.05 significant level.***

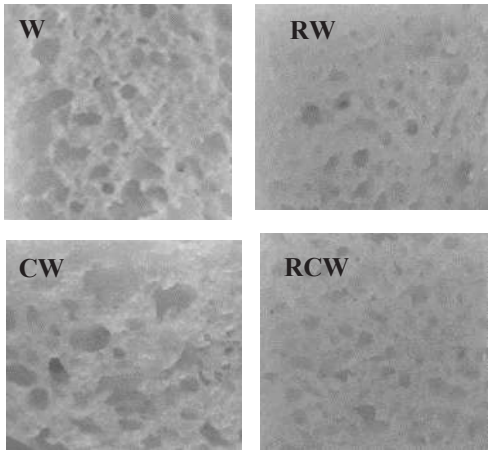


Figure5: Porous crumb structure of the each sample

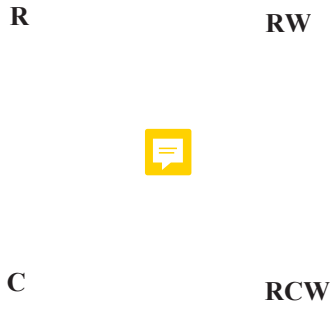


Figure 4: Gas cell distribution of the samples

As per the results in Table 3, crumb porosity (%) and the average pore sizes (cm^2) of the sample CW (21.990%, 0.0787 cm^2) and control (22.331%, 0.0787cm^2) does not significantly differ ($P \geq 0.05$) from each other. And when considering the pore structure and cell wall properties (fig 4 and 5), sample CW had somewhat gummy and dense pores compared to the other three samples. Since cassava starch has a higher amylopectin to amylose ratio than cereal starches, it has the gummy and sticky properties which can entrap leavened gas within the dough mass that results in more stable but flexible gas cells. (Onyango et al, 2009).

The crumb porosityis significantly lower ($P \leq 0.05$) in sample RW (16.840 %) compared to the control and CW. Crumb porosity was increased in sample RCW (18.561%) compared the RW and the obtained value is not significantly different ($P \geq 0.05$) from the porosity of CW and W. As shown in fig 4 and 5, RCW had stable, dense and flexible gas cells compared to RW resulting in a product that is less breakable and easier to slice due to the incorporation of cassava flour.

4. Conclusion

Incorporating cassava flour for rice-related composite flour mixtures can result in a product with better physical properties and porous structure. Composite flour mixture of wheat and cassava flour resulted in more flexible and well developed porous structure compared to the control.Therefore future studies will be conducted to find a method to obtain a leavened baked product with well-developed porous crumb structure (improved gas retention capacity) from rice-based composite flour with various nutritious flour types (with best ratios of flour combination). Further, those products will be tested for microbial stability and hardness during storage to obtain a secured and nutritious product with an improved shelf life that is suitable for children, elders and patients.

5. Acknowledgement

The authors wish to offer their gratitude towards all the academic and non-academic staff of the Department of Food Science and Technology, University of Sri Jayewardenepura and National Science Foundation, Sri Lanka (Grant no: TG/2017/Tech-D/03) for funding the project.

References

1. AACC International. (2000). Approved Methods of American Association of Cereal Chemists,10th ed. St. Paul, MN: American Association of Cereal Chemists.
2. Angioloni, A., & Collar, C. (2009). Bread crumb quality assessment: a plural physical approach. *European Food Research and Technology*, *229* (1), 21-30.
3. Central Bank of Sri Lanka. (2016), Sri Lanka Socio-Economic Data 2016-VOL. XXXIX.[Online]
4. Cornejo, F., & Rosell, C. M. (2015). Influence of germination time of brown rice in relation to flour and gluten free bread quality. *Journal of food science and technology*, *52* (10), 6591-6598.

5. Curic, D., Novotni, D., Skevin, D., Rosell, C. M., Collar, C., Le Bail, A., & Gabric, D. (2008). Design of a quality index for the objective evaluation of bread quality: Application to wheat breads using selected bake off technology for bread making. *Food research international*, *41* (7), 714-719.
6. Department of Census and Statistics. (2016). Statistical Data Sheet 2016. [ONLINE]:
7. Eduardo, M., Svanberg, U., Oliveira, J., & Ahrné, L. (2013). Effect of cassava flour characteristics on properties of cassava-wheat-maize composite bread types. *International journal of food science*, *2013*.
8. Eriksson, E., Koch, K., Tortoe, C., Akonor, P. T., & Oduro-Yeboah, C. (2014). Evaluation of the physical and sensory characteristics of bread produced from three varieties of cassava and wheat composite flours.
9. Fari, M. J. M., Rajapaksa, D., & Rana - weera, K. K. D. S. (2010). Effect of rice variety on rice based composite flour bread quality.
10. Gujral, H. S., & Rosell, C. M. (2004). Functionality of rice flour modified with a microbial transglutaminase. *Journal of Cereal Science*, *39*(2), 225-230.
11. He, H. A. N. D., & Hoseney, R. C. (1991). Gas retention of different cereal flours. *Cereal Chem*, *68*(4), 334-336.
12. Hager, A. S., & Arendt, E. K. (2013). Influence of hydroxyl propyl methyl cellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain charact - eristics of gluten-free breadsbased on rice, maize, teff and buckwheat. *Food Hydrocolloids*, *32* (1), 195-203.
13. Hamada, S., Aoki, N., & Suzuki, Y. (2012). Effects of water soaking on bread-making quality of brown rice flour. Food Science and Technology Research, 18(1), 25-30.
14. Heo, S., Lee, S. M., Shim, J. H., Yoo, S. H., & Lee, S. (2013). Effect of dry-and wet-milled rice flours on thequality attributes of gluten-free dough and noodles. Journal of Food Engineering, 116(1), 213-217.
15. Hui YH, Corke H, De Leyn I. (2008). Bakery Products : Science and Technology. Iowa: Blackwell Publishing, pp. 57-60.
16. López, A. C. B., Pereira, A. J. G., & Junqueira, R. G. (2004). Flour mixture of rice flour, corn and cassava starch in the production of gluten-free white bread. Brazilian Archives of Biology and Technology, 47(1), 63-70.
17. Maktouf, S., Jeddou, K. B., Moulis, C., Hajji, H., Remaud-Simeon, M., & Ellouz-Ghorbel, R. (2016). Evaluation of dough rheological properties and bread texture of pearl millet wheat flour mix. Journal of food science and technology, 53(4), 2061-2066.
18. Meterei, A., Syendrei, E., Fekete, A. (2004). Prediction of Bread crumb texture quality. In: Int. Conf. Engineering and Food. Montpelier, France.
19. Nawaratne, S. B. (2010). Exploring Possibility of Using Davulkurundu (Neolitsea involurate) Leaf Extract to Improve Leavening Action and Crumb Structure of Wheat Bread. Tropical Agricultural Research and Extension, 10.
20. Onyango, C., Unbehend, G., & Lindhauer, M. G. (2009). Effect of cellulose-derivatives and emulsifiers on creep-recovery and crumb properties of gluten-freebread prepared from sorghum and gelatinised cassava starch. Food Research International, 42(8), 949-955.
21. Pa, N. C., Chin, N. L., Yusof, Y. A., & Aziz, N. A. (2013). Measurement of bread crumb texture via imaging of its characteristics. Journal of Food, Agriculture & Environment, 11(2), 48-55.
22. Park, J. H., Kim, D. C., Lee, S. E., Kim, O. W., Kim, H., Lim, S. T., & Kim, S. S. (2014). Effects of rice flour size fractions on gluten free rice bread. Food Science and Biotechnology, 23(6), 187-188.
23. Rathnayake, H. A., Navaratne, S. B., & Navaratne, C. M. (2018). Porous Crumb Structure of Leavened Baked Products. International Journal of Food Science, 2018.
24. Skendi, A., Biliaderis, C. G., Papage - orgiou, M., Izydorczyk, M. S. (2010). Effects of two barley b -glucan isolates on wheat flour dough and bread properties. Food Chem. 119 (3), pp.1159–67
25. Svec, I., & Hruskova, M. (2004). Image data of crumb structure of bread from flour of Czech spring wheat cultivars. Czech Journal of Food Sciences-UZPI (Czech Republic).

26. Tonukari, N. J. (2004). Cassava and the future of starch. Electronic journal of biotechnology, 7(1), 5-8.
27. Wang, J., Rosell, C. M., & de Barber, C. B. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality. Food chemistry, 79 (2), 221-226.
28. Zghal, M. C., Scanlon, M. G., & Sapirstein, H. D. (1999). Prediction of bread crumb density by digital image analysis. Cereal Chemistry, 76(5), 734-742.

8. QUALITY AND SAFETY ENHANCEMENT OF CASSAVA-BASED FOOD PRODUCTS IN THE PHILIPINES

Julie D. Tan, Genesis Jared A. Cutamura, Rogelio Q. Clavite, Jr., Sarah Jean C. Sugano, Mary Rose M. Maniego, and Inish Chris P. Mesias
Philippine Root Crops Research and Training Center (PhilRootcrops), Visayas State University (VSU), Baybay City, Leyte 6521, Philippines
Corresponding author's Email: julietan@vsu.edu.ph

Abstract
Assessment of the existing processing methods for cassava-based food products in the Philippines was conducted for improvement of techniques to enhance food quality and safety for commercialization. About 350 cassava food processors from different regions were interviewed. There are 26 different kinds of cassava-based food products, majority of which are processed using traditional or inherited technologies. Non-compliant to Good Manufacturing Practices (GMP), inefficient processing, lack of materials and equipment and inadequate supply of raw materials are the major problems of the cassava processors. The safety and quality levels of cassava-based food products were determined through microbiological analyses and risk assessment using the predictive modelling for the growth of *Escherichia coli.* Some products failed in coliform, total and fecal coliform counts, and presumptive *E. coli* count which indicates contamination from water sources and unhygienic processing. Results on predictive modelling using Combase for the growth of *E. coli* implies thatconsumer awareness in proper food product handling must be promoted. After 2 days, the products exceeded acceptable limit for *E. coli* concentration based on Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods of the Food and Drug Administration (FDA). Risk assessment study revealed high exposure to the infective dose of *E. coli* with temperature abuse thus, this factor must be controlled. Trainings on GMP and principles and approaches in foodprocessing were conducted. Five selected processors were assisted for Licence-To-Operate (LTO) application based on the availability of processing area and potential of the product for commercialization.

Keywords: Cassava processing, food safety, good manufacturing practices (GMP), risk assessment

1. Introduction

Rootcrops in general have been considered as poor man's crop. The production of these crops is usually associated with farmers with low income and small areas of land for production. But recently, the demand for these crops are increasing because of their health benefits. In particular, cassava has been identified as good source of energy, vitamins and micronutrients with low glycemic index (Trinidad et al, 2009). In line with the pressing need of the consumers, Philippine Root Crops Research and Training Center (PhilRootcrops) is mandated to develop different high-yielding cassava varieties and postproduction technologies specifically on cassava processing and utilization. Rootcrops, particularly cassava, is now being tapped as functional food and raw material for processing due to rising incidence of diseases such as diabetes, high blood pressure, and other malfunctions in the body caused by nutrient deficiencies.

PhilRootcrops has developed complete processing systems for cassava flour and grates which serve as raw materials for a number of food products. Wheat flour is an imported raw material containing gluten which can cause celiac diseases. As a training center, PhilRootcrops has disseminated a number of postproduction technologies on cassava through trainings. However, there is still no concrete data on the adoption of these technologies or commercialization of cassava food products. In general, the main problem of processed products in the country is non-conformity to the quality standards set by the local and global market. The establishment of processing plants that are compliant to Good Manufacturing Practices (GMP) will contribute greatly in product commercial- ization. Local and global market for foods has defined rules and regulations, foremost of which is the region's compliance to Good Manufacturing Practices (GMP). To ensure safe and quality foods, a minimum sanitary and processing requirement as defined in GMP is necessary. It is aimed to assess and evaluate the existing processing methods for cassava-based food products in the Philippines and recommend improvement in the processing techniques through food safety practices for product commercialization.It is hoped that this project can help elevate the production of some

selected cassava-based food products at commercial scale level for local and international market.

2. Materials and methods

Survey on the different cassava-based food products and different techniques used in processing in the Philippines

The identification of the different cassava based-food products, current processing techniques and methods, and equipment used in processing was done in the different regions in the Philippines through survey using a pre-formed questionnaire. The different cassava food products in the Philippines were compiled in a brochure. Based on the survey, the extent of adoption of the different PhilRootcrops post production technologies was also determined. Problems and issues on cassava processing and the adoption of technologies were solicited from the cassava food processors.

Technological Assistance for Some Selected Cassava Processors in the Philippines

GMP checklist was developed using the FDA and DOST (Department of Science and Technology) Food Safety guidelines and other published literatures on food safety practices. Selected cassava food processors across the country were surveyed and evaluated for their compliance to good manufacturing practices. Microbiological analyses of some selected cassava food products were also conducted. Aerobic Plate Count and Yeast and Mold Count were performed following the method described by Maturin and Peeler (2001) and Tournas et al (2001). E. *coli* and coliform counts were performed using the standard multiple fermentation tube technique or most probable number (MPN) method (Hitchins et al, 2001 and Garthright, 2001).

Growth Assessment of E. coli

The growth behaviour of *E. coli* in the production to consumption continuum of the selected cassava-based food products was modelled using *ComBase*. It is a tool which can predict either microbial growth or survival. The model pathway comprised of 6 nodes (Figure 1). For model simulation, @Risk software package version 6 (Palisade USA) was used.

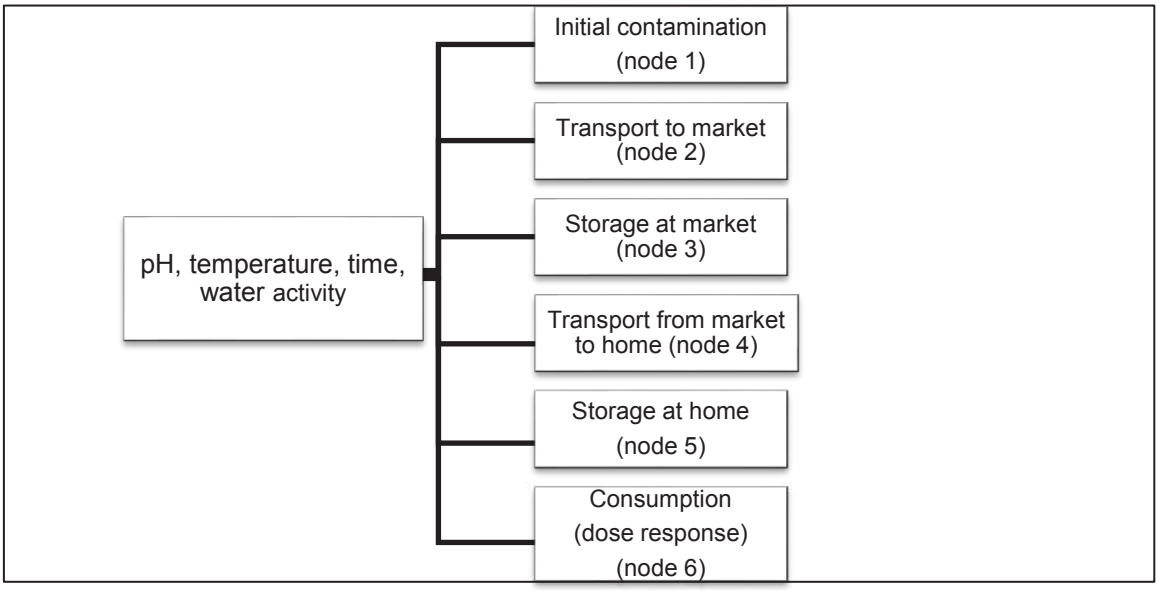


Figure 1: Post-production to consumption model pathway of cassava-based food products

Trainings on processing techniques and food safety practicesfor cassava-based food products processors

The GMP Checklist that was developed served as guide in assessing processors' practices. Technological assistance in terms of lectures and seminars were done to enhance the knowledge and skills of cassava food processorsin methods and approaches in processing and food safety practices. The trainings on the principles and approaches in food processing, GMP, Labelling and Packaging of Processed and Hazard Analysis and Critical Control Point (HACCP), were provided to cassava processors. Trainings were assisted by the Local Government Unit

(LGU), Department of Agriculture (DA) and Department of Social Welfare and Development (DSWD).

3. Results and discussion

Cassava Food Products in the Philippines

A total of 350 cassava food processors from the different regions in the Philippines were interviewed.A wide variety of products are produced in the different regions. There are 26types of cassava-based food products. These are wine, cakes, pudding, pies, chips and fritters, muffin, cookies, biscuit, crunch, noodles, rolls and balls. Ingredients, formulations and names of the products vary in different regions. They are boiled, steamed, fried, roasted or fermented. The description and ingredients of each food product are found in the brochure that was developed for dissemination.

Cassava food processing and procedures differ with regions and localities according to food cultures, environmental factors and the types of processing equipment and technologies available. Similarly in Nigeria, processing methods vary from place to place and the processing method determines the product (Lancaster et *al*., 1982). The techniques and methods for processing generally involve peeling and slicing, chipping or grating, pressing, spreading, blanching, drying, fermenting, boiling, roasting, frying, steaming, and baking. Fresh and dried cassava grates were commonly used in processing. Same products are also modified in other regions and given different names.
Materials like polypropylene and polyethylene bag, paper cups, boxes, plastic cellophane, styrofoam, aluminum foil, roll bags and wilted banana leavesare used as packaging materials for cassava-based food products. Labelling of the products is seldom observed. Some products with labels were assisted by government agencies (Department of Trade and industry, DTI and the Department of Science and Technology, DOST.

Post-Production Technologies
Post production technologies are an important intervention for efficient processing of cassava. Table 1 shows the source of processing techniques or methods for cassava processing in different regions. Most of the processors practice traditional or inherited techniques (69.4%) from their parents and ancestors which are done manually and labor intensive.Similarly in Nigeria, practicing this method by majority of the processors become a constraint in cassava processing (Oyewole and Sanni, 1995 and Fefa, et *al*., 2012). In rural areas in South-west Nigeria, traditional cassava processing is also observed (Adebayo, 2009).

About 12% of the processors learned the technology from the trainings conducted by the Department of Agriculture (DA). About 0.9% of the processors were also trained by DA after they have been trained by PhilRootcrops. The latter has also contributed 3.9% of the processors who have gained knowledge on the technology. Some processors learned the technology from schools (Technical Education and Skills Development Authority, TESDA and other universities) who conducted trainings on cassava processing, while others learned through viewing from the internet and newspapers (5.4%).Other processors learned it from agencies like DTI (1%), DOST (1.5%), DA through ATI (0.9%) and other agencies (5%).

Table 1: Source of knowledge of processing techniques or methods

Agency/Institution	**Percent (%)**
Traditional or Inherited Techniques	69.4%
Department of Agriculture (DA)	12%
DA through ATI	1%
PhilRootcrops	3.9%
PhilRootcrops through DA	0.9%
Schools (TESDA, Universities)	3.3%
DTI	1.0%
DOST	1.5%
Mass Media	2.1%
Other Agencies (Non-government Organizations)	5%

Majority of the cassava processors operate as sole proprietor (84.1%), and about 15.3% and 0.6% through association and partnership respectively (Table 2). These sole proprietors usually operate with very small capital where production is done only in their kitchen. Associations and partners in cassava processing are generally producing their products in commercial level.

Table 2: Type of Business of Cassava Processors in the Country

Type of Business	Percentage (%)
Sole Proprietorship	84.1
Association	15.3
Partnership	0.6

Problems Encountered in Cassava Processing

The four major problems of the processors are inadequate supply of raw material such as cassava tubers (31.2%), lack of materials and equipment (16.5%), inefficient process (14.4%) and inconsistent product quality (10.2%) [Table 3]. This resulted to products in low quality, small quantities and low profit. Inadequate funds are a major challenge for the cassava processors (Asinobi*et al*., 2009). The processors also complained that the price of their processed products were low due to inadequate storage facilities in which, products must be sold at once to prevent spoilage. These constraints and challenges of the cassava food processors must be addressed in order to increase profit and make the enterprise more attractive and conducive for processors (Ehinmowo et al, 2015). Adoption of technologies is difficult to some processors because of the lack of materials and equipment in processing like mechanical cassava grater. Most of the processors use manual grating of cassava.

Table 3: Problems Encountered in Cassava Processing

PROBLEMS ENCOUNTERED	Percentage (%)
Inadequate Supply of Raw materials	31.2
Lack of Materials and Equipment	16.5
Inefficient Process	14.4
Inconsistent Product Quality	10.2
Lack of Capital	7.8
Low Market	1.2
Lack of Processing Area	1.8
Lack of Training	0.60

Assessment of Cassava Processing Areas in the Philippines

To assess the knowledge and awareness to Good Manufacturing Practices (GMP), it is beneficial for the food processors to undergo GMP inspection and training. During the survey, ocular inspection of the processing areas was also conducted to determine the level of compliance of the processors to GMP using the developed GMP checklist. Table 4 shows the summary of the result of the evaluation with respect to the eight (8) areas of GMP. Results showed that higher percentage of the cassava food processors did not comply with the GMP standards in all the areas such as Premises (67%), Transportation and Storage (57%), Equipment (84%), Personnel (69%), Manufacturing Controls (57%), Product Recall (70.3%), and Records (94%). Majority of the processors however, are partially compliant on sanitation and pest control (66%).

Table 4: Summary of GMP Inspection of the Cassava Food Processors

GMP Areas	GMP Compliant	Not Compliant	Partially Compliant
Premises	2%	67%	31%
Transportation and Storage	9%	57%	34%
Equipment	1%	84%	15%
Personnel	14%	69%	17%
Manufacturing Controls	2%	57%	41%
Sanitation and Pest Control	2%	32%	66%
Product Recall	2.3%	70.3%	27.4%
Records	2%	94%	4%

GMP Compliant (adherence to the regulations set by cGMP); Not Compliant (non-adherence to the regulations set by cGMP); Partially Compliant (partial observance to the regulations set by cGMP.

The processing activities are done mostly at home particularly in the kitchen (Table 5). According to the GMP guidelines, such activities must be performed in an area solely for the processing of food products only. A provision for any part of a house is allowable provided that no other household activities are done in the area.

Table 5: Processing Area of Cassava Food Processors

Processing Area	**Percentage (%)**
Commissary/ Bakeshop	15
Home	85

Microbiological Analyses of Cassava-based food products

All cassava processors in general do not practice Good Manufacturing Practices (GMP) or any in-depth food safety measures. Only 33.33% and 45.83% of the products comply with APC and mold and yeast counts respectively. Although APC cannot directly contribute to the safety assessment of ready-to-eat foods, it can indicate quality which determine product storability (National Food Handling Survey Evaluation Report (2007). Results also reveal poor hygiene and handling of foods. Similarly, mold and yeasts in the product should be atallowable level in order to have a safe product. High yeast and mold counts are indications of improper food preparation. Unlike bacteria, molds and yeasts grow rapidly at low moisture content like bread and other baked products.

The presence of coliform bacteria in food products is an indication of contamination of the water source or during the packaging process. Coliform bacteria generally come from fecal contamination or environment.The presence of E. *coli* in ready-to-eat foods is undesirable because it indicates inadequate heat treatment and poor hygienic conditions during processing. Ideally, E. *coli* should not be detected and a level of <3 per gram which is the limit of the MPN test is a satisfactory criteria for product safety (Guidelines for the microbiological examination of ready-to-eat foods, 2001).Water Treatment to eradicate the contamination of coliform bacteria in the food processing area would offer a good solution.

Table 6: Percentage of products with acceptable level of microbial counts based on GMP guidelines

Type of Microorganism	Percentage of acceptable level, %
Aerobic Plate Count, APC (CFU/g)	33.33
Mold and Yeast Count (CFU/g)	45.83
Total Coliform, (MPN/g)	56.25
Fecal Coliform (MPN/g)	56.25
E. coli Presumptive	62.25

Based on 16 product samples analysed; Limits were based on the Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods, FDA, 2013; At <3.0 MPN/g (no growth in the indicated microbiological parameter at 9-tube Most Probable Number (MPN); Different set of samples analysed for APC and mold and yeast counts.

Growth Assessment of E. coli

Predictive modelling for the growth of *E. coli* revealed that after 2 days, all products exceeded the acceptable limit according to FDA's Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods (Table 8).

Table 8: Predictive modelling for the growth of *E. coli* during the storage and marketing of ready-to-eat cassava-based food products at ambient room temperature (26 ^{0}C)

Product	pH*	Aw	Maximum growth rate (log.conc/h)	Doubling time (hour)	Concentration after 1 day (log_{10} cells/g)	Concentration after 2 days, (log_{10} cells/g)
Special Binagol de Salcedo	5.39	0.96	0.070	4.316	3.46	4.96
Hub-hub	5.85	0.96	0.088	3.436	3.79	5.82
Cassava (Hanged)	6.25	0.96	0.102	2.961	4.09	6.48
Cassava Cheese Roll	6.55	0.96	0.103	2.911	4.13	6.57
Chippy with Malunggay	5.55	0.96	0.075	4.040	3.54	5.19
Cassava Pretzel	6.92	0.96	0.100	3.025	4.04	6.38
Chippy Plain	5.25	0.96	0.065	4.638	3.39	4.73

** Assumption – initial load of 3 log cfu/g Effect of Temperature on the E. coli Concentration*

At 29^0 C, the maximum and minimum *E. coli*concentration were found to be 9.71and 6.34 log CFU/serving respectively. The model predicted that 100% of the population is at risk of being exposed to the infective dose of *E. coli* (Fig 2a). At 22^0 C, a maximum concentration of 7.67 log CFU/serving and minimum concentration of 5.29 log CFU/servingwere observed. About 90.6% exposures to the infective dose were indicated (Fig 2b). At 10^0 C, 30.6% of the population is at risk of being exposed to the infective dose having a maximum and minimum *E. coli*concentration of 6.90 and 4.71 log CFU/serving respectively.

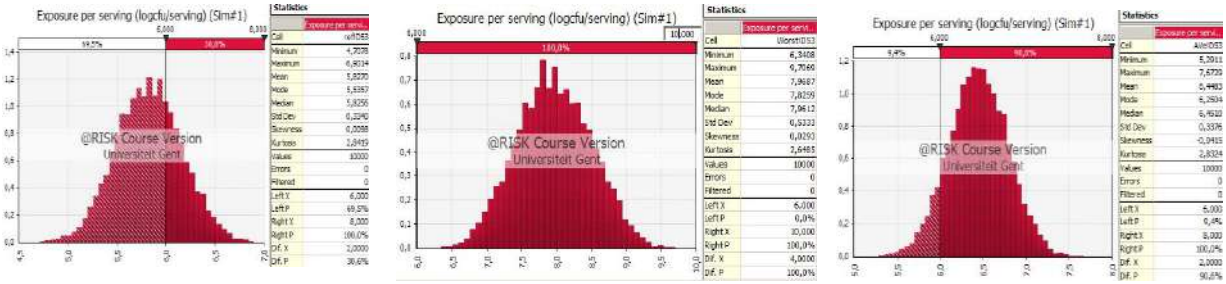


Figure 2: Frequency distribution of *E. coli* at a) 29^0 C b) 22^0 C and C) 10^0 C
Note: Values in x axis are in log CFU/serving

Assistance to Local Cassava Food processors

Five processors with available fund and areas for processing and with potential product for commercialization were selected from processors who were trained on processing and food safety (Table 11). A total of 21 trainings on processing and food safety were conducted in different regions in the Philippines (Table 11). About 677 local processors were trained. To ensure follow-up of the applications of knowledge gained during the training, participation from LGU, DA and DSWD in every region was encouraged.

The selected processors were assisted in making of plant layout or floor plan, location map, product description, manufacturing practices which includes GMP Checklist and compliance to FDA physico-chemical and microbiological standards.
Additional lectures on the health benefits of root crops, Labelling and Packaging of Processed Foods, HACCP, Sanitation Standard Operating Procedures (SSOP) and Licence-to-Operate Application were also provided to some processors. The knowledge on the health benefits of root crops gave the participants' fresh insights ofthe importance of root crops to human health and a higher level of importance on cassava which motivated them to continue processing cassava food products.

Table 11: Selected processors assisted for GMP compliance and LTO application

Identified processors for LTO Assistance	Region
PhilRootcrops, VSU, Leyte	8
Bubon Rural Improvement Club, Leyte	8
Farmer's Entrepreneurs Association, Eastern Samar	8
Joji's Bakeshop, Bohol	7
Cassava Growers and Processors Association, Zamboanga del Norte	9

Note: Total number of Trainings Conducted – 21; Number of Participants Trained: 677 Local Processors and 110 DA/LGU/DSWD Personnel

Summary, Conclusion and Recommendation
A total of 350 cassava food processors were interviewed in the different regions in the Philippines. There are 26 cassava-based food products that are commonly produced in the country with varying formulations and names.Majorityof the processors are still using traditional processing technologies and operate as sole proprietors. Problems such as inadequate supply of raw materials, lack of materials and equipment, inefficient process and inconsistent product are identified by the processors. Majority of the cassava food processors were non-GMP compliant based on food safety guidelines. Predictive modelling for the growth of *E. coli* revealed that after 2 days, all of the products exceeded the acceptable limit for *E. coli* concentration. The risk assessment study revealed high exposure to the infective dose of *E. coli* with temperature and time abuse. Thus, both temperature and time should be controlled to reduce the risk of food borne illness from street-vended cassava delicacies. A total of 21 trainings were conducted in different regions in the Philippines. Brochures on cassava food products were produced and GMP Checklist was developed as Guide to all cassava processors. Five local food processors were selected for further assistance for LTO application in order for them to fully commercialize their products.

4. Acknowledgment

The authors would like to thank the Department of Agriculture-Bureau of Agricultural Research (DA-BAR) for providing funds for the conduct of this research project; the Visayas State University (VSU) through the Philippine Root Crop Research and Training Center (PhilRootcrops) for the counterpart funds; the Regional Office of the Department of Agriculture, Local Government Units (LGUs), and Department of Social Welfare and Development (DSWD), for their assistance during the conduct of the surveys and trainings.

References

1. Asinobi, C. O., Ndimantang, B., & Nwajiuba, C. U. (2005). Cassava Production, Processing Trends and Constraints in Ohaji –Egbema Local Government Area of Imo State, Nigeria. In 14th Annual Africa/ Diaspora Conference (pp. 28-30).
2. Combase. (2017). A Web Resource for Quantitative and Predictive Food Microbiology.
3. Ehinmowo, O. O., Afolabi, J. A., & Fatuase, A. I. (2015). Determinants of profitability among small scale cassava processors in South Western Nigeria. Russian Journal of Agricultural and Socio-Economic Sciences, 37(1).
4. Fefa, J., Obute, C. O., & Ucherwuhe, S. I. Cassava Processing Technology Adoption

and Poverty Reduction among Operators in Benue State, Nigeria.
5. Garthright, W. E. (2001). Most Probable Number from Serial Dilutions. Bacteriological Analytical Manual Online; US Food and Drug Administration. Center for Food Safety and Applied Nutrition.
6. Guidelines for the microbiological examination of ready-to-eat foods. (2001). Food Standards Australia New Zealand.
7. Hitchins, A. D., Feng, P., Watkins, W. D., Rippey, S. R., and Chandler, L. A. (2001). Escherichia coli and the Coliform Bacteria. Bacteriological Analytical Manual Online. US Food and Drug Administration. Center for Food Safety and Applied Nutrition.
8. Lancaster, P. A., Ingram, J. S., Lim, M. Y., & Coursey, D. G. (1982). Traditional cassava-based foods: survey of processing techniques. Economic Botany, 36(1), 12-45.
9. Maturin, L. J., and Peeler, J. T. (2001). Aerobic Plate Count. Bacteriological Analytical Manual Online. US Food and Drug Administration. Center for Food Safety and Applied Nutrition.
10. National Food Handling Survey, Evaluation Report. (2007). New Zealand Food Safety Authority. Series No. 19.
11. Oyewole, O. B., & Sanni, L. O. (1995). Constraints in traditional cassava processing: the case of" fufu" production les contraintes au cour de la transformation traditionnelle du manioc: le cas de la production de foufou. Agbor-Egbe, Tom.
12. Revised Guidelines for the Assessment of microbiological Quality of Processed Foods. 2013. Food and Drug Administration Philippines. Circular No. 3013-010. Annex J. Risk Software Package Version 6 (Palisade USA) [Software].
13. Tournas, V., Stack, M. E., Mislivec, P. B., Koch, H. A., and Bandler, R. (2001). Yeasts, Molds and Mycotoxins. Bacteriological Analytical Manual Online. US Food and Drug Administration. Center for Food Safety and Applied Nutrition.
14. Trinidad, T. P., Mallillin, A. C., Loyola, A. C., Encabo, R. R., & Sagum, R. S. (2010). Nutritional and Health Benefits of Root Crops. In Bioactive Foods in Promoting Health (pp. 717-728).

9. HEAVY METAL AND HEALTH RISK ASSESSMENT OF PARAGIS (*Eleusineindica*) GRASS CONCOCTION FROM DIFFERENT PARTS OF LAGUNA NEAR THE LAGUNA LAKE SHORE LINE

Dannielle Theo Martinez, and Mariero H. Gawat
College of Food Nutrition and Dietetics, Laguna State Polytechnic University, Philippines
Corresponding author's Email: gawatmarieo@gmail.com

Abstract
Paragis **(***Eleusineindica*) grass is ubiquitously distributed throughout the Philippines is becoming popular in the Philippines as herbal medicine, claimed to cure several medical conditions when processed and consumed as a concoction. In this study, concentration of three heavy metals namely, Lead (Pb), Cadmium (Cd), and Chromium (Cr) in Paragis Grass concoction from the three towns (Los Baños, Bay, and Calamba) along Laguna Lake was determined. Also, health-risk effects, specifically non-carcinogenic, due to the esposure of the three heavy metal studied for adult consumers were estimated. Heavy metal analysis was carried out through Flame Atomic Absorption Spectrophotometry (AAS). Results show that the highest concentration of Pb, Cd, and Cr were found to be from the samples gathered in Bay, Laguna. Also, for the Estimated Daily Intake of Pb, Cd, and Cr, Paragis grass concoction from Bay, Laguna exceeds the maximum permissible limit set by FAO. For the non-carcinogenic risks associated with the consumption of the samples, estimated Target Hazard Quotient (THQ) for the three samples, for Pb, Cd and Cr did not exceed the limit (THQ>1). For the estimation of the the potential health risk posed by more than one metal, the computed Hazard Index (HI) isless than 1, thus no adverse health effects are expected because of exposure for Pb. Cd and Cr, and the risk of the person getting a disease from exposure to the sample is low.

Keywords: Lead, Cadmium, Chromium, THQ, Hazard Index

1. Introduction

Paragis (*Eleusineindica*) grass is also known as goose grass, is ubiquitously distributed throughout the archipelago, and native in the tropics (Haber and Semaan, 2007). At present, this plant isbecoming popular in the Philippinesbecause of its medicinal claims. The plant is used as a remedy for kidney problems and is used for the treatment forhypertension; influenzaetc (Cooper, 2005; Lans, 2006; Leach et al 1995). All parts of the plant are required to be boiled to get its medicinal effect. However, there are no studies conducted for the safety of drinking Paragis concoction, specifically for its heavy metal content.

Heavy metals are found naturally on earth and plays vital role for several lifeprocesses; however, it can be toxic if taken in excess and can bind to and affect vital functions of cellular components. Among the common heavy metals, Chromium, Arsenic, Cadmium, Mercury, and Lead are potentially very harmful when taken extensively (Baird and Cann 2012; Singh et al.
2010). For this study, the heavy metal to be determined arePb, Ch and Cd, considering their high potential in imparting harmful effects to human. Furthermore, non-carcinogenic risks associated with the exposure of the studied heavy metal and estimation of its potential health risk when consuming paragis concoction will also be assessed.Emphasis on choosing Laguna lake as the site of the study will be stressed accordingly because Laguna lake is the largest Lake in the Philippines, known to be heavily contaminated due to its strategic location of being at the center of industrial hub in the country (Molina, 2011). Moreover, the area is considered as the "industrial contamination hotspot" by Greenpeace (2011).Thus, determining the heavy metal content of paragis concoction along the areas of Laguna lake will shed some light to the impact of the lake's environmental condition to health. Furthermore, this study will provide useful information about the possible risks in taking in paragis grass a medical remedy.

2. Materials and methods

Sample Collection

Paragis grass was gathered from three towns namely; Calamba, Bay and Los Baños, located on the south bay areain Laguna Lake. In each town, three representative Barangays were

chosen as the sampling site. For Bay, Barangay Santo Domingo, San Antonio, and Tagumpay. For Calamba, Barangay Lingga, Pansol, and Masiit and lastly, for Los Baños, Barangay Baybayin, Malinta, and Bayog. Grass samples were collected 10 meters away from the shoreline. The samples were placed in a sealed plastic bag and were brought to the lab. Each sample was provided with identification tag accordingly.

Sample preparation

The collected grass samples were thoroughly washed using distilled water. After cleaning the grass, it was air dried for 20 minutes to remove excess water. In homogenizing the samples, equal proportions of grass from the three barangays for one site were mixed, to represent one sampling site for Bay, Calamba and Los Baños respectively. In preparing the concoction, twenty grams of paragis grass were added with 1L of water and was boiled for 15 minutes. After boiling, the concoction was cooled down and filtered through a fluted paper filter and the resulting filtrate was transferred to a clean bottle.

Heavy Metal Analysis

The total heavy metal contents were determined by digesting 1 mL of paragis concoction filtrate in a 12 ml mixture of concentrated Nitric acid and Per Chloric acid (3:1 v/v). All the digestion procedure was adopted from the methods of Garba et al. (2015).Heavy metal analysis was done Using AAS (SPECTR AA 55B), using standards for Cd, Ch and Pb. *Health Risk Assessment (Non-Carcinogenic Health Effects)*

The estimated daily intake was calculated using the formula: EDI= (MC x IR/BW). The IR is the ingestion rate, based on the ingestion rate of tea in the Philippines is 0.027 kg or 27 g (The Statistics Portal,); the MC is the metal concentration of the heavy metals (Pb, Cd, and Cr) and BW is the body weight of an average human; 60.5 kg for males and 52.2 kg for females (Philippine Dietary Reference Intake, 2015). Target Hazard Quotient (THQ) of the Paragis concoction was determined using the formula by (Zhuang et al, 2013).

where, Exposure Frequency (EFr) is the number of days in a year for an average consumer, which is set to be 365 days/year; Exposure Duration (ED) is the life span of an average Filipino (68 years). Ingestion Rate (IR) of tea in the Philippines is 27 g (The Statistics Portal); Metal Concentration (MC) of Pb, Cd, and Cr in samples (mg/L); Oral reference dose (RfDo). For the reference doses, the following are used: 0.2 mg/kg day for Pb, 0.005 mg/kg day for Cd and 0.009 mg/kg day for Cr (Sakar et al 2016). The average Body Weight (BW) for females is 52.5 kg and 60.5 kg for 25 males (Philippine Dietary Reference Intake, 2015). The average time for Noncarcinogenic risk considered is 30 years or 10,590 days.

Health Risk (HI) is the sum of every Target Hazard Quotient for every heavy metal (USEPA, 2011). This is used to determine the potential health risk when considering the exposure to more than one heavy metal.

Statistical Analysis

All the values reported were the calculated average value and standard error for triplicate analyses performed. All data were analyzed with the SPSS software, version 17.0 (SPSS Inc., Chicago, USA). Statistical significance was determined by variance analysis (ANOVA). Values found to be significantly different were subjected to post-hoc analysis using Tukey's honestly significant difference (HSD) test to compare and separate the means, and significance was accepted at the 5% level ($P<0.05$).

3. Result and discussion

Heavy Metal Concentration

The concentrations of heavy metals from paragis concoction samples collected from three towns located in the South bay area of Laguna Lake is shown in Table 1.

$$[THQ= [(EFr \times ED \times IR \times MC) / (RfDo \times BW \times AT)] \times 10^{-3}],$$

Table 1: Levels of heavy metal

Sample Source	Heavy metal Concentration (mg/mL)		
	Pb	Cd	Cr
Bay	1.605±.01[a]	0.309±0.02[a]	0.065±0.00[a]
Calamba	0.340±0.02[b]	0.086±0.01[b]	0.062±0.01[a]
Los Baños	0.313±0.02[b]	0.089±.001[b]	0.033±0.01[b]

*values with the same letter across a column are not significantly different from each other ($p \leq 0.05$)

Table 1 shows that for the concentration of Cd, samples from Los Baños and Calamba show no significant difference and did not exceed the recommended limit set by WHO which is 0.10 µg/g (Food and Agriculture Org., 2001), However samplescollected from Bay got a significantly higher Cd concentration of 0.309 mg/mL.Also, for the concentration of Lead, samples from bay had a significantly higher value (1.605 mg/mL), compared to the concentrations of Lead from Calamba and Los Baños. All concentrations of Lead from the three sampling areas exceeded the allowable limit set by WHO which is 0.3 µg/g (FAO 2002), thus this implies an issue of concern in the consumption of the studied samples. When it comes to the concentration of Ch, results show that samples from Calamba and Bay exceeded the allowable limit which is 0.05 (FAO, 2002). The values for Ch for Calamba and Bay are significantly higher than the concentration of Ch for samples from Los Baños.

Based on the results discussed above, it was shown that samples from Bay exceeded the recommended limit for the three heavy metals studied compared to its neighboring towns.Theresults can be attributed to the geographical location of Bay, Laguna, where it is identified as an area with high flood retention service (the capacity of the lake to store water) along Laguna lake (Faraon et al. 2016). Furthermore, bigger areas in Bay, Lagunaare prone to flooding dueto more flood plains along the lakeshore area (Ardales, 2015). Flooding in these areas causes high depositions of minerals such as heavy metal contaminants of anthropogenic nature from the contributing nearby industrial areas.

Human Health Risk Assessment

The Estimated Dietary Intake (EDI) of metals, a common index for the transfer of heavy metal transfer from plant to humans were computed based on rate of tea consumption in the Philippines are shown in Table 2, while Table 3presented information on the total hazard quotient (THQ) and hazard index (HI) of metals from consumption of paragis concoction. In this study, the computed concentrations of heavy metals from the paragis concoction samples were used for the EDI, THQ and HI computation. At present, there are relatively no values for the ingestion rate of paragis concoction, thus ingestion rate used for the computations was for tea consumption in the Philippines.

Table 2: Estimated daily intake

Sample Source	(EDI mg/kgbw)					
	Lead		Cadmium		Chromium	
	Male	Female	Male	Female	Male	Female
Bay	0.71[b]	0.83[b]	0.14[b]	0.16[b]	0.03[a]	0.03[a]
Calamba	0.08[a]	0.09[a]	0.04[a]	0.04[a]	0.01[a]	0.02[a]
Los Baños	0.19[a]	0.22[a]	0.04[a]	0.05[a]	0.02[a]	0.02[a]
Maximum Permissible Limit (MPL)	10mg/kgbw		0.3 mg/kgbw		1.3 mg/kgbw	

*values with the same letter across a column are not significantly different from each other ($p \leq 0.05$)

Table 2 shows the computed EDI for each heavy metal. For Pb and Cd, EDI for samples collected from Bay were significantly higher for male and female, than in samples collected from Calamba and Los Baños. The result was expected due to the significantly higher concentration of Pb and Cd from the samples from Bay. On the other hand, for the Ch, no there were significant differences in EDI values from different sampling areas. The

computed EDI for the samples shows that all paragis concoction from the three areas had an EDI value not exceeding the MPL for Pb, Cd and Cr set by WHO, thissignifies that for the studied paragis concoction, its consumption does not indicate potential health risks to consumers (Zhuang et al., 2013; Islam et al., 2016).

Table 3: Total Hazard Quotient

	THQ											
	Lead			Cadmium			Chromium			HI		
Sample Source	M	F	Ave	M	F	Ave	M	F	Ave	M	F	Ave
Los Baños	0.02	0.03	0.025	0.004	0.005	0.005	0.001	0.002	0.001	0.105	0.037	0.071
Bay	0.08	0.09	0.085	0.016	0.018	0.017	0.003	0.003	0.003	0.099	0.111	0.104
Calamba	0.01	0.01	0.01	0.004	0.005	0.005	0.002	0.002	0.002	0.016	0.017	0.016

M-Male F-Female HI-Hazard Intake

Using the THQ guidelines set by USEPA, the computed THQ for the individual heavy metals in the paragis concoction studied were all below the limit (<1). Correspondingly, the effect of the consumption of more than 1 heavy metal (Pb, Cd and Cr) expressed as HI value for the three metals were all less than one. The results suggest for the given of heavy metal concentration, there is no significant health implications to the exposed population during a given lifetime (IRIS 2011).The present study reveals that for the health risk assessment, paragis concoction from the different parts of Laguna lake showed no possible health risk. Currently, the result adds up to the health risk studies conducted for the consumption of tea and infusions, having the same result of no potential health risk (Zhang et al, 2018; Patrick-Iwuanyanwu and Udowelle, 2017).

4. Acknowledgement

This paper was made into completion through the support of Laguna State Polytechnic University Research and Development Office and the College of Food Nutrition and Dietetics.

References

1. Ardales, G. Y., Espaldon, M. V. O., Lasco, R. D., Quimbo, M. A. T., and Zamora, O. B. (2015).Trends in Rainfall and the Causes of Flood Events in the Municipalities of Los Baños and Bay, Laguna, Philippines. Journal of Nature Studies. 14 (2): 40-53.
2. Baird, C., and Cann, M. (2012). Environmental Chemistry, 5th ed. W. H. Freeman and Company, New York
3. Cooper, E. L. (2004). Drug discovery, CAM and natural products. *Evidence Based Complementary and Alternative Medicine*, *1*(3), 215-217.
4. FAO/WHO. (2002). Codex Alimentarius General standards for contaminants and toxins in food. Schedule 1 maximum and guideline levels for contaminants and toxins in food Joint FAO/WHO food standards programme, Codex Committee, Rotterdam. Reference CX/FAC 02/16
5. FAO/WHO. (2011). Joint FAO/WHO Food Standards programme Codex committee on contaminants in foods. Food CF/5 INF/1, pp 1–89
6. Faraon, A., Santos-Borja, A., Varcas, N., Angeles, B., and Mercado, E. K. (2016). Valuationo of Ecosystem Serviceo of the Laguna Lake Basin: Erosion Control and Flood Water Retention. Laguna Lake Development Authority.
7. Garba, Z. N., Ubam, S., Babando, A. A., & Galadima, A. (2015). Quantitative assessment of heavy metals from selected tea brands marketed in Zaria, Nigeria. *Journal of Physical Science*, *26*(1), 43.
8. Haber, R. M., & Semaan, M. T. (2007). Two new records from Lebanon: Chamaesyce nutans (Lag.) Small (Euphorbiaceae) and Eleusine indica (L.) Gaertner (Poaceae). *Turkish Journal of Botany*, *31*(4), 341-343.
9. Integrated Risk Information System (IRIS). (2011). U.S EPA hazards and dose response assessments available from

https:// www.epa.gov/iris. Cited on 29 May 2015.
10. Labunska, I., Brigden, K., and Johnston, P. (2011). Laguna Lake, the Philippines: industrial contamination hotspots. Green peace Research Laboratories Technical Note
11. Lans, C. A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal of ethno biology and ethno medicine*, *2*(1), 45.
12. Leach, G. E., Devine, M. D., Kirkwood, R. C., & Marshall, G. (1995). Target enzyme-based resistance to acetyl coenzyme A carboxylase inhibitors in Eleusine indica. *Pesticide Biochemistry and Physiology*, *51*(2), 129-136.
13. Molina, V. B. (2011). Health risk assessment of heavy metals bio accumulation in Laguna de Bay fish products, Philippines. In *14th World Lake Conference, Austin Texas. p* (pp. 1-23).
14. Patrick-Iwuanyanwu, K. C., & Udowelle, N. A. (2017). Monitoring of essential and toxic metalsin imported herbal teas marketed in selected cities in Southern Nigeria: A health risk assessment study. *Journal of Applied Sciences and Environmental Management*, *21*(6), 1189-1196.
15. Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Tropical Ecology*, *51*(2), 375-387.
16. USEPA. (2000). Risk-Based Concentration Table Environmental Protection Agency, Philadelphia PA, Washington DC, United States
17. USEPA. (2011). USEPA Regional Screening Level (RSL) Summary Table: November 2011.
18. World Health Organization. (2011). Lead Compounds.
19. Zhang, J., Yang, R., Chen, R., Peng, Y., Wen, X., & Gao, L. (2018). Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China. *International journal of environmental research and public health*, *15*(1), 133.
20. Zhuang, P., Li, Z. A., McBride, M. B., Zou, B., & Wang, G. (2013). Health risk assess-ment for consumption of fish origina-ting from ponds nearDabaoshan mine, South China. *Environmental Science and Pollution Research*, *20*(8), 5844-5854.

10. POTENTIAL POSTHARVEST IMPROVEMENT IN THE SUPPLY CHAIN OF FRESH BITTER GOURD IN SOUTHERN LUZON PHILIPINES

Perlita A. Nuevo[1], Matilde V. Maunahan[1], ReginRuis B. Oliveros[2] and Jennelyn M. Resorez[1]
[1]Postharvest Horticulture Training and Research Center (PHTRC)/College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines, 4031
[2]Formerly with the PHTRC
Corresponding author's Email: perly4246@gmail.com

Abstract
Bitter gourd (*Momordicacharantia* L.) is widely grown and popular in the Philippines due to its rich nutritional content and culinary use. It is highly perishable and gets bruised very easily, with postharvest losses ranging from 2% to 70%. Traders in Southern Luzon, the region with the largest hectarage planted to the crop, reported yellowing and mechanical damage as the main causes of these losses. Visual quality and safety are concerns for consumers. The study aimed to identify the critical points in the supply chain and the potential postharvest improvements to ensure quality and safety of the produce. The postharvest handling of bitter gourd was documented from the farm to the market, and quality evaluation subsequently done. Microbial testing and simulation trial of bulk dropping were also conducted. Postharvest handling included hand-picking, field sorting, bulk packing, hauling to the packing shed, resorting, packing in 10-kg capacity polyethylene bags and transport over land to the market. Mechanical damage was incurred at some points along the chain due to rough handling, lack of appropriate postharvest facilities and improper stacking during transport. Microbial contamination was detected after harvest and at retail. Careful handling and stacking inside the transport vehicle only up to 1.35 meters high is advisable. Horizontal dividers will minimize damage on bottom fruits and refraining from loading on top of the roof of the vehicle will prevent high moisture loss. To avoid contamination, clean liners must be used during harvesting and collection, and sanitation be practiced at the retail level.

Key words: Mechanical damage, microbial contamination, quality, postharvest handling.

1. Introduction

Bitter gourd (*Momordicacharantia* L.), locally known as *ampalaya*, is a widely grown and popular vegetable in the Philippines due to its nutritive value and use as ingredient in various dishes. Southern Luzon has the largest area allocated for planting and harvesting of the crop (2,311 hectares) with a total production of 21,323 metric tons (PSA, 2016).

Local traders reported yellowing and mechanical damage as the main causes of losses in bitter gourd. These may range from 2% to 70%. At different stages of the handling chain, fruits are frequently dropped and over-stacked which results in impact bruises and compression damage, respectively. Physical injuries affect the physiological behavior of the fruits which in turn, can hasten the degradation of quality that will eventually lead to the unmarketability of the commodity (Valerio etal, 2001).

Microbial contamination also raises great concerns for processors and consumers, hence the sources of foodborne pathogens must be identified to develop effective control measures that will ensure the safety of the commodity for consumption (Sant'Anaet al, 2014).

This study aimed to identify the issues in the supply chain of bitter gourd and the potential postharvest improvement to ensure quality and safety for consumption.

2. Materials and methods

The handling chain of bitter gourd ('Galaxy' var.) harvested from Liliw, Laguna and transported to a wholesale market in Tanauan, Batangaswas documented. As per traders' practice, fruitspacked in 10-kg capacity polyethylene bags were stacked inside and on top of the transport vehicle.

To determine the extent of mechanical damage on bitter gourd in relation to stacking position in the transport vehicle and container used, samples from different positions (top, middle, bottom) were procured. Fruits packed in crates were loaded in the same vehicle for comparison. Quality evaluation after transport was doneat the PHTRC laboratory. To simulate the condition in the retail market,

fruits were subsequently stored at ambient condition and quality evaluated.

Microbial testing for *Salmonella* and *E. coli*in samples collected after harvest and from the retail market was done by the Microbiology Division of the Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños.

A trial simulatingbulk dropping of bitter gourd fruits on bamboo floor from a height of 0.75 meter was done. Each fruit from the bulk was evaluated based on the severity of bruising and cracking. Samples were then packed in 10-kg polyethylene bag, stored at ambient condition and evaluated thereafter for weight loss, and visual and textural changes.

A laboratory study was conducted to determine the effect of mechanical injury on the postharvest behavior of bitter gourd. Fruits, of good quality and minimal defects, were used in this study. Bruising was introduced by slightly scratching the fruits on rough surface. Both injured and non-injured fruits were stored at ambient condition (28.4 ± 1.3°C, 79.3 ± 6.0% RH) and evaluated for weight loss, visual quality and textural changes. Respiration rate and ethylene production were measured using Gas Chromatography equipped with Thermal Conductivity Detector (GC-TCD) and Gas Chromatography equipped with Flame Ionization Detector (GC-FID), respectively.

Data were analyzed using analysis of variance (SAS v9) and means separation was carried out using LSD at 5% level of significance.

3. Results and discussion

Handling system of bitter gourd from Liliw, Laguna (Fig1)

Bitter gourd fruits were harvested early in the morning. They were cut from the vine using a pair of scissors, placed directly on the ground, collected in sacks, carried to a shaded area and placed on the ground covered with tarpaulin for field sorting. Damaged, diseased and ripened fruits were removed and left in the farm while good quality ones were loaded in two metal baskets lined with rubber pads. These baskets were hauled by horse to an assembly area or packing shed along the roadside approximately 2 km away. Fruits were poured from the padded baskets to the covered bamboo flooring of the shed and resorted based on quality. Sorted bitter gourd were packed in 10-kg capacity polyethylene (PEB) bag, and stacked or loaded inside and on top of transport vehicle called a jeepney, together with other vegetables bound for the wholesale market in Batangas (50.6 km in distance). Travel time took approximately 2½ hours over mixed rough and paved roads. Fruits for nearby markets were transported via tricycle.

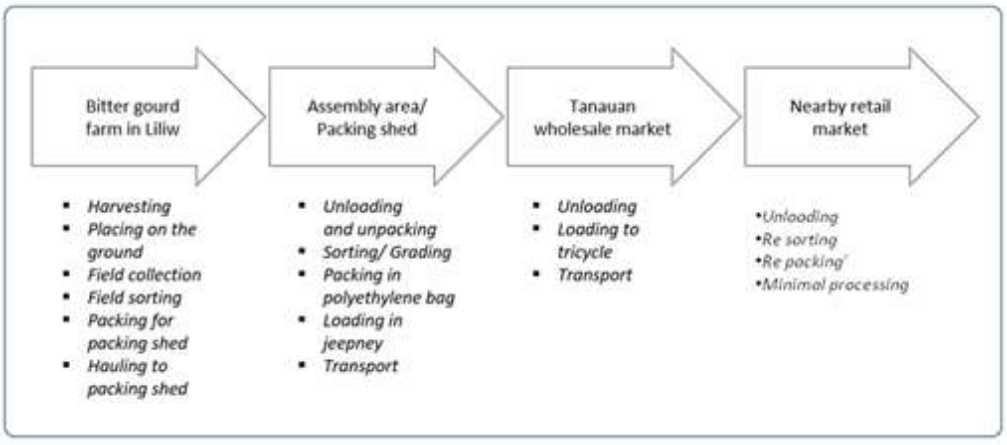


Figure 1: Handling chain of bitter gourd harvested from Liliw, Laguna and transported to the wholesale market in Tanauan City, Batangas.

Identified problems and possible improvement

Microbial contamination in the farm and retail levels

Direct contact of harvested fruits to the soil prior to collection could have resulted in

microbial contamination as presence of *Salmonella* was detected in samples collected after harvest. Increased risk of contamination during harvesting might be due to field environment and contact with harvesting equipment (Drissner and Zuercher, 2014). Use ofclean harvesting tools and clean liner as ground cover to avoid direct contact of fruits to the soil should be emphasized to the farmers. Directly placing the harvested fruits inclean collection containers can also be done. Meanwhile, samples from the retail market tested positive for *E. coli* (400mpm, most probable number) could have been caused by cross-contamination from handlers.

contamination may occur during handling, storage and transport through contaminated A simulation trial of bulk dropping showedan increase in the cracking and bruisingdamage (Fig. 2) which negatively affected thevisual quality of fruits (Fig. 3).
Provision of soft padding on the bamboo floor and careful placing of fruits instead of pouring

containers and ill workers (Drissner and Zuercher, 2014). Thus, thorough washing and proper cooking of bitter gourd is recommended to ensure its safety.

Dropping of fruits on bamboo floor

Bitter gourd is very susceptible to moisture loss and physical injury due to its high moisture content, large surface area to volume ratio, and a relatively thin cuticle (Mohammed and Wickham, 1993). Poor handling such as dropping of the fruits resultsin physical injury or impact damage which consequently affects its visual quality during storage. This is commonly observed in the handling chain.

them harshly is suggested to minimize mechanical damage on the fruits. The provision or use of sorting tables in the packingshed is most appropriate to minimize impact damage on fruits.

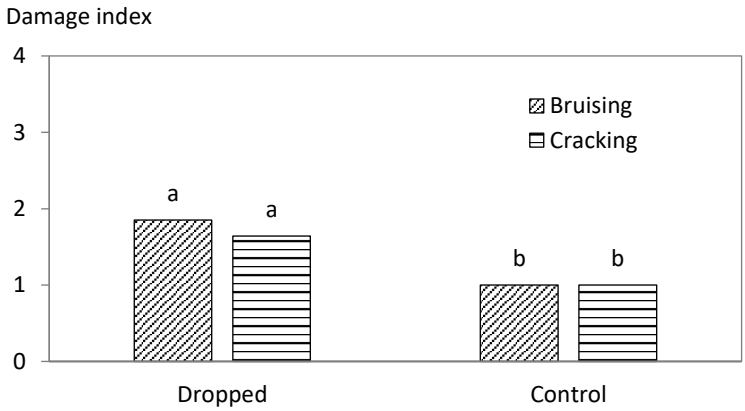


Figure 2: Bruising and cracking indices of bitter gourd after dropping in bulk. Means with the same letter are not significantly different (LSD, 5%).

Cracking /bruising index: 1 – none; 2 – slight; 3 – moderate; 4 - severe

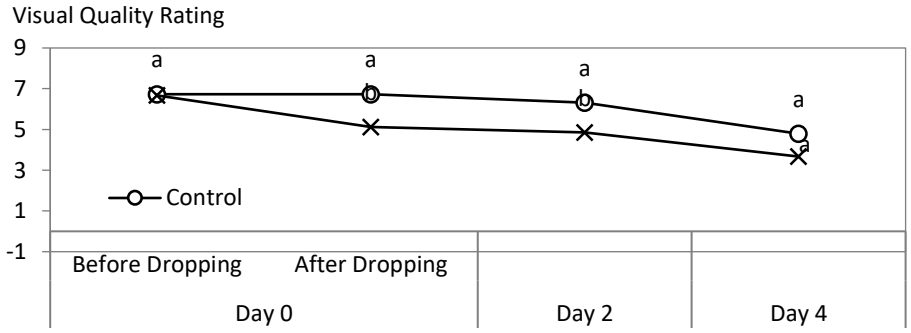


Figure 3: Visual quality rating of bitter gourd after dropping in bulk and stored at ambient condition. Means with the same letter are not significantly different (LSD, 5%).

Visual Quality Rating: 9,8 – excellent quality, field fresh; 7,6 – good, minor defects; 5,4 – fair, moderate defects; 3 – poor, serious defects, limit of marketability; 2 – limit of edibility; 1 – inedible

Stacking condition during transport

Quality evaluation of bitter gourd after transport to the wholesale market showed that mechanical damage in fruits from different stackingpositions was comparable (Fig. 4), but affected the quality during storage at ambient condition (Table 1). Bitter gourd fruits were still marketable up to 2 days in ambient storage. Loading on top of the vehicle during transport increased weight loss due to the fruits' direct exposure to heat and wind. This resulted in moderate shriveling of bitter gourd. Samples at the top of the stack inside the vehicle had reduced weight loss and shriveling than thoseat the middle and bottom portions where fruits are more prone to compression damage. Bitter gourd transported in crates had comparable weight loss and visual changes with samples at the middle and bottom of the stack (Table 1).

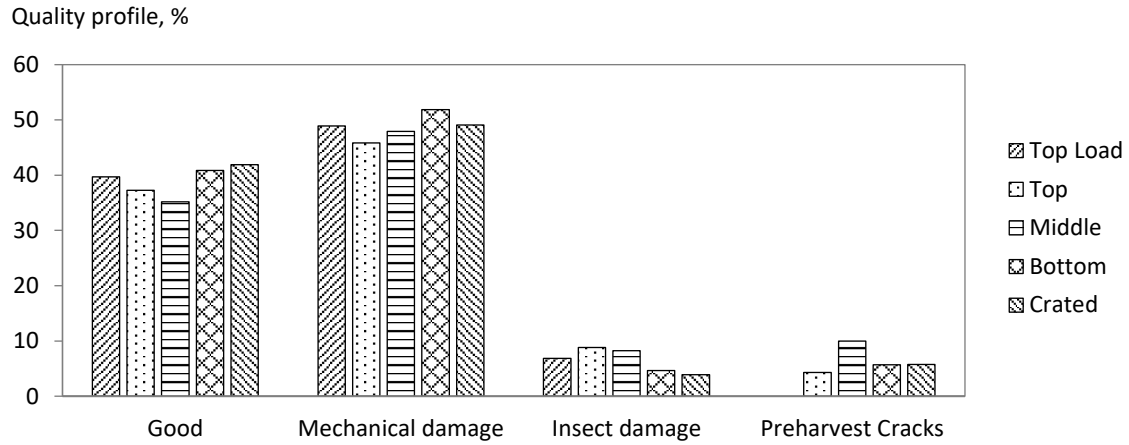


Figure 4: Quality profile of bitter gourd fruits in different stacking positions after transport.

Table 1: Cumulative weight loss, visual quality rating and shriveling of bitter gourd fruits from different stacking positions during ambient storage

Stacking position	***Weight loss, %***				***Visual Quality Rating[1]***				***Shriveling[2]***			
	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3
Top load	0	2.34	7.82^{a}	11.88	6.89^{b}	6.85	4.00^{c}	2.57	1.00	1.56	2.63^{a}	3.20^{ab}
Top	0	1.83	6.44^{b}	10.34	7.15^{a}	7.04	4.89^{a}	2.96	1.00	1.31	2.04^{b}	3.15^{ab}
Middle	0	2.04	7.60^{ab}	11.37	6.96^{ab}	6.96	4.11^{bc}	2.59	1.00	1.67	2.59^{a}	3.07^{ab}
Bottom	0	1.99	7.09^{ab}	11.08	7.04^{ab}	6.96	4.41^{abc}	2.84	1.00	1.57	2.46^{ab}	3.26^{a}
Crated	0	1.92	7.09^{ab}	10.55	7.07^{ab}	7.07	4.74^{ab}	2.69	1.00	1.57	2.20^{ab}	2.83^{b}

Means with the same letter within a column are not significantly different (HSD, 5%)
[1]Visual Quality Rating: 9,8 – excellent quality, field fresh; 7,6 – good, minor defects; 5,4 – fair, moderate defects; 3 – poor, serious defects, limit of marketability; 2 – limit of edibility; 1 – inedible
[2]Shriveling: 1 – none; 2 – slight; 3 – moderate; 4 - severe

Given the physical and mechanical properties of bitter gourd fruits, the recommended stacking height is 1.35 meters (Lualhati, RA, 2018). Stacking above this height will result in permanent and irreversible mechanical damage in fruits at the bottom of the stack. A jeep has approximately 1.75 meters space inside the vehiclewhere fruits are loaded. The use of horizontal divider could minimize damage on fruits at the bottom of the stack.

Postharvest physiology of non-injured (good) vs. mechanically injured bitter gourd fruits

Bitter gourd fruits with mechanical injury had higher respiration rate than those without injury.The respiration and ethylene production rates of non-injured fruits initially decreased while those of mechanically injured ones slightly increased on the first day of storage (Fig 5).Rapid increase in the respiration of the fruits was observed on the second dayduring

the onset of ripening, followed by a decline on the third day.Ethylene production continuously increased until the end ofstorage or until fruits reached the limit of marketability.The higher ethylene evolved from the injured fruits though not significant, could also be due to the presence of decay causing microorganism possibly evolving ethylene (Qadir*et. al*., 1997; Ilag and Curtis, 1968 as cited by Misaghi, 2012).

Mechanical injury also increased weight loss in fruits although the textural changes in both injured and non-injured fruits were comparable (Fig 6). Bitter gourdstarted to soften and exhibited slight shriveling on the first day. Presence of decay and moderate shriveling of fruits negatively affectedits visual quality. Non-injured fruits can be stored or marketed up to three days while the mechanically injured ones can only last forup to two days under ambient condition (28.4 ± 1.3°C, 79.3 ± 6.0% RH).

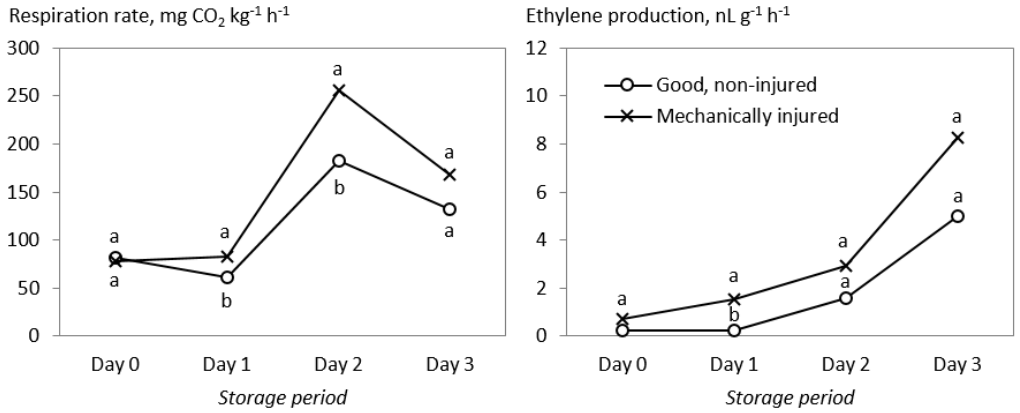


Figure 5: Respiration rate and ethylene production of non-injured (good) and mechanically injured bitter gourd stored at 28.4±1.3°C, 79.3±6.0% RH.Means with the same letter within a day are not significantly different (LSD, 5%)

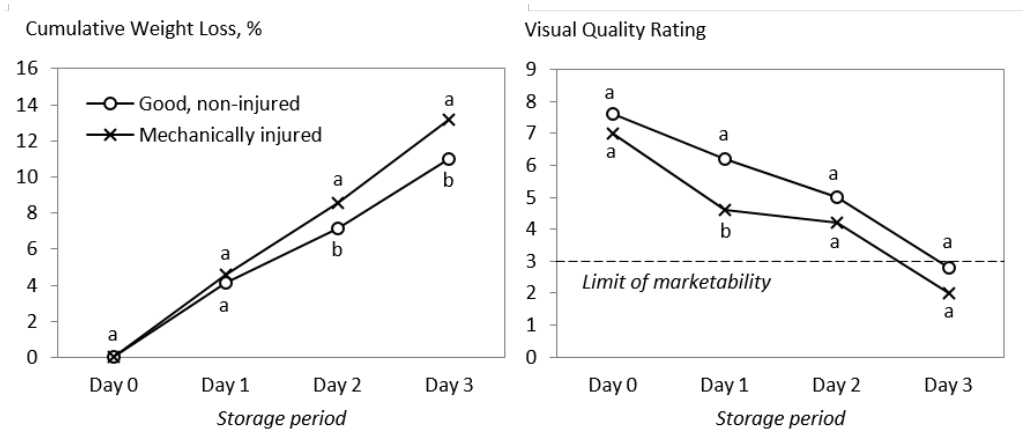


Figure 6: Cumulative weight loss and visual quality changes in non-injured (good) and mechanically injured bitter gourd stored at 28.4±1.3°C, 79.3±6.0% RH. Means with the same letter within a day are not significantly different (LSD, 5%)

4. Conclusion

Proper handling at any point in the supply chain is very important as bitter gourd is very susceptible to moisture loss and physical injury. Frequent dropping of the fruits resulted in cracks and bruises which negatively affected its visual quality. Thus, provision of soft padding on the sorting floor and careful placing of fruits instead of pouring them harshly is advisable to minimize impact damage. Use of sorting tables in the packingshed is most appropriate.

Bitter gourd should be properly stacked inside the transport vehicle at not more than 1.35 meters. Use of horizontal divider will minimize mechanical damage on fruits at the bottom of the stack. It is also advisable to refrain from loading the fruits on top of the

vehicle as this will result in increased moisture loss that will negatively affect the fruit quality during storage.

Mechanical injury in bitter gourd resulted in increased respiration rate and weight loss which, in turn, limited the storability and marketability of the fruit.

Microbial contamination of *Salmonella* and *E. coli* was detected at the farm and retail levels, respectively. At the farm, some possible solutions to prevent contamination include use of clean harvesting tools, and provision of clean liner as ground cover to avoid direct contact offruits to the soil. Another option is directly placing the bitter gourd in clean collection containers. At the retail level, cross-contamination from handlers could have resulted in contamination with *E. coli* thus, thorough washing and proper cooking of bitter gourd are recommended to ensure safety among consumers. Food safety awareness through the production of information, education and communication materials; trainings and seminars should likewise be done.

5. Acknowledgment

The authors would like to express their gratitude to the Philippines' Department of Agriculture Bureau of Agricultural Research (DA-BAR) for the research grant.

References

1. Drissner, D., & Zuercher, U. (2014). Safety of food and beverages: Fruits and vegetables.
2. Lamb, C. (1983). Physiology and biochemistry of plant-pathogen interactions: edited by IJ Misaghi, Plenum Press, 1982. ISBN 0 306 41059 1. *Trends in Biochemical Sciences*, *8*(12): 464-465.
3. Lualhati, R. A. (2018). Mechanical Properties of Selected Fresh Fruits and Vegetables. Unpublished report. PHTRC-UPLB.
4. Mohammed, M., & Wickham, L. D. (1993). Extension of bitter gourd (Momordica charantia L.) storage life through the use of reduced temperature and polyethylene wraps. Journal of food quality, 16(5), 371-382.
5. Philippines Statistics Authority (PSA). (2016). countrystat.psa.gov.ph.
6. Qadir, A., Hewett, E. W., & Long, P. G. (1997). Ethylene production by Botrytis cinerea. Postharvest Biology and Technology, 11(2), 85-91.
7. Sant'Ana, A.S., Silva, F.F.P., Maffei, D.F., and Franco, B.D.G.M. (2014). Fruits and Vegetables: Introduction. In: Batt, C.A. (ed.) Encyclopedia of Food Microbiology (2nded.) USA: Elsevier Ltd. 972-982.
8. Valerio, R. F., Peralta, E. K., & Esguerra, E. B. (2001). Rheological properties of mango (Mangifera indica L.) fruits in relation to handling and transport. Philippine Agricultural Scientist (Philippines).

11. ANTIOXIDANT PROPERTIES OF MANGO VARIETIES IN BANGLADESH

S.M. Maruf Kabir
Head of Quality Control, PRAN-RFL Group
Corresponding author's Email: pblqc@pip.prangroup.com,marufqc@gmail.com

Abstract
The study is concentrated with the role of inherent antioxidants content of mango varieties on the quality and shelf life of developed mango products. Three mango varieties as gutti, ashina, and Langra and two mango products; mango bar and mango fruit drinks were used in this study. Antioxidant contents of three varieties of mango pulp and microbial loads of mango bar and mango fruit drinks were assessed. All three varieties contained vitamin A, beta carotene, and vitamin C as an antioxidant and their contents were higher in gutti variety than other two varieties; Asina and Langra. Antioxidants were made an effective role to resist microbial load in the mango products. No microbial growth was observed within three months in the products (mango bar and mango fruit drink) of gutti mango variety except total plate count (TPC), which showed slight growth after four months of storage. The other two products from Asina and Langra were affected by microbial growth as TPC within one and two months consecutively. The study discloses that the antioxidant content of mango varieties had significant effect on the quality and shelf life in mango products.

1. Introduction

Antioxidants are chemicals (both naturally occurring and man-made) that can prevent or slow cell damage. Fruits and vegetables contain many antioxidant compounds including phenolic compounds, carotenoids, anthocyanins and tocopherols ((Bartosz, 1997; Naczk & Shahidi, 2006). Antioxidants can also be produced artificially and consumed in supplement form. Antioxidants are one of the first lines of defense that the body employs to keep free radicals in check and prevent them from causing a domino effect of damage on other cells. Antioxidant compounds can "donate" electrons to unstable free radicals so they don't have to snatch electrons from unsuspecting nearby cells. Antioxidants can also help repair cell damage caused by free radicals. Studying the relationship between antioxidant status and disease has proven to be a highly profitable line of research. It has expanded our knowledge concerning the etiology of numerous diseases and the means by which they might be prevented. But it is essential to take a balanced perspective and avoid the danger of over-enthusiasm for the potential of antioxidants (Temple, 2000). Mango (Mangifera indica L.) is one of the most important commercial crops worldwide in terms of production, marketing and consumption (Ribeiro *et al.,* 2007). The mango is indigenous to Bangladesh, India, Pakistan and Southeast Asia. The item is cultivated in many tropical regions and distributed widely in the world. Mangoes can be considered a good source of dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds (Sheiber *et al.,* 2000). Mango is one of the most extensively exploited fruits for food, juice, bar, chutney, color, making it a common ingredient in functional foods often called super fruits. Mango is a seasonal fruit and it is highly perishable. The improper handling, processing and preservation technique can losses large amount of mango in peak season in Bangladesh. The prevention of the losses of the seasonal surplus of mango could be possible by processing and preservation of them using proper techniques at farmer's level as well as industrial level. There are various techniques that will help the farmer to utilize the surplus fruits in peak season (Bose, 2000). The processing of mango into bar and drinks are the common techniques for utilizing the surplus fruits. The most common and convenient mode in which fruits are processed and preserved is in the form of fruit juices/pulps (Ahmed, 2014). In Bangladesh, fruit bar prepared from mango is commercially available. In laboratory, investigations were carried out to prepare fruit bars from banana (Hossain and Amin, 2008), mixed fruit bar (Karmokar, 2009) and pineapple (Emdad, 2014). On the other hand mango juice is prepared by diluting mango pulp and adding required amount of sugar, acidulants and preservatives. Both the products are susceptible to oxidation during storage due to sugar content. Hence, for better keeping

quality antioxidant as well as preservative need to be added. Besides, addition of potassium metabisulphite in mango leather gave better sensory qualities and longer shelf-life (Gujral and Brar, 2003). It is generally known that fruits are rich in antioxidants that help in lowering degenerative diseases (Feskanich, et al., 2000). Thus, it is considered important to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food with antioxidant. Processing food with antioxidant enrich raw materials are preferred to addition of pure antioxidants to it from economic point of view. It is reported that there are few mango varieties which are higher in antioxidant content compared to other varieties. Therefore, the present study was undertaken to evaluate antioxidant profile of selected mango varieties as well as to assess the effect of antioxidant on the shelf life of selected mango products prepared from those varieties.

2. Materials and methods

Samples of mangoes in three varieties like gutti, asina, Langra are collected from various area of the country like southern part of country named Jessore, Northern part named Chapinawabgoanj, Eastern side of Bangladesh named Chittagong. All mangoes were mature and ripe. Average weights of the experimental mangoes were 180g -200g of gutti, 300g-350g of asina and 190g-250g of Langra. All were checked visually and only defect free mangoes were allowed for study. Two products, dehydrated product named mango bar and mango fruit drink were considered to experiment.

Sample preparation (mango pulp)

Mangoes are washed by treated water, initially normal and finally hot water (45^0C). After peeling and deseeding the mangoes of three varieties (gutti, asina, and Langra), these are kept in separate stainless-steel vessel with covers. Then they were transferred into refiner having mesh-350 one by one to remove fibers from the mango pulp. Right after samples were transferred in the steam jacket kettle and heated to 95^0C-100^0C for 30 minutes to pasteurize the samples. Finally, pulps were filled into screw cap bottles sealed and stored at room temperature for further use. Now some parameters like vitamin A, beta carotene, vitamin C, colour, and flavor are tested on these three separate mango pulps of gutti, asina and Langra variety.

Sample preparation of mango bar

The prepared stock mango pulp of the three varieties was heated up to obtain the ^{0}brix 28^0 and poured by 4kg in each tray into the three separate SS-trays. All trays were cleaned and greased by applying food grade paraffin oil on the surface of the trays. Then the trays with mango pulps of gutti, asina and Langra were transferred into a drier for drying by 55^0C-60^0C for 48 hours and completion of dehydrating the first layer of the bar then again 4kg of mango pulps of the experimental three varieties were poured on the trays and dried by using same temperature (55^0C-60^0C) and time (48 hours) in the same drier for drying the second layer. After completion of drying the two layers of those products of three varieties were turned into one layer. Now they were sliced by SS-knife having size of length8.0cm, width 2.0cm and thickness0.45cm and again the slices of the mango bars were dried for further 24 hours in the drier till achieving the moisture 15%. The prepared sliced bars were packed in the layered flexible foil which was made of PET (polyethylene terothalate), MPET (metalize polyethylene terothalate) and LLDPE (Linear low-density polyethylene) and kept in an ambient temperature for further various experiments. Samples were analyzed for microbial loads at o, 30, 60, 90, 150, 180, 240 and 270 days.

Sample preparation of mango fruit drink

Mango pulp of three varieties like gutti, asina and Langra were taken from stock and refined by refiner with mesh-350 separately to remove fibers and other foreign particles and preserved in three separate master vessels. In another three separate SS-vessel, juice ingredients like citric acid 4.94%, sugar as syrup13%, mango emulsion 0.015%, mango flavor 0.045%, mango pulp 10% and water 72% were taken and blended for 30 minutes. Then the blended fruit drinks were transferred into three separate saucepans and heated them at 85^0C-90^0C for sterility the fruit drinks and filled in the cleaned and washed PET (polyethylene terrothalate) bottles and closed the filled bottles with plastic closures and kept in an ambient temperature for further study.

Apparatus for analysis of vitamin A, beta-carotene and vitamin C

For chemical test: Liquid Chromatography Mass Spectrometer (LCMS) (model no: LCMS-8040, origin: Shimadzu, Japan); Balance (model no: BSA 124S, origin: Sartorius scientific instruments (Beijing) Co, Ltd, China). Test parameters (vitamin A, Beta carotene and vitamin C) were tested by method AOAC.

Microbial apparatus and media description

Microbial parameters as coliform, TPC, Yeast and Mold are tested. Eosin methyl blue (EMB) agar and MacConkey broth w/neutral red (HiMedia Laboratories Pvt. Ltd., India) for coliform; Plate count agar (HiMedia Laboratories Pvt. Ltd., India) for TPC; Potato dextrose agar (HiMedia Laboratories Pvt. Ltd., India) for yeast & mold testing.

Methyl Red Indicator; Iodin; Methyl Orange; Ferroin Indicator Sol; Glycerol; Methylene Blue; Oil emersion; Chrystal violet; Safranin; Covacs Indole reagent; Vogusproscure; MIU Medium; Lactophenol cotton blue; Microbial testing of parameters (Coliform, TPC, yeast and mould) were tested by using the method BDS ISO 4832:2009, BDS ISO 4833:2009 and ISO 21527-1: 2008 consecutively.

Statistical analysis

All the experiments were carried out triplicates. The experimental data were compiled, tabulated and analyzed. Data analysis was carried out using commercial statistical software Stat graphics Centurial XVI.

3. Results and discussion

Antioxidant content of different variety of mango

The pulp prepared from three varieties of mango was analyzed for vitamin A, beta-carotene and vitamin C and the results are shown in Table 1.

Table 1: Antioxidant profile of mango pulp prepared from three mango varieties (Gutti, Ashina, Langra)

Sample	Vitamin A (µg /100g) ± RSD			Beta carotene (µg /100g) ± RSD			Vitamin C (µg /100g) ± RSD		
	Gutti	Ashina	Langra	Gutti	Ashina	Langra	Gutti	Ashina	Langra
Sample-1	45±0.12	33±0.05	40±0.08	480±0.85	420±0.72	410±0.69	32±0.05	25±0.04	20±0.03
Sample-2	45±0.11	32±0.05	42±0.09	475±0.76	420±0.71	420±0.71	33±0.06	24±0.03	21±0.04
Sample-3	44±0.90	34±0.06	41±0.08	480±0.84	425±0.74	415±0.70	32±0.04	24±0.04	20±0.03
Sample-4	45±0.11	33±0.07	40±0.07	470±0.75	430±0.76	410±0.68	31±0.04	25±0.05	19±0.02
Sample-5	46±0.15	33±0.06	42±0.08	480±0.83	420±0.70	410±0.67	32±0.05	25±0.04	20±0.03
Sample-6	46±0.14	32±0.05	40±0.08	475±0.75	430±0.77	410±0.66	34±0.07	25±0.05	22±0.04
Sample-7	45±0.15	34±0.06	41±0.09	476±0.77	425±0.75	420±0.72	32±0.06	26±0.06	21±0.03

RSD: Relative Standard Deviation

As shown in Table 1, irrespective of mango variety beta-carotene is the dominant antioxidant in mango followed by vitamin A and vitamin C is the least dominant components. When comparing among variety, gutti contained the highest amount of vitamin A (45µg/100g), closely followed by Langra (40µg/100g) whereas asina contained the lowest amount of vitamin A (33µg/100g). In case of beta carotene, the content was the highest in gutti variety, followed by Asina and then followed by Langra. The same trend was observed in case of vitamin C. Due to close value of beta carotene, the color of pulp of all varieties remained yellowish and hardly able to differentiable with naked eyes. Lim et al. (2007) reported antioxidant activity of different tropical fruits like mango, jackfruit (Sultana et al., 2009), papaya, banana etc. They reported the mango as a potential source of antioxidant.

Correlation with mango pulp antioxidants and microbial count in Mango barKinetics of microbial counts in mango bar prepared with mango pulp from three mango varieties is Shown in Figure 1.

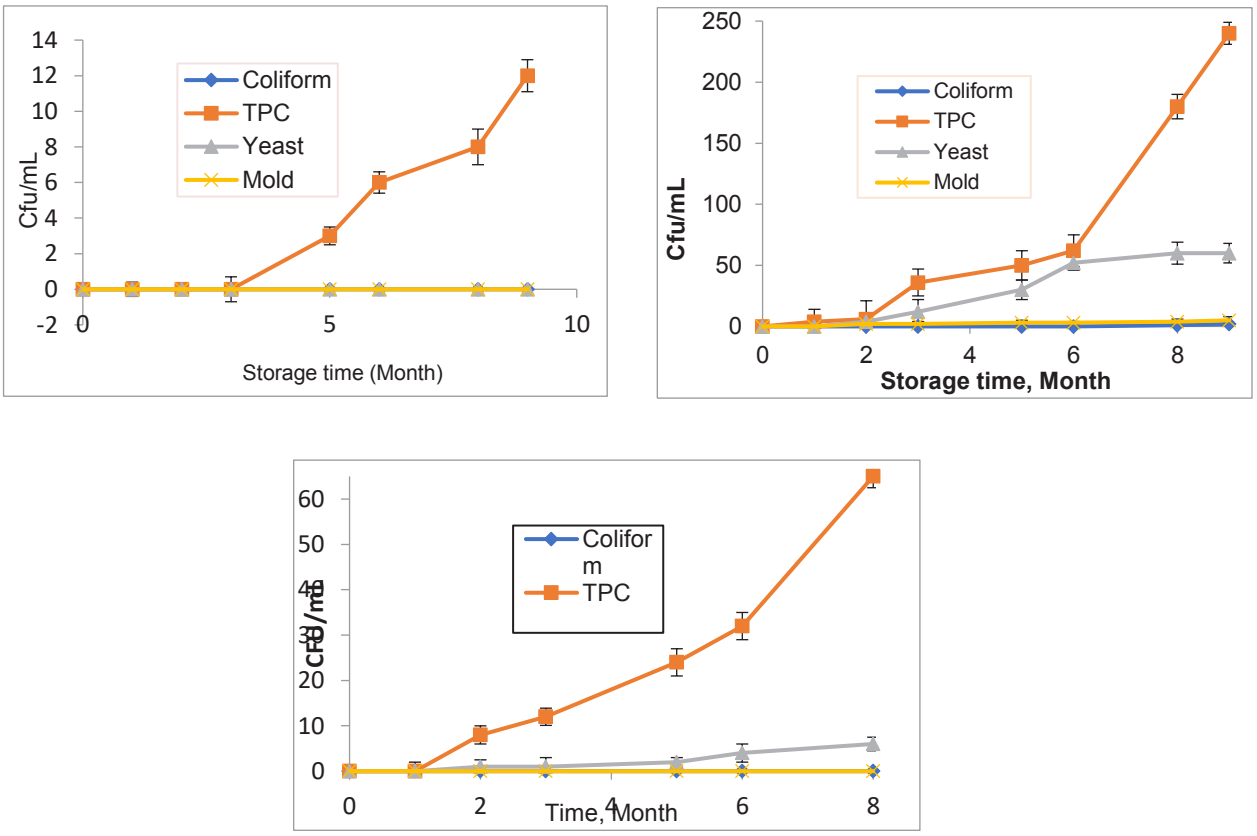


Figure 1: Kinetics of microbial growth in mango bar prepared from pulp of (a) Gutti variety (left) (b) Asina (right) and (c) Langra (bottom).

As shown in Figure 1, different microbial counts with storage was highly correlated with the mango variety. As shown in Figure 1(a), the least microbial count was observed in mango bar prepared with gutti variety. Except TPC, no other microbial growth was observed. Even after three months of storage, TPC growth was started and its count was increased with quite slow rate up to 9 months of storage. During this storage, no other microbes like coliform, yeast and mold were observed. In case of mango bar prepared from Asina variety, microbial growth was started from very beginning of storage and their population were constantly increased with increasing storage period up to end of storage study (Figure 1b). Up to first 2 months, TPC growth was slow and then its rate was increased and this trend was continued up to 6 months and then this rate increased abruptly. However, mold and coliform growth remained quite slow. A significant growth rate was observed in case of yeast. In case of mango bar prepared from Langa variety, a moderate growth of TPC and yeast was observed. This growth is slightly high compared to that in mango bar from gutti variety and pronounced low compared to that in mango bar from Asina variety.

This is mainly due to antioxidant content in pulp from where mango bar. As shown in Table 1, gutti variety contained the highest amount of antioxidant followed by Asina and the lowest amount in Asina. Consequently, mango bar prepared from gutti variety inhibited potentially the growth of differently microorganism. On the other hand, antioxidant content of Asina variety and consequently its effect on the growth of microorganism was also neglected. The antimicrobial potential of many antioxidants was reported by many researchers (Nusrat, 2015). Jagtap et. Al. (2009) reported the antioxidant capacity of mango pulp and showed that its elevated content helped to increase the shelf life of mango pulp.

Correlation with pulp antioxidants and microbial count in Mango fruit drinkKinetics of microbial counts in mango drinks prepared with mango

pulp from three mango varieties is shown in Figure 2.

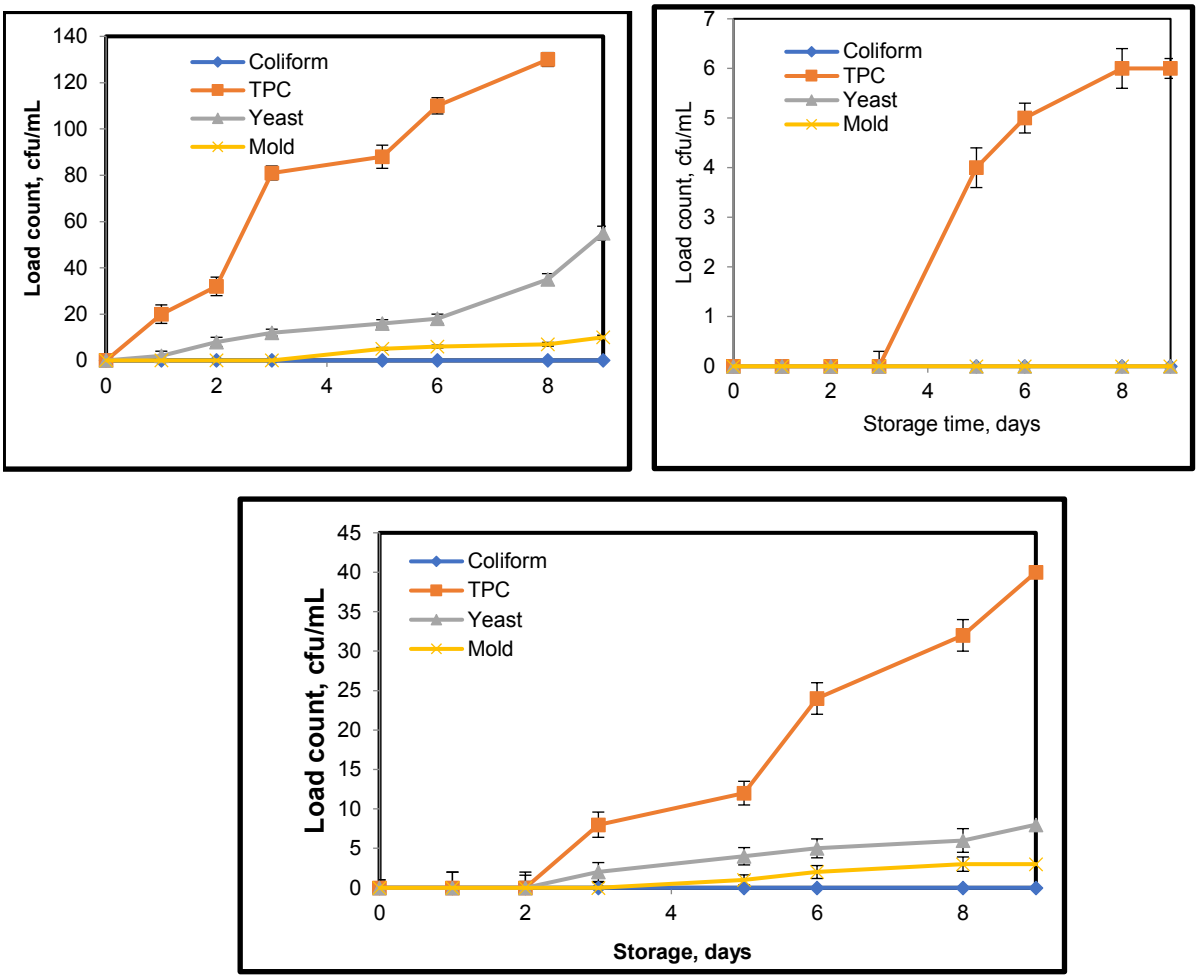


Figure 2: Kinetics of microbial growth in mango drinks prepared from pulp of (a) Gutti variety (top-left) (b) Asina (top-right) and (c) Langra (bottom).

As shown in Figure 2, different microbial counts with storage in mango drinks showed the same behaviour as that of mango bar, i.e. the content of antimicrobial components varied with mango variety and accordingly inhibited growth of microbes. As shown in Figure 2(a), the least microbial count was observed in mango drinks prepared with gutti variety. Except TPC, any other microbial growth was hardly observed. Even TPC growth was started after three months of storage and its count was increased at quite slow rate up to 9 month of storage. During this storage, no other microbes like coliform, yeast and mold were observed. In case of mango drinks prepared from Asina variety, except coliform, all other microbial counts were started from the very beginning of storage and their population were constantly increased with increasing storage period up to end of storage study (Figure 2b). Up to first 3 months, TPC growth was slow and then its rate was increased at quite faster. However, no coliform growth was observed throughout the storage period even though growth of mold exhibited at a quite slow rate. In case of mango drinks prepared from Langra variety, a moderate growth of TPC and yeast was observed. This growth is slightly high compared to that in mango bar from gutti variety and pronounced low compared to that in mango drinks from Asina variety. No coliform growth was also observed in mango drinks prepared with Langra variety thoughout the storage period.

As shown in Table 1, gutti variety contained the highest amount of antioxidant followed by Asina and the lowest amount in Asina. Consequently, mango drinks prepared from gutti variety inhibited potentially the growth of differently microorganism. On the other hand,

antioxidant content of Asina variety was low and consequently its inhibition capacity on the growth of microorganism was also neglected. The antimicrobial potential of many antioxidants was reported by many researchers (Nusrat, 2015). Jagtap *et. al*. (2009) reported the antioxidant capacity of mango pulp and showed that its elevated content helped to increase the shelf life of mango pulp.

4. Conclusion

The work exposes that the antioxidant content of mango has an enormous effect on the quality and shelf life of mango products. In this study gutti variety of Bangladesh contains the highest antioxidant content and hence of products like mango bar and mango fruit drink developed from this variety showed the best quality and the longest shelf life compared to ashina and Langra varieties.

References

1. Ahmed, B., Uddin, M. B., & Jubayer, M. F. (2014). Extraction and standardization of selected fruit juices by enzymatic process. Pak Journal of Food Science and Technology, 2(2), 18-27.
2. Bose, S. (2000). Studies on the development of mixed mango-Pineapple Juice. MS Thesis. Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh.
3. Emdad. (2014). Kinetics of non-enzymatic browning of mango bar. MS Thesis. Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh.
4. Hossain, A. A., and Jahan, R. (2014). Studies on the Processing of mixed fruit bar from mango, banana andpapaya Project Work. Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh.
5. Inzé, D., & Van Montagu, M. (1995). Oxidative stress in plants. Current opinion in Biotechnology, 6(2), 153-158.
6. Jagtap, U. B., Panaskar, S. N., & Bapat, V. A. (2010). Evaluation of antioxidantm capacity and phenol content in jackfruit (Artocarpus heterophyllus Lam.) fruit pulp. Plant foods for human nutrition, 65(2), 99-104.
7. Karmokar, P. (2009). Studies on the Processing of mixed fruit bar from mango, pineapple, papaya. MS Thesis. Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh.
8. Lim, Y. Y., Lim, T. T., and Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry103: 1003-1008.
9. Naczk, M., and Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. Journal of pharmaceutical and Biomedical Analysis41: 1523–1542.
10. Nushrat, Y. (2014). Effects of extraction methods on the antioxidant activity of tarmarind seed. MS Thesis. Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh.
11. Ribeiro, S. M. R., Queiroz, J. H., de Queiroz, M. E. L. R., Campos, F. M., & Sant'Ana, H. M. P. (2007). Antioxidant in mango (Mangifera indica L.) pulp. Plant Foods for Human Nutrition, 62(1), 13-17.
12. Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. Journal of the National Cancer Institute, 92(22), 1812-1823.
13. Schieber, A., Ullrich, W., & Carle, R. (2000). Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innovative Food Science & Emerging Technologies, 1(2), 61-166.
14. Temple, N. J. (2000). Antioxidants and disease: more questions than answers.

12. ASIA FOOD PROJECT: TECHNOLOGY OVERVIEW FOR FOOD SECURITY

Sermkiat Jomjunyong[1], Nobutaka Ito[2*]
[1]Industrial Engineering Department, Faculty of Engineering, Chiang Mai University, Thailand;[2]Mechanical Engineering Department, Faculty of Engineering, Chiang Mai University, Thailand
Corresponding author's Email: ito@eng.cmu.ac.th

Abstract
We, human beings are facing two immerging global issues of energy and environment to tackle and solve. Agriculture is importantly closed up recently from the viewpoints of various eco-friendly indispensable primary resource production available for food, energy, environment, material etc. Asia is one of the most active well-known regions for agriculture in the world. Food resource production is one of the main roles of agriculture to play as known well. ASEAN Economic Community was established in December 2015. Ito proposed the basic framework and concept of Asia Food Project for the purpose of promoting regional economy and peace keeping. The proposed project scheme and related programs for On the Job Training) and FFA, (Future Farmers of Asia) growing are introduced. Food security is one of the most important issues to consider at the first priority in food production. Technologies are therefore overviewed for food security in this paper.

Keywords: Asia food project, food security, FFA growing program, traceability, 2QSL, smart agri.

1. Introduction

Asia is one of the biggest foods producing regions in the world especially for rice and most of the countries in this region are relying on the national economy obtained from agricultural production more or less. It can be therefore notified from this background that Asia can be classified into two types of countries, they are 1) Resource oriented and 2) Technology oriented ones named ASEAN plus 3 such as China, Japan and South Korea. Ito proposed Asia Food Project in 2014, in which those two types of countries should collaborate together and compete each other to achieve the final goal of regional economy promotion and sustainable peace keeping for mutual prosperity.

Asia Food Project

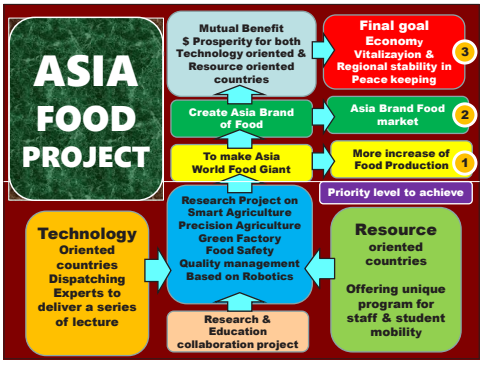


Figure 2: Asia food project.

Asia can be characterized and qualified as one of the world food pantries from the viewpoint of its huge amount of production. Rice is especially produced more than 90 % of the world total production, however it should be notified that the production is being done based on small scale, therefore the farmers are always forced to stay on farm as working poor due to low income in spite of heavy physical labor and needed the official support by the national government for subsidy.Something should be done for improving the current situation even for promoting Asian agriculture. Asia Food Project is one of the concepts proposed by Ito which can be practically available for application to the site. This project is prepared and proposed mainly focusing on the regional community-based agriculture how and which way Asia should be going for economic promotion and regional peace keeping.

Figure 1: Asian agriculture growth strategy.

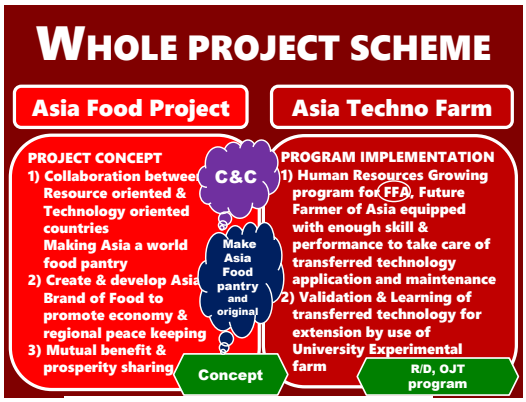


Figure 3: Whole project

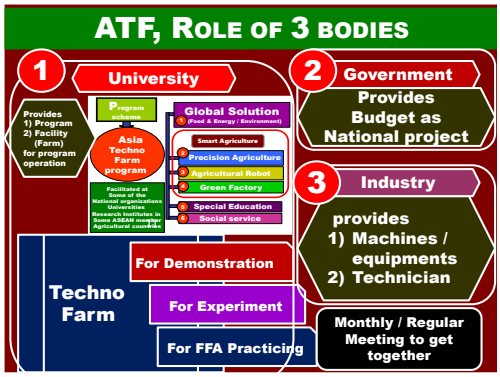


Figure 4: Asia techno farm.

Strategy

ASEAN Economic Community was established in December 2015. What is the main purpose of it? What industrial sector should be considered and picked up as the main framework for community development? How it can be promoted and achieved? Ito's answer for these questions are the followings

1) Main purpose of establishment is regional economic promotion regional keeping in ASEAN
2) Industry sector should be focused on Agriculture, because most of the countries in Asia are still relying on the economy obtained from agricultural production
3) Farming scale up is the most important first priority to compete at the world market and achieve the value-added product production satisfied with condition of high quality safe and reasonable price in which anyone can obtain easily. Farming population should be reduced and recommend the farmers to change the job in the other industry sector. Part-time farmer is not an essential solution just for income increase. Agriculture can't be promoted by part-time farming. (See Fig. 1)

Project scheme (See Fig. 2)

With the promotion of farming population decrease, farming scale can be expanded per one full time farmer, then the mechanization can be promoted, Asia has a huge amount of food production, however the quality is not well controlled. In addition, the food safety should be secured and guaranteed. Considering the level of agriculture in Asia, the high-tech based machine and equipment are needed to secure the food safety and quality. In Asia there are two kinds of countries such as technology oriented and resource-oriented countries. Those two kinds of countries should be collaborated for technology transfer and human resources development. Targets should be achieved one by one as shown below. [2]

a) Efforts should be made for making Asia as one of the world food pantries in production and supply
b) Unique, original most reliable Asian food brand should be created for stable economy development
c) Regional peace keeping can be maintained by two target achievement
d) Mutual benefit and prosperity can be shared

Asia techno farm

This is OJT (On the Job Training) program attached to Asia Food Project. The program consists of mainly three sections of smart agriculture, such as precision agriculture, robotics and green factory. Highly educated trainees are accepted and requested to engage the practical farming at least more than one season of objective crop cultivation. Special program is also offered and prepared for the farmers to change the job to the other industrial sector in addition to the recommendation and introduction. Programs are prepared not only agriculture, but also the other issues**.** (See Fig. 4),

Future farmers of Asia growing program

With the farming scale expansion, higher level of performance is requested to learn and equip enough to access and negotiate. Program should be opened not only for Thailand, but also for neighbor ASEAN countries. Participants / trainees should be at the level enough to communicate in English, good at familiar with data processing and analysis, high tech machine control and operation.

Food security

Ito defines that the food security consists of the following four factors to be considered and product liability is one of the most difficult ones among them, because it can be caused mainly by the shortage / lack of responsibility of social sense of morality and ethics mainly arose from the management difficulty.

2QSL: Ito thinks that food security consists of the following four factors:

Quantity: Should beenough for feeding the rapidly and drastically increasing human population which is already around 7.5 billion and still continuously increasing at the rate of 80 million per year. Further efforts should be needed to cover for feeding those newly increasing population, where actually newly born population per year is totally 140 million, but almost 60 million die on the other hand. Technology is needed to increase the food production more to meet this background condition urgently. It was generally thought that food per capita might be enough if the food production can be possibly maintained almost 400 kg per capita, however this figure is now going down recently. It takes time to newly develop the new farmland. In addition, those newly developed lands are not stably used for the original purpose for few years until getting stabilized condition.

Quality: Food should be high quality controlled and managed with reasonable price enough to compete in the world market, This condition should be satisfied commonly not only for agricultural products, but also industrial ones. High tech mechanization may be one of the solutions to make it possible.

Safety: This must be secured with first priority especially for human food, Traceability is also one of the systems for securing this issue attached with logistics and supply chain.

Liability: This is one of the most difficult factors to distinguish regulations from human intervention between producer and consumer. No solutions are unfortunately found and proposed yet for liability issue. The other three factors mentioned above look comparatively easier to secure, because machine and equipment to be used for checking the quality don't show the fake data normally as far s they are calibrated correctly. Mechanically or systematically the data can be shown by the instrumentation equipment or device imme - diately if the food products could clear the pre-set safety standard level. Even if accurate information is obtained, if a person falsely displays or processes the information, reliability will be completely lost, then once mutual trust is lost, it will not return to the original level easily. Everybody already knows this; however even large companies display a fake about products as management runs out. It looks very difficult to prevent this kind of issue at this moment. Education and training for level up the social morality and ethics including corporate social responsibility to be fulfilled may not solve this product liability problem as we can see almost the same in car industry too.

Technology overview for food security

From this background point of view, the discussion on what technology can be needed and developed should be discussed. The following shows some of the hopeful technologies seemed to be effectively applied especially for food safety management. They are introduced and explained one by one later attached with more detailed explanation, however here shows the list of them tentatively.

a) Carbon Nano Fiber – applied already to aircraft material& water purification
b) Cellulose Nano Fiber - 5~7 times Stronger than metal and hopefully applied to many industrial sectors not only agriculture, but also the others
c) OHMASA gas: Electric Generation, LPG blended fuel for energy saving
d) Oxygen Nano Bubbles – Fisheries
e) Ozone Nano Bubbles - Sterilization of agricultural products, weed control without using chemical herbicides, but almost same amount of yield under use of chemicals
f) Nitrogen Nano Bubbles - Freshness preservation, Deoxygenation, Reduce the time to be exposure with oxygen.

These above-mentioned technologies could be applied in the following industrial sectors:

a) Food safety – Vegetable sterilization
b) Aquaculture (Fishery) – Oyster sterilization
c) Dentistry – Periodontology / Periodontics
d) Medical science - Cancer cell control
e) Plasma technology – Waste to Energy

High temperature treatment to hydrogen production

Low temperature- Waste oil to fuel

Agricultural production consists of mainly two production system such as primary production and secondary one. The former one covers the operation process from seed sowing to harvesting and the latter one covers the processing operation after harvest, normally called post-harvest technology which can be applied for value adding onto the final products. If the management of objective agricultural product could be done so well in the primary production, the quality of the product can be controlled and finally the total production loss can be reduced, therefore the pre-harvest technology can reduce the post harvest technology process and it leads to the final loss reduction in total production process. Technologies such as Precision agriculture, Remote sensing (NDVI), Machine vision, Image processing, Green Factory (Programmed control of plant growing & cultivation) are some of the examples to be applied for extension in near future completely.

The following shows some of the research projects seemed to be usefully applied to agriculture, medicine and environment which are mostly related to human survival such as food, energy, environment etc.

4. Conclusion

The conclusions derived from the discussion can be shown as shown below.

a) As far as concerned with a small-scale farming, farmers must be staying on farm as working poor due to low income in spite of heavy hard work.
b) For further promotion of agriculture, the community-based project should be launched as soon as possible focusing on high technology transfer for FFA (Future Farmer of Asia) growing to be in time for a rapid increase of human population at the rate of 80 million people per year.
c) Micro-Nano bubbles technology may be one of the hopeful higher potentialities for sterilization of products and growing plants under the condition very close to organic farming.
d) Precision agriculture secures the stable yield of agricultural products reducing total loss from growing soil with constant fertile soil, improve productivity, and also produce high quality managed value-added products combined with GIS
e) Nano bubble water can deeply penetrate cells and wash out bacteria and a little virus, therefore nano level bubble water has a key for problem solving

References

1) Nobutaka, I. (2015). Asian Agriculture Growth Strategy, Japanese Society of Agricultural Machinery and Food Engineering, No. 4. Vol.77, p.226-230.

2) Nobutaka, I. (2015). How ASEAN Economic Community should play the Role for Asia Sustainability,Proceedings of the 5th International Conference on Green and Sustainable Innovation ICGSI 2015, p.O-124, November 7 - 10, Pattaya, Thailand,

3) Noutaka, I. (2018). FFA (Future Farmers of Asia) growing program, Proceedings of the 11th TSAE International Conference, IM04-04 FFA (Future Farmer of Asia) Growing, Program, Proceedings of the 11th TSAE International Conference, pp.13 - 23, April 26~27, Chulaborn International Convention Center (Wora Wara Hua Hin Hotel & Convention) Hua Hin, Prachuap Khin Khon, Thailand

13. PHYTOCHEMICAL SCREENING AND *in vitro* ANTIMICROBIAL ACTIVITY ANALYSIS OF *Acmella oleracea* L. FLOWER POD EXTRACTS EXPOSED TO DIFFERENT DRYING TECHNIQUES

T G G Uthpala[1], H H Munasinghe[2], L D C Peiris[3], N M M G S B Navarathne[1]
[1]Department of Food Science & Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda , Colombo, Sri Lanka
[2]Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Colombo, Sri Lanka
[3]Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Colombo, Sri Lanka
Corresponding author's Email: gimhani@sci.sjp.ac.lk

Abstract
Acmella oleracea L.(Asteraceae) is a flowering herb commonly known as tooth ache plant. This study was aimed to investigate the effect of four drying techniques on extraction of *A. oleracea* pods using ethanol as the solvent by evaluating extraction yield, phytochemical availability and *in vitro* antibacterial activity. Ethanol extracts were obtained by maceration (<45 ^{0}C). Acmella pods were exposed to four drying [Sun drying (SD), Air drying (AD), Oven drying (OD) and Cooling with dehumidifying (CD)] methods and extracts were subjected to phytochemical screening and tested against food pathogenic gram positive (*Staphylococcus aureus* ATCC 25923) and negative (*Escherichia coli* ATCC 25922*)* strains using disc diffusion method. The extraction yield given from the oven drying method was higher than that of CD, AD and SD. Phytochemical screening revealed the presence of alkaloids, tannin, quinone and total phenols in all dried extracts while flavonoid was detected only in both SD and CD extracts. Phlobatanin, saponins and gum mucilage were not detected in none of the extracts. Only the extracts obtained by CD, AD and OD were exhibited antimicrobial activities with zones of inhibitions 10.2±0.2, 7.0±0.5, 7.2±1.0 mm respectively against *S. aureus* while 8.0±1.1, 6.2±0.2, 6.1±0.8mm respectively against *E. coli*. CD dried extracts showed higher effectivity against bothbacteria while lowest given by SD. Availability of phytochemicals and ability of *A. oleracea* to inhibition of both gram negative and positive bacteria is an indication of its antimicrobial potential which may be employed in management of microbes in food contact surfaces.

Keywords: Acmella, cooling with dehumidifying, antimicrobial activity, phytochemical screening

1. Introduction

*Acmella oleracea*is a flowering herb in the family of Asteraceae. It is an important medicinal plant with rich source of antimicrobial and antifungal constituents and commonly known as toothache plant which is found in various parts of the world. Paracress, Sichuan buttons, Buzz buttons, Ting flowers and Electric daisy are some of the other common names used for this plant. This plant growing abundantly in the wild and still unexploited which is only restricted to Ayurvedic medicine. It is found in tropical and subtropical regions around the world (Jansen,1985). Other important traditional uses of this herb are treatment of rheumatism, as a sialagogue for stammering, tongue paralysis, antipyretic, sore throat, and gum infections. Moreover leaves and flowers of this plants have sensorial properties (pungency, tingling, numbing, mouth-watering) that make it a popular spice and ingredient in several Brazilian dishes (Barbosa et al.,2016).Major cause (80%) of food borne illnesses are caused by pathogenic bacteria and people living in the South-East Asian Region fall ill and die from this every year than in any other Region (WHO, 2015).Food borne illnesses are very common in Sri Lanka and major causes for this national issue are pathogens multiplication in food products.

Therefore, the aim of this study is to investigate potential useof *Acmella oleracea* flower bud extract in controlling of microbial growth related to food borne pathogens. Flower inflorescences of *Acmella oleracea (L.)* R. K. Jansen were harvested and subjected to dehydration using four types of drying techniques. Those dried pods were grounded and subjected to ethanoicextractions. Then the extraction yield was measured and extracts were subjected to phytochemical screening

and antimicrobial susceptibility testing for the selected bacterial strains in lieu to identify the effect against microbes.

2. Materials and methods

Sample collection and preparation

Fresh flower pods of *Acmella oleracea*L. was collected from home garden in Haldummulla ,Haputale City, Badulla district, Uvaprovincre, Sri Lanka (7654° N, 80.9526° E) in the month of May ,2017. The plant was identified by the Botanical garden, Peradeniya, Sri Lanka and a voucher specimen was deposited in the herbarium for reference.

Then collected *Acmella* flower pods were washed with distilled water and dried using four different drying methods [Sun drying (40-44^{0}C), Dehydration by oven drying (40^{0}C), Air drying (30-34^{0}C) and Cooling with de-humidifying (0-4^{0}C)] for a period of two days. Then the dried pods were grounded in a domestic grinder until a fine powder was obtained.

Extraction yield

The powder obtained from four drying methods were extracted by maceration at room temperature for 72 hourswith ethanol (1: 20 w/v). The extracts were filtered through Whatmann No:1 filter paper and concentrated using a rotary evaporator set at 40˚C. Extraction yield was recorded as dry weight basis.

Phytochemical screening

The extracts were subjected to phytochemical tests for plant secondary metabolites of tannin, alkaloids, flavonoids, quinone, Phlobatanin, Saponin, Total phenols Gum and mucilage accordance with Harborne (1998) and Trease and Evans (1998).

Antimicrobial susceptibility test

Antimicrobial activity of plant extracts was determined in accordance with disk diffusion method described by Kirby-bauer (Hudzicki, 2009; Jorgensen and Turnidge**,** 2015).

The bactericidal experiments were carried out with gram negative bacteria *Escherichia coli* (ATCC 25922) and gram-positive bacteria *Staphylococcus aureus (*ATCC 25923) in Muller Hinton agar. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 cfu/ml). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar. Then 6 mm diameter sterile disks were placed into the agar after impregnation of 30 µl of each extract at 20 mg/ml concentration and plates were incubated at 37°C.The effects of extracts were compared with Gentamicin (10 µg) as the positive control and relevant solvent as the negative control. The plates were observed for zones of inhibition after 24 h.

Data analysis

Statistical analysis was done using Microsoft excel 2010 data sheets and Minitab 17. The differences between the control and the treatments in these experiments were tested for statistical significance by ANOVA tests and Turkey comparison. A value of $p \leq 0.05$ was considered to indicate statistical significance. Values were expressed as mean ± SD

3. Results and discussion

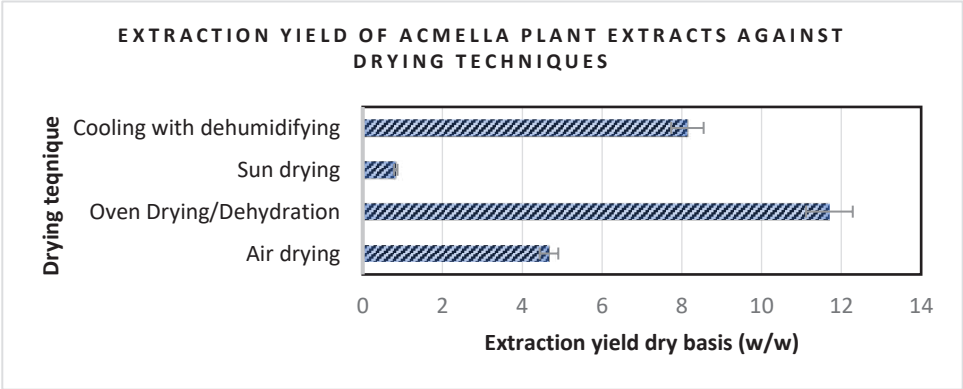


Figure 6: Extraction yield of Acmella extracts using different. drying methods, values are mean ± SD, n= 6

Results of the experiment carried out to determine the extraction yield of Acmella extracts using different drying methodsis shown by the figure 1. The extraction yield given by drying method of oven dehydration showed to be higher followed by Cooling with dehumidifying, Air drying and Sun drying. All four types of drying methods showed significantly different extraction yield at 0.05 of level of significance.

Extraction is the separation of active portions of herbs using selective solvents through standard procedures (Handa, 2008). The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular residuals. These drying methods [Sun drying (40-44^{0}C), Dehydration by oven drying (40 ^{0}C), Air drying (30- 34 ^{0}C) and Cooling with dehumidifying (0- 4^{0}C)]as well as the maceration techniquedo not force dried plant materials using high temperature and therefore heat-labile compounds are preserved. Oven drying is considered as one of the easiest andrapid thermal processing that can preserved phytochemicals which causes removal of moisture from the samples by thermal energy.

Extractable solid content or the extraction yield is affected by several factors including time taken for the maceration, temperature applied, solvent type, initial drying method etc. (Cunha et al., 2004; John et al.,2006). It is visible that the flower pod extractswhich were obtained under higher temperature presser-vation (SD) showed lower extraction yield while the others are having higher yields (CD, AD) with irrespective to the oven dried sample. Also it revealed that drying method has a significant impact on the extraction yield.

Phytochemical screening results for the ethanoic extracts obtained by different drying methods.

The initial crude extracts using four drying methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids.

Table 1 shows the results of the phytochemical screening of ethanoic extracts from pods of Acmella oleracea. It revealed that the presence of alkaloids, tannin, total phenols and quinone in all the dried techniques applied extracts while flavonoidwas detected only in the ethanoic extract subjected to Cooling with dehumidifying and sun drying methods. Phobatanin, saponins and gum mucilage were not detected in the any of the ethanoic extracts. Most of the gum and mucilage are said to be soluble on water but not in alcohol (Smith, 1959). The solubility of phytochemicals are strongly affected by both the nature of the solvent and the structure of the phytochemical (Felhi et al., 2017). Results revealed that the availability of phytochemicals in the ethanoic extracts have the impact of drying method subjected to the raw material.

Table 1: Phytochemical availability of flower pod extracts of Acmella plant using different drying methods and solvents.(+) mark indicates the availability of relevant phytochemical in the extract while (–) mark indicates unavailability

Phytochemicals / Drying Method	Alkaloids	Flavonoids	Tannin	Total phenol	Quinone	Phlobatanin	Saponin	Gum and mucilage
Cooling with dehumidifying	+	+	+	+	+	-	-	-
Sun drying	+	+	+	+	+	-	-	-
Oven drying	+	-	+	+	+	-	-	-
Air dryng	+	-	+	+	+	-	-	-

Antimicrobial activity of flower pod extracts

The results (Table 2) indicated that antibacterial activity profile of ethanoic extracts from pods of *Acmella oleracea*plant with zones of inhibitions ranging from 10.2 to 6.1 which were comparable to the standard drug gentamicin (23.7 to 22.0). Among the extracts, the extracts obtained by cooling with dehumidifying showed both higher effectivity against both *Staphylococcus aureus* and *Escherichia coli* bacteria. For both of the bacterial strains extracts obtained by sun dried technique was unable to show inhibition zone

indicating that the unavailability of antibacterial compounds through this drying method. There were no any noticeable significant difference ($p < 0.05$) between Air dried and Oven dried extracts for zone of inhibition of both types of bacterial strains. Cooling with dehumidifying method has obtained higher inhibition zone of 10.2±0.2 for *Staphylococcus aureus*which causes one of the most common worldwide food-borne diseases (Jhalka et al., 2014). Phytochemicals which are available in the plants of Asteraceae family can exert a variety of biological and pharmacological effects including antioxidant, antimutagenic, anti-cancer, anti-inflammatory, antimicrobial and insecticidal activities (Barbosa et al, 2016) may be the reason for the antimicrobial activity shown against above microbes.

Table 2: Antimicrobial activity of flower pod extracts using ethanol as the solvent against *Staphylococcus aureus* and *Escherichia coli*. Values are Mean ±SD, n = 5 a,b,c,d values in the same row with different superscripts are significantly different at 0.05 level

	Zone of Inhibition (mm) Mean ±SD					
Drying methods / Type of bacteria	**Cooling with dehumidifying**	**Air dried**	**Sun Dried**	**Dehydration/ Oven dried**	**+ve control**	**-ve control**
Staphylococcus aureus	10.2±0.2^{b}	7.0±0.5^{c}	ND^{d}	7.2±1.0^{c}	23.7±0.3^{a}	ND^{d}
Escherichia coli	8.0±1.1^{b}	6.2±0.2^{c}	ND^{d}	6.1±0.1^{c}	22.0±1.2^{a}	ND^{d}

4. Conclusion

Results obtained from the extraction yield revealed that drying method had significant impact on the extraction yield even though same solvent used for the extraction. Therefore, thereis an impact of drying method subjected to the raw material on the phytochemical availability in the solvent. Anti-microbial properties were detected only from the extracts subjected to drying by cooling with dehumidifying, Air dried and oven dried while sun drying method was unable to show inhibition zones for both gram negative bacteria *Escherichia coli* (ATCC 25922) and gram-positive bacteria *Staphylococcus aureus (*ATCC 25923) in Muller Hinton agar.

Availability of phytochemicals and ability of *A. oleracea*extractsto inhibition of both gram negative and positive bacteria is an indication of its antimicrobial potential which may be employed in management of microbes in food contact surfaces.

5. Acknowledgement

The authors wish to offer their gratitude towards University of Sri Jayewardenepura for providing Financial Assistance (ASP/01/RE/ SCI/2017/22) for the research.

References

1) Barbosa, A. F., Carvalho, M. G. D., Smith, R. E., & Sabaa-Srur, A. U. (2016). Spilanthol: occurrence, extraction, chemistry and biological activities. Revista Brasileira de Farmacognosia, 26(1), 128-133.
2) Chung, K., Wang, & Peng, (2008). Notes on Acmella (Asteraceae: Heliantheae) in Taiwan. Botanical Studies, 73 82.
3) Cunha, I., Sawaya, A. C., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., & Carvalho, P. D. O. (2004). Factors that influence the yield and composition of Brazilian propolis extracts. Journal of the Brazilian Chemical Society, 15(6), 964-970.
4) Dubey, S., Maity, S., Singh, M., Saraf, S. A., & Saha, S. (2013). Phytochemistry, pharmacology and toxicology of Spilanthes acmella: a review. Advances in pharmacological sciences, 2013.
5) Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., & Kadri, A. (2017). Solvent extraction effects on phytochemical constituents' profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Science and Technology, 37(3), 483-492.
6) Handa, S.S., Khanuja, S.P.S., Longo, G., Rakesh, D.D. (2008). Extraction Technologies for Medicinal and Aromatic Plants, (1stedn), no. 66. Italy: United Nations Industrial Development Org and

the International Centre for Science and High Technology.
7) Harborne, A. (1998). Phytochemical methods a guide to modern techniques of plant analysis: Springer Science & Business Media.
8) Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol.
9) Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed research international, 2014.
10) Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. In Manual of Clinical Microbiology, Eleventh Edition (pp. 1253 1273). American Society of Microbiology.
11) John, K. M., Vijayan, D., Kumar, R. R., & Premkumar, R.(2006). Factors influencing the efficiency of extraction of polyphenols from young tea leaves. Asian J. Plant Sci, 5(1), 123-126.
12) Smith, F., & Montgomery, R. (1959). The chemistry of plant gums andmucilages. Reinhold Publishing Corporation; New York; Chapman and Hall Ltd; London.
13) Trease, G. E., & Evans, W. C. (1989). Phytochemical screening. Pharmacognsy. 11th edn. Brailliar Tiridel Can.
14) WHO. (2015). News Release, GENEVA, online http://www.who.int/newsroom/detail/03-122015. WHO first ever global estimates of foodborne diseases find children under 5 account for almost one-third-of-deaths

14. EFFICACY OF pH LEVELS AND MALTED RICE TO STARCH RATIONON BROWNING IN MALTOSE SYRUP

Srey, T.[1*], Dizon, E.I.[2], Castillo-Israel, K.A.T.[2], Raymundo, L.C.[2], Hurtada, W.A.[3], and Serrano, E.S.[4]
[1]Faculty of Agro-Industry, Royal University of Agriculture, Dangkor District, Phnom Penh, Cambodia
[2]Institute of Food Science and Technology, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines, 4031
[3]Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, College, Laguna, Philippines, 4031
[4]Postharvest Horticulture Training and Research Center, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines, 4031
Corresponding author's Email: theavysrey@gmail.com

Abstract
In thermal processing of food, for instance, maltose syrup, different qualities of maltose is produced. In this process, Maillard reaction normally takes place due to presence of reducing sugars and protein. In some case, browning is of preference such as in bakery products; however, it is not of preference in canned fruits. Browning was observed in maltose syrups produced from different adjustments of pH levels (unadjusted pH, 4.0, 4.5, 5.0, 5.5, 6.0 & 6.5) and amounts of malted rice (32 g and 48 g). Cassava Rayong 9 cv. was used as a source of starch and rice Sen Pidao cv. was germinated and used as a source of amylase enzymes. Germination of rice Sen Pidao cv. was done for 10 days. Amylase activity in germinated rice was assayed daily. Amylase activity in germinated rice was significantly increased on day 8. Two different ratios of malted rice to starch with different levels of pH were used in the processing of maltose syrup. The resulting syrups were analyzed for color, pH, moisture content, total soluble solids (TSS), % reducing sugar (RS), dextrose equivalent (DE), crude ash, and crude protein and subjected to sensory evaluation. Yield of maltose syrups from different treatments was recorded. The resulted syrups had pH value within the range of 5.0 - 5.5 and the higher the amount of malted rice, the higher the value of RS and DE. Extracted juice after 8 hours saccharification with additional overnight storage resulted in decreasing pH value both in treatment with lower and higher amount of malted rice. Moisture content of syrups ranged from 4.65% – 11.79%. Crude protein in syrups may have contributed to browning reactions. The *L*-values signified that the more acidic the syrup, the lighter is the color. Color of syrups ranged from light brown to dark brown; and browning intensity increased when pH value and amount of malted rice increased. Samples with pH 4 showed significantly higher yield and had brighter color than other samples; as well as the lower the ratio of malted rice to starch of the syrup, the brighter the color.

Keywords: Maltose syrup, starch, malted rice, dextrose equivalent, α-amylase activity, browning, saccharification

1. Introduction

Maillard reactions occur between reducing sugars and protein or amino acid during thermal processing and storage of foods. Color and colorless can be produced by Maillard reactions in some foods. Formation of brown color represents one feature of these diverse reactions. Browning must be controlled to avoid undesirable products, for instance, in canned fruits. However, in some case, browning is highly desirable, for instance, in baking of bread (Davies et al., 1997). Maltose syrup is a di-saccharide with the molecular formula $C_{12}H_{22}O_{11}$. Maltose is the second important product in biochemical series of glucose chains. By adding of another glucose unit yields malto-triose and a four-glucose chain is maltos-tetrose, etc. The next product is dextrins when more units of glucose are added; it is also called maltodextrins later is starch. Maltose syrups processing is similar to glucose syrups through enzymatic saccharification of liquefied starch (DSSE, 2018).

The pH value of medium can either lower browning or boost browning in product. Generally, browning is increased with increasing pH value (Wolform & Rooney, 1953). When pH of the medium is between <3 to >9, some enzymatic reactions occur complete with the Maillard reaction. A change in pH leads to a change in mechanism of the reaction, therefore, leads to formation of

different volatile and colored products (Kroh & Westphal, 1988).

The study generally aimed to evaluate the browning and quality of maltose syrup that produced from cassava Rayong 9 cv. and Cambodian indigenous rice Sen Pidao cv. specifically, the study aimed to i) identify the best germination time of paddy rice to obtain the highest amylase production; ii) determine the best processing parameters such as pH, saccharification time, and ratio of substrate (cassava starch) to enzyme (malted rice) for maltose syrup production; iii) evaluate the physicochemical properties [color, pH, % moisture content, total soluble solids (TSS), % reducing sugar, % dextrose equivalent, % crude ash content and % crude protein] and yield (%) of the resulting maltose syrups; and iv) determine the acceptability of maltose syrups from cassava starch.

2. Materials and methods

Experimental materials

Paddy rice Sen Pidao cv. produced by the Cambodia Agricultural Research and Development Institute (CARDI), and Cassava Rayong 9 cv. commonly grown by Cambodian farmers were used in this study. The enzyme activity assay was conducted at Postharvest Horticulture Training and Research Center, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines and cassava starch and maltose syrup processing were conducted at faculty of Agro-Industry, Royal University of Agriculture, Cambodia.

Germination of paddy rice

Rice Sen Pidao cv. grains were cleaned, and then soaked in sufficient amount of water for 3 days. During soaking, water was changed twice a day to remove fermented odor. Soaked rice grains were spread (approximately 2cm thick) uniformly on plastic tray that was stand slope and covered with black plastic bag. Rice grains were sprayed with just enough amount of water twice a day. Amylase enzyme activity in germinated rice grains was checked every day for 10 days as well as length of roots and shoots of the representative grains. The time (day) which produced highest activity of α-amylase in the germinated rice was chosen for processing of maltose syrup.

Preparation of malted rice powder

Malted rice was dried in a convection oven dryer (kilning) until approximately 20% moisture content, ground and packed in PE bags and stored properly at room temperature for future use.

Determination of α-amylase activity in malted rice

Preparation of acetone powder

Acetone powder was prepared by homogenizing 1 part fresh rice seedlings tissue with 8 parts frozen acetone in mortar and pestle for 5 min. The homogenate was filtered using vacuum filtration and the pellet was washed several times with cold acetone. The residue was air-dried, ground to a fine powder and stored inside the freezer for future use.

Extraction of enzyme

A 0.2 g acetone powder was homogenized with 10 mL of 0.02 M sodium phosphate buffer (pH 6.9) by using a pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 10 min at 5^{o}C and the supernatant was dialyzed overnight against distilled water.

Enzyme assay

The activity of α-amylase was determined by the loss in the ability to give a blue color with iodine. The assay mixture (7.5 mL) contained 0.5 mL enzyme extract, 1.0 mL soluble starch wherein 1 mg/mL was dissolved in 0.02 M phosphate buffer (pH 6.9), and 5.0 mL 0.005% I_2-0.05% KI solution.

The reaction mixture was incubated for 10 min at 20^{o}C after which the reaction was stopped with the addition of 1.0 mL 0.1 M HCl. The mixture was read using spectrophotometer at 700 nm. The enzyme activity was calibrated against a standard curve made by plotting known units of purified α-amylase versus 700 nm using the method adopted from Serrano (1991). However, due to the unavailability of the purified α-amylase, the method used was modified as 1 unit α-amylase activity defined as to cause change of the mixture's absorbance by 0.001/min (1 unit = ΔAbs/min) to the plot of the α-amylase activity at the 700 nm at different germination time (day) after which the reaction was stopped by addition of 1.0 mL of 0.1 M HCl.

Preparation of cassava starch

Newly harvested, 12-month old fresh cassava root of Rayong 9 cv. obtained from a farm at Preahvihea province was used in the study. Fresh cassava roots were washed, peeled, washed again, chopped into small pieces, mixed with water and ground, the mixture was filtered and the residue was disposed, the filtrate was then placed into a plastic pail, filled up with tap water, covered well, and then left at room temperature over night. On the following day, water was drained slowly, and the white muddy starch (at the bottom of the container) was collected into another container. The washing process was repeated for several days to obtain more pure and white starch. The wet washed starch was spread on aluminum trays and dried using a solar dryer. Dried starch was powdered and passed through a 20-mesh sieve to obtain fine starch powder; packed and sealed properly in PE bags.

Preparation of maltose syrup

The preparation of maltose syrup using malted rice was adopted from Quynh and Cecil (1996). The study was done with two different proportions of dried crushed rice seedlings. First treatment was made up of 200g dried cassava starch and 32g dried crushed rice seedlings (T1: 200: 16+16). Second treatment was made up of 200g dried cassava starch and 48g dried crushed rice seedlings (T2: 200 : 24+24).The hydrolysis of cassava starch slurry was divided into two batches based on malted rice addition. The first batch was composed of 200g cassava starch each, with 16g (T1) and 24g (T2) dried crushed rice seedlings separately and 400 ml of distilled water was added into stainless steel pot each and stirred well to avoid lumping of starch. Then, pH was adjusted at varying levels (not adjusted, pH 4, pH 4.5, pH 5, pH 5.5 pH 6 and pH 6.5). This is referred to as the reaction step. Another 800 ml of distilled water was prepared separately with different pH levels and then boiled and added into the first batch of starch slurry.

The second batch of crushed rice seedlings (malted rice) composed of 16g (T1) and 24g (T2) were added into each pot and mixing was continued. After addition of malted rice and water, the temperature dropped to approximately 60ºC and then it was increased and maintained to approximately 80ºC with continuous stirring until the starch are cleared. The mixture was cooled at room temperature to approximately 60-62ºC and were left for saccharification in an oven set at 60ºC for 8 hours. For complete starch hydrolysis, the mixtures were stand further overnight. Starch test was done to check whether the starch is completely hydrolyzed (complete hydrolyzed starch is when there is no blueness after addition of iodine solution). The mixture was taken out of the oven on the following day. The saccharified starch was briefly boiled and immediately filtered using cheese cloth. The juice was boiled until the viscous syrup was obtained. When the syrup was thick enough, the pot was removed from the heat and the final product was cooled down. The determination of end point depends on the experience of the processor by observing the way the syrup falls from the stick. This is called gelatinization. In this set up, the gelatinization stage took around 40 minutes. The viscous syrups produced were tested for reducing sugar content, physicochemical properties and acceptability by panel of judges.

Physico-chemical analysis

pH

The pHof samples was determined using a calibrated Orion 2 Star Bench-top pH meter.

Total soluble solids (TSS) (°Brix)

The resulting maltose syrups were analyzed for TSS by using handheld refractometer.

Color measurement

Chromameter, CapSure^{TM} was used to read the color of the maltose syrups. In 1976, the CIE officially adopted the modified system as CIELAB with the parameters L^*, a^*, b^*. The L^* indicates lightness (0 to 100) with 0 being black and 100 being white. The coordinate a^* is for red (+) and green (−), and b^* is for yellow (+) and blue (−).

Reducing sugar determination

Reducing sugar content of the samples was determined using Dinitrosalicylic Acid (DNS) method (Miller 1959).

The intensity of dark red color was read using UV-Vis spectrophotometer, UVmini-1240 at 570 nm. A series of standards was prepared using glucose (0–500 µg) and a standard graph was plotted. The reducing sugar of samples was determined from a standard curve prepared from a glucose solution with the following concentrations (ug/mL): 100, 200, 300, and 400.

Dextrose equivalent
Dextrose equivalent of the sample was calculated using the equation:

$$\text{DE} = \frac{(\%\ \text{Reducing Sugars})(100)}{\%\ \text{Dry Substances}}$$

Moisture content (Oven-drying method)
Moisture content of samples was analyzed using the method adopted by Horwitz (2000). Percentage moisture (w/w) content was calculated as:

$$\%\ MC\ = \frac{(initial\ mass - final\ mass)}{initial\ mass_{sample}} \times 100$$

Crude ash
Crude ash content of samples was analyzed using dry ashing method of AOAC (2007).

$$\%\ \text{Ash (dry basis)} = \frac{\text{Weight after ashing} - \text{tare weight of crucible}}{\text{Original sample weight x Dry matter coefficient}}\ \text{x } 100$$

Crude protein
Crude protein content of sample was analyzed by using Kjeldahl method (Horwitz, 2000; WHO, 1973; Greenfield & Southgate, 1992). The crude protein content was calculated using the equation:

$$\text{N (g\%)} = \frac{(\text{mL 0.1N HCl sample} - \text{mL 0.1N HCl blank}) \text{x } 0.0014 \text{ x NHCl x } 100}{\text{Weight of sample}}$$

$$\text{Protein (g /100g)} = \%\ \text{Total nitrogen x appropriate nitrogen conversion factor}$$

Acceptability of the maltose syrup
A 9-point Hedonic scale scoring test was employed to test the acceptability of the products through rating on an appropriately defined scale. Twenty-eight (28) judges familiar with maltose syrup were randomly chosen to evaluate the products. Score sheet and a glass of water were provided to the judges during evaluation.

Statistical analysis
All experiments were done in triplicates. Analysis of Variance (ANOVA) and Least Significant Different (LSD) were performed to find out the significant difference between treatments and variables and the significant difference of general acceptability between treatments at $p \leq 0.05$.

4. Results and discussion
Changes in temperature and relative humidity (RH) of paddy rice at different germination period
The germinating paddy rice seeds temperature ranged from 32.8°C (day 4) to 34.4°C (day 6); while the values for relative humidity ranged from 47% (day 5) to 59% (day 9) with an average of 34°C and 53%, respectively. Results revealed no significant difference both in the changed of temperature and relative humidity with the progressed of germination period. The effect of temperature on the development of amylase activity in germinating wheat was reported by Kneen *et al*. (1942). They concluded that changes in amylase level were similar at all temperatures studied and differed chiefly in the length of time required to attain equal levels of amylase. α-Amylase activity increased steadily with seedling growth at all temper-atures, essentially paralleling increases in shoot length. In the present study, the activity of enzyme did not increase during 3

days of germination but then increased markedly after 6 days with a peak at day 8.

Shoot and root development of rice seedlings at different germination period

Day 1 of the germination period started from the first day that the soaked seeds were taken out from water. The shoot of paddy rice seedlings had yellow color during the first week then turned light green to green after the plastic bag was removed. The length of shoots and roots of the rice seedlings from day 0 to day 10 was found significantly different ($P \leq 0.05$). The roots were found slightly longer than the shoots throughout the germination period. Both the roots and the shoots started to increase in length on day 2 with an average values of approximately one (1) cm per day until day 10. Expectedly, the longest shoots and roots were obtained after 10 days of germination period with values of 13±0.25 cm and 13.17 ± 0.35 cm, respectively. A study conducted by Owusu-Mensah *et al*. (2010) showed that shoot lengths of the rice seedlings from four steeping periods increased with increasing malting periods. The differences in the shoot lengths showed that steeping period has significant influence on germination and shoot development of rice grains. The longer the shoot, the higher is the diastatic power of the rice malt (Hammond and Ayernor, 2001).

Alpha-amylase activity of rice seedlings during germination period

Daily α-amylase activity of the paddy rice seeds was observed to be significantly different ($P \leq 0.05$). The rate of amylase activity increased very slowly during the first 3 d and shoot up (366 ± 1.53 unit/ml/min) on the 8th d which was found significantly higher than days 1 to 7. From day 4 to day 8, there was a sharp increase of α-amylase activity and then the rate was slowed down from day 8 to day 10. With this result, rice seedlings germinated for 8 days was selected for the processing of maltose syrup from cassava starch. In a related study conducted by Ameko (2013) on maize malt crude extracts for the artisanal production of maltose syrup from fresh cassava starch, reported that activity of α-amylase reached its peak on day 4-5 and again on day 10 in germinating seeds. However, in another study by Lenz (1978), amylase activity in intact maize seeds started 2-3 days after absorption of water and reached its peak at day 6. The α-amylase activity was very low at the early part of germination period. This generally involved protein mobilization in the germinating seeds. During the course of germination, α-amylases broke down starch into simple sugars (Kruger, 1972a-b). The monitoring of the starch degradation during the germination of cereals, including wheat and oats, revealed an increase in α-amylase activity which was accompanied by a rise in free sugars in all cereals examined (Lineback and Ponpipom, 1977).

Reducing sugar and dextrose equivalent of maltose syrup

To determine the efficiency of malted rice as enzyme source, percent reducing sugar (%) and dextrose equivalent (%) of maltose syrup from liquefied cassava starch were carried out at different pH levels and results are summarized in Table 1.

Reducing sugar (RS, %)

A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group. The "liquefaction" step is where the α-1,4 linkages are hydrolyzed randomly which led to decrease in the viscosity of the gelatinized starch resulting in increase dextrose equivalent (Kearsley and Dziedzic, 1995). Petersen (1975) explained that the starch granules gelatinize in water when the temperature reached 60^{o}C to 70^{o}C. The granules will normally swell to form a paste and the shorter molecules dissolve. The insolubility of starch granule in cold water at room temperature is transformed to more soluble starch at high temperature due to the loss of low molecular weight amylose granules, consequently, the solution turns viscous and sticky (Collison, 1968). The loss of the stickiness of cooked starch after addition of the second batch of crushed rice seedling was due to the presence of α-amylase enzymes in the crushed malted rice seedlings. β-Amylase (α-1,4-glucose-maltohydrolase) is known to cause a slow viscosity reduction but higher sugar formation and thus so called as saccharogen-amylase because it has weak liquefying ability but strong in sugar production. It cleaves the 1,4-glucosidic linear chain of amylose and exoenzyme from the non-reducing end asides

from the amylose and amylopectin (Bruchmann and Fauveau, 2010).

Results of the study suggested that the treatment without any pH adjustment (pH 5.3) had the highest RS for both T1 (91.75 ±0.50%) and T2 (106.83±0.80%) as compared to other treatments with the same amount of rice seedlings. When T1 and T2 were compared, samples with higher amount of malted rice seedling obtained significantly higher RS (%). This work agreed with the research findings of Bruchmann and Fauveau (2010) wherein the optimum pH of β-amylase activity was reported to be around 5.2 and generally between 4.8 and 5.5. The highest values of RS (%) among samples was observed in samples with pH ranged from pH 5.0-5.5 regardless of the amount of malted rice added. The treatment without any adjustment of pH (pH 5.3) is within the ranged of pH 5.0 and 5.5 which was reported most appropriate during starch hydrolysis in literatures.

Dextrose equivalent (DE, %)

The sweetness of syrup depends on the degree of hydrolysis. The term used in UK and USA is that a complete hydrolysis results in the production of only glucose and dextrose. The higher is the sugar content, the higher the viscosity (10,000 centipoises at 50 ^{o}C) and vice versa. The higher the DE of syrup, the more of reducing sugars content. The browning reaction, flavor enhanment, and sweetness of foods were reported to increase while the viscosity decrease as DE increase and vise versa (Hull 2011). Dextrose equivalent of viscous syrups from T1 and T2 were significantly different among each others. In Treatment 1, DE (%) of sample with unadjusted pH (83.28 ± 2.04%) was not significantly higher than the rest of the samples. Likewise, the DE (%) values of T2 in unadjusted pH was also significantly higher (97.07 ± 0.44%) from other samples within the same treatment and also highest among samples from both treatments. However, there was no clear trend observed on the effect of pH levels on the values obtained for DE.

According to van der Maarel *et al*. (2002), the DE of syrup depends on the incubation time and enzyme added. The DE obtained from the present study ranged from 64.46 to 97.07 which are higher than 53, the DE of maltose reported by van der Maarel *et al*. (2002), higher than 40% reported by Ameko (2013), and majority of treatment had DE higher than 50-70% reported by Arasaratnam *et al*.,(1998) of syrup obtained from corn starch.

According to van der Maarel *et al*. (2002) unhydrolyzed starch paste has a DE of zero, maltrotriose has a DE of 36, maltose has a DE of 53 and a completely hydrolyzed starch (glucose or known as dextrose) has a DE of 100. Therefore, based on the DE values (64.46 – 97.07%) obtained in this study, the cassava starch was almost completely hydrolyzed to glucose.

Overall, treatments with unadjusted pH (5.3) both revealed higher DE values than the rest of the samples within treatment. Maltose syrup from pH adjusted to 5.0 to 5.5 obtained higher DE (%) consistent with samples that showed high RS (%) values. Results suggest that pH adjustment of cassava starch and malted rice seedlings mixtures (T2) within the range of 5.0-5.5 and the use of higher malted rice is necessary to obtain high quality maltose syrup.

Chemical properties of maltose syrup

pH

All maltose syrup obtained pH values which are significantly different from each other. In treatment 1, samples with unadjusted pH and pH 5.0 both obtained significantly higher pH values of 5.27 ± 0.06 than the rest of the samples. In treatment 2 with higher proportion of dried crushed rice seedlings, samples adjusted to pH 6.5 (5.30 ± 0.00) had significantly higher pH than the other samples. Overall, there was no clear trend observed on the effect of initial pH of various treatments on the final pH of the products, maltose syrup (Table 2).

The pH of maltose syrups from Treatment 1 ranged from 4.57 to 5.27 while Treatment 2 obtained pH which ranged from 4.53 to 5.30. These values were comparable to the pH values of maltose syrup made from wet cassava starch with different varieties of maize reported by Ameko (2013), which ranged from 4.6 to 5.30 but lower than the values of 5.5 to 6.5 of glucose syrup made from cassava flour and malted rice reported by Dziedzoave *et al*. (2004). According to Marie and Piggott (1991) the pH values of 3.5-5.5 of corn syrup were good enough to minimize color and flavor development. The pH value of the final product of syrup indicates the quality of the starch used in the production. Meanwhile, the pH of the treatment without any adjustment of pH was 5.3. This pH value is acceptable for the hydrolysis of the starch by enzymatic method based on literatures. Based on the

obtained pH values of all extracted juice after 8 hr saccharification and extended storage over night of the mixtures, the pH values mostly decreased for both T1 and T2. The slight decreased in pH value after 8 h saccharification and overnight storage may be due to the fermentation that could have taken place during this delaying period before the juice was placed in the refrigerator for further processing (boiling) prior to filtration and gelatinization.

Total soluble solid (TSS)

Total soluble solid (TSS) of maltose syrups produced with different pH levels and amount of crushed rice seedlings was significantly different from each other. In Treatment 1, TSS of samples adjusted to pH 6.5 obtained the highest (83.67 ± 0.58 ^{o}Brix); the same value was obtained from Treatment 2 in samples adjusted to pH 5.0. The rest of the samples, regardless of the treatment, were significantly lower from the above samples. The TSS of the maltose syrups was higher than 66.6 ^{o}Brix reported by Ameko (2013). Results showed that the treatments with different pH levels and concentration of rice seedlings did not show any significant effect on the TSS of the final syrups (Table 2). The variation of the TSS values of the syrup depends on the processing time and therefore, it is related to the moisture content in the final product of syrup. Based on moisture content of the maltose syrup, the lower the moisture content, the higher is the TSS value.

Moisture content (MC)

Moisture content of syrups from both T1 and T2 was significantly different from each other. In treatment 1, sample adjusted to pH 6 had the highest moisture content (11.79%) and significantly higher than the rest of the samples which ranged from 4.65% (pH 4.5) to 10.09% (pH 4.0). In treatment 2, the highest MC was observed in sample adjusted to pH 6.5 (11.34%) and also significantly higher than the rest of the samples which ranged from 6.58% (pH 5-5.5) to 10.14% (pH 6.0) (Table 2).

Overall, it can be concluded that the pH and the proportion of cassava starch to rice seedlings have no substantial effect on the moisture content of the maltose syrup as exhibited by unclear trend of the obtained values. Moisture content of all maltose syrups ranged from 4.65 – 11.79% lower than 33.4% reported by Ameko (2013) and the 15-20% reported by Dziedzoave *et al.* (2004); the final moisture content of the syrup depends on the degree of evaporation process.

Crude ash (%)

Crude ash content of syrups from both treatments was significantly different from each other. In Treatment 1, crude ash from pH 6.0 (0.63 ± 0.02%) and pH 6.5 (0.64 ± 0.03%) were not considerably different from each other but significantly higher than the rest of the samples within the same treatment. Similarly, crude ash content of samples in Treatment 2 showed no significant difference in samples adjusted to pH 6.0 (0.66 ± 0.02%) and pH 6.5 (0.64 ± 0.05%) but also was significantly higher than the other samples within the same treatment. Results showed that regardless of the treatment, based on proportion of cassava starch and rice seedlings, samples with pH 6.0 and 6.5 obtained significantly higher crude ash content than the rest of the samples.

Percent crude ash content of maltose syrup from both Treatments 1 and 2 ranged from 0.43 – 0.66% which is lower than 0.9% of the syrup obtained from banana starch reported by Bello-Perez *et al.* (2002), and some treatments contained higher percentage of crude ash than 0.45% obtained from hydrolysis of wet starch with different kinds of maize reported by Ameko (2013); ash content in the final product depends on the quality of the starch used. On the other hand, it might be attributed to the NaOH used during pH adjustment. According to Kent (2010), acid hydrolysis by mineral acids results in a syrup with a high ash content due to neutralization process done with sodium or potassium hydroxide. In order to obtain pH of 6.0 and 6.5 higher amounts of NaOH was added.

Crude protein (%)

Syrup samples show no clear trend of crude protein content but values ranged from 0.75% to 1.82%. The presence of small amount of crude protein in the samples can be due to the incomplete washing during cassava starch preparation. It should be noted that washing of cassava starch was done by just draining the upper part liquid (composed of protein) during soaking; hence complete removal of protein was not attained. The presence of crude protein can also contribute to the degree of browning of syrups through reaction with the hydrolyzed starch or simple sugars resulting in Maillard browning (Davies *et al*., 1997).

Color

*Color L**. The *L*-values ranged from 0 to 100 such that lighter samples have values approaching 100.In treatment 1, *L*-value of sample adjusted to pH 4 (51.60 ± 1.66) obtained significantly higher value than the rest of the samples within treatment. The values decrease with increase in pH values. The same trend was noticed for Treatment 2). This shows that the more acidic the sample, the lighter is the color (Table 3). While the pH level had significant effect on the lightness of maltose syrup color, varying the amount of rice seedlings (source of amylase enzyme) did not affect the color lightness of the products (Figure 1). Raisii and Aroujalianii (2010) studied the effect of pH on the color formation during storage of glucose syrup. They reported that browning reaction rate was enhanced with increasing pH and vice versa. The effect of pH may be attributed to three facts: 1) increasing the pH enhances the reactivity of amino acids and sugars because the open chain form of sugar and the unprotonated form of the amino groups considered to be the reactive forms, are favored at higher pH; 2) formation of HMF, one of the main intermediates in color pigment formation in Maillard reactions, decreases with increasing pH; and 3) enolization of glucose to fructose at higher pH may also increase the rate of non-enzymatic browning.

*Colora**. The coordinate *a* is for red (+) and green (−). Obviously, the maltose syrup has no tinge of green color hence most of the values obtained refer to the slight redness of the samples. In treatment 1, *a*-values from pH 4 – 6.5 increased as pH increased. Therefore, the change of pH had an effect on the *a*-value of the product. The higher the pH value, the higher is the amount of redness detected on the product (dark brown) (Figure 1). Meanwhile, reducing sugar content in the syrup also contributes to the color of the product. According to Marie and Piggott (1991), reducing sugar content of syrup such as in glucose, fructose and maltose are involved in the occurrence of Maillard or browning reactions. Moreover, temperature during gelatinization of the syrup is also important because it contrbutes a lot to the color development in the syrup. Between two treatments, T1 with higher amount of malted rice used had higher a-value. The impurity of the starch also contributes to the color development of the product.

Color development that occurs in the absence of nitrogenous compounds with the application of heat or acids is the result of caramelization. Excessive heating of starch-derived sweeteners will result in partial caramelization and development of undesirable flavors.

*Color b**. The *b* value is for yellow (+) and blue (−) color. The values obtained signified that maltose syrup had slight yellow color and not on the blue color side. The *b*-value of maltose syrup produced at different pH range and different combinations of starch and malted rice was highly significant. The lowest *b*-value was obtained from the sample with the highest pH value of 6.5, with 20.30±2.91 for T1 and 14.70±0.46 for T2. Visual observations of the maltose syrups show less yellow color intensity at lower pH values. This means that browning reaction can be minimized by lowering the pH of the solution. In addition, the differences in color of the products can be attributed to the subjective estimation of end point in boiling the syrups. The longer the boiling time, the darker the syrups it becomes due to time of exposure to heat resulting in caramelization.

Yield of maltose syrup

Three distinct steps are involved in enzymatic hydrolysis of starch to maltose. Firstly, cooked starch is dissolved into viscous solution, which is called gelatinization; secondly, there is a partial hydrolysis and a loss in viscosity, which is knowns as liquefaction; and thirdly, glucose and maltose are produced through further hydrolysis, which is called saccharification (Souza and Magalhaes, 2010). Gelatinized starch is converted into maltodextrins by α-amylase and mainly oligosaccharides and dextrins are remained during liquefaction. Maltodextrins are then converted into maltose by β-amylase during saccharification (Sigma Process Technologies, 2012). The process of maltose syrup production from Treatments 1 and 2 yielded approximately 1100 ml clarified juice. The yield of maltose syrup within treatment was significantly different among samples. Treatment 1 with pH 4 yielded the highest (100.50 ± 2.50% w/w dried starch) among samples. Treatment 2 samples adjusted to pH 4 yielded significantly the highest maltose syrup (122.33% w/w dried starch) as compared to other samples including those with unadjusted pH. In general, samples from treatment 2, higher amount of rice seedlings, exhibited higher yield of maltose syrup than treatment 1. The yield of all maltose syrups were observed higher than 23.59% reported by Ameko *et al.* (2013) on hydrolysis of

uncooked wet starch from different types of maize; also higher than 86.71% reported by Zainab *et al* (2011) on hydrolysis of gelatinized starch from maize, millet and sorghum with commercial amyloglucosidase.
In this study, the proportion of dried starch and dried crushed rice seedlings used in treatments 1 and 2 followed the same set-up of Quynh and Cecil (1996). However, the present study used 8 h saccharification time instead of 6 h in their set up. Starch test was first conducted after 6 hours saccharification time but found out that there was still blueness in the iodine solution and thus the saccharification time was extended for another 2 hours. As suggested by the authors, the saccharification time can be extended if the starch test indicates the presence of blueness with iodine solution.

General acceptability of maltose syrup
General acceptability is usually based on the flavor or taste of the product. Other attributes may also affect acceptability but flavor represents the overall quality of the food. All syrups from T1 at different pH levels and lower amount of malted rice added were not significantly different with the local product (Table 4). However, when Less Significant Different (LSD) was done, syrup at pH 4.0 had significantly higher acceptability (6.39±2.04) than the unadjusted pH 5.3 (4.7±2.66) and the local product (4.96 ± 2.40) but not significantly different from samples adjusted to pH 4.5 to 6.5. This shows that the local product and the syrup from unadjusted pH have been evaluated to be of the same level of acceptability but syrup from pH 4 is more acceptable than the local product. For treatment 2, with higher malted rice, acceptability of all samples from different pH levels was not significantly different from the locally produced syrup (Table 4). This means that regardless of pH level, malted syrup can be produced with comparable acceptability to the local syrup when the amount of malted rice is increased.

5. Conclusion

Results revealed that cassava starch from Rayong 9 cv. is a potential substrate for maltose syrup production using malted rice as source of enzyme. Significant increase in α-amylase activity can be attained on day 8 of germination period and found efficient in the conversion of cassava starch to maltose syrup at this stage. The pH adjustment within the range of 5.0-5.5 and the use of higher malted rice resulted in high reducing sugar (%) dextrose equivalent of maltose syrup. An 8-hour saccharification period of extracted juice with additional overnight storage resulted in almost complete hydrolysis of the starch.
The presence of crude protein in syrups contributed to the intensity of browning reactions of malted syrups. The color of the maltose syrups ranged from light brown to dark brown; and browning intensity increases with increasing pH and malted rice. Samples from pH 4 had higher yield than the rest of the treatments.

6. Acknowledgement

The authors wish to thank IDRC-SEARCA for the scholarship support and the Royal University of Agriculture for an opportunity to finish the research successfully.

References

1) Ameko, E., Achio, S., Alhassan, S., Gyasi, G., & Sackey, R. (2013). A procedure to determine the germination period for optimum amylase activity in maize malt crude extracts for the artisanal production of maltose syrup from fresh cassava starch. Innovative Romanian Food Biotechnology, 12.
2) AOAC International. (2007). Official Methods of Analysis. 18th ed. 2005. Current through revision 2, 2007 (On-line). AOAC International, Gaithersburg, MD. In: Nielsen, S.S. 2010. Food Analysis. 4th ed. ISBN 978-4419-1477-4; DOI 10.1007/978-1-4419-1478-1; Springer New York Dordrecht Heidelberg London.
3) Nand, A. V., Charan, R. P., Rohindra, D., & Khurma, J. R. (2008). Isolation a property of starch from some local cultivars of cassava and taro in Fiji. The South Pacific Journal of Natural and Applied Sciences, 26(1), 45-48.
4) Bello-Pérez, L. A., Sánchez-Hernández, L., Moreno-Damían, E., & Toro Vazquez, J. F. (2002).Laboratory scale production of maltodextrins and glucose syrup from banana starch. Acta cientifica venezolana, 53(1), 44-48.
5) Bruchmann, A., and Fauveau, C. (2010). Enzyme in potable alcohol and wine production. In Whitehurst, R.J. (Ed). Enzymes in Food Technology, p.194-209. USA: Wiley-Blackwell.
6) Collison. (1968). Corn syrup analysis. Analytical Methods of the Member

Companies of the Corn Refiners Association, Inc. Accepted 5-27-68.
7) Davies, C. G. A., Wedzicha, B. L., & Gillard, C. (1997). Kinetic model of the glucose-glycine reaction. Food Chemistry, 60 (3), 323 -329.
8) Danish Sugar & Sweetener Engineering (DSSE). (2018). Maltose syrups. http://www.dsse.biz/starchsweetner/maltose-syrup
9) Dziedzoave, N. T., Graffham, A., & Boateng, E. O. (2003). Training manual for the production of glucose syrup.
10) Greenfield, H., & Southgate, D. A. (2003). Food composition data: production, management, and use. Food & Agriculture Org.
11) Hammond, T. K., & Ayernor, G. S. (2001). Characteristics of malted rice for the production of sugar syrup. Journal of the Ghana Science Association, 3(3), 91-99.
12) Horwitz, W. (2000). Official Method of Analysis of AOAC International. 17th Edition. AOAC International, Maryland, USA. In: Puwastien, P.; Siong, T.E.; Kantasubrata, J.; Craven, G.; Feliciano, R.R.; Judprasong, K. 2011. ASEAN Manual of Food Analysis. 1st ed. Institute of Nutrition, Mahidol University, Thailand.
13) Hull, P. (2010). Glucose syrups: technology and applications. John Wiley & Sons.
14) Kearsley, M. W., & Dziedzic, S. Z. (1995). Handbook of starch hydrolysis product and their derivatives. Blackie academic & professional.
15) Kent, J.A (ed). (2007). Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology. James A. Kent (11th ed), Vol 1; ISBN: 978-0-387-27842-1; Springer Science+Business Media, LLC.
16) Kneen, E. R. I. C., Miller, B. S., & Sandstedt, R. M. (1942). The influence of temperature on the development of amylase in germinating wheat. Cereal chem, 19(1), ll-27.
17) Kroh, L., & Westphal, G. (1989). Die Reaktion in Lebensmittel. Chemische Gesellschaft, 35, 73-80
18) Kruger, J. E. (1972). Changes in the amylases of hard red spring wheat during germination. Cereal chemistry.
19) Kruger, J.E. (1972). Changes in the amylase of hard spring wheat during germination. Cereal Chemistry 49: 379.
20) Lenz, D.E. (1978). Hydrolysis of maize endosperm. Master of Science Thesis. McMaster University, Hamilton, Ontario.159 pp.
21) Lineback, D. R., & Ponpipom, S. (1977). Effects of Germination of Wheat, Oats, and Pearl Millet on Alpha-Amylase Activity and Starch Degradation. Starch-Stärke, 29(2), 52-60.
22) Marie, S., and Piggott, J.R. (1991). Handbook of sweeteners. New York: Springer Science Business Media, LLC. 69:664.
23) Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31(3), 426-428.
24) Owusu-Mensah, E., Oduro, I., & Sarfo, K. J. (2011). Steeping: a way of improving the malting of rice grain. Journal of food biochemistry, 35(1), 80-91.
25) Patil, S. K. (1991). Starch Properties, Modification, and Applications in Foods. Part 1. Eur. Food Drink Rev, 72-84.
26) Petersen, N. (1975). Edible starches and starch-derived syrup. New Jersey: Noye Data Corporation.
27) Nguyen, K. Q., Cecil, J. E., & Guyhn, N. (1996). Sweetness from starch: a manual for making maltose from starch. Food & Agri Org.
28) Serrano, E.P. (1991). Anaerobiosis and the etiology of internal breakdown in the mango (Mangifera indica L. cv Carabao). PhD. Thesis. Postharvest Horticulture Training and Research Center, Library. University of the Philippines Los Banos; Philippines.
29) Souza, P. M. D. (2010). Application of microbial α-amylase in industry-A review. Brazilian Journal of Microbiology, 41(4), 850-861.
30) Van Der Maarel, M. J., Van der Veen, B., Uitdehaag, J. C., Leemhuis, H., & Dijkhuizen, L. (2002). Properties andapplications of starch- converting enzymes of the α-amylase family. Journal of biotechnology, 94(2), 137-155.
31) Veen, M. V. D., Goot, A. V. D., & Boom, R. M. (2005). Production of glucose syrups in highly concentrated systems. Biotechnology progress, 21(2), 598-602.
32) World Health Organization (WHO). (1973). Report of a Joint FAO/WHO Ad

Hoc Expert Committee on Energy and Protein Requirements, WHO Technical Report Series No. 522. Geneva: WHO. In Puwastien, P. Siong, T.E., Kantasubrata, J., Craven, G., Feliciano, R.R. and Judprasong, K. 2011. ASEAN Manual of Food Analysis (1st Ed.). Thailand: Institute of Nutrition, Mahidol University.

33) Wolfrom, M.L., & Rooney, C.S. (1953). J. Amer. Chem. Soc., 75, 5435-3436
34) Zainab, A., Modu, S., Falmata, A. S., & Maisaratu, A. (2011). Laboratory scale production of glucose syrup by the enzymatic hydrolysis of starch made from maize, millet and sorghum. Biokemistri, 23(1).

Table 1: Percent reducing sugar (%) and dextrose equivalent (%) of maltose syrup made from different combination of cassava starch and rice seedlings at different pH levels

COMBINATION OF CASSAVA STARCH AND RICE SEEDLINGS	pH	REDUCING SUGAR* (%)	DEXTROSE EQUIVALENT* (%)
T1: Cassava Starch (g) : Rice Seedlings (g) (200 : 16+16)	Unadjusted (pH 5.3)	91.75 ± 0.50^{b}	83.28 ± 2.04^{bc}
	pH 4.0	82.67 ± 0.38^{d}	79.12 ± 1.88^{cd}
	pH 4.5	68.83 ± 3.19^{f}	64.46 ± 3.01^{f}
	pH 5.0	87.92 ± 5.35^{bc}	81.36 ± 11.29^{bc}
	pH 5.5	87.83 ± 2.89^{bc}	80.38 ± 1.41^{bc}
	pH 6.0	75.58 ± 3.32^{e}	71.29 ± 3.60^{e}
	pH 6.5	87.42 ± 1.38^{bc}	80.21 ± 1.59^{bc}
T2: Cassava Starch (g) : Rice Seedlings (g) (200 : 24+24)	Unadjusted (pH 5.3)	106.83 ± 0.80^{a}	97.07 ± 0.44^{a}
	pH 4.0	75.75 ± 0.25^{e}	86.61 ± 1.88^{b}
	pH 4.5	66.67 ± 0.63^{f}	64.77 ± 2.85^{f}
	pH 5.0	88.83 ± 3.76^{bc}	85.76 ± 3.80^{bc}
	pH 5.5	89.58 ± 0.14^{bc}	80.71 ± 1.34^{bc}
	pH 6.0	77.33 ± 0.29^{e}	73.16 ± 0.81^{de}
	pH 6.5	86.58 ± 0.14^{c}	80.40 ± 1.54^{bc}

Note: Means in the same column with the same superscripts are not significantly different at P≤0.05

Figure 1: Maltose syrups from hydrolysis of cassava starch at various amount of malted rice adjusted at different pH levels.

Table 2: Chemical properties of maltose syrup from different combinations of cassava starch and malted rice at various pH levels

COMBINATION OF CASSAVA STARCH AND RICE SEEDLINGS	pH	pH AFTER 8HRS SACCHARI-FICATION	FINAL pH*	TOTAL SOLUBLE SOLIDS (°Brix)*	MOISTURE CONTENT (%)*	CRUDE ASH (%)*	CRUDE PROTEIN (%)*
T1:Cassava Starch (g) : Rice Seedlings (g) (200 : 16+16)	Unadjusted (pH 5.3)	5.6	5.27 ± 0.06^{ab}	76.33 ± 0.58^{e}	8.97 ± 0.47^{d}	0.46 ± 0.03^{c}	1.31 ± 0.08^{cde}
	pH 4.0	4.8	5.03 ± 0.06^{d}	76.67 ± 0.58^{e}	10.09 ± 0.11^{c}	0.46 ± 0.05^{c}	1.43 ± 0.08^{bc}
	pH 4.5	4.9	4.63 ± 0.06^{fg}	80.13 ± 0.23^{c}	4.65 ± 0.56^{g}	0.43 ± 0.03^{c}	1.03 ± 0.05^{f}
	pH 5.0	5.4	5.27 ± 0.06^{ab}	77.33 ± 1.15^{e}	6.20 ± 0.05^{f}	0.45 ± 0.03^{c}	0.79 ± 0.11^{g}
	pH 5.5	5.5	4.70 ± 0.00^{ef}	73.00 ± 1.00^{f}	8.80 ± 0.05^{d}	0.48 ± 0.01^{c}	1.26 ± 0.06^{e}
	pH 6.0	5.9	4.77 ± 0.06^{e}	78.67 ± 0.58^{d}	11.79 ± 0.17^{a}	0.63 ± 0.02^{a}	1.82 ± 0.13^{a}
	pH 6.5	6.2	4.57 ± 0.06^{gh}	83.67 ± 0.58^{a}	7.37 ± 0.22^{e}	0.64 ± 0.03^{a}	1.03 ± 0.05^{f}
T2:Cassava Starch (g) : Rice Seedlings (g) (200 : 24+24)	Unadj. (pH 5.3)	5.2	4.77 ± 0.06^{e}	80.67 ± 1.15^{bc}	8.77 ± 0.12^{d}	0.44 ± 0.03^{c}	1.45 ± 0.06^{b}
	pH 4.0	4.6	4.53 ± 0.06^{h}	80.47 ± 0.50^{bc}	8.93 ± 0.27^{d}	0.47 ± 0.02^{c}	1.30 ± 0.03^{cde}
	pH 4.5	4.8	4.57 ± 0.06^{gh}	78.67 ± 0.58^{d}	8.89 ± 0.11^{d}	0.55 ± 0.04^{b}	1.52 ± 0.03^{b}
	pH 5.0	5.1	5.20 ± 0.00^{bc}	83.67 ± 0.58^{a}	6.58 ± 0.09^{f}	0.55 ± 0.02^{b}	1.40 ± 0.11^{bcd}
	pH 5.5	5.2	5.13 ± 0.06^{c}	81.67 ± 0.58^{b}	6.58 ± 0.24^{f}	0.58 ± 0.04^{b}	1.28 ± 0.02^{de}
	pH 6.0	5.5	5.03 ± 0.06^{d}	76.33 ± 0.58^{e}	10.14 ± 0.09^{c}	0.66 ± 0.02^{a}	1.05 ± 0.08^{f}
	pH 6.5	5.7	5.30 ± 0.00^{a}	76.33 ± 0.58^{e}	11.34 ± 0.18^{b}	0.64 ± 0.05^{a}	0.75 ± 0.07^{g}

Note: Mean value is ± SD (n=14). Means in the same column followed by the same superscripts are not significantly different ($P < 0.05$) by Duncan's multiple range test; *Significantly different at $P < 0.01$; a > b > c > d >….

Table 3: Color values of maltose syrups using chromameter

COM.TION OF CASSAVA STARCH & RICE SEEDLINGS	pH	COLOR VALUES		
		L *	*a* *	*b* *
T1: Cassava Starch (g) : Rice Seedlings (g) (200:16+16)	Unadj. pH	36.33 ± 1.15^{ef}	16.33 ± 0.06^{bcd}	25.43 ± 1.75^{d}
	pH 4.0	51.60 ± 1.66^{a}	8.63 ± 0.15^{j}	29.70 ± 0.44^{bc}
	pH 4.5	45.77 ± 2.31^{b}	9.90 ± 0.62^{i}	30.00 ± 0.60^{bc}
	pH 5.0	43.97 ± 0.49^{b}	10.90 ± 0.10^{h}	28.90 ± 0.17^{bc}
	pH 5.5	38.27 ± 2.95^{de}	15.67 ± 0.23^{de}	26.93 ± 3.75^{cd}
	pH 6.0	40.47 ± 2.45^{cd}	15.87 ± 0.67^{cd}	29.33 ± 2.15^{bc}
	pH 6.5	33.13 ± 1.86^{fg}	16.83 ± 0.58^{b}	20.30 ± 2.91^{e}
T2: Cassava Starch (g) : Rice Seedlings (g) (200:24+24)	Unadj. pH	44.57 ± 1.87^{b}	13.33 ± 0.64^{f}	31.93 ± 0.71^{ab}
	pH 4.0	47.07 ± 3.01^{b}	11.87 ± 0.75^{g}	34.43 ± 1.68^{a}
	pH 4.5	44.60 ± 3.08^{b}	11.90 ± 0.62^{g}	31.47 ± 2.18^{ab}
	pH 5.0	43.70 ± 1.44^{bc}	11.87 ± 0.21^{g}	30.63 ± 1.10^{b}
	pH 5.5	30.90 ± 0.44^{g}	18.50 ± 0.30^{a}	17.70 ± 0.92^{ef}
	pH 6.0	31.23 ± 0.85^{g}	16.77 ± 0.76^{bc}	17.13 ± 1.27^{f}
	pH 6.5	30.10 ± 0.52^{g}	14.90 ± 0.79^{e}	14.70 ± 0.46^{f}

Note: Mean value ± SD (n=14). Means in the same column followed by the same superscripts are not significantly different (P ≤ 0.05); *Significantly different at P ≤ 0.01; a > b >…; unadjusted pH was 5.3

Table 4: Mean score of general acceptability of maltose syrup from cassava starch and malted rice

pH	T1: CASSAVA STARCH (g) : RICE SEEDLINGS (w/w; g) (200 : 16+16)	T2: CASSAVA STARCH : RICE SEEDLINGS (w/w; g) (200 : 24+24)
Unadj. (pH 5.3)	4.79 ± 2.66 ^{b}	5.21 ± 2.42
pH 4.0	6.39 ± 2.04 ^{a}	5.46 ± 2.32
pH 4.5	5.43 ± 2.33 ^{ab}	5.32 ± 2.33
pH 5.0	5.57 ± 2.08 ^{ab}	5.43 ± 2.28
pH 5.5	5.00 ± 2.48 ^{ab}	6.04 ± 2.15
pH 6.0	5.07 ± 2.29 ^{ab}	4.82 ± 2.16
pH 6.5	5.11 ± 2.42 ^{ab}	5.82 ± 2.68
Local product	4.96 ± 2.40 ^{b}	4.96 ± 2.40
p-Value	0.2*	0.6**

Note: N = 28. Means in the same column followed by the same superscripts are not significantly different ($p \leq 0.05$); *LSD was significantly different among some treatments; Duncan's multiple range test a > b > …; ** not significantly different ($p \leq 0.05$)

Range of scores: General acceptability: 1 = Very unacceptable 9 = Very acceptable

15. ANTAGONISTIC PROPERTY OF LACTIC ACID BACTERIA STARTER CULTURES AGAINST Staphylococcus *aureus* IN FERMENTED CARABEEF

Vernabelle L. Balmori[1], and Francisco B. Elegado[2]
[1]Food Science and Technology Department, Southern Leyte State University – Main Campus, Sogod Southern Leyte, Philippines
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, 4031, College Laguna, Philippine
Corresponding author's Email: balmorivernabelle@gmail.com

Abstract

The effect of selected starter cultures (*Lactobacillus plantarum* and *Pediococcus acidilactici*) on the growth of pathogenic microorganism *Staphylococcus aureus*in fermented carabeef at different fermentation temperatures (32°C and 10°C) was evaluated. BothLAB starters showed antagonism against *Staphylococcus aureus*. The decreasing pH-conditioned LAB growth ensured a negative polynomial growth rate of inoculated *S. aureus* during fermentation. Highest decrease rate was exhibited by meat inoculated with *P. acidilactici* 3G3, followed by meat inoculated with a combination of *P. acidilactici* 3G3 and *L. plantarum* BS with decrease rates of 3.21 and 1.73 log, cfu/g, respectively. Temperature also played a significant role on the reduction of *S. aureus* counts in the samples. Fermentation at 32°C was more effective in controlling *S. aureus*. Negative correlation between LAB and *S. aureus* growth as well as the significant positive correlation between pH and *S. aureus* counts demonstrate the role of LAB starter cultures in controlling *S. aureus.* The study establishes the potential of LAB starter cultures in fermented carabeef production in improving product quality in terms product safety. These LAB strains can potentially be good inocula in producing a fermented carabeef, as their effect on the microbial properties of the product proved to be beneficial.

Keywords: Meat fermentation, carabeef, *Lactobacillus plantarum*, *Pediococcus acidilactici*, bacterial antagonism

1. Introduction

Fermented meats provide a suitable environment for proliferation of meat spoilage microorganisms. The increase in resistance of food spoilage microorganisms to current preservatives, the consumer's demand for safe and minimally processed foods and the hazards associated with the use of high doses of chemical preservatives have led to the need for finding safer alternatives in food preservation. As a consequence, bacterial antagonism has received considerable attention in food preservation.

The antimicrobial effect responsible for safety in fermented meats is mainly ascribed to the rate of acidification of the raw meat. However, certain antimicrobials such as bacteriocins have been looked into as a factor that contributes to the elimination of undesirable microorganisms that display acid tolerance, particularly in slightly acidified products (Kröckel, 2013). Bacteriocins often have narrow inhibitory spectra and are most active towards closely related bacteria likely to occur in the same niche or environment (Eijsink et al., 2002 as cited by Kröckel, 2013). Lactics produce a diversity of bacteriocins that are generally active towards other LAB. This contributes to the competitiveness of the bacteria to thrive in the food against other microorganisms including foodborne pathogens. The application of bacteriocin-producing LAB in the meat industry is seen as something that offers a way of natural food preservation (Kröckel, 2013).

The use of *L. sakei* as starter culture was able to decrease *Listeria* levels in fermented sausage. Antilisterial effects have also been demonstrated with bacteriocinogenic *L. curvatus* and *L. plantarum* sausage starter cultures (Dicks et al., 2004). Bacteriocin-producing pediococci have also been used in certain products including American-style sausages fermented at elevated

temperatures. Bacteriocin-producing *L. lactis* strains have been used as new, functional starters for fermented sausage manufacture, despite the fact that they are not particularly adapted to sausage technology (Kröckel, 2013). Inoculation of LAB in meat fermentation was also found to have an effect on the inhibition of spoilage and pathogenic microorganisms. Inoculation of the sausage batter with a starter culture composed of selected LAB and Gram-positive, catalase-positive cocci (GCC), have been found to improve the quality and safety of the final product and standardizes the production process (Leroy et al., 2006).

*Staphylococcus aureus*is a Gram-positive, non-spore forming spherical bacterium that produces a staphylococcal enterotoxin (SE) responsible for almost all staphylococcal food poisoning (FDA, 2012). *S. aureus* is widely distributed in meat, is very salt and nitrite tolerant, and produces a heat stable enterotoxin that can survive a heat treatment even though the organism is killed. This organism could be a problem when high water activity (aw), and/or pH are encountered (Montville and Matthews, 2008). Suggested control methods of this microorganism include using clean raw materials and control of pH decline with starter cultures or direct addition of an organic acid.

The use of new, functional starter cultures with industrially or nutritionally important functionality is recently, being explored. This study aimed to evaluate the antagonistic effect LAB starter cultures, particularly *Lactobacillus plantarum* (BS) and *Pediococcus acidilactici* (3G3) cultures on the growth of *Staphylococcus aureus* pathogen in meat fermentation.

2. Materials and methods

The study was conducted at the Food and Feed Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH) U.P. Los Banos, College Laguna.

Preparation of lactic acid bacteria starter culture

LAB starter cultures (*Lactobacillus plantarum* BS and *Pediococcus acidilactici* 3G3) were obtained from BIOTECH-UPLB. Both LAB cultures were spray-dried prior to inoculation to meat mixture. Spray-dried stock cultures were prepared by cultivating both LAB strains in MRS broth, incubated at 37˚C for 24 hours. After incubation, broth cultures were centrifuged (6000 rpm at 12˚C) for fifteen minutes. The supernatant was discarded, while the cells were aseptically transferred to a feed solution composed of 10% lactose, 20% maltodextrin and 70% distilled water. The solution was placed in a flask and subjected to spray dying at 0.66 $L.h^{-1}$, 37-42 ˚C using a low-temperature spray dryer (True Ten Industrial Co., Ltd.; model YK-100-1) (Figure 2). The process flow for the production of spray-dried LAB cultures is shown in Figure 1 (Elegado, et. al., 2014).

LAB population counts of the powdered starter culture were also determined and monitored by standard plate count method. LAB counts for the powdered cultures were found to be 6.30 $x10^6$ cfu/g for *L. plantarum*BS and 6.47 $x10^6$cfu/gfor *P. acidilactici*3G3. Powdered starter culture was added to the meat mixture at 0.1% of the meat system.

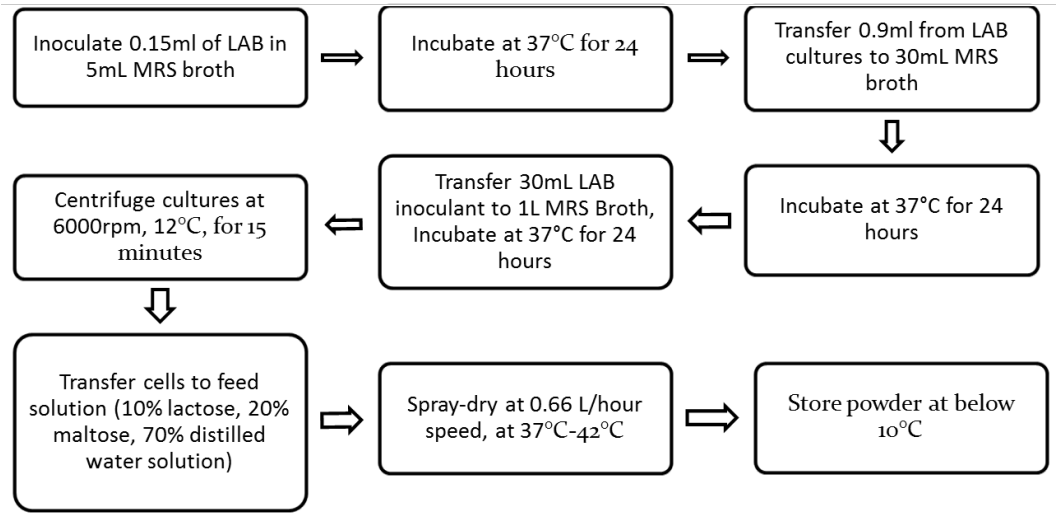


Figure 1: Production of spray-dried LAB starter cultures.

Preparation of fermented Carabeef

Lactic acid bacteriapowder suspensions along with fermentation ingredients were added to previously sterilized meats prior to fermentation. The curing formulation for every kilogram of carabeef included 150g sugar (15%), 20g salt (2%), 2.5g prague powder (0.25%), 3g potassium phosphate (0.30%) and 100mL water (10%). Treatments include *S. aureus* inoculated samples, with different LAB starter cultures and fermented at different temperatures (32°C and 10°C) following a completely randomized design. A control treatment was included wherein the meat was not sterilized and LAB and *S. aureus* were spiked. Five treatments were set-up for each fermentation temperature: T_0: Control; Meat Unsterilized; No starter culture, No *S. aureus,* T_1: No Starter culture; With *S. aureus,* T_2: *S. aureus* + *L. plantarum* BS, T_3: *S. aureus* + *P. acidilactici* 3G3, and T_4: *S. aureus* + Combination of *L. plantarum* BS and *P. acidilactici* 3G3 (50:50). Meat mixtures were placed in food grade polypropylene bags at 250 grams per bag and sealed. Meats were fermented for 5 days at two different temperatures (32°C and 10°C), with each treatment-temperature combination done in triplicate. Initial microbial analysis was done prior to fermentation.

Data gathering

Microbial analysis

Initial counts for LAB starter cultures and S. aureus were determined using plate count method. The growths of S. aureus and LAB in the samples were enumerated and monitored using plate count method. Observations were done on a 48-hour interval starting from day 1 to day 7 (days 1, 3, 5, 7). Meat samples (25 g) of each batch were homogenized with 225 mL of sterile 0.85% NaCl solutions and decimal dilutions of 10-1 to 10-8 were prepared. Viable counts for S. aureus were determined on Baird-Parker Agar with 1% tellurite solution and egg yolk after incubation at 37°C for 48 hours. Counts for LAB were determined by plating in MRS agar with 1% CaCO3 incubated at 37°C for 24 hours.

pH

Meat samples were tested for pH using a digital pH meter. The electrode of pH meter was calibrated using two buffer solutions of known pH, 4 and 7. Ten grams of finely ground sample were taken and blended in 50ml of distilled water in a test tube and mixed in a vortex mixer. The extract was filtered through Whattman No.1 filter paper and the pH of the sample was recorded.

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) of SPSS 17.0 software (SPSS, Inc., Chicago, IL). Significantly different results were further analyzed using Tukey's HSD test to further determine which groups in the samples differ significantly.

3. Results and discussion

Spray-dried starter culture

Solids recovery for both starter cultures (calculated as grams powder per liter of feed solution) was only about 50% as shown in Table 1. Maximum recovery of powdered samples was hardly achieved since adherence on the wall and on the filter, cloth pose problem in collecting the spray-dried samples.

Table 1: Solids recovery of spray-dried starter cultures

STARTER CULTURE	YIELD (g/L)	% RECOVERY	LAB COUNT (CFU/g)
Lactobacillus plantarum BS	455.9	45.49	6.30×10^6
Pediococcus acidilactici 3G3	546.0	54.60	6.47×10^6

The growth inhibition of S. aureus can be attributed to the decrease in pH of the meat samples during fermentation, however, it is observed that in the control treatment (T0) in which no LAB starter culture and spiked with S. aureus (although the pH of the meat decreased during fermentation) viable S. aureus counts were still detected at the end of fermentation.

This may indicate that natural fermentation of carabeef is not sufficient to render the product safe from S. aureus. This indicates that addition of LAB starter cultures to meat samples have positive effect to product safety. A much better growth and survival of LAB is expected in samples inoculated with starter culture as growth and survival of LAB is also affected by the initial counts the start of fermentation. Papamanoli et al. (2003) reported that LAB, including probiotic strains, have a synergistic antioxidant and nitrite effect of inhibiting pathogenic bacteria.

LAB growth, pH reduction and Staphylococcus aureus counts

LAB growth and pH reduction were also determined along with S. aureus growth in meat samples. Growth of S. aureus and LAB as well as pH were plotted for treatments 2, 3 and 4 fermented at 32°C as these demonstrated the most significant reduction of S. aureus growth among the treatments (Figure 2).
Results show that there was an increase in the LAB counts for the first 3 days of fermentation. Stationary phase started at about the third day of fermentation. LAB counts remain relatively high after seven days of fermentation ranging from 8. 44 to 8.69 (log, cfu/g). pH reduction was also evident with the samples during fermentation, with final pH ranging from 3.27 to 3.58. The correlations between parameters including S. aureus and LAB counts, pH, fermentation temperature and fermentation time for treatments inoculated with LAB starter cultures are presented in Table 2. Spray-dried starter cultures (Figure 7) were subjected to viable cell count analysis prior to inoculation. A relatively high viable cell count was observed for both LAB strains after spray-drying. This demonstrates that spray-drying with the use of lactose and maltodextrin as carriers (protective agents) is able to preserve the LAB cultures and produce a stable powdered starter culture for fermentation. A study by Elegado et al. (2014) suggested that these sugars replace water molecules around proteins and polar residues of membrane phospholipids thereby acting as water substitutes and protecting dehydrated biomaterials. They also prevent protein denaturation of bacterial cells by forming hydrogen bonds with proteins upon water removal. Powdered cultures were packed in metallized pouch (prepared by DOST packaging section) and stored in the chiller at 10°C.

Antagonistic property of LAB against Staphylococcus aureus
Figure 1 shows the regression trends of the viable S. aureus counts for the different treatments during fermentation at different temperatures. The best fit trend of regression of S. aureus count during all time investigated is shown by a negative polynomial graph curve. This means that, although generally decreasing, the trend does not follow a strict linear curve as the decrease in counts is not constant at any given time. Results show that for treatments spiked with S. aureus, the initial inoculation concentration (7.45 log, cfu/g) was significantly ($p<0.05$) reduced after 7 days of fermentation in treatments inoculated with starter cultures.

Table 2: Correlation (r) values and significance (p) levels between measured parameters and changes of inoculated *S. aureus* counts.

PARAMETERS	CORRELATION TO *S. aureus* GROWTH					
	T2		T3		T4	
	r	p	R	p	r	p
Temperature	-0.46	0.18	-0.48	0.16	-0.49	0.15
Time	-0.70*	0.03	-0.70*	0.03	-0.70*	0.03
LAB	-0.36	0.31	-0.48	0.16	-0.20	0.58
pH	0.71*	0.06	0.72*	0.02	0.72*	0.02

*Correlation is significant at 5% level of significance.

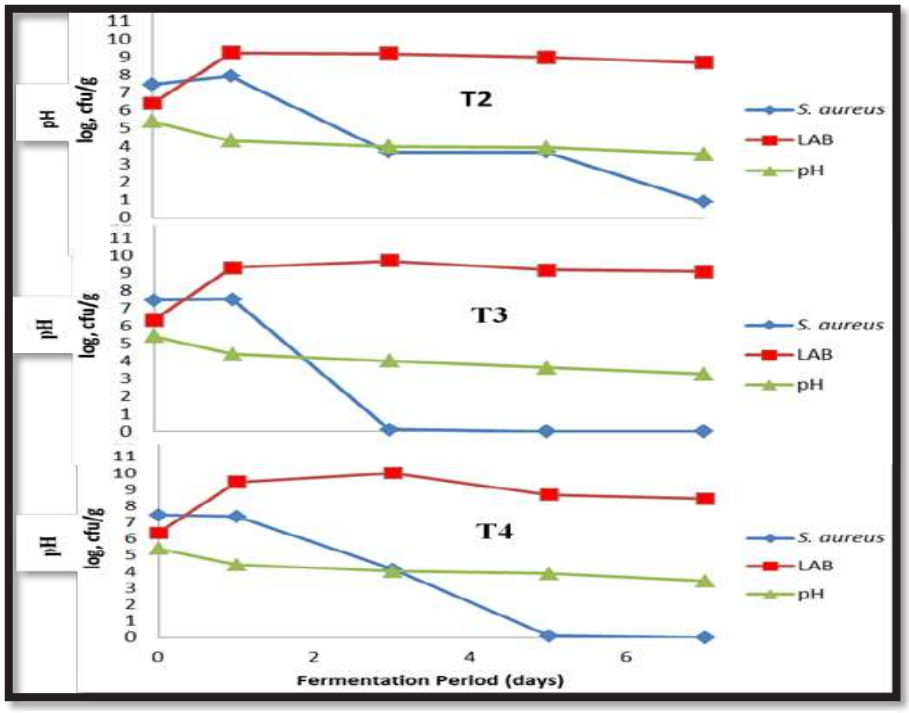


Figure 1: Changes in pH, LAB counts and *S. aureus* counts in carabeef samples during fermentation at 32°C. T2= *S. aureus* + *L. plantarum* BS; T3= *S. aureus* + *P. acidilactici* 3G3*;* T4= *S. aureus* + Combination of *L. plantarum* BSand *P. acidilactici* 3G3.

In all treatments, the decrease of detected S. aureus counts showed a negative polynomial regression curve during the first 7 days with the decrease rate ranging from 0.06 to 3.21 log, cfu/g per day. For T1 which was spiked only with S. aureus and no LAB starter, it is expected that the S. aureus counts were the highest after 7 days. It is observed that the highest decrease rate was exhibited by meat inoculated with P. acidilactici 3G3 (T3), followed by meat inoculated with a combination of P. acidilactici 3G3 and L. plantarum BS (T4) with decrease rates of 3.21 and 1.73 log, cfu/g, respectively. For the naturally fermented meat with no LAB and spiked with S. aureus, S. aureus counts were detected at the start and the end of fermentation, although the counts also followed decreasing trend. Temperature also played as significant role on the reduction of S. aureus counts in the samples. Fermentation at a higher temperature was more effective in controlling S. aureus in the meat samples. This can be attributed to faster pH reduction and near-optimum LAB activity at 32°C. Time and temperature have negative correlations with S. aureus count. This means that as fermentation time and temperature is increased viable counts of S. aureus decreased. A negative correlation was also observed between LAB counts and S. aureus counts. As viable counts for LAB increases, the number S. aureus counts decreases. This demonstrates the inhibitory effect of LAB against S. aureus. Although the main factor that contributes to the inhibition of S. aureus is pH reduction as shown in the significant correlation between pH and S. aureus growth, L. plantarum and P. acidilactici are also known to produce antimicrobial compounds such as bacteriocin that helps control the growth of spoilage and pathogenic bacteria including S. aureus.

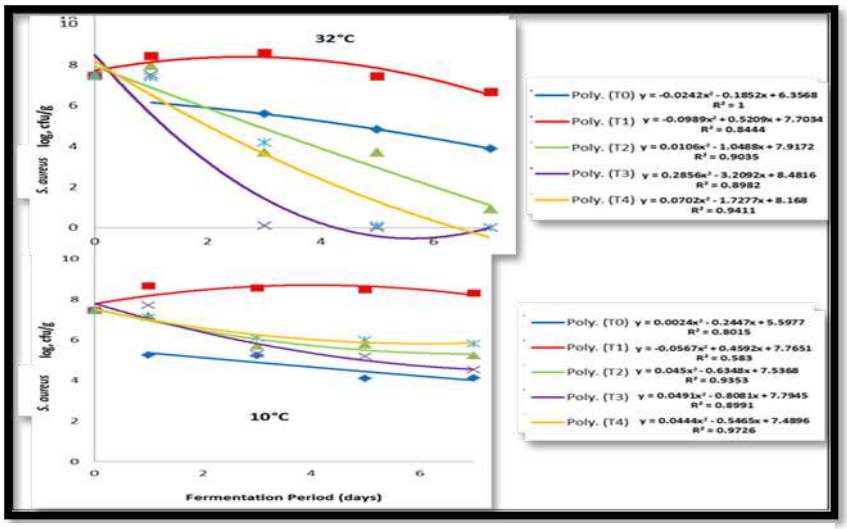


Figure 2: Polynomial regression analysis on the changes of *Staphylococcus aureus* counts during fermentation of carabeef as affected by LAB starter cultures and fermentation temperature. T0= Control; Natural meat microflora; T1= No Starter culture; Spiked with *S. aureus*; T2= *S. aureus* + *L. plantarum* BS; T3= *S. aureus* + *P. acidilactici* 3G3; T4= *S. aureus* + Combination of *L. plantarum* BSand *P. acidilactici* 3G3.

Elegado et al. (2004) reported that a slight bacteriocinogenic activity through "spot-on-lawn" assay was detected with L. plantarum BSagainst S. aureus and E. coli O157: H7. In a study by Nedelcheva et al. (2010), it was reported that the addition of L. plantarum BSin raw sausage mass caused a rapid increase in acidity along with synthesis of metabolites which inhibited the growth of S. aureus. P. acidilactici 3G3 is also known to produce pediocins which are reported to be bactericidal to sensitive gram-positive pathogenic micro organisms (Chen and Hoover, 2003). This LAB is also generally recognized as safe (GRAS) organism and is commonly used in fermented sausage production. They are also common in fermented sausages from the United States where they are deliberately added as starter cultures to accelerate acidification of the meat batter (Papamanoli et al., 2003).

LAB can proliferate in fermented carabeef and can produce lactic and other organic acids, bacteriocins and other biologically active compounds which inhibit the development of pathogenic, toxigenic and saprophytic microorganisms to a large extent. This helps ensure the safety of the end product.

4. Conclusion

The results of the study is an indication that using starter cultures, particularly L. plantarum BS and P. acidilactici 3G3 in carabeef fermentation is a potential measure in controlling the pathogenic microorganism S. aureus in fermented meat. Temperature also has a significant effect on the reduction of S. aureus counts. Fermentation at a higher temperature is more effective in controlling S. aureus in fermented carabeef samples. Furthermore, this is an indication that using these starters is beneficial in terms of product safety as it could be useful for maintaining hygienic quality of fermented carabeef, which may allow a good preservation of meat products and consequently improve its shelf life. The LAB starters can be added individually or combined, however, with the strong potential of P. acidilactici 3G3 in terms microbial antagonism against S. aureus, this microorganism can be a good starter for carabeef fermentation.

5. Acknowledgement

The study would have not been completed without the generous help of the Department of Food Science and Technology (DOST), the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños (BIOTECH-UPLB) and the Institute of Food Science and Technology (IFST-UPLB).

References

1) And, H. C., & Hoover, D. G. (2003).
7) Bacteriocins and their food applications. Comprehensive reviews in food science and food safety, 2(3), 82-100.
2) AOAC. (2000). Official Method of Analysis. Vol. I and II. Association of Official
8) Analytical Chemist. Washington, D.C.
3) Dicks, L. M. T., Mellett, F. D., & Hoffman, L.C. (2004). Use of bacteriocin-producing starter cultures of Lactobacillus plantarum and Lactobacillus curvatus in production of ostrich
9) meat salami. Meat Science, 66(3), 703-708.
4) Elegado, F. B., Guerra, M. A. R. V., Macayan, R. A., Mendoza, H. A., & Lirazan, M. B.
10) (2004). Spectrum of bacteriocin activity of Lactobacillus plantarum BS and fingerprinting by RAPD-PCR. International Journal of food microbiology, 95(1), 11-18.
5) Elegado, F. B., Mercado, S. M., Calapardo, M. R., Perez, M. T. M., Mercado, M. R., and Saguibo, J. D. (2014). Development of Probiotic Starter Cultures and Adoption of
11) Technology for Functional Food. Terminal report. National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines, Los Banos (UPLB).
6) FDA. (2012). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring.

Kröckel, L. (2013). The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products. In Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes. InTech.

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. International journal of food microbiology, 106(3), 270-285.

Montville, T. J., and Matthews, K. R. (2008). Food microbiology: An introduction. 2nd ed. ASM Press, Washington D.C.

Nedelcheva, P., Denkova, Z., Denev, P., Slavchev, A., & Krastanov, A. (2010). Probiotic strain Lactobacillus plantarum NBIMCC 2415 with antioxidant activity as a starter culture in the production of dried fermented meat products. Biotechnology & Biotechnological Equipment, 24(1), 1624-1630.

Papamanoli, E., Tzanetakis, N., Litopoulou Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat science, 65(2), 859-867.

16. EFFECT OF LED LIGHT EXPOSURE IN DELAYING BROWNING INCIDENCE AND MAINTAINING POSTHARVEST QUALITY OF FRESH-CUT PINEAPPLE (*Ananascomosus* L.) STORED AT TWO STORAGE TEMPERATURES

Ng Wan Jing, Yusnita Hamzah, and Wan Zaliha Wan Sembok
School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
Corresponding author's Email: wzaliha@umt.edu.my

Abstract

Fresh cut pineapple has a high popularity nowadays because of its fresh like quality, ready-to-eat properties and high nutritional values. However, prior to peeling and cutting processes, fresh cut pineapple is usually susceptible to browning incidence, which was catalyzed by polyphenol oxidase (PPO) enzyme. Browning incidence (BI) generally degraded the overall attributes of fresh cut pineapple, and simultaneously, limited its shelf life. In the present study, the effects of LED lights (white, blue, red) on fresh cut pineapple were evaluated under two storage temperatures (5˚C and 10˚C) for 18 days. The parameters being evaluated were browning index, color, percentage weight loss, firmness, soluble solids concentration, total phenolic content (TPC) and ascorbic acid (AA) concentration. A significant interaction between storage temperatures and types of LED lights was recorded in BI, percentage weight loss, TPC and AA. Interestingly, blue LED light (470nm) at 5˚C had a significant effect ($p \leq 0.05$) in reducing BI of fresh cut pineapple. This might be due to the increasing value of AA which inhibit the activity of PPO enzyme. In conclusion, fresh cut pineapples treated with blue LED light and further stored at 5˚C has a tendency in delaying BI without adversely affecting other quality attributes.

Keywords: Minimally processed, chiller, postharvest, Josapine

1. Introduction

Due to its organoleptic quality and morphological structure that makes the direct consumption difficult, pineapple is suitable to be commercialized as fresh-cut product (Antoniolli et al., 2012). However, fresh cut pineapple has shorter shelf life than whole fruit due to the fresh cut processing that promotes a faster physiological deterioration, biochemical changes and microbial degradation of the products which lead to the degradation of organoleptic quality and nutritional value (Corbo et al., 2010).The typical symptoms of fresh cut products due to cut processing operation include tissue softening, cut surface browning, decrease nutritional value, presence of off-flavour and microbiological spoilage during storage (Ma et al., 2017).Surface browningin fresh cut fruit closely related to polyphenol oxidase (PPO) action (Martinez and Whitaker, 1995), some action of phenol peroxidase (POD) (Toivonen and Brummell, 2008) and phenylalanine ammonia lyase (PAL) (Kang and Saltveit, 2003). Generally, browning incidence (BI) occur on fresh cut pineapple after 6 days even under 4˚C of storage temperature (O'Connor-Shaw et al., 1994). Many postharvest treatments have been used in delaying BI. Currently, light emitting diodes (LED) is reported able to preserve and improve quality of fruit and vegetables during storage. LED light treatment delaysBI by increasing the total phenolic content and ascorbic acid concentration which enhances the antioxidant defence system. Besides that, LED light treatment also inhibits the activity of PPO which catalyse BI. Manzocco et al. (2009) claimed that PPO enzyme inactivated by light due to direct photo-oxidation of absorbing residues of PPO amino acids to form radicals. Besides that, temperature management is also one of the important tools for extending the shelf life of fresh cut pineapple. Benitez et al. (2012) claimed that storage temperature for fresh cut product should be controlled and maintained during refrigerated storage as optimum temperature able to maintain the visual quality, reduce their respiration rate, tissue softening and microbial spoilage.Therefore, the impacts of LED light at varying types(white, blue, red) in reducing browning incidence and maintaining other quality attributes of fresh cut pineapple were evaluated under two storage temperature (5˚C and 10˚C) for 15 days.

2. Materials and methods

Experimental location and Experimental design

A total of 40 *Ananascomosus* L. cv. Josapinewith maturity index of three were purchased from Gong Pauh wholesale market, Terengganu. The experiment was conducted in the Post Harvest Technology Laboratory, School of Food Science and Technology, Universiti Malaysia Terengganu.The experiment was arranged in completely randomized design (CRD) with two factors viz.; two storage temperatures which were 5˚C and 10˚C and three LED light colours; white (390 nm-700 nm), blue (430-470 nm) and red (630-640 nm). The treatments include (i) White LED light (control) in 5˚C storage temperature, (ii) White LED light (control) in 10˚C storage temperature, (iii) Blue LED light in 5˚C storage temperature, (iv) Blue LED light in 10˚C storage temperature, (v) Red LED light in 5˚C storage temperature and (vi) Red LED light in 10˚C storage temperature with three replications. The duration of the experiment was 15 days continuously and the parameter were evaluated for every 3 days interval as 0, 3, 6, 9, 12 and 15 days.

Preparation of sample and parameter evaluations

Postharvest parameters evaluation were percentage of weight loss (PWL), browning index score, fruit colour and firmness, soluble solids concentration (SSC), total phenolic content (TPC) and ascorbic acid (AA) concentration.For the PWL determination, the fruit samples were weighted during the sampling days and calculated using formulae:PWL (%)=(Initial weight-Final Weight)/(Initial weight)×100.The BI of fresh cut pineapple was evaluated visually according to the score proposed by Ding et al. (2007). The score was given from 0 to 5, which 0=None (0-20% browning), 1=Trace (20-40% browning), 2=Slight (40-60% browning), 3=Moderate (60-80% browning), 4=Severe (80% of browning) and 5=Extremely severe (>100% of browning). The flesh colour of fresh cut pineapple was measured using Kinoca Minolta CR-400 (Minolta camera Ltd, Japan) as according to CIELAB colour parameter. Fruit colour data were expressed in lightness (L*), chromaticity value a* and chromaticity value b* (McGuire, 1992). L* is represent the lightness coefficient which ranges from 0 (black) to 100 (white) while a* represent the position between red (+) and green (-) while b* scale between blue (-) and yellow (+). Chromaticity value a* and b* were further used to calculate chroma [$C^* = (a^2 + b^2)^{1/2}$] and hue angle ($h^\circ = \tan^{-1} b^*/a^*$) for colour interpretation. Chroma corresponded to the intensity or colour saturation, in which low values represent dull colour while high values represent vivid colour. Hue angle represented red-purple (0˚), yellow (90˚), bluish green (180˚) and blue (270˚). The colour measurements were taken on the upper, middle and bottom part of pineapple wedges.

The firmness of fresh cut pineapple was determined using TX plus texture analyser (Stable Micro Systems, United Kingdom). The probe of P/2 stainless needle was used to penetrate the flesh of the pineapple wedges with a test speed of 1 mm/sec. The pre-test speed and the post-test speed were set as 5 mm/sec and the target distance was adjusted to 10 mm. The firmness readings were taken on the upper, middle and bottom part of pineapple wedges and the reading expressed in Newton (N). SSC was measured individually by means of a hand-held refractometer (AOAC, 1990). The samples were sliced into smaller pieces and squeezed into juice by using muslin cloth and the readings were expressed as % Brix. Total phenolic content of fresh cut pineapple determined according Wan Zaliha and Koh (2016). The total phenolic content was determined by using standard curve obtained and expressed as milligrams of gallic acid equivalents per 100 grams of fresh weight (mg GAE $100g^{-1}$ FW). Ascorbic acid concentration was being determined by using indophenol titration method according to AOAC (2004). The concentration of ascorbic acid was determined by using the standard curve obtained and expressed as mg per 100g fresh weight.

Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) using General Linear Models (GLM) procedures with SAS 9.1 software package, SAS Institute Inc, Cary, NC, USA. Treatments means were further separated by Tukey (HSD) for at least significance at $p \leq 0.05$ (SAS Institute Inc, 1999).

3. Results and discussions

A significant interaction ($p \leq 0.05$) was observed on different types of LED lights and storage temperatures on BI of fresh cut pineapple (Fig. 1). In general, the BI increased throughout 15 days of experimental periods which ranging between score 0 and score 5. The treated fruit under blue light in 5˚C able to extend the shelf

life to day 15 and had lowest browning index which was remain score 0 throughout experimental period. Meanwhile, red light treated fruit and further stored in 10˚C that had highest browning index (score 5) and had the shortest shelf life (6 days) (Fig 1 and Table 1). Similar to BI, there was a significant interaction ($p \leq 0.05$) between different LED lights and storage temperatures on PWL of fresh cut pineapple as shown in Fig2. In general, the PWL of all fresh cut pineapples had an increase trend throughout 15 days of experimental period. Interestingly, fresh cut under blue light in 5˚C had the lowest PWL throughout the whole experiment. In contrast, blue light in 10˚C had highest PWL. The higher storage temperature of 10˚C accelerated and activated the rate of weight loss as it increased the rate of metabolism that cause the loss of pigment and natural compounds. In contrast, lower storage temperature of 5˚C able to slow down the fruit metabolism that induced by fresh cut processing (Garcia and Barret, 2002). Thus, the treatment of blue LED light in 5˚C had the lowest percentage of weight loss.

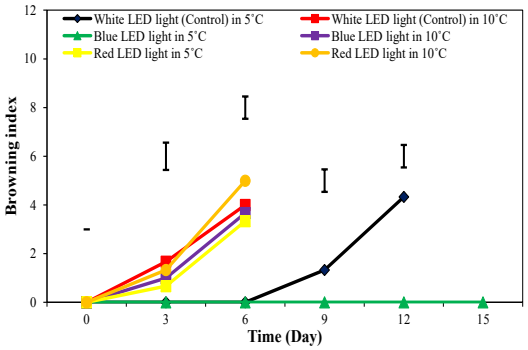


Figure1: Effects of different LED lights exposure on browning index of fresh cut pineapple stored at two storage temperatures. Vertical bars represent HSD at 5% level. (HSD value on Day 0=0, Day 3=1.12, Day 6=0.91, Day 9=0.93 and Day 12=0.93)

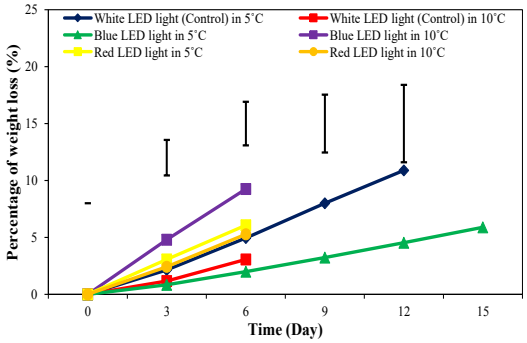


Figure2: Figure 4.12: Effects of different LED lights exposure on percentage weight loss (%) of fresh cut pineapple stored at two storage temperatures. Vertical bars represent HSD at 5% level. (HSD value on Day 0=0, Day 3=3.12, Day 6=3.83, Day 9=5.09 and Day 12=6.80)

Table1: Effects of different LED lights exposure on browning index of fresh cut pineapple stored at two storage temperatures. Number in the box denoted to browning score

Treatment/Day	0	3	6	9	12	15
White LED light (control) in 5˚C	0	0	0	1	4	
White LED light (control) in 10˚C	0	0	4			
Blue LED light in 5˚C	0	0	0	0	0	0
Blue LED light in 10˚C	0	0	3			
Red LED light in 5˚C	0	1	2			
Red LED light in 10˚C	0	1	5			

The lowest BI in fruits treated with blue light in 5˚C was affected by the increasing value of AA. In the present work, AA concentration of fresh cut pineapple rapidly declined from day 0 after fresh cut processing except sample that under blue LED light in 5˚C (Fig 3). The AA in fruits under blue light in 5˚C increased from day 0 to day 9 and then decreased continuously until day 15. This phenomenon can be explained by Zhang et al. (2015) who claimed that blue light able to increase the AA concentration by activating the expression related to metabolism of gene and increasing the enzyme in AA biosynthesis. They also claimed that the red

LED light did not affect the AA metabolism while the continuous irradiation with blue LED light at intensity of 100µmol m^{-2} s^{-1} effectively enhanced the content of AA in the three citrus varieties. Thus, the blue LED light able to induce the AA concentration. Queiroz et al. (2008) reported that AA acts as an antioxidant because it reduced the quinones produced before it undergoes secondary reactions that leading to browning. Other than that, AA also decreased the pH. Since the optimum pH to PPO ranged from 5 to 7.5, lower value of pH inhibited the enzymatic activity of PPO to induce browning (Queiroz et al., 2008). Thus, the higher the concentration of AA, the lower the browning index. Furthermore, Ibrahim (2016) reported that, low temperature could slow down the degradation of AA generally while higher temperature may be as result of oxidation reaction by residual oxygen then followed by decomposition which have been accelerated due to storage temperature.

Meanwhile, for TPC, all the treatments significantly showed an increase trend and blue light in 5˚C able to extend the shelf life to day 15 with the increasing value of TPC (Fig 4). According to Kim et al. (2014), tomato seedlings grown under the blue LED light were significantly higher in total phenolic concentration, total flavonoid concentration and antioxidant capacity compared to those grown under other wavelengths and the control light. This is because the wavelength of the blue LED light was close to that ultraviolet light. Thus, it is expected that blue LED light had a similar effect to ultraviolet light which able to activate the biosynthesis of phenolic compounds (Kim et al., 2014). Similar result with Manivannan et al. (2015) as monochromatic red and blue LED light both able to enhance the antioxidant capacities, total phenolic and flavonoids in Chinese foxglove as compared to white fluorescent light while blue light more efficient than red light. Thus, blue light induced and increased the amount of TPC. However, the higher amount of TPC did not affect the BI as shown by fresh cut fruit treated with blue LED light and stored at 5°C. This was in agreement with the report of Rocha and Morais (2001) who proved that there were no correlations found between the browning index and TPC and L* value and hue angle. There were very weak correlations were obtained between TPC and a*, b* and chroma values.

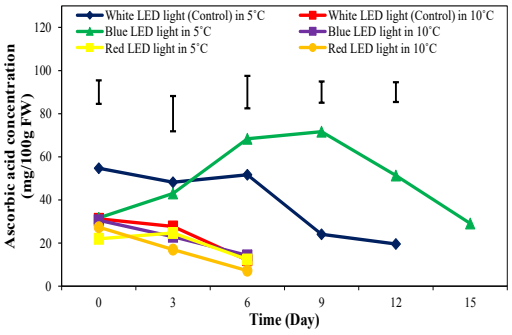


Figure 3: Effects of different LED lights exposure on browning index of fresh cut pineapple stored at two storage temperatures. Vertical bars represent HSD at 5% level. (HSD value on Day 0=0, Day 3=1.12, Day 6=0.91, Day 9=0.93 and Day 12=0.93).

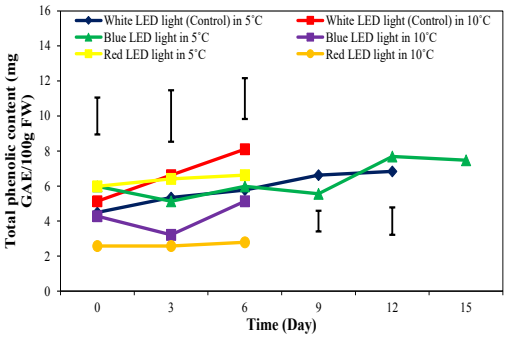


Figure 4: Effects of different LED lights exposure on percentage weight loss (%) of fresh cut pineapple stored at two storage temperatures. Vertical bars represent HSD at 5% level. (HSD value on Day 0=0, Day 3=3.12, Day 6=3.83, Day 9=5.09 and Day 12=6.80).

For fruit colour, no significant interaction was recoded in all colour attributes of fresh cut pineapples (Fig 5, 6, 7, 8, 9, 10, 11 and 12). Ding and Yap (2014) reported that the fresh cut fruit experienced BI when it haslow L* value which denotes to the loss of brightness, high b* denotes to yellowing, low h˚ value to 0 indicates redder fleshduring ripening. In the present study, treated fruit under red light had lowest value of L* and highest value of a* and b*among the LED light irradiation indicated that it experienced thesevereBI on fresh cut pineapple. Regardless of types of LED light, 10˚C which had lower value of L* and higher value of a* and b* as compared to 5˚C showed that treated fruits under 10˚C had higher browning index. This was supported by the report of Kulkarni and Karadbhajne, (2015) where the treated fruit with lower L* value and higher a* and b* value was influenced by the enzymatic browning in fresh cut pineapple.

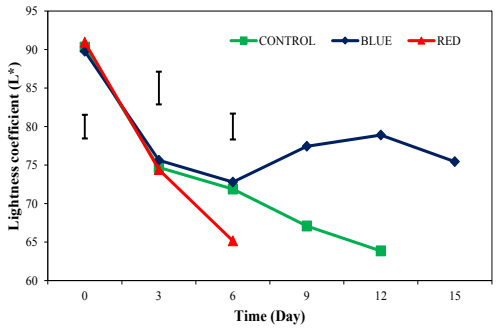


Figure 5: Effects of different LED lights on lightness coefficient (L*) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=3.07, Day 3=4.24 and Day 6=3.37)

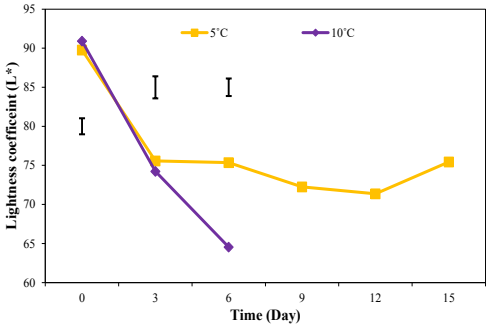


Figure 6: Effects of two storage temperatures on lightness coefficient (L*) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=2.05, Day 3=2.83 and Day 6=2.24)

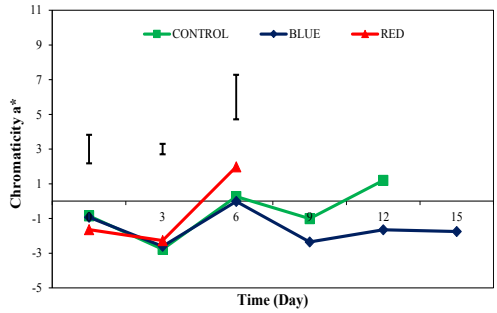


Figure 7: Effects of different LED lights on chromaticity a* of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.65, Day 3=0.60 and Day 6=2.56)

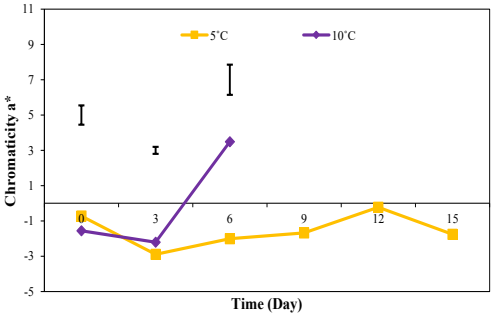


Figure 8: Effects of two storage temperatures on chromaticity a* of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.10, Day 3=0.40 and Day 6=1.71)

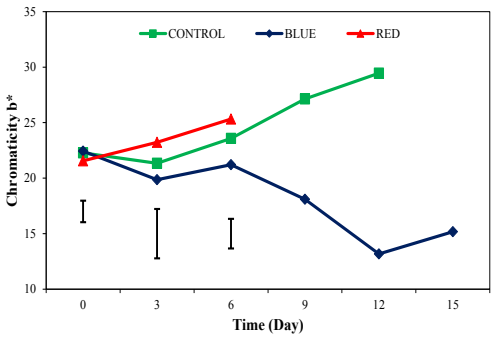


Figure 9: Effects of different LED lights on chromaticity b* of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.95, Day 3=4.45 and Day 6=2.66)

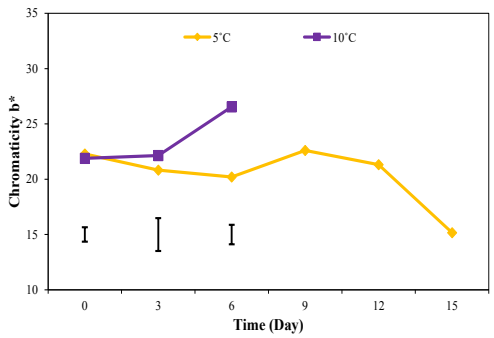
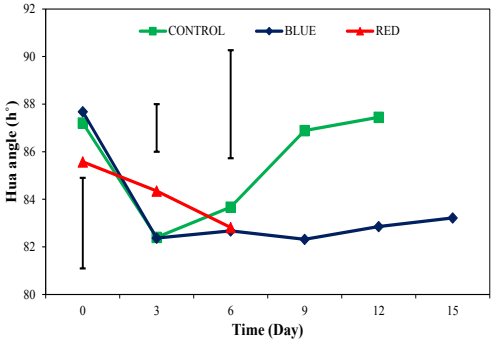


Figure 10: Effects of two storage temperatures on chromaticity b* of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.30, Day 3=2.96 and Day 6=1.77)



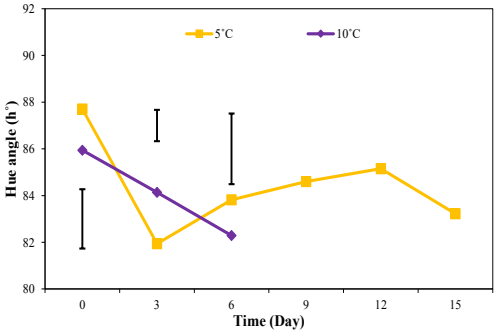


Figure 11: Effects of different LED lights on hue angle (h˚) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=3.81, Day 3=2.00 and Day 6=4.54).

Figure 12: Effects of two storage temperatures on hue angle (h˚) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=2.54, Day 3=1.34 and Day 6=3.03).

Other quality attributes such fruit firmness and SSC were not significantly interacted between the two factors (Fig 13,14, 15 and 16).Although the effect of LED light on firmness of fresh cut pineapple showed fluctuated and non-significant trend observed, but the treated fruits under blue light had higher firmness as compared to white and red light (Fig 15). Dhakal and Baek (2014) claimed that blue light able to maintain the highest level of firmness as compared to red light and darkness on tomato as blue LED light with a specific wavelength that ranging from 440-450 nm could be an integral approach to maintenance of the quality of fresh produce during storage. However, the mechanism is unknown and need further investigation. On the other hand, there was no significant effect on firmness of treated fruit under two different storage temperatures regardless LED light. However, it is believed that treated fruit in 10˚C storage temperature had lower firmness as compared to 5˚C as higher temperature accelerated the rate of metabolism to breakdown the cell wall component (Garcia and Barret, 2002) by activated pectinase enzymes such as pectin methylesterase and polygalacturonese that played role in softening and texture loss of plant tissues (Kulkarni and Karadbhajne, 2015).

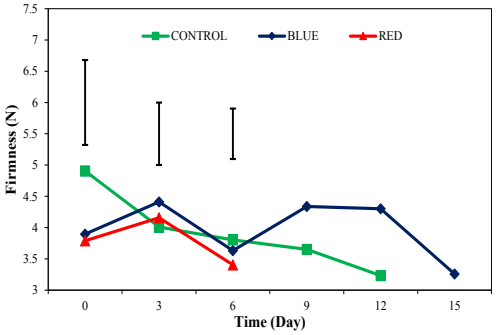


Figure 13: Effects of different LED lights on firmness (N) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.36, Day 3=1.00 and Day 6=0.80)

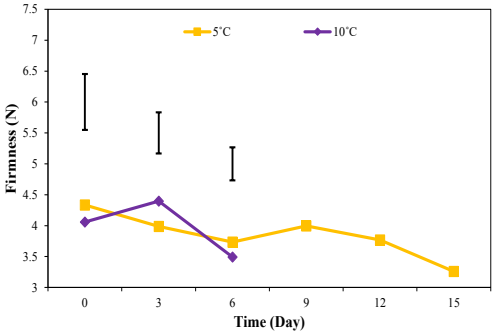


Figure 14: Effects of two storage temperatures on firmness (N) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=0.90, Day 3=0.66 and Day 6=0.54)

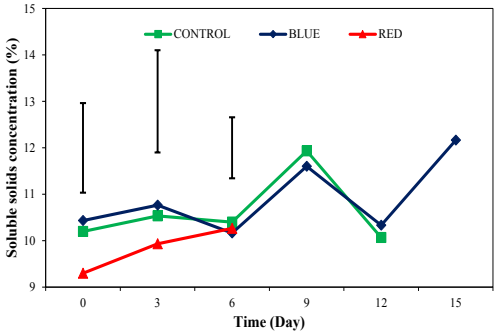


Figure 15: Effects of different LED lights on soluble solid concentration (%) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.93, Day 3=2.2 and Day 6=1.32).

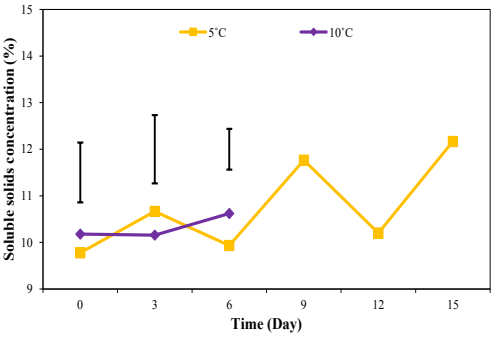


Figure 16: Effects of two storage temperatures on soluble solid concentration (%) of fresh cut pineapple. Vertical bars represent HSD at 5% level. (HSD value on Day 0=1.29, Day 3=1.47 and Day 6=0.88).

In conclusion, blue LED light in 5˚C can inhibit the enzymatic browning by enhancing the bioactive compounds and increasing the antioxidant capacity through increasing the AA

concentration in Josapine pineapple. Therefore, blue LED light exposure at 5˚C has a tendency to delay BI and maintain the postharvest quality of fresh cut pineapple as well as prolong its shelf life. For future study, it is recommended to conduct the experiment on other fresh produces such as bidara and carambola to delay the occurrence of browning and prolong the shelf life of fresh produces. Sensory evaluation test is suggested to conduct to determine the effect of LED light irradiation on organoleptic quality of fresh cut pineapple other than nutritional value and appearance. Besides that, comparison with the exposure to fluorescence light and stored in darkness can conduct to further determine the effect of LED light exposure.

4. Acknowledgement

The authors wish to thank the Universiti Malaysia Terengganu for the financial support.

References

1) Antoniolli, L. R., Benedetti, B. C., Souza Filho, M. D. S. M., Garruti, D. D. S., & Borges, M. D. F. (2012). Shelf life of minimally processed pineapples treated with ascorbic and citric acids. Bragantia, 71(3), 447-453.
2) AOAC (1990) Official Methods of Analysis. 15th ed. Arlington: Association of Official Analytical Chemists. p 394.
3) Barrett, D. M., & Garcia, E. (2002). Preservative treatments for fresh-cut fruits and vegetables. In Fresh-cut fruits and vegetables (pp. 273-309). CRC Press.
4) Benítez, S., Chiumenti, M., Sepulcre, F., Achaerandio, I., & Pujolá, M. (2012). Modeling the effect of storage temperature on the respiration rate and texture of fresh cut pineapple. Journal of food engineering, 113(4), 527-533.
5) Corbo, M. R., Speranza, B., Campaniello, D., D'amato, D., & Sinigaglia, M. (2010). Fresh-cut fruits preservation: current status and emerging technologies. Current research, technology and education topics in applied microbiology and microbial biotechnology, 2, 1143-1154.
6) Dhakal, R., & Baek, K. H. (2014). Short period irradiation of single blue wavelength light extends the storage period of mature green tomatoes. Postharvest biology and technology, 90, 73-77.
7) Ding, P., & Ling, Y. S. (2014). Browning assessment methods and polyphenol oxidase in UV-C irradiated Berangan banana fruit. International Food Research Journal, 21(4), 1667.
8) Halim, N. A. (2016). Policy intervention for the development of the pineapple industry in Malaysia [online]. Available from http://ap.fftc.agnet.org/ap_db.php?id=716&print=1 [Accessed on 20th March 2018).
9) Hassan, A, Othman Z., and Siriphanich. (2011). Pineapple (Anannascomosus L. Merr). In: Postharvest Biology and Technology of Tropical and Subtropical Fruits. Woodhead Publishing. Pp 194-217.
10) Ibrahim, M. A. (2016). Effect of different storage condition on pH and vitamin C content in some selected fruit juices (pineapple, pawpaw and watermelon). International Journal of Biochemistry Research and Review, 2, 1-5.
11) Kang, H. M., & Saltveit, M. E. (2003). Wound induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions. Postharvest biology and technology, 29 (3), 271-277.
12) Kim, E. Y., Park, S. A., Park, B. J., Lee, Y., & Oh, M. M. (2014). Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under mono-chromatic light emitting diodes. Horticulture, Environment, and Biotech-nology, 55(6), 506-513.
13) Kulkarni, S., & Karadbhajne, S. (2015). Influence of uv light exposure on shelf Life extension of fresh-cut fruits. International Journal, 3(5), 1296-1306.
14) Kumar, K., Chandra, S., Kumar. V., and Prince. (2016). Medico-nutritional importance and value-added products of pineapple –A Review. South Asian Journal of Food Technology and Environment 2: 290-298.
15) Loeillet, D. (1995, February). The world pineapple market: the importance of Europe. In II International Pineapple Symposium 425 (pp. 37-48).
16) Ma, L., Zhang, M., Bhandari, B., & Gao, Z. (2017). Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. Trends in Food Science & Technology, 64, 23-38.
17) Manivannan, A., Soundararajan, P., Halimah N., Ko, C. H., & Jeong, B. R. (2015). Blue LED light enhances growth, phytochemical

contents, and antioxidant enzyme activities of Rehmannia glutinosa cultured in vitro. Horticulture, Environment, and Biotechnology, 56(1), 105-113.
18) Manzocco, L., Quarta, B., & Dri, A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. Innovative Food Science & Emerging Technologies, 10(4), 506-511.
19) McGuire, R., G. (1992). Reporting of colour objective measurements. HortScience 27: 1254-1255.
20) O'Connor-Shaw, R. E., Roberts, R., Ford, A. L., & Nottingham, S. M. (1994). Shelf life of Minimally processed honeydew, kiwifruit, papaya, pineapple and cantaloupe. Journal of Food Science, 59(6), 1202-1206.
21) Queiroz, C., Mendes Lopes, M. L., Fialho, E., & Valente-Mesquita, V. L. (2008). Polyphenol oxidase: characteristics and mechanisms of browning control. Food reviews international, 24(4), 361-375.
22) Rocha, A. M. C. N., & Morais, A. M. M. B. (2002). Polyphenoloxidase activity and total phenolic content as related to browning of minimally processed 'Jonagored'apple. Journal of the Science of Food and Agriculture, 82(1)120-126.
23) SAS Institute Inc (1999). SAS Online Document. Version 8. Cary. North Carolina, USA.
24) Toivonen, P. M. (2004). Postharvest storage procedures and oxidative stress. HortScience, 39(5), 938-942.
25) Wan Zaliha, W. S., and Koh, S. Y., (2016). Effects of nitrogen gas fumigation on postharvest quality of minimally processed carambola (Averrhoacarambola L.) stored at low temperature. Malaysian Applied Biology 46: 189-198.
26) Xu, F., Cao, S., Shi, L., Chen, W., Su, X., & Yang, Z. (2014). Blue light irradiation affects Anthocyanin content and enzyme activities involved in postharvest strawberry fruit. Journal of agricultural and food chemistry, 62(20), 4778-4783.
27) Zhan, L., Li, Y., Hu, J., Pang, L., & Fan, H. (2012). Browning inhibition and quality preservation of fresh cut romaine lettuce exposed to high intensity light. Innovative Food Science & Emerging Technologies, 14, 70-76.

17. EFFECTS OF DIFFERENT FORMS OF PALM KERNEL SHELL CHARCOAL IN REDUCING ETHYLENE PRODUCTION AND MAINTAINING POSTHARVEST QUALITY OF BERAGAN BANANA (*Musa* sp. AAA BERAGAN BANANA)

Ang Yew Ching, Yusnita Hamzah, and Wan Zaliha Wan Sembok
School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
Corresponding author's Email: wzaliha@umt.edu.my

Abstract
Banana is a climacteric fruit that produces enough ethylene to change its physico-chemical characteristics such as colour, texture, aroma, chemical composition, respiration rate and senescence. Ethylene, a colourless gas, is the main regulator of ripening in climacteric fruits which leads to the short marketable life and increase postharvest losses. The usage of non-commercial charcoal from agricultural wastes such palm kernel shell (PKS) has been proved able to absorb ethylene and delay ripening of Berangan banana. In line with that, this present study is conducted to evaluate the effects of PKS at varying sizes (0.5 mm, 1.5 mm and 0.3 mm PKS charcoal) in reducing ethylene production in Berangan banana stored for 12 days. No apparent effect of various sizes of PKS charcoal on ethylene production and also other postharvest quality parameters were observed. Moreover, irrespective of sizes, PKS charcoal had the ability to prolong the shelf life of banana by maintaining the skin greenness and reducing starch pattern index (SPI) score. In conclusion, PKS charcoal without regard to sizes can delay ripening and maintain postharvest quality of berangan banana.

Keywords: Biochar, pyrolysis, ripen, soluble solids concentration, size

1. Introduction

Berangan Banana (*Musa* sp. AAA Berangan) is from Musaceae family (Rieger, 2006). Banana is considered as the eight most important food crop worldwide while in developed countries, it ranks as the fourth most important fruit (Ploetz, 2015). Banana can be consumed both in flesh and cooked form as ripe and raw fruit (Yakub et al., 2015). Yakub et al. (2015) reported that banana helps in reducing the risk of heart diseases and recommended for patients suffering from high blood pressure, arthritis, ulcer, and gastroenteritis and kidney disorders. About 12% of total production banana in Malaysia exported to overseas such as Singapore, Brunei, Middle East (Jain and Swennen, 2014), Hong Kong and Japan (Jamaluddin, 2014). However, there are average between 10 to 40% postharvest losses in many country due to poor infrastructure for storage, processing and marketing (Anon, 2006). This will increase the ethylene production and cause rapid deterioration especially in climacteric fruits such as banana because it reacts well with ethylene. Moreover, high production of ethylene will fasten the ripening of climacteric fruit. Previously, many postharvest treatments have been used to delay ripening of banana by using chemical based products such as 1-Methylcyclopropene (1-MCP), salicylic acid, gibberellic acid, sodium dehydroacetate (Na-DHA) and Indole-3-acetic acid (Sajib et al., 2015). However, most of these chemical based product are expensive and harmful to human if excess dose are used. One of the approaches is to use a cheaper, light, and easy to handle materials and abundant source such as Palm Kernel Shell (PKS) charcoal to delay the ripening of banana and maintain its quality. Kong et al. (2014) reported that total exports of palm oil and palm oil products reached 14.7 million t in 2010 and there are one-third of fresh fruit bunch derived by-product such as PKS and empty fruit bunch. These abundant PKS will become agriculture waste and lead to pollution problem. In order to reduce pollution, PKS undergo pyrolysis process and become PKS charcoal which is one of the possible way to reduce ethylene gas and postharvest losses.Siti Amirah et al. (2017) claimed that non-chemical-based-product such as PKS charcoal has the ability to reduce the ethylene production in Berangan banana. According to Nor Afifah et al. (2018), 75g of PKS charcoal has the ability to delay the occurrence of ethylene climacteric peak, retain the lowest score of SPI, however, not able to retard the changes of skin colour. In the present study, based on Nor Afifah et al. (2018), 100g of PKS charcoal was used but in different forms. It is assumed that the finer the size of PKS charcoal, the higher the ability to absorb ethylene as reported by Meteku (2013). Furthermore, adsorption is higher with smaller

particle size as there is a positive correlation between particle size and activity (Meteku, 2013). Therefore, this study aimed at evaluating the effects of different forms of PKS charcoal in reducing ethylene production of Berangan banana as well as delaying ripening and maintaining its postharvest quality.

2. Materials and methods

Experimental location and Experimental design

The study was conducted at the Postharvest Technology Laboratory, School of Food Science and Technology, Universiti Malaysia Terengganu. Berangan banana and PKS charcoal were purchased from local supplier in Kuala Terengganu and Malaysian Palm Oil Board (MPOB), Bangi, Selangor, respectively. The experiment was arranged in a Complete Randomized Design (CRD), with four treatments, i) control (normalform of PKS charcoal + silica gel, ii) 0.5 mm of PKS charcoal + silica gel,iii)1.5 mm of PKS charcoal + silica gel and iv) 3.0 mm of PKS charcoal + silica gel. Each treatment was replicated three times and each replicate consist of 6 banana fingers

Preparation of sample and parameter evaluations

A total of 60 hands of Berangan banana at maturity stage 2 were used and washed with 200 mg/L of sodium hypochlorite to remove dirt and left to be air dried. The ripening process of banana were initiated using 200 mg/L of ethylene for 15 seconds.Each hand of banana then placed in polyvinylchloride (PVC) air tight containers as according to the treatments. PVC containers were first sanitized with 70% alcohol to avoid decay microorganism. After that, the PVC containers contained assigned treatments were stored at ambient environment (26 ± 2°C) for 16 days.

The postharvest parameters assessment were ethylene production, starch pattern index (SPI), fruit colour, fruit firmness, soluble solids concentration (SSC), titratable acidity (TA) and ascorbic acid concentration. All the parameters were evaluated on four days interval except for ethylene production. Endogenous ethylene production from banana fruit was determined by using gas chromatography (GC). The GC was fitted with 2m long stainless steel Supleco column and a flame ionization detector (FID). Ethylene was estimated using software program and calculated from integrated areas of the sample and corresponding standard (Wan Zaliha, 2009). Fruit firmness was recorded using TA. XT plus Texture Analyzer, a stable micro system with flat steel plate mounted on the machine. The texture analyzer was calibrated together with Probe P/2N Stainless steel. Five millimeters of penetration were achieved at 1.00 mms-1 pre test speed, 0.50 mms-1 test speed and 5.00 mms-1 post test speed in (Wan Zaliha et al., 2014). The firmness values were expressed in Newton (N). While, SSC was recorded following the method of Dadzie and Orchard (1997).A 30g of pulp tissue was blended in 90 mL of distilled water for 2 minutes and filtered using muslin cloth. A single drop of the filtrate was placed on the prism of a hand-held refractometer and the reading was measured in percentage (%).Titratableacidity (TA) of banana was measured by using potentiometric method as outlined by Dadzie and Orchard (1997). The measurement of TA was expressed in percentage of malic acid. For SPI, banana was cut into 2 to 3 cm thick and the peel was separated from the pulp. One side of the cut surface of the pulp was immersed in iodine solution for a few minutes. The starch present in the pulp was reacting with iodine causing a dark blue color change. Assessment of starch pattern of each banana was observed by comparing the stain cut surface with the Starch Pattern Chart (Kader and Barett, 2005). The starch patterns indicated the relative amounts of starch and sugars. Total phenolic content was evaluated by using a modified colorimetric method as described by Singleton and Rossi (1965). Meanwhile, ascorbic acid concentration was based on the method of AOAC (1984). Banana skin colour was measured by using Konica Minolta Colorimeter. Banana skin colour was expressed as L*, chromaticity a* and chromaticity b* (McGuire, 1992). L* represents the lightness coefficient which range from 0 (black) to 100 (white), a* ranges from -60 to +60, in which +60 indicates red colour and -60 indicated green colour. b* also ranges from -60 to +60, but +60 represents yellow colour while -60 represents blue colour. a* and b* will be further used to calculate chroma, C* and hue angle, h°. Chroma, C* refers to colour intensity while hue angle rerpresents red-purple (0°), yellow (90°), bluish-green (180°) and blue (270°). Ascorbic acid concentration was measured using standard curve and express as milligram per 100g (mg/100g) fresh weight (AOAC, 1984).

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using GLM (General Linear

Models) procedures with SAS 9.1 software package, SAS Institute Inc, Cary, NC, USA. Treatments means were further separated by Tukey for least significance at P≤0.05 (SAS Institute Inc, 1999).

3. Results and discussions

Ethylene production in Berangan banana treated with different forms of PKS charcoal were similar to control (Fig. 1). The climacteric peak of ethylene production of 0.5mm PKS charcoal (0.46µg/mL) and 1.5mm PKS charcoal (0.36µg/mL) were observed on day 15 while climacteric peak of ethylene production of normal form PKS charcoal (0.23µg/mL) on day 11 and 3.0mm PKS charcoal (0.20 µg/mL) on day 13. Shukor et al. (1988) reported that climacteric peak of ethylene production tends to occur earlier with banana which have been ripen with ethylene. The earlier the occurrence of climacteric peak, the faster the ripening process, the rapid the deterioration process. PKS charcoal with different forms were not significantly affected the ethylene production of Berangan banana, however, it has been proved that it has the ability to absorb ethylene (Siti Amirah et al., 2017 and Nor Afifah et al., 2018). They claimed that PKS charcoal had the ability to absorb ethylene gas and thus reduce the ripening process in Berangan banana. Moreover, Zagord (1995) reported that ethylene can be absorbed by a number of substances including activated charcoal where the effectiveness of the adsorption was depends on the type of agricultural wastes and the porosity structure of the charcoal. Interestingly, PKS charcoal consists of porous structure and large surface area per volume so when the nature of the forces holding a solid together, it will produce a force such as Van der Waal interaction around each ion, atom, or molecule (Bansal and Goyal, 2005). Thus, non-polar ethylene was adsorbing by the porous structure of PKS charcoal due to van der Waals force.

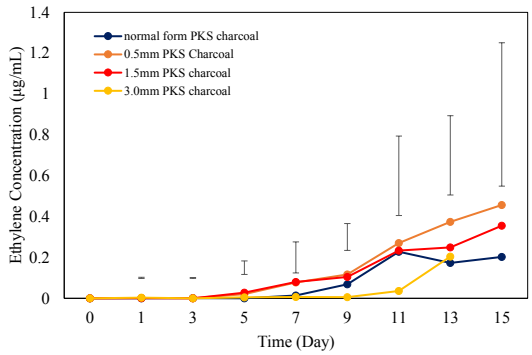


Figure 1: Effects of different forms of palm kernel shell (PKS) charcoal on ethylene concentration of Berangan banana. Vertical bars represent $HSD_{0.05}$.

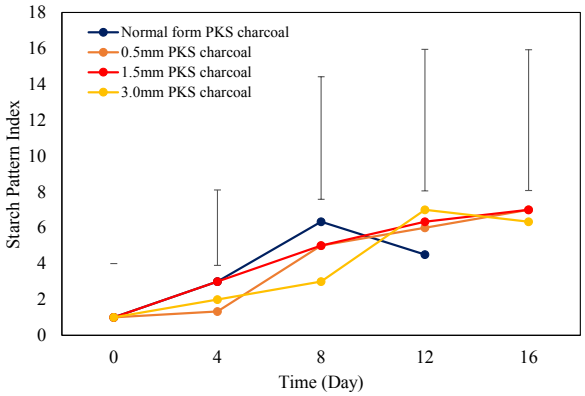
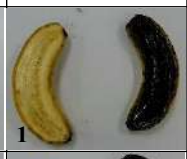
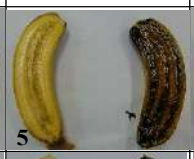
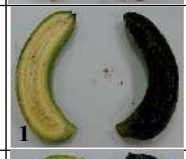
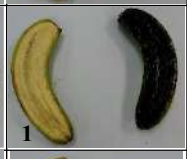
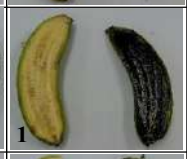
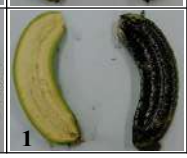
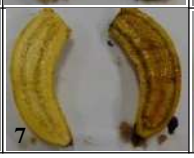
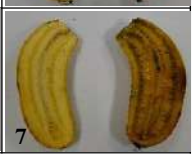


Figure 2: Effects of different forms of palm kernel shell (PKS) charcoal on starch pattern index (SPI) of Berangan banana. Vertical bars represent $HSD_{0.05}$.

For SPI, the changes of starch hydrolysis in Berangan banana stored for 16 days can be clearly seen in Fig. 2 and Table 1. However, different forms of PKS charcoal did not significantly affect the starch hydrolysis in Berangan banana stored for 16 days. The conversion of starch into sugarwas pronounced in control fruit followed by 1.5mm PKS charcoal, 3.0 PKS charcoal and 0.5mm PKS charcoal fruits (Table 1).

Normal form (control), 1.5mm and 3.0mm PKS charcoal treated fruits were fully ripen on day 12 (score 7) indicated that the starch was fully degrade into sugar. Banana treated with 0.5mm PKS charcoal had score 6 and 7 on day 12 and 16, respectively. Meteku (2013) claimed that the finer the size of PKS charcoal, the higher the ability to absorb ethylene. The conversion of starch into sugar closely related to the production of ethylene as shown in Fig 1.

Table 1: Effects of different forms of palm kernel she KS) charcoal on the starch pattern index of Berangan banana (Musa AAA Berangan). Number in the small box indicates SPI score

Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
Normal form PKS charcoal					
0.5mm PKS charcoal					
1.5mm PKS charcoal					
3mm PKS charcoal					

The production of ethylene closely related to the changes in starch hydrolysis, fruit skin colour, fruit firmness, soluble solids concentration and titratable acidity during ripening. Berangan banana fruit change its skin colour from green to yellow during ripening due to stimulation of ethylene on chlorophylls losses and resulted yellow colour of banana fruit and promotes the ripening of the pulp (Salviet, 1999). In addition, the changes in fruit colour might be ascribed to the degradation of chlorophylls to phytol which catalyzed by chlorophylase enzyme. However, the colour changes can be slow down by removing ethylene or inhibition of its action (Salviet, 1999). In the present study, no significant effect of different forms of PKS charcoal on the L*, chromaticity a*, chromaticity b* and h° were observed (Table 2). Similar results were reported by Siti Amirah et al., (2017) who claimed that PKS charcoal has the ability to remove the ethylene and thus will retain the colour changes in Berangan banana. Based on Table 2, the fruit colour changes from green to yellow represented by the lower to higher value of lightness (L*), chromaticity b* and higher to lower value of chromaticity a* and hue angle (h°) throughout the experimental period.

Table 2: Effects of different forms of palm kernel shell (PKS) charcoal on Berangan banana fruit skin colour stored at 26°C ± 2°C

Treatment/Day	0	4	8	12	16
Lightness (L*)					
Normal form (control)	55.82^{a}	72.64^{a}	68.82^{a}	67.05^{a}	-
0.5mm PKS charcoal	50.92^{a}	66.38^{a}	66.59^{a}	64.94^{a}	68.53^{a}
1.5mm PKS charcoal	56.95^{a}	64.42^{a}	66.18^{a}	68.75^{a}	67.24^{a}
3.0mm PKS charcoal	51.84^{a}	63.59^{a}	61.63^{a}	69.87^{a}	67.21^{a}
$HSD_{0.05}$	5.42^{ns}	19.10^{ns}	17.23^{ns}	20.90^{ns}	3.69^{ns}
Chromaticity a*					
Normal form (control)	-16.80^{a}	-2.88^{a}	1.83^{a}	-1.08^{a}	-
0.5mm PKS charcoal	-17.25^{a}	-4.65^{a}	-3.20^{a}	4.45^{a}	4.68^{a}
1.5mm PKS charcoal	-17.51^{a}	-5.36^{a}	-5.17^{a}	3.62^{a}	5.40^{a}
3.0mm PKS charcoal	-17.83^{a}	-9.53^{a}	-8.17^{a}	3.55^{a}	4.77^{a}
$HSD_{0.05}$	3.13^{ns}	16.28^{ns}	21.01^{ns}	15.18^{ns}	5.86^{ns}
Chromaticity b*					
Normal form (control)	31.40^{a}	52.20^{a}	49.02^{a}	48.00^{a}	-
0.5mm PKS charcoal	31.11^{a}	47.41^{a}	48.15^{a}	48.43^{a}	49.11^{a}
1.5mm PKS charcoal	31.64^{a}	44.37^{a}	44.88^{a}	50.66^{a}	46.84^{a}
3.0mm PKS charcoal	31.37^{a}	40.08^{a}	41.08^{a}	51.21^{a}	43.23^{a}
$HSD_{0.05}$	3.21^{ns}	14.66^{ns}	16.99^{ns}	15.46^{ns}	8.33^{ns}

**ns= not significant (p>0.05). Means with the same letter are not significantly different at P > 0.05

Other fruit quality attributes such as fruit firmness, SSC and TA of Berangan banana were not affected with different forms of PKS charcoal (Table 3). Although no apparent effect of different forms of PKS charcoal on the aforementioned attributes were recorded, the fruit firmness and TA tend to show a decreasing trend throughout the experimental period. The lower firmness values were recorded in fruit treated with normal form of PKS charcoal. The possible reason might be attributed to the breakdown of starch and cell wall to form sugar and soluble pectin substances are two enzymatic processes (Maftoonazad and Ramaswamy, 2005). Meanwhile, for TA, the organic acids, mainly malic acid were reduced as experimental period extended. Heather and James (1964) found a marked increase in malic acid during the earlier of banana ripening and tend to fall during ripening stages. Hailu et al., (2013) reported the same occurrence level of organic acids in fruits where the acidity is increased during ripening and as ripening advances the acidity is decline due to the utilization as respiratory substrate. During ripening, the starch of banana is fully converted into sugar and the SSC value increased. The increment of SSC is an important trait of hydrolysis starch into soluble sugars such as glucose, sucrose and fructose (Soltani et al., 2010). In the present study, SSC in fruits treated with 0.5mm to 3.0 mm PKS charcoal increased gradually which reflects to the production of ethylene during ripening process. PKS charcoal has the ability to absorb the ethylene and slow down the respiration rate of Berangan banana. Das et al. (2013) reported that a suppressed respiration rate slows down the synthesis and the use of metabolites, resulting in lower soluble solids due to the slower hydrolysis of carbohydrates to sugars. Moreover, Jafarizadeh Malmiri et al. (2011) claimed that following the decrease in the respiration rate, metabolic process and production of substrate for respiration process such as organic acid will decreased. Thus, PKS charcoal can absorb the ethylene, slow down respiration rate, slow down the hydrolysis of starch to sugar, maintain the percentage of malic acid and delay the ripening of Berangan banana. Ascorbic acid concentration (AA) in Berangan banana was not significant affected by the application of different forms of PKS charcoal except day 8 (Table 3). Regardless of PKS charcoal, AA concentrations were maintained range from 0.23 to 0.48mg/100 g fresh weight throughout the experiments.

Table 3: Effects of different forms of palm kernel shell (PKS) charcoal on fruit firmness, soluble solids concentration (SSC), titratable acidity (TA) and ascorbic acid concentration (AA) of Berangan banana stored at 26°C ± 2°C

Treatment/Day	0	4	8	12	16
Fruit firmness (N)					
Normal form (control)	5.86^{a}	2.00^{a}	1.27^{a}	2.06^{a}	-
0.5mm PKS charcoal	6.37^{a}	3.06^{a}	2.50^{a}	1.06^{a}	0.66^{a}
1.5mm PKS charcoal	6.48^{a}	4.04^{a}	2.80^{a}	1.05^{a}	0.99^{a}
3.0mm PKS charcoal	5.90^{a}	4.72^{a}	3.58^{a}	0.85^{a}	1.17^{a}
$HSD_{0.05}$	2.30^{ns}	4.15^{ns}	5.29^{ns}	3.58^{ns}	2.57^{ns}
SSC (% Brix)					
Normal form (control)	2.60^{a}	14.00^{a}	17.00^{a}	12.00^{a}	-
0.5mm PKS charcoal	2.80^{a}	9.60^{a}	16.06^{a}	17.50^{a}	18.00
1.5mm PKS charcoal	2.93^{a}	8.33^{a}	13.46^{a}	16.00^{a}	19.50^{a}
3.0mm PKS charcoal	2.93^{a}	6.86^{a}	8.80^{a}	18.35^{a}	18.83^{a}
$HSD_{0.05}$	0.56^{ns}	11.7^{ns}	17.50^{ns}	16.90^{ns}	6.10^{ns}
TA (%malic acid)					
Normal form (control)	0.16^{a}	0.51^{a}	0.42^{a}	0.38^{a}	-
0.5mm PKS charcoal	0.18^{a}	0.45^{a}	0.36^{a}	0.32^{a}	0.25^{a}
1.5mm PKS charcoal	0.21^{a}	0.45^{a}	0.36^{a}	0.37^{a}	0.35^{a}
3.0mm PKS charcoal	0.25^{a}	0.35^{a}	0.30^{a}	0.37^{a}	0.31^{a}
$HSD_{0.05}$	0.12^{ns}	0.25^{ns}	0.16^{ns}	0.29^{ns}	0.40^{ns}
AA (mg/100g FW)					
Normal form (control)	0.23^{a}	0.33^{a}	0.37^{b}	0.29^{a}	-
0.5mm PKS charcoal	0.24^{a}	0.34^{a}	0.32^{c}	0.30^{a}	0.32^{a}
1.5mm PKS charcoal	0.36^{a}	0.34^{a}	0.26^{d}	0.36^{a}	0.38^{a}
3.0mm PKS charcoal	0.27^{a}	0.36^{a}	0.48^{a}	0.30^{a}	0.38^{a}
$HSD_{0.05}$	0.14^{ns}	0.25^{ns}	0.10^{ns}	0.34^{ns}	0.64^{ns}

**ns= not significant (p>0.05). Means with the same letter are not significantly different at P > 0.05

As a conclusion, the application of PKS charcoal at various forms had the ability to prolong the shelf life of Berangan banana without significant reduction on the postharvest quality. All postharvest attributes of different forms PKS charcoal exhibited a comparable value to control forms PKS (normal form of PKS charcoal) fruits. For further study, more researches should be conducted on PKS charcoal in term of absorption of ethylene in various types of climacteric fruits and maintaining its postharvest shelf life. Research on PKS charcoal packaging would also recommendedin order to have better absorption of ethylene.

4. Acknowledgement

The authors wish to thank the Universiti Malaysia Terengganu for the financial support.

References

1) AR, N. A., MZ, S. A., MR, H. H., & WS, W. Z. (2018). The effects of charcoal from different agricultural wastes in reducing ethylene production of berangan banana (Musa sp. AAA Berangan). *UNEJ e Proceeding*, 201-210.
2) Anon (2006). Postharvest Management of Fruit and Vegetables in the Asia-Pacific Region. Asian Productivity Organization: 15-23.
3) Association of Official Analytical Chemists (AOAC) (1984). Official methods of analysis. 14th edition, pp: 844-846.
4) Bansal RC and Goyal M (2005). Adsorption Energetics, Models, and Isoterm Equations. Activated carbon Adsorption. New York: CRC Press. Pp. 67-69
5) Dadzie BK and Orchard JE (1997) Routine post-harvest screening of banana/ plaintain hybrids: Criteria and Methods. INIBAP Technical Guidelines 2. International Plant Genetic Resources Institute, Rome, Italy.
6) Das, D. K., Dutta, H., & Mahanta, C. L. (2013). Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature. *LWT Food Science and Technology*, *50*(1), 272-278.
7) Hailu, M., Workneh, T. S., & Belew, D. (2013). Review on postharvest technology of banana fruit. *African Journal of Biotech*, *12* (7).
8) Jafarizadeh, M. H., Osman, A., Tan, C. P., & Abdul, R. (2011). Development of an edible coating based on chitosan-glycerol to delay' Berangan 'banana (Musa sapientum cv. Berangan) ripening process. *International food research journal*, *18*(3).
9) Jain, S. M., and Swennen, R., (2014). Biotechnology and in vitro mutagenesis for banana improvement. Banana improvement 6: Cellular, Molecular Biology, and Induced Mutations. Agriculture and Consumer Protection, Science Publishers, Inc. Enfield, NH, USA. Pp 120-130.
10) Jamaluddin, S, H., (2014). Banana production and development for export in Malaysia. Advancing banana and plantain R&D in Asia and the Pacific 10: 123.
11) Kader, A, A., and Barrett, D, M, (2005). Classification, composition of fruits and postharvest maintenance of quality. In: Processing Fruits Science and Technology. Eds: Barett, D. M. Somogyi, L and Ramaswamy, H. 2nd edition. CRC Press Boca Raton, London. p:124
12) Kong, S. H., Loh, S. K., Bachmann, R. T., Rahim, S. A., & Salimon, J. (2014). Biochar from oil palm biomass: A review of its potential and challenges. *Renewable and sustainable energy reviews*, *39*, 729-739.
13) Maftoonazad, N., & Ramaswamy, H. S. (2005). Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *LWT-Food Science and Techn*, *38*(6), 617-624.
14) McGuire, R. G. (1992). Reporting of objective color measurements. *HortScience*, *27*(12), 1254-1255.
15) Meteku, B. E. (2013). Production of activated carbon from palm kernel shell for gold absorption using leachates from cocoa husk ask (crude potash) as activating agent. M. Sc. Thesis, School of Graduate Studies, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Pp. 12-20.
16) Ploetz, R. C. (2015). Management of Fusarium wilt of banana: A review with special reference to tropical race 4. *Crop Protection*, *73*, 7-15.
17) Rieger, M. (2006). Introduction to fruit crops. New York: The Haworth Press. Pp. 75-87.
18) Sajib B, Rahi T, Ullah E, Ghosh D and Ahmed S (2015) Delay in fruit ripening: a promising approach for reduction of spoilage and use of hazardous chemicals in Bangladesh. International Journal of Agronomy and Agricultural Research (IJAAR) 6: 163-173.
19) Salviet ME (1999) Effect of ethylene on quality of fresh fruits and vegetables.

Postharvest Biology Technology 15: 279-292.
20) SAS Institute Inc (1999) SAS Procedures Guide, Version 9.1. Cary, NC.
21) Shukor AR, Abd Abdullah H and Ahmad Shokri O (1988) Influence of exogenous ethylene on the ripening of banana (*Musa sapientum* cv. Mas). Journal of Tropical Agricultue and Food Science 16: 1-6.
22) Siti Amirah MZ, Nor Afifah AR, Husni Hayati MR and Wan Zaliha WS (2017). The effects of charcoal from different agricultural wastes in reducing ethylene production of berangan banana (*Musa* sp. AAA Berangan). Proceedings of TheInternational Conference of Food Sovereignty and Sustainable Agriculture. Jember-Indonesiapp: 201-210.
23) Smith AWJ, Poulston S, Rowsell L, Terry LA and Anderson JA (2009) A New Palladium- Based Ethylene Scavenger to Control Ethylene-Induced Ripening of Climacteric Fruit. Platinum Metals Review 53: 112-122.
24) Soltani M, Alimardani R and Omid M (2010) Prediction of banana quality during ripening stage using capacitance system. Australian Journal of Crop Science 4: 443.
25) Singleton VL and Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144–158..
26) Wan Zaliha WS (2009) Regulation of fruit colour development, quality, and storage life of 'Cripps Pink' apples with deficit irrigation and plant bioregulators. PhD Thesis. 246p.
27) Wan Zaliha WS, SitiHajar A,Yusnita H and Zuraida AR (2014) Effect of different ethylene removals on Berangan Banana (*Musa sp*. AAA Berangan). Transaction of Malaysia Society of Plant Physiology 22: 69-73.
28) Wyman, H., & Palmer, J. K. (1964). Organic acids in the ripening banana fruit. *Plant physiology*, *39*(4), 630.
29) Yakub SA, Jena SCR and Ghosh A (2015) Post harvest profile of banana: 2015 [online]. Available from https://agmarknet.gov.in/Others/CPBANANA.pdf [Accessed on 28 October 2017]
30) Zagord D (1995). Ethylene-removing packaging. In Active food packaging. Ed: M. L. Rooney. Glassgow, Blackie Academic and Professional: 38-54.

18. DETERMINATION OF IMPACT OF SOCIO-DEMOGRAPHIC DYNAMICS ON FOOD SAFETY, HYGIENIC PRACTICES AND HEALTH UNDERSTANDING OF INDIVIDUAL HOUSEHOLD IN SOUTHERN PART OF SRILANKA

S.B.Navaratne*, Rumesh Liyanage and Indira Wickramasinghe
Department of Food Science and Technology, Faculty of Applied Sciences,
University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
Corresponding author's Email: rumesh@sci.sjp.ac.lk

Abstract

Four socio-demographic dynamics age, gender, education and income each at 4 levels, except gender (At 2 levels) were selected to determine the influence of these on the food safety awareness, hygienic practices and health understanding of individual house hold in three geographic segments urban, semi urban and rural areas in southern part of Sri Lanka. A self-administrated questionnaire was developed while addressing each dynamic with 7 variables as given below.For awareness on food safety - cleaning of food, handling of raw and cooked food, proper cooking, keeping food in safe temperature, drinking safe water, proper hand washing and checking food labels.For hygienic practices – house cleaning, pest control, keeping foot ware, handling of pet animals and waste management.For health understanding - knowledge on danger zone, microorganisms, food additives, poisons compound, agrochemicals, food allergens and water quality.Two types of questions direct and descriptive were asked and 5 marks as well as 5-point scale were used to quantify the correct answers. ISO sampling plan was resorted to select the sample size and collected data were statistically analyzed. Results revealed that there was a significant difference between educational levels and income levels except age and gender of the respondents on the awareness of food safety and hygienic practices. There was also a significantdifference between educational levels, income levels and age of the respondents except gender for health understanding. Further there was a significant difference between urban and rural community on the food safety, hygienic practices and health understanding.

Keywords: Food safety, hygienic practices, health understanding, socio-demographic dynamics

1. Introduction

Food safety is an important aspect in public health and it was estimated that around 9.4 million cases of foodborne diseases causing 55,961 reported hospitalizations and 1,351 deaths are been reported in USA each year (WHO, 2004). Moreover previous studies also reveal that knowledge, attitude, understanding and practice are the key elements in preventing the occurrence of food safety hazards and health difficulties in the world. (Low *et al.*, 2016)

In Sri Lankan (SL) context, SL is also not spare from these food borne diseases; because we frequently here this social malady through social media, health bulletins and other mode of communications. This situation has alarmingly been increased among the SL consumers due to consumption of fast food, ready to cook food and cooked foods. Gravity of this situation has further been aggravated as a result of poor income, level of education and other down grading sociel issues. Thus a study on the knowledge, attitudes and practice pertaining to food safety is warranted in view of the lack of awareness on the importance of food safety knowledge (Aunger *et al.*, 2016). The major scope of this research study was to evaluate the knowledge on foodsafety, hygienic practices and health understanding and to identify the relationships between socio- demographic and aforesaid factors of the respondents in different demographical area of southern province, Sri Lanka. Since, southern province of SL representing diverse socio-demographic phenomenon along with the sheer number of population possibly around 2 500 000 people (around 12% of the country - (Department od sensus and statistics data, 2012) the selected area is well qualified to conduct this study.

2. Materials and methods

This study was conducted in southern part of Sri Lanka; because where social cross profile has

been diversified remarkably in compliance with envisage social variables of the study pertainingto the three geographic segments such as urban, semi urban and rural. After selecting the area, cross sectional data collection was performed on three important health dimensions namely Food safety awareness, Hygienic practices and Health understanding by distributing pre-prepared self-administered questioner. The number of households related to the aforesaid geographic segments was collected from Department of Census and Statistics, Sri Lanka as well as from the District Secretariat, Matara. Google Earth map was also resorted to identify topography of the study area particularly in identifying possible shortest route to approach the household of the respondents in conducting the survey.

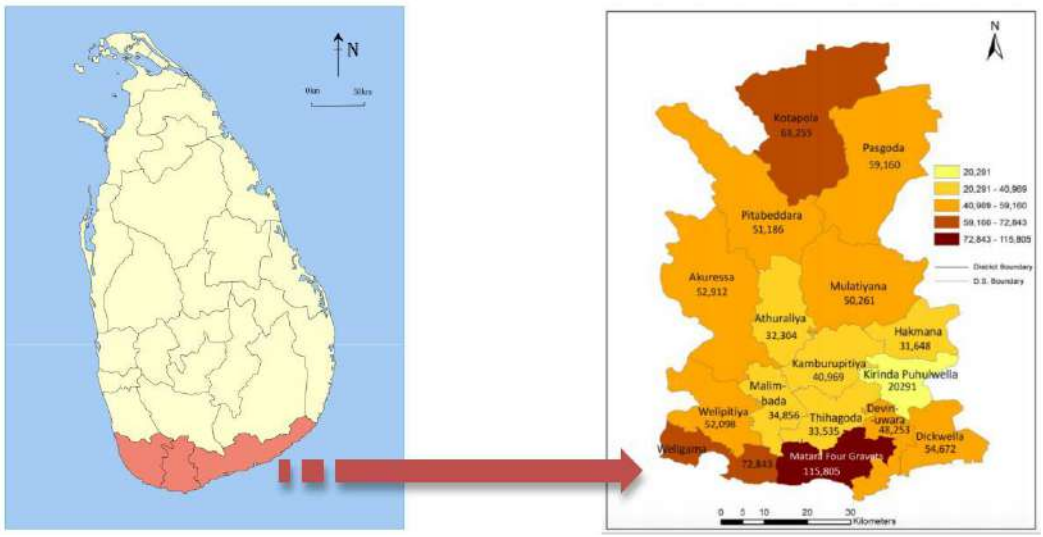


Figure 1: Detailed map of the selected geographical area.

Development of the structured questioner

The questioner was developed by incorporating and modifying questions based on the previous research to evaluate the influence of four socio-demographic dynamics age, gender, education and income each at 4 levels, except gender (At 2 levels) on the 3 objectives of the study namely food safety awareness, hygienic practices and health understanding of individual house hold in three geographic segments urban, semi urban and rural areas in southern part of Sri Lanka. Furthermore, the questionnaire was developed by addressing the three aforesaid study objectives pertaining to the **seven** variables as given below.

For awareness on food safety: cleaning of food, handling of raw and cooked food, proper cooking, keeping food in safe temperature, drinking safe water, proper hand washing and checking food labels.

For hygienic practices: house cleaning, pest control, keeping foot ware, handling of pet animals and waste management.

For health understanding: knowledge on - danger zone, micro organisms, food additives, poisons compound, agrochemicals, food allergens and water quality.

Two types of questions, Direct (based on Yes or No answers) and Descriptive (Eg: frequency of hygienic, safety and awareness practices performed) were asked and 5 marks were given for direct question and 5-point scale was used for descriptive questions.

Moreover, the questioner consists with some other personal details such as name, address, occupation, contact number, e-mail address etc.

Sampling, Data collection and analysis

Self-administered questioners were distributed among the selected household in 3 geographical locations and respondents were given 10 days interval to complete the questioner wilfully.

Before distributing the questioner, the respondents were adequately educated “how to fill thequestioner” and they were also assured that the collected data were not divulged for any purposeof a third party without their consent other than using to accomplish the objectives of the study.

So also the respondents were emphasised that the collected data would be protected and confidentiality of the data will be maintained strictly.

Nevertheless, the questioneries were distributed among all the family members of the household except the age less than 20 and morethan 70

years; because previous studies revealed that less than 20 would be vocabulary poor andstill playful and more than 70, memory power would be very low and indifference for theanswers. Hence, vague answers would be possible.
Further, ISO 2859-1: 1989 (E) sampling plan was resorted to select the number of house-hold preferably 150 from each geographical segment and also to select location of these house hold, a random table was used. Responses of the respondents obtained from direct and descriptive questions were calculated as a percentage and finally these data were analyzed using Minitab 17.0 statistical package at 95% confidence level. Desired margin errors for the percentage of outcomes were also calculated by using the test statistics given below; *at 95% confidence*

$$SE = z\sqrt{\left(\frac{p(1-p)}{n}\right)}$$

3. Results and discussion

Relationship between food safety awareness and four socio-demographic variables of respondents in 3 geographic segments

Food safety awareness of individual household pertaining to three geographical segments namely urban, semi urban and rural were determined in relation to four demographic variables such asage, gender, education and income levels. Percentages of the responses of the respondents (Inrelation to 4 socio-demographic dynamics as well as 7 variables) are given in table 1.

Relationship between food safety awareness and age

The percentage values of the responses of the respondents were statistically analyzed according to two-way parametric ANOVA and results revealed that there is a significant difference between geographic areas and food safety awareness: because calculated F value 13.7 is higher than the table value (F_8^2= 4.46). However, there is no significant difference between age and food safety awareness as calculated F value 2.37 is lower than the table value (F_8^2 = 3.34). Reason for this consequence may be due to family members in households at different age levels are used to live together in the same house or individual houses adjacent to each other. Therefore, knowledge of the person pertaining to food safety is unknowingly exchanging among the family members in spite of their age gaps. Hence, it is inevitable, in developing somewhat even knowledge in food safety among the family members in the same household or houses nearby. On the other hand, if one family member is educated on the food safety awareness, the knowledge gained pertaining to it can easily be transmitted among the family members easily. In the case of three different geographical locations, there is a less possibility to exchange the knowledge among the three communities because they are living considerably apart from each other. Hence, there is a slim possibility to exchange knowledge pertaining to the food safety among the people in three communities.

Relationship between food safety awareness and gender

The outcome of the statistical analyzed revealed that there is no significant difference between gender and food safety awareness; because calculated F value 1.0 is lower than the table value (F_2^1 = 18.5). Reason for this consequence is both genders used to live in the same house or nearby. Hence, knowledge pertaining to food safety is informally or unknowingly passing among the members. However, there is a significant difference between geographic location and food safety awareness as calculated F value 49.70 is higher than the table (F^2_2 = 19.0). Reason for this consequence is as also same as description given in the previous paragraph with respect to the section 3.1.1 “Food safety awareness and age”

Relationship between food safety awareness and educational level

Statistically analyzed data relating to educational level and food safety awareness revealed that there is a significant difference (F_8^4Cal= 14.86 >F_8^4Table=3.84) between educational level and food safety as well as geographical segments (F_8^4Cal = 13.18 > F_8^4Table=4.46) and food safety. Reason for this phenomenon is when elevating the level of education, the knowledge pertaining to food safety awareness is also concurrently increasing according to existing educational programmes and also there is a good tendency that educated persons pay more attention towards information related to food safety as well as grabbing them with a proper understanding. In the case of geographical locations where rural determinants having comparatively low knowledge due to dearth of information flows, cultural belief and

considerable number of school dropouts just after primary education.

Table 1: Relationship of food safety awareness of respondents in 3 geographic segments on four socio-demographic variables

Awareness of food safety %

Socio-demographic variables	Geographical segment		
	Urban %	Sub-urban%	Rural%
Age			
20-30	54±4.4	50±4.2	52±4.0
30-40	69±3.6	63±3.8	48±3.8
40-50	67±2.7	60±4.6	48±2.7
50-60	59±3.2	56±3.2	42±3.2
60-70	55±4.1	58±4.1	38±3.3
Gender			
Male	63±3.7	56±2.7	49±3.2
Female	61±3.7	57±4.1	47±4.2
Education level			
Primary	51±2.2	48±2.3	32±4.0
G.C.E.Ordinary Level	50±3.1	47±4.1	30±3.3
G.C.E. Advanced Level	58±3.3	57±3.3	52±3.7
Graduate	62±4.1	63±3.7	57±2.9
Post graduate	61±4.3	64±3.0	54±3.1
Income level (* 1000)			
<10	38±2.7	42±3.2	36±2.7
10-20	46±3.1	47±4.1	52±3.3
20-40	54±2.5	52±4.3	54±4.1
40-60	59±3.3	60±3.6	49±4.0
>60	61±2.7	58±3.9	53±4.4

Relationship between food safety awareness and level of income

By considering the relationship between food safety awareness and income level along with the geographical locations, there is a significant difference between income level and food safety awareness but there is no significant difference for that in geographical segments; because calculated F value 11.17 for level of income is higher than the table value (F_8^4 = 3.84); and calculated F value for location (0.88) is lower than the table value (F_8^2 = 4.46). Most probable reason for this outcome is most of high- and middle-income persons used to live either in rural or semi urban or urban locations spend a handsome amount of money (Corresponding to their income level) in spite of their geographical location for various requirements including family health too. Thus, the affluent family members in either location having an adequate knowledge in food safety awareness and most instances, which is almost par with the three communities (Urban, semi urban and rural). In addition, family members with high income levels frequently used to move with other communities (rich) in local and international as well. However, this situation becoming contrast with the low income as low-income persons generally tend to pay a little attention towards the food safety awareness. Nevertheless, this situation is further aggravated by their law literacy rate because; literacy rate is also usually subsiding along with the declining of the income.

According to the study carried out by (Low *et al.*, 2016), Overall mean knowledge score 1⁄469.5 ± 6.3 (personal food hygiene 73.0; causes of foodborne diseases 71.9; symptoms of foodborne disease 62.1; food handling practices 70.7). And overall knowledge was significantly associated with gender, level and field of study and father's educational level. Significant association was found between personal food hygiene and field of study with science students being more knowledgeable. (Low *et al.*, 2016).

Relationship between health awareness and four socio-demographic variables of respondents in 3 geographic segments

Health awareness of individual household relevant to three geographical segments namely urban, semi urban and rural were determined with respect to the four demographic variables namely age, gender, education and income levels. Percentages of the responses of the

respondents in relation to 4 socio-demographic dynamics as well as 7 variables are given in table 2.

Table 2: Relationship of health awareness of respondents in 3 geographic segments onfour socio-demographic variables

Socio-demographic Variables	Health awareness of the respondents % Geographical segments		
	Urban	Sub-urban	Rural
Age			
20-30	73±3.1	70±3.3	66±2.5
30-40	75±3.5	70±3.0	68±3.8
40-50	72±4.4	69±2.2	70±4.0
50-60	70±4.6	68±2.6	65±2.6
60-70	66±2.7	70±2.6	60±3.5
Gender			
Male	80±2.6	78±3.4	68±2.5
Female	82±3.6	80±3.8	72±3.0
Education level			
Primary	55±4.5	53±3.2	40±3.2
G.C.E.Ordinary Level	60±4.0	58±4.5	53±3.4
G.C.E. Advanced Level	74±3.5	73±4.3	60±3.5
Graduate	76±3.2	75±4.1	70±4.5
Post graduate	77±4.1	77±3.7	69±4.1
Income level (* 1000)			
<10	42±3.3	44±3.1	42±3.0
10-20		46±3.7	48±4.2
20-40	63±4.1	59±4.3	54±3.7
40-60	65±4.7	65±2.7	64±3.6
>60	73±4.1	70±2.8	70±4.1

Relationship between health awareness and age of the respondents

The percentage values of the responses of the respondents were statistically analyzed according to two-way parametric ANOVA and results revealed that there is a significant difference between geographic areas and health awareness: because calculated F value 6.5 is higher than the table value (F_8^2= 4.46). However, there is no significant difference between age and health awareness as calculated F value 2.7 is lower than the table value (F_8^2 = 3.84). Reason for this consequence is as same as the description given in the section 3.1.1.

Relationship between health awareness and gender

The statistical analysis revealed that there is no significant difference between health awareness and gender because calculated F value 10.40 is lower than the table value (F_2^1 =18.5) at 95% confidence level. Reason for this outcome is both gender is used to living together or living adjacent to each other. Under this circumstance, the knowledge relevant to the health awareness is mutually transmitting among the family members inadvertently. Thus, know-how pertaining to the health awareness among both genders alike. Therefore, when they are going to educate on the health aspects, either gender can be selected, educated and deployed to educate others. In the case of geographical segments, there is a significant difference between health knowledge and 3 locations (semi urban and rural) as calculated F value 68.70 is higher than the table value (F_2^2 =19.0) at 95% confidence level. Reason for this outcome is as same as the description given in the section 3.1.1.

Relationship between health awareness and education level

The statistical analysis revealed that there is a significant difference between educational level and health awareness as well as geographical segments and health awareness because calculated F value for both parameters 76.12 and 21.60 were higher than the table F values (F_8^4=3.84) and (F_8^2=4.46) respectively (<0.05). Reason for this outcome is as also same as the description givenin the section 3.1.3.

Relationship between health awareness and income level

The statistical analysis disclosed that there is a significant difference between income level and health awareness as calculated F value for the income level 63.70 is higher than the table F value (F_8^2 =3.84) at 95% confidence level. However, there is no significant difference between geographical segments and health awareness (<0.05) as calculated F value 0.62 is

lower than the table F (F_8^2=4.46). Reason for this phenomenon is that the income level of respondents either in urban or semi urban or rural areas are entangled with the adequate knowledge in health awareness. Thus, respondents with high or moderate-income levels in either geographic location pay more attention towards the knowledge pertaining to the health awareness.

Relationship between food hygienic practices and four socio-demographic variables of respondents in 3 geographic segments

Food hygienic practices of individual household relevant to three geographical segments namely urban, semi urban and rural were determined with respect to four demographic variables namely age, gender, education and income levels. Percentages of the responses of the respondents in relation to 4 socio-demographic dynamics as well as 7 variables are given in table 3.

Table 3: Relationship of food hygienic practices of respondents in 3 geographic segmentson four socio-demographic variables (Food hygienic practices of the respondents %)

Socio-demographic Geographical segment variables

	Urban	Sub-urban	Rural
Age			
20-30	76±3.4	74±3.3	67±3.1
30-40	85±3.6	89±3.7	70±3.3
40-50	85±3.3	88±3.6	70±3.3
50-60	78±3.5	82±2.9	73±3.2
60-70	76±3.5	74±3.5	68±3.0
Gender			
Male	83±3.3	81±3.0	76±3.4
Female	81±3.2	83±3.4	75±3.0
Education level			
Primary	75±3.3	71±3.2	60±3.0
G.C.E. Ordinary Level	83±3.7	85±3.4	72±3.1
G.C.E. Advanced Level	80±3.0	86±3.3	74±3.2
Graduate	82±3.1	80±3.7	78±3.5
Post graduate	83±3.3	84±3.2	80±3.3
Income level (* 1000)			
<10	64±2.8	65±3.0	42±3.2
10-20	72±3.7	71±3.1	66±3.5
20-40	85±3.6	83±3.3	72±3.7
40-60	84±3.7	81±3.2	85±3.7
>60	78±3.4	82±3.4	80±3.3

Relationship between food hygienic practices and age

The calculated values of the results mentioned in table 3 reveals that there is a significance difference between location, age and hygienic practices of the respondents. Because both calculated F value for age and location are (F_8^4 =4.91 and F_2^8 = 18.08) are higher than the tablevalue (F_8^4 =3.84 and F_2^8 = 4.46) respectively. Reason for this phenomenon is decline of the sanitary practices of the respondents with the aging. And also with the aging preferably above 50years, tend to disregard important hygienic practices due to their belief with experience, memory power, lethargic attitudes and numbness. Reasons for the significance differences betweenhygienic practices and location are same as description given in the section 3.1.1.

According to (Miko *et al.*, 2012), They have conducted 11 studies in Africa, Asia, and Latin America in this regard and discovered 3 categories of hygiene behavior such as, habitual, motivated, and planned. Habitual hygiene means practices learned in early childhood, often through modeling and reinforcement by the primary caregiver. (Miko *et al.*, 2012) Motivational determinants include disgust over contamination, the wish to fulfill a social norm, the desire to care for one's child, or fear of an infectious threat. Planned hygiene is the least common behavior observed such as hand washing practices. Because habitual hygiene behaviors are learned well before adulthood, they are unlikely to be useful targets for improving hand washing among college students. Both motivated and planned hygiene activities do provide opportunities for intervention in the dormitory setting. (Curtis, Danquah and Aunger, 2009)

Relationship between food hygienic practices and gender

Gender is again negligible for food hygienic practices because persons of both genders mutually exchange knowledge on food hygienic practices as they used to live same house or nearby. And also there is no significance difference in between location and hygienic practices. Reason for this out come is three communities in 3 geographical locations are followed somewhat same food hygienic practices because Sri Lankans are culturally enriched with to implement good housekeeping practices such as frequently cleaning cooking & living areas, keeping garbage aside, properly managing kitchen wastes, not allowing pest animals to come into food preparation areas etc. And also most of occasion respondents in 3 locations used to use cooked foods, clean and boiled water, etc.

Relationship between knowledge on hygienic practices and education level

Education level is very important for hygienic practices of the respondents. The study was also revealed that there is a significant difference between education level and knowledge on hygienic practices of respondents as calculated F value 8.00 higher than the table F value (F_8^4 =3.84). In addition, when considering the hygienic practices of the people in different locations of southern part of Sri Lanka, they tend to practice it into different ways. In this study also revealed that calculated F value for hygienic practices 10.00 is higher than the table F_8^2=4.46 value. However, reason for this outcome is also same as the description given in the section 3.1.3.

According to literature, a cross sectional survey to determine the food safety knowledge among youths was carried out using a questionnaire which is composed of four types of knowledge reveals, overall knowledge level of students on food safety was moderate with a mean value of 69.5%. Knowledge on Personal Food Hygiene accounted the highest score (73.0%) while knowledge on Symptoms Foodborne Diseases showed the lowest score (62.1%). Furthermore, they have poor knowledge on personal food hygiene because only 32.1% were correctly answered for question regarding hygienic practices. (Low *et al.*, 2016)

And postgraduate students had significantly higher mean score on overall knowledge compared to matriculation/diploma and undergraduate students. But, by examining the level of knowledge according to the knowledge on causes of foodborne diseases, it was found that the undergraduate students significantly had higher mean score. (Angelillo *et al.*, 2000)

Relationship between knowledge on hygienic practices and income

By considering the relationship between knowledge on hygienic practices and income levelalong with the geographical location, there is a significance difference between income level and knowledge on hygienic practices but no significance difference for geographical locations. Because calculated F value for income level 9.82 is higher than the table F value (F_8^4 = 3.84). And also calculated F value for location 2.63 is lower than the table F value (F_8^2 = 4.46). Since most of the high income generated persons (Even if they are living in rural, semi urban or urban areas) are highly sensitive for their external environments as well as their internal environments, they tend to gather more information and upgraded their knowledge relevant to their day today business activities including food hygienic practices too.

A study carried out by (El Azar *et al.*, 2009) under the theme of "Effect of Women's Perceptions and Household Practices on Children's Waterborne Illness in a Low-Income Community" showcase that housework is traditionally perceived to be a female responsibility in poor communities and they

examined the role of household practices and women's perception of health as determinants of diarrhea among children of a semi-rural, low-income community. Furthermore, the study findings suggest that diarrhea like food borne illnesses are gendered health problems, such that female children are at higher risk of suffering than male children. And female caretakers' personal hygiene, household practices, lack of awareness of health and perceptions have been identified as risk factors for food borne illnesses. (El Azar *et al.*, 2009)

4. Conclusion

The study reveals that there is no relationship between food safety awareness and age within the geographical segments (GS); but there is among the segments. There is also no relationship between food safety awareness and gender within and among GS. However, there is a relationship between food safety awareness and education within and among GS. Finally, there is a relationship between food safety awareness and income level within the segments but no among the segments.

There is a relationship between food health awareness and age within and among the GS. But there is no relationship between health awareness and gender. However there is a relationship among GS. There is also a relationship between health awareness and education level within and among GS. Lastly, there is a relationship between healthawareness and income level within the segments but no among the segments.

There is a relationship between hygienic practices and age within and among the GS. However, there is no relationship between hygienic practices and gender within and among GS. But there is a relationship between hygienic practices and education level within and among the GS and also there is a relationship between hygienic practices and income level within and among the GS. At last, when they are going to educate on the health aspects as mentioned above, either gender can be selected, educated and deployed to educate others.

References

Angelillo, I. F., Viggiani, N. M., Rizzo, L., & Bianco, A. (2000). Food handlers and foodborne diseases: knowledge, attitudes, and reported behavior in Italy. *Journal of food protection*, *63*(3), 381-385.

Aunger, R., Greenland, K., Ploubidis, G., Schmidt, W., Oxford, J., & Curtis, V. (2016). The determinants of reported personal and household hygiene behaviour: A multi country study. *PloS one*, *11*(8), e0159551.

El Azar, G. E., Habib, R. R., Mahfoud, Z., El Fadel, M., Zurayk, R., Jurdi, M., & Nuwayhid, I. (2009). Effect of women's perceptions and household practices on children's waterborne illness in a low-income community. *EcoHealth*, *6*(2), 169-179.

Curtis, V. A., Danquah, L. O., & Aunger, R. V. (2009). Planned, motivated and habitual hygiene behaviour: an eleven-country review. *Health education research*, *24*(4), 655-673.

Department of Sensus and Statistics, S. L. (2012). *No Title*. Southern province. Available at:http://www.statistics.gov.lk/PopHouSaCPH2011/Pages/Activities/Reports/Southrn.pdf.

Low, W. Y., Jani, R., Halim, H. A., Alias, A. A., & Moy, F. M. (2016). Determinants of food hygiene knowledge among youths: a cross-sectional online study. *Food Control*, *59*, 88-93.

Miko, B. A., Cohen, B., Conway, L., Gilman, A., Seward Jr, S. L., & Larson, E. (2012). Determinants of personal and household hygiene among college students in New York City, 2011. *American Journal of Infection Control*, *4 0*(10), 940-945.

WHO. (2004). *Facts and figures: Water, sanitation and hygiene links to health. Availablefrom*. Available at: http://www.who.int/water_sanitation_healthpublications/ factsfigures04/en/ (Accessed: 1 August 2014).

19.
QUANTIFICATION OF MAJOR PHYTOCHEMICAL COMPONENTS OF NONI (*Morindacitrifolia* L.) FRUIT JUICE DURING NATURAL FERMENTATION

Feliz Louie D. Salazar[1*], Erlinda I. Dizon[1], Lotis E. Mopera[1], and Maria Cynthia R. Oliveros[2]
[1]Food Science Cluster, College of Agriculture, University of the Philippines Los Baños, 4031, College Laguna
[2]Animal and Dairy Science Cluster, College of Agriculture, University of the Philippines Los Baños, 4031, College Laguna
Corresponding author's Email: flsalazar@feucavite.com.ph

Abstract
Noni, in the ancient tradition, is believed to cure variety of illnesses. Since then, number of studies existed to prove these claims. Subsequently, noni was found to contain variety of potent compounds responsible for curing diseases, among of which are anthraquinone, flavonoids, alkaloids, and tannins. Noni juice, a fermented product of noni has been a topic of interest since then. A lot of literatures calls for studies about quantification of the compounds present in noni, and its behavior during the process of fermentation. Hence, the objective of the study is to quantify the major phytochemical components, the total phenolics and DPPH-radical scavenging activity of the noni fruit juice at pre-determined time of fermentation. Results showed a decrease in the total alkaloid, flavonoid, and tannin content drop down from the initial stage up to the final stage of fermentation, with values of 141.44 ± 6.09 µg CE/mL down to 5.97 ± 0.36 µg CE/mL, 50.64 ± 3.58 µg QE/mL down to 36.89 ± 1.23 µg QE/mL, and 1656.20 ± 38.65 µg TAE/mL down to 57.56 ± 32.14 µg TAE/mL, respectively. Anthraquinone content increased from week 0 (43.79 ± 0.78 µg AE/mL) to week 3 (135.03 ± 2.93 µg AE/mL). However, a significant decrease was observed from week 4 (70.12 ± 1.92 µg AE/mL) down to week 8 (30.51 ± 1.69 µg AE/mL) of fermentation. Antioxidant and total phenolics also decreased during fermentation. Majority of the phytochemicals dropped down in amount; hence it is recommended that the fermentation condition will be controlled.

Keywords: Phytochemicals, fermentation, phenolic, antioxidant, noni juice

1. Introduction
Noni is the common name for *Morinda citrifolia*L., a tropical tree native to Australia, South East Asia like Indonesia and India, and is cultivated as well in Polynesian countries such as Tahiti and Hawaii (Chan-Blanco *et al.* 2005). The noni plant has been utilized by the ancient people to sustain their needs, whether as a source of shelter, foodstuff or home remedy to various illnesses (Nelson and Elvitch 2006). The unproven claims on the medicinal aspect, specifically the fruit's positive effect to human health led to its popularity and marketing success (Yang *et al.* 2007). These claims caught the attention of some researchers, and it was reported subsequently that there were more than 150 phytochemicals present in noni fruit, and most of which were biologically active compounds. Singh (2012), Krishnaiah*et al.* (2012), and Chan-Blanco *et al.* (2005) stated that noni fruit contains biologically active compounds that are antimicrobial, antifungal, antiviral, anthelmintic activity, antioxidant activity, hepato protective, anti-obesity and hypoglycaemic, analgesic, anxiolytic, anti-inflammatory, wound healing, hypotensive, cardiovascular, estrogenic, immunological, anticancer, and antioxidant. The major phytochemicals in noni fruit are phenolic compounds, organic acids, flavonoids, tannins, alkaloids, and anthraquinones (Alwala*et al.* 2014).Fermented noni juice has been a notable product derived from the plant, as the demand for functional beverage increase.
Despite several studies showing that noni contains various nutritional and functional compounds, most of them are still not quantified. Research on the effects of fermentation on the major bioactive chemical components is a desperate need of noni industry as of the moment (Hui and Evranuz 2012; Chan-Blanco *et al.* 2006). Hence, this study aimed to quantify the major phytochemical components of the fermented noni juice, determine the physico-chemical properties (pH, titratable acidity, and total soluble solids) at predetermined time intervals during natural fermentationand to determine the antioxidant activity and total phenolic contents at initial and final stage of fermentation.

2. Materials and methods

Procurement and Preparation of Experimental Material

The noni fruits were collected along the coastlines of Calauag, Quezon Province, Philippines. The fruits were allowed to ripen for 2 days (until soft and the light green color turned to brown or amber color) prior to bagging and fermentation.

Fermentation of Noni Fruit

Traditional production of fermented noni juice was based on the method of Kantachote*et al.* (2008). The fruits were homogenized prior to bagging. Three batches were prepared for fermentation. The fruits in each batch were packed and distributed equally into 10 polypropylene bags (0.01 mm). Each bag contained 2.75 Kg of the fruits. The bags were securely closed to protect it from the entrance of insects. The bags were then placed into a plastic bucket container where the fruits were allowed to ferment for 2 months (8 weeks) at room temperature (28-32 ^{o}C).

Determination of Phytochemical Contents

Alkaloids

The modified method of John *et al.* (2014) was used to determine the total alkaloids. Aliquots of caffeine standard solution (0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, and 2 ml) were measured. Each was transferred to different separatory funnels. A 5 mL of pH 4.7 phosphate buffer and 5 mL of bromcresol green solution were added. The mixtures were added with 1, 2, 3, and 4 mL chloroform and were vigorously shaken. The extract was then collected and the absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without caffeine. The concentration was computed using a standard calibration curve equation. Total alkaloids were expressed as µg Caffeine Equivalent (CE)/ mL of sample.

Flavonoids

The method of Pallab*et al.* (2013) was used in determining the total flavonoid content of the noni juice. The prepared stock solution was diluted to the following concentrations: 0, 6.25, 12.5, 25, 50, 80 and 100 µg/ml. The test samples were made by adding 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water and mixed well. Sample blank was prepared in similar way by using the 0 µg/mL concentration and replacing aluminium chloride with distilled water. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a Shimadzu UV-1800 spectrophotometer. The concentration was computed using the standard calibration curve equation. Total alkaloids were expressed as µg Quercitin Equivalent (QE)/ mL of sample.

Tannins

The method of Ahmad *et al.* (2012) was used in determining the total tannin content of the fermented noni fruit. In this method, 100 mg of tannic acid was placed into a 100 mL volumetric flask and was diluted into volume, 5 mL from this concentration was obtained and was diluted to 100 mL using distilled water to make 5 mg/mL stock solution. The solution was then diluted to the following concentrations: 4.5, 4, 2, 1.5 1, 0.5, and 0 mg/mL. The test samples were made by adding 0.1 mL of the standard, 7.5 mL distilled water, 0.5 mL follinciocalteu, and 1 mL sodium carbonate. Same procedure was used in the sample using 1 mL of juice extract (10 mL of juice in 10 mL of water). A calibration curve was made by measuring the absorbance of the dilutions at 700 nm with a Shimadzu UV-1800 spectrophotometer. The concentration was computed using the standard calibration curve equation. Total tannin was expressed as µg Tannic Acid Equivalent (TAE)/ mL of sample.

Anthraquinones

Total anthraquinone content was determined using the modified method of Soladoye and Chukwuma (2012).A 5 mg of alizarin standard was weighed and diluted to 50 mL of distilled water. The solution was allowed to stand for 16 hours and was then heated in a water bath at 70^{o}C for 1 hour. After the solution was cooled, 50 mL of 50% methanol was added to it. The stock solution having a concentration of 0.050 mg/mL was then used to make concentrations of 0, 0.01, 0.015, 0.020, and 0.025 mg/mL. On the other hand, the sample was prepared by diluting 1 mL of juice in 9 mL of 50% methanol. The absorbance was read at 450 nm. The concentration was computed using the standard calibration curve equation. Total anthraquinone were

expressed as µg Anthraquinone Equivalent (AE)/ mL of sample.

Determination of Total Phenolic Content

The modified method of Seruga*et al.* (2010) was used in determination of total phenolic content of the fermented noni fruit juice. A 3 mL of sample (0.01 mL of noni juice in 9.99 mL ethanol). was added to 2.25 mL Folin-ciocalteu reagent (1 part of folinciocalteu reagent and 10 parts of distilled water). The absorbance was measured at 750 nm, against a blank consisting the same reagents and 1 mL of distilled water. On the other hand, a standard curve was prepared using varying concentration of Gallic Acid (0-250 and µg/mL). The total polyphenol was expressed as gallic acid equivalents (GAE) in µg/mL product.

Determination of Antioxidant Activity

The method of Garcia et al. (2012) was used in determining the antioxidant activity of the noni fruit juice sample. The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL sample, 3 mL of absolute ethanol, and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read (Absorbance at 517 nm after 100 minute of reaction using a UV Vis Spectrophotometer). The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was expressed using the formula below:

$$AA\ \% = \frac{Abs_{sample} - Abs_{blank}}{Abs_{control}} x100$$

3. Results and discussions

Phytochemical Contents of Fermented Noni juice

The phytochemicals in noni are known to execute certain biological importance in human. Limited studies are available in the quantification of these phytochemicals specifically during the natural fermentation process of noni. In this study, the major phytochemicals in noni juice (flavonoid, alkaloid, tannin, and anthraquinone) were quantified during natural fermentation.

Flavonoids

A significant difference (Table 1) was observed from week 0 (50.64 ± 3.58 µg QE/mL) to week 1 (46.89 ± 0.70 µg QE/mL), and further decreased until the 8th week of fermentation (36.89 ± 1.23 µg QE/mL). The decrease in total flavonoid content is attributable to the oxidation brought about by the polyphenol oxidases Oxidation may occur after senescence or plant stress (wounding, elicitation by phytopathogenic microorganisms). Autoxidation of flavonoid, quercitin in particular, leads to activated oxygen and subsequently to the formation of quinones, a substrate for peroxidases, which enhances the autoxidation and induces the formation of 3, 4-digydrobenzoic acid, a well-known antifungal agent, which explains the antifungal property of noni (Konig*et al.* 2009; Osbourn and Lanzotti 2009).

Alkaloids

Shown in Table 1 is the continued decrease in the alkaloid content from week 0 to week 8 of fermentation, 141.44 ± 6.09 and 5.97 ± 0.36 µg CE/mL, respectively. Many factors influence the decrease in alkaloid content, which includes: a) *N-demethylation* (Waller and Nowacki 2012; Stumpf and Conn 1981) the most frequently described degradative step in alkaloid catabolism. For many secondary compounds including alkaloids, demethylation generally serves to increase their water reactivity, and it probably initiates more extensive process of degradation of the molecules. N-demethylation has been found to be connected with senescence of the relevant plants; b) *The alkaloids, containing an amino group, may form salts with mineral and organic acids* (Bahl and Bahl 2008; Kakhia 2016), the rise in the total titratable acidity (as shown in Table 2) could have induced salt formation, thereby decreasing the alkaloid compounds. In the case of noni fermentation, the total acid present (expressed as lactic acid) and the alkaloid

(base) present in the noni juice are both weak acid and base, respectively.

Tannins

A significant decrease in tannin was noted from week 0 (1656.20 µg TAE/mL) to week 8 (657.56 ± 32.14 µg TAE/mL) as shown in Table 1. The result suggested that along fermentation, tannic acid degrades into smaller component (Nazarni*et al.* 2016). Osman (2010) observed a decrease in the same result in the fermentation of pearl millet attributed by the microbial phenyl oxidase action. Khalifa and Tinay (1994) also observed the same result in the fermentation of low- and high-tannin cultivars of sorghum, which was due the effect of microorganisms. In the study about the assessment of the effects of microbial fermentation on selected antinutrients in local cassava from Nigeria, Etsuyankpa et al. (2015) also observed a decrease in tannin content which was attributed to the degradation of polyphenols as a result of different microorganisms that are involved in fermentation and the corresponding microbial enzymes that are released during fermentation period.

Anthraquinone

As presented in Table 1, a significant increase was observed in the anthraquinone content from week 0 followed by weeks 1, 2, and 3 with concentrations of 43.79, 64.83, 88.37, and 135.03 µg AE/mL.

Quinones are product of quercitin and polyphenol oxidation (Simic et al., 2007; Boots et al., 2005). This could explain the rise of anthraquinones in the product up to the third week of fermentation. Drop in the total anthraquinone content started on the 4th week, and continuously decrease thereafter until the final week of fermentation. This could be attributed to prolonged exposure to light, as it was bagged in a plastic material which is susceptible to contact with the light. Elizabeth (2013) reported that in microbial cell culture production of anthraquinone, the presence of light had an adverse effect on the amount of anthraquinone.

Table 1: Changes in the total flavonoid, alkaloid, tannin, and anthraquinone content of noni juice during 8-week fermentation

FERMENTATION TIME (Week)	FLAVONOID (µg QE/mL)	ALKALOID (µg CE/mL)	TANNIN (µg TAE/mL)	ANTHRAQUINONE (µg AE/mL)
0	50.64 ± 3.58^{a}	141.44 ± 6.09^{a}	1656.20 ± 38.65^{a}	43.79 ± 0.78^{f}
1	46.89 ± 0.70^{b}	62.87 ± 1.55^{b}	1613.79 ± 14.43^{a}	64.83 ± 0.44^{d}
2	43.24 ± 1.83^{c}	60.36 ± 4.33^{b}	1324.60 ± 56.34^{b}	88.37 ± 0.53^{b}
3	42.29 ± 1.08^{c}	47.01 ± 3.51^{c}	1251.34 ± 24.54^{c}	135.03 ± 2.93^{a}
4	42.06 ± 1.92^{c}	39.46 ± 0.36^{d}	1216.64 ± 49.98^{c}	70.12 ± 1.92^{c}
5	38.97 ± 0.61^{d}	25.61 ± 2.57^{e}	1099.04 ± 46.35^{d}	57.16 ± 2.83^{e}
6	37.83 ± 2.26^{d}	23.59 ± 3.17^{e}	848.42 ± 59.04^{e}	43.68 ± 1.32^{f}
7	37.50 ± 2.58^{d}	8.74 ± 1.23^{f}	709.61 ± 31.44^{f}	43.06 ± 0.76^{f}
8	36.89 ± 1.23^{d}	5.97 ± 0.36^{f}	657.56 ± 32.14^{g}	30.51 ± 1.69^{g}

*Means within the same column having the same superscript are not significantly different at P>0.05

Physico-chemical Properties of Fermented Noni Juice

Table 2 shows that total soluble solids decreased significantly in week 3 (8.07 ± 0.09 ^{o}Brix) and 4 (8.87 ± 0.04 ^{o}Brix), which explains the sugar consumption of the microorganisms. No Significant change was observed afterwards. Along with the decrease in total soluble solids is the increase in the total acidity from week 0 (1.07±0.05 %) to week 1 (4.51±0.14 %), and gradually continued to increase until the 8th week of fermentation (5.95±0.10 %), thus implicating that the sugars that is consumed by the microorganisms is converted into acids. On the other hand, despite the increasing production of acid, the total soluble solid maintained its value from week 5 to week 8. This is explained by the significant change in the total tannin content, wherein glucose is the primary breakdown product (Rodriguez *et al.* 2008). Although sugars are the major soluble solids in the fruit, there are still other factors that may contribute significantly to the TSS reading like organic acids, amino acids, and soluble pectins (Heuvelink 2005).

In line with the increasing acidity, the pH value has significantly decreased from week 1 (4.1 ± 0.00) to weeks 2 (3.96 ± 0.04), 3 (3.8 ± 0.00) and 5 (7.73 ± 0.05) until week 6 (3.7±0.00). The pH and titratable acidity did not simultaneously change, the rise in the acidity was observed in week 1, while the pH dropped at week 2. Amerine and Joslyn (1970) explained that there is no direct correlation between titratable acidity and pH, as affected by the widely varying buffer capacity of the fruit must.

Table 2: Changes in total soluble solids, pH value, total titratable acidity of noni (*Morindacitrifolia*) juice during 8-week fermentation

FERMENTATION TIME (week)	TOTAL SOLUBLE SOLIDS (°Brix)	TITRATABLE ACIDITY (% lactic acid)	pH
0	8.18 ± 0.03^{a}	1.07 ± 0.05^{f}	4.1 ± 0.00^{a}
1	8.18 ± 0.03^{a}	4.51 ± 0.19^{e}	4.1 ± 0.00^{a}
2	8.18 ± 0.04^{a}	4.83 ± 0.54^{de}	3.96 ± 0.04^{b}
3	8.07 ± 0.09^{b}	4.94 ± 0.54^{cd}	3.8 ± 0.00^{c}
4	7.87 ± 0.04^{c}	5.30 ± 0.32^{b}	3.8 ± 0.00^{c}
5	7.8 ± 0.00^{c}	5.28 ± 0.45^{bc}	3.73 ± 0.05^{d}
6	7.8 ± 0.00^{c}	5.51 ± 0.27^{b}	3.7 ± 0.00^{e}
7	7.8 ± 0.00^{c}	5.53 ± 0.26^{b}	3.7 ± 0.00^{e}
8	7.8 ± 0.00^{c}	5.95 ± 0.13^{a}	3.7 ± 0.00^{e}

Total Phenolics Content and Antioxidant Activity

As shown in Table 3, the noni fruit juice initially has enormous amount of total phenolic (2320.76 µg GAE/mL) and exhibited a high antioxidant activity (70.83%). Noni juice was reported to contain various phenolic compounds which include the alkaloids, flavonoids, tannins, and especially anthraquinones. A significant decrease was observed in values in the final stage of fermentation, both in the total phenolic (1584.49 µg GAE/mL) content and antioxidant activity (57.57%). Several publications have found a correlation between phenolic content and the antioxidant activity of various products: chinese medicinal plants (Song *et al*., 2010), red wine (Buyuktuncel*et al.,* 2014), herb spices like kesum, ginger, and turmeric (Maizura*et al*., 2011), and some root crops like yellow and purple flesh potatoes (Lachman *et al*., 2008).

Table 3: Total phenolic content and DPPH-free radical scavenging activity of noni juice at initial and final stage of fermentation

FERMENTATION TIME (week)	TOTAL PHENOLIC CONTENT (µg GAE/ mL)	DPPH-Free Radical Scavenging Activity (%)
0	2320.76 ± 4.54^{a}	70.83 ± 0.36^{a}
8	1584.49 ± 12.96^{b}	57.57 ± 2.17^{b}

Based on the result of the study, it can be concluded that the natural fermentation of noni could decrease its major phytochemical component specifically the polyphenolics and the antioxidant potential of the juice due to various factors mentioned. The following are recommended for future studies:

- Optimization of the fermentation method.
- Screening of microorganisms/ starter cultures and their effect on the phytochemical compounds in fermented noni juice.
- Comparative study on the effect of the opacity of storage material on the anthraquinone content of the juice.

4. Acknowledgement

The author would like to acknowledge the College of Agriculture and Food Science particularly the Faculties and Personnels in the Institute of Food Science and Technology of University of the Philippines Los Baños, for their intellectual and physical support for the success of this study.

References

1) Ahmad, M. S., SHEEBA, A. A., & RAI, K. B. (2012). Cancer preventive Effect of Morinda citrifolia (Noni) fruit juice against the Aflatoxin B1-inducedgenotoxicity in human peripheral lymphocytes in vitro. *IOSR J. Pharm*, *2*(2), 228-234.
2) Alwala, J., Manisha, D. R., Mandla, V. K., Bojja, S., Sreelakshmi, C., Nalvothula, R., and Rudra, M. P. (2014). Interprtative in vitro phytochemical, TLC, synthesis of silver nanoparticles and their antibacterial screening of aqueous and ethanolic extracts of *Morindacitrifolia* L. (Noni) fruit and their comparative study. World Journal of Pharmaceutical Research. 3 (6): 989-1007
3) Amerine, M. A., Amerine, M. A., & Joslyn, M. A. (1970). *Table wines: the technology of their production*. Univ of California Press.
4) Aniszewski, T. (2007). Biological significance of alkaloids. *Alkaloids-Secrets of Life. Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier. Oxford, RU*, 141-180.
5) Bahl, A., and Bahl, B. S. (2008). Advanced Organic Chemistry. Rajendra Ravindra Printers Pvt. Ltd. New Delhi, India. 1381
6) Boots, A. W., Balk, J. M., Bast, A., & Haenen, G. R. (2005). The reversibility of the glutathionyl-quercetin adducts spreads oxidized quercetin-induced toxicity. *Biochemical and biophysical research communications*, *338*(2), 923-929.
7) Büyüktuncel, E., Porgalı, E., & Çolak, C. (2014). Comparison of total phenolic content and total antioxidant activity in local red wines determined by spectrophotometric methods. *Food and nutrition sciences*, *5*(17), 1660.
8) Chan-Blanco, Y., Vaillant, F., Perez, A. M., Reynes, M., Brillouet, J. M., & Brat, P. (2006). The noni fruit (Morinda citrifolia L.): A review of agricultural research, nutritional and therapeutic properties. *Journal of Food Composition and Analysis*, *19*(6-7), 645-654.
9) Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.
10) Elizabeth, A.O. (2013). Shade conditions prevents anthraquinone production in leaves of *Vernonia amygdalina* plants. International Journal of Science and Nature. 4 (4): 713-715.
11) El Khalifa, A. O., & El Tinay, A. H. (1994). Effect of fermentation on protein fractions and tannin content of low-and high-tannin cultivars of sorghum. *Food chemistry*, *49*(3), 265-269.
12) Etsuyankpa, M. B., Gimba, C. E., Agbaji, E. B., Omoniyi, I., Ndamitso, M. M., & Mathew, J. T. (2015). Assessment of the Effects of Microbial Fermentation on Selected Anti-Nutrients in the Products of Four Local Cassava Varieties from Niger State, Nigeria. *American Journal of Food Science and Technology*, *3*(3), 89-96.
13) Garcia, E. J., Oldoni, T. L. C., Alencar, S. M. D., Reis, A., Loguercio, A. D., & Grande, R. H. M. (2012). Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Brazilian dental journal*, *23*(1), 22-27.
14) Heuvenlink, E. (2005). Tomatoes. CABI Publishing. Wageningen University, Netherlands. 157.
15) Hui, Y. H., and Evranuz, E. O. (2012). Handbook of Plant Based Fermented Food and Beverage Technology 2nd Edition. CRC Press. United States of America. 327-337.
16) John, B., Sulaiman, C. T., George, S., & Reddy V. R. K. (2014). Spectrophotometric estimation of total alkaloids in selected Justicia species. *International journal of pharmacy and pharmaceutical sciences*, *6*(5), 647-648.
17) Kakhia, T. K. (2016). Alkaloids and Alkaloids Plants. http://tarek.kakhia.org/books_eng/Alkaloids.Tarek_Kakhia.pdf
18) Kantachote, D., Charernjiratrakul, W., & Umsakul, K. (2008). Antibacterial activities of fermented plant beverages collected in southern Thailand. *Journal of Biological Sciences*, *8*(8), 1280-1288.
19) König, H., Unden, G., & Fröhlich, J. (Eds.). (2009). *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 330). Heidelberg: Springer.
20) Krishnaiah, D., Nithyanandam, R., and Sarbatly, R. (2012). Phytochemicals: A Global Perspective of

their Role in Nutrition and Health. InTech Publishers. Shanghai China. 128-146
21) Lachman, J., Hamouz, K., Sulc, M., Orsák, M., & Dvorak, P. (2008). Differences in phenolic content and antioxidant activity in yellow and purple-fleshed potatoes grown in the Czech Republic. *Plant Soil and Environment*, *54*(1), 1.
22) Maizura, M., Aminah, A., & Wan Aida, W. M. (2011). Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract. *International Food Research Journal*, *18*(2).
23) Marin, B. (Ed.). (2013). *Plant vacuoles: their importance in solute compartmentation in cells and their applications in plant biotechnology* (Vol. 134). Springer Science & Business Media.
24) Nazarni, R., Purnama, D., Umar, S., & Eni, H. (2016). The effect of fermentation on total phenolic, flavonoid and tannin content and its relation to antibacterial activity in jaruk tigarun (Crataeva nurvala, Buch HAM). *International Food Research Journal*, *23*(1).
25) Nelson, S. C., & Elevitch, C. R. (2006). *Noni: the complete guide for consumers and growers*. PAR.
26) Oliveira, M. D. C., Negri, G., Salatino, A., & Braga, M. R. (2007). Detection of anthraquinones and identification of 1, 4 naphthohydro quinone in cell suspension cultures of Rudgea jasminoides (Cham.) Müll. Arg. (Rubiaceae). *Brazilia Journal of Botany*, *30*(1), 167-172.
27) Osbourn, A. E., and Lanzotti, V. (2009). Plant Derived Natural Products: Synthesis, Function, and Application. Springer Science + Business Media, LLC. London New York, United States of America. p. 274
28) Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences*, *10*(1), 1-6.
29) Kalita, P., Tapan, B. K., Pal, T. K., & Kalita, R. (2013). Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of Biophytum sensitivum Linn. *Journal of Drug delivery and Therapeutics*, *3*(4), 33-37.
30) Saroya, A. S. (2015). Herbal Drugs as Therapeutic Agents. CRC Press Taylor and Francis Group. New York, United States of Amrica. 153.
31) Seigler, D. S. (1998). Plant Secondary Metabolism. Springer Science + Business Media. New York, United States of America. 207.
32) Šeruga, M., Novak, I., & Jakobek, L. (2011). Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectro-photometric methods. *Food Chemistry*, *124*(3), 1208-1216.
33) Simić, A., Manojlović, D., Šegan, D., & Todorović, M. (2007). Electrochemical behavior and antioxidant and prooxidant activity of natural phenolics. *Molecules*, *12*(10), 2327-2340.
34) Singh, D. R. (2012). Morinda citrifolia L.(Noni): A review of the scientific validation for its nutritional and therapeutic properties. *Journal of Diabetes and Endocrinology*, *3*(6), 77-91.
35) Soladoye, M. O., & Chukwuma, E. C. (2012) Quantitative phytochemical profile of the leaves of Cissus populnea Guill. Perr.(Vitaceae)–an important medicinal plant in central Nigeria. *Archives of Applied Science Research*, *4*(1), 200-206.
36) Song, F. L., Gan, R. Y., Zhang, Y., Xiao, Q., Kuang, L., & Li, H. B. (2010). Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. *International Journal of Molecular Sciences*, *11*(6), 2362-2372.
37) Stumpff, P. K., and Conn, E. E. (1981). The Biochemistry of Plants: A Comprehensive Treatise. Academic Press. New York, United States of America. 709-710
38) Waller, G. R., and Nowacki, E. K. (2012). Alkaloid Biology and Metabolism in Plants. Plenum Press, New York. p. 184
39) Wang, M. Y., & Su, C. (2001). Cancer preventive effect of Morinda citrifolia (Noni). *Annals of the New York Academy of Sciences*, *952*(1), 161-168.
40) Yang, J., Paulino, R., Janke-Stedronsky, S., & Abawi, F. (2007). Free-radical-scavenging activity and total phenols of noni (Morinda citrifolia L.) juice and powder in processing and storage. *Food Chemistry*, *102*(1), 302-308.
41) Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R., & Chen, S. S. (2004). Flavonoids in food and their health benefits. *Plant foods for human nutrition*, *59*(3), 113-122.

20. UTILIZATION OF TARO (Colocasia esculenta [L.] Schott) FOR INCREASED VIABILITY OF LACTIC ACID BACTERIA IN YOGHURT PROCESSING

Hazel Alena D. Tan[1], Julie D. Tan[2], Erlinda I. Dizon[3], Virginia L. Barraquio[4], Susana M. Mercado[1]
[1]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna
[2]Philippine Root Crops Research and Training Center (PhilRootCrops), Visayas State University, Visca, Baybay City, Leyte
[3]Institute of Food Science and Technology, University of the Philippines Los Baños, College, Laguna
[4]Dairy Training and Research Institute, University of the Philippines Los Baños, College, Laguna
Corresponding author's E-mail: hdtan@up.edu.ph

Abstract
Yogurt is a semi-solid fermented milk product which is considered as probiotic due to the presence of beneficial microorganisms known as lactic acid bacteria (LAB). Probiotic is defined as live microorganisms that beneficially affects the host by improving the balance of the intestinal microflora. However, for a product to be considered as probiotic, the viability of LAB must reach the optimum microflora balance of 10^6 to 10^9 cfu.ml^{-1}. Moreover, proper selection of substrates must be taken into consideration to maximize the ability of LAB for fermentation. The incorporation of taro (*Colocasia esculenta* [L.] Schott) in yoghurt is a promising development due to its high starch content and prebiotic potential. It was proven that taro was able to encourage the growth and proliferation of beneficial microorganisms. Studies involving the use of taro starch as wall material for the encapsulation of yoghurt starter cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, and as substrate, in the form of puree and syrup, encouraged the growth, proliferation and activity of the beneficial microorganisms. In addition, the encapsulation of yoghurt starter cultures using taro starch was effective in maintaining the high viability of the bacterial cells as well as protect the cells against the environment without inhibiting the activity of the lactic acid bacteria to degrade fermentable sugars into lactic acid.

Keywords: Probiotic, prebiotic, enzymatic degradation, encapsulation

1. Introduction

Yoghurt is a semi-solid fermented milk product that has been originated centuries ago. It is produced with the aid of some beneficial microorganisms called lactic acid bacteria (LAB) (Lacroix and Yildirim, 2007). Due to the presence of these microorganisms, yoghurt was considered as probiotic. Probiotic is a term which was defined as live microorganisms that beneficially affects the host by improving the balance of the intestinal flora.However, a product may only be considered as probiotic which confers health benefits to the consumer if the viability ofLAB in the product is at the optimum level, which is 10^6 to 10^9 cfu.ml^{-1} (Lacroix and Yildirim, 2007). Moreover, proper selection of substrates must be taken into consideration to maximize the ability of LAB for fermentation.

The incorporation of a potential prebiotic material in yoghurt processing has been done to improve the viability of lactic acid bacteria in yoghurt. Prebiotics have been described as non-digestible food substances that selectively stimulate the growth of favorable species of bacteria in the gut, thereby benefiting the host (Gibson and Roberfoid,1995).Various factors can affect LAB in yoghurt by decreasing the viability or by inhibition of growth. One promising technology that could maintain the viability of LAB in yoghurt is through encapsulation in calcium-alginate beads as it protects the LAB cells from adverse environmental condition. Alginate is the most commonly used biopolymer for encapsulation due to its non-toxicity, formation of gentle matrices with calcium chloride to trap sensitive materials and low cost (Krasaekoopt, *et. al.*, 2003; Sheu and Marshall, 1993).In addition, the prebiotic material can be added to the calcium-alginate matrix as it serves as stabilizer. Utilizing the prebiotic material as an alternative substrate for yoghurt promising is also a promising discovery. Given the right amount of prebiotics, the good bacteria (probiotics) would improve the viability of LAB by increasing the number and suppress the growth of undesirable microorganisms.

According to FAO (1997), the Philippines has the largest area devoted to taro in Asia proper, apart from China, with about 34, 000 hectares of land producing 117,000 tons in 1996. Though relatively taro *(Colocasia esculenta* [L.] Schott*)* has been considereda minor crop here in Philippines, it has been consumed as a staple in the diet due to its high carbohydrate content and dietary fiber (Gibson, 1995; Onwueme, 1994).Starch is the predominant carbohydrate in taro amounting to 70-80% and are highly digestible due to small-sized granules. On the other hand, the dietary fibers in taro, like prebiotics, are non-digestible carbohydrates which are typically fermented by gut bacteria. Thus, this study aimed to utilize taro in yoghurt processing and determine its effect on the viability of lactic acid bacteria as an encapsulating agent with calcium alginate and as an alternative substrate.

2. Materials and methods

Procurement of raw materials

Purple-fleshed taro variety (VG-9) was used in this study. Both the ready-made taro starch for the encapsulation and the taro root for alternative substrate were purchased at PhilRootCrops-VSU, Baybay, Leyte.

Yoghurt stater culture propagation

About 2.5-3.0% (w/v) of yoghurt starter culture was inoculated to sterile 12% reconstituted skim milk and incubated at 42°C for 3 to 6 hours.

A. Yoghurt production utilizing enzymatically-degraded Taro Puree

Production of Taro Puree through enzyme activation

Taro was prepared for enzyme activation technique in two sets – using heating alone and addition of commercial enzyme, Alpha Amylase manufactured by Sinobios (Shanghai) Imp. & Exp. Co., Ltd. Baiyin Sino Bio-Science Co., Ltd. The taro was washed, peeled, sliced into small cubes (approximately 1 cm^2) and water was added at the ratio of 1:2 (w/v). The mixture was made into puree using blender. The first set of taro puree was subjected to heat at boiling temperature for 30 minutes with constant stirring. On the second set of taro puree, 1 gram of commercial amylase was added for every 100 grams of sample to increase starch conversion and thorough blending was done to mix the enzyme with the puree. It was allowed to stand for 30 minutes and then, subjected to heat at boiling temperature for 20 minutes.

Yoghurt processing

Yoghurt was processed using different set of treatments (Table 1). The yoghurt formulations were pasteurized at 72°C for 20 seconds and cooled down to 42°C before inoculating the propagated starter culture. Afterwards, the inoculated mixtures were dispensed into glass jars as per treatment and incubated at 42°C for 4 to 6 hours. Sampling was done at 0, 2, 4 and 6 hour-fermentation for lactic acid bacteria viability, and physicochemical properties.

Table 1: Different formulations for yoghurt production

TREATMENT	FORMULATION
T_1 (*Control*)	1,200 ml yoghurt mixture + 67 g refined sugar
T_2	1,200 ml yoghurt mixture + 67 g taro puree (subjected to heat only)
T_3	1,200 ml yoghurt mixture + 67 g taro puree (treated with commercial amylase)
T_4	1,200 ml yoghurt mixture + 67 g taro-rice syrup
T_5	1,200 ml yoghurt mixture + 67 g muscovado sugar

*yoghurt mixture: *500 ml milk, 95 g skim milk, 40 ml starter culture, 632 ml distilled water*

*taro-rice syrup: *taro-red rice wine distilled to remove alcohol and concentrated to thick syrup*

B. Yoghurt starter culture encapsulation in Alginate-Taro starch matrix

Propagated starter culture was encapsulated using the emulsion technique done by Chen *et. al.* (2006). The calcium alginate-taro starch gel beads were prepared using different levels of taro starch (Table 2). Lactic acid bacteria viability determination as well as the physicochemical properties (pH and %TTA) were done at duplicate during the initial time of incubation at 42°C, after 6 hours of incubation and after 4, 8, and 12 days of storage at 4°C.

Table 2: Levels of alginate and taro starch solutions for the encapsulation of yoghurt starter culture

TREATMENT	ALGINATE (% w/v)	TARO STARCH (% w/v)
T_1 (*Control*)	0.0	0.0
T_2	4.0	0.0
T_3	4.0	0.5
T_4	4.0	1.0
T_5	4.0	1.5

Microbial and physicochemical analyses

Lactic acid bacteria viability

Serial dilution and pour plating was employed to determine the microbial count in yoghurt from the first set of experiment. Glucose Yeast Peptone-Bromcresol Purple (GYP-BCP) Agar and de Man, Rogosa and Sharpe-Bromcresol Purple Agar (MRS-BCP) were used. On the 2nd set of experiment, the viability of LAB in the calcium alginate-taro starch gel beads was determined by suspending the beads in phosphate buffer followed by gentle shaking in vortex mix for 30 minutes to destabilize the gel bead and release the entrapped bacterial cells. Serial dilution and pour plating method were also done using de Man Rogosa and Sharpe Agar with 1% Calcium Carbonate. After incubation of plates, microbial colonies were counted, computed and reported as colony forming unit (CFU) per ml of sample.

CFU/ml = (Average colonies)*(reciprocal of the dilution factor)*(reciprocal of volume plates)

Physicochemical properties

The pH of yoghurt and the yoghurt starter culture was determined using pH meter. On the other hand, the %TTA (total titratable acidity) was determined by the addition of 3 drops of 0.5% phenolphthalein into the sample and titrating the sample with 0.1 N NaOH. The %TTA was calculated using the formula below:

$$\% \text{ TTA} = \frac{\text{V1 x N x meq.wt.}}{\text{V2}} x\ 100$$

Where:

V1 = volume of NaOH added, ml
V2 = volume of sample to be titrated, ml
N = concentration of NaOH, normality, 0.1 N
Meq. Wt. = milliequivalent wt. of predominant organic acid
= Lactic Acid (0.09)

Experimental design and statistical analysis

Data on the microbial and physicochemical analyses were subjected to Analysis of Variance (ANOVA) following the Complete Randomized Design (CRD) to determine the effects. Treatments were then subjected to Duncan's Multiple Range Test (DMRT) and Tukey's-b range test to locate significant difference among treatments for the 1st and 2nd set of experiments, respectively.

3. Results and discussion

A. Yoghurt production utilizing enzymatically-degraded Taro Puree

Lactic acid bacteria viability

Generally, incubation time and microbial growth are directly proportional with each other. Longer fermentation time would increase the microbial load of the product. Maximum number of viable lactic acid bacteria was attained by treatments with refined sugar, heat-treated taro puree and enzyme-treated taro puree with microbial counts of 1.9×10^8, 1.7×10^8 and 9.7×10^9, respectively (Figure 1).

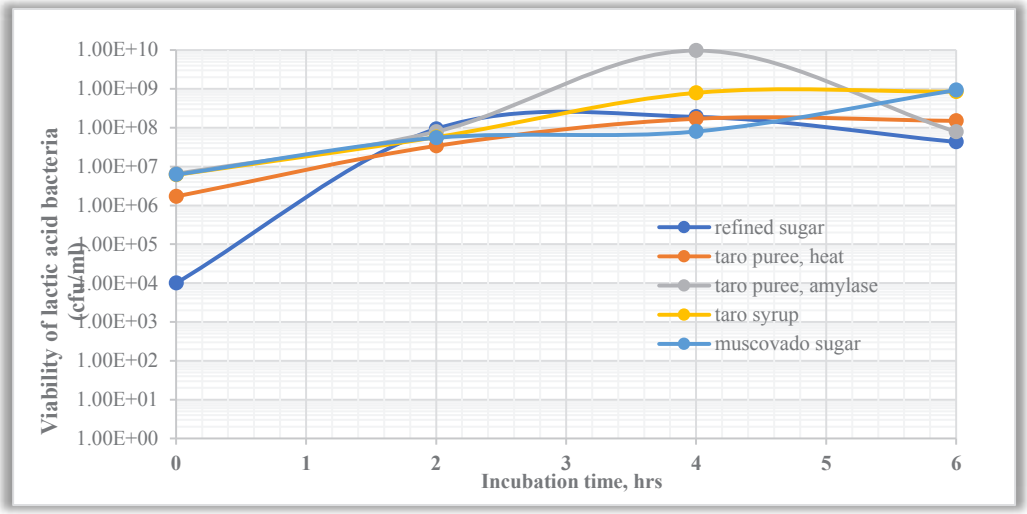


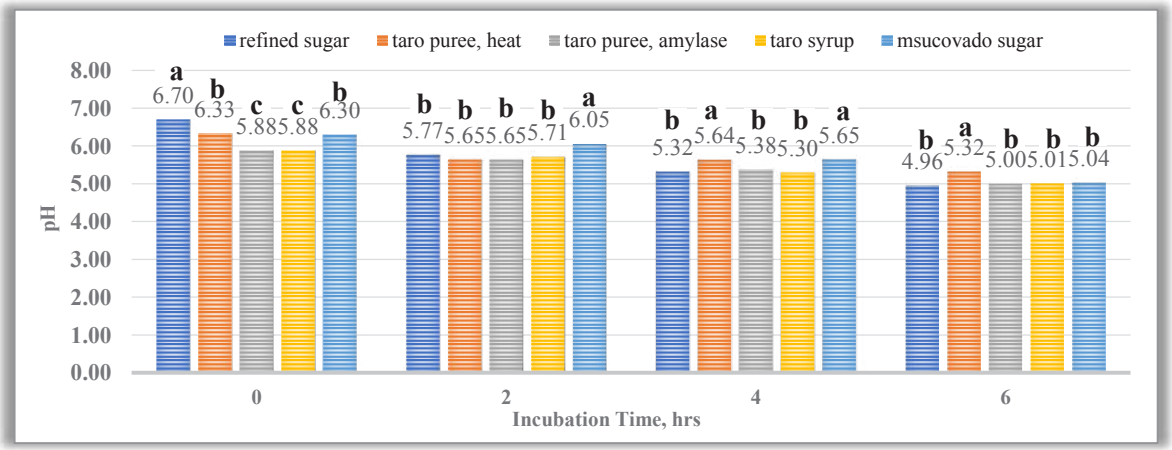
Figure 1: Lactic acid bacteria count (cfu/ml) of different treatments at different incubation time.

According to Romanowski (2010), the optimum incubation time for yoghurt is three to four hours. In addition, one possible reason for the decrease of LAB viability on the three treatments is the elaboration of various types of compounds by LAB which can be lethal to the cells themselves. In a batch fermentation process like *poi* production, upon reaching the optimum bacterial growth, decrease in cell count follows due to the production of compounds that destroys the cells and loss of cell's vigor as explained by the normal bacterial growth curve. In the case of yoghurt with taro syrup and muscovado sugar attained the maximum number of viable cells on the 6th hour of incubation with a microbial load of 8.5 x 10^8 and 9.3 x 10^9, respectively.

Physicochemical properties

The pH and the % TTA (total titratable acidity) of different yoghurt formulations were determined as well (Figures 2 and 3) to support the microbial count.In relation with the viability of lactic acid bacteria, as microbial count increases, pH decreases and acidity increases due to the production of lactic acid. According to the Australian Food Standards (Standard H8) as well as Codex Alimentarius (2008), yoghurt or any fermented milks must have an optimum pH of 4.5. Moreover, the acidity of any fermented milk must have a minimum of 0.8%, especially lactic acid.

Figure 2: Mean scores for the pH of different treatments at a given period of time.



Though the pH of all the yoghurt formulations did not reach the optimum pH after 6 hours of incubation, there was no significant difference between treatments except with the pH of yoghurt with heat-treated taro puree. This implies that the amylase-treated taro puree, taro syrup and muscovado sugar have comparable effect on the pH of yoghurt added with refined sugar.

In the case of % total titratable acidity (%TTA), there was no significant difference in the acidity of yoghurt with heat-treated taro puree after 6 hours of incubation which implies that heat-treated taro puree and refined sugar had the same effect on the acidity of yoghurt. In addition, the physicochemical properties of yoghurt with taro puree can be affected by the taro corms used at different maturities (Tan, 2010). It might be possible that corms at different maturity vary in nutrients and microbial load which may have affected the growth and proliferation of LAB, thereby, altering the physicochemical properties.

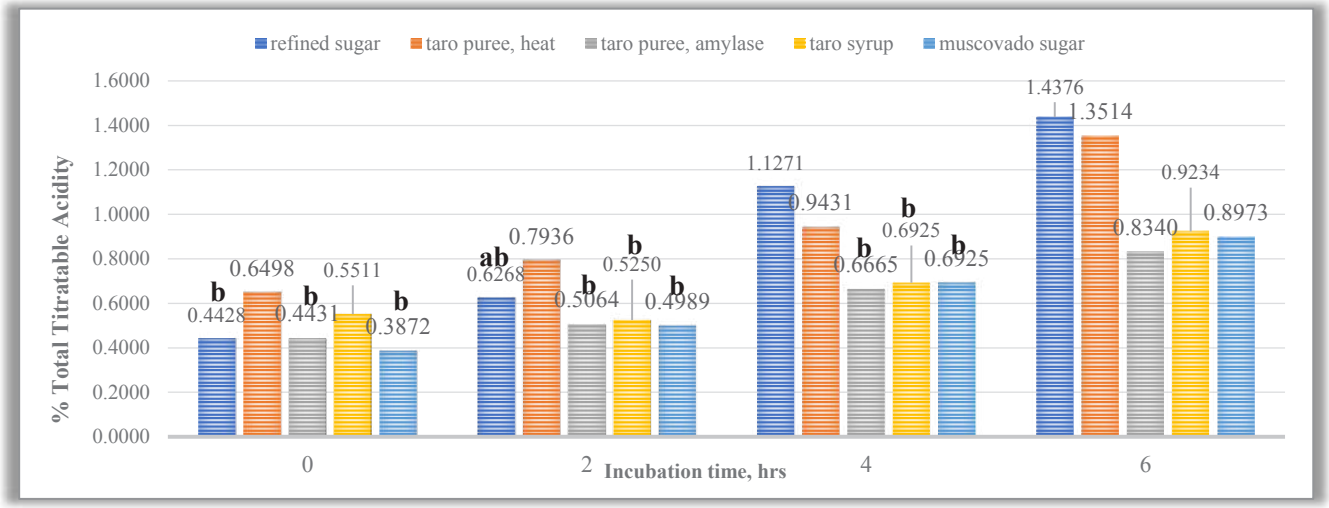


Figure 3: Mean scores for the percent total titratable acidity (% TTA) of different treatments at a given period of time.

B. Yoghurt starter culture encapsulation in Alginate-Taro starch matrix

Lactic acid bacteria viability

Microencapsulation techniques have been successfully used to enhance the fermentation of milk products for the production of concentrated lactic acid bacteria and to improve the survival of microorganisms in the product (Yoo *et.al.,* 1996). Figure 4 shows the calcium alginate-taro starch produced through emulsion technique. Figures 5 and 6 show the viability of lactic acid bacteria in the encapsulated starter culture.

Figure 4: Calcium alginate-taro starch gel beads with yoghurt starter culture.

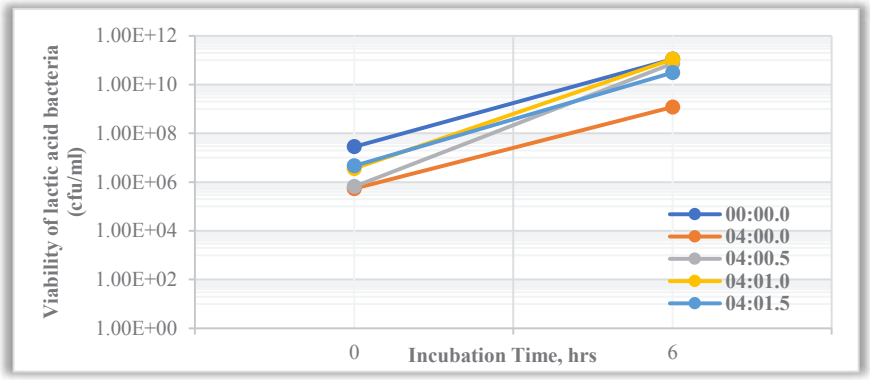


Figure 5: Viability of lactic acid bacteria in different treatments (alginate: taro starch ratio) of encapsulated yoghurt starter culture (cfu/ml) during incubation period.

The microbial load of lactic acid bacteria encapsulated with 4% alginate-1.0% taro starch after incubation period was comparable with that of the control treatment with non-encapsulated starter culture with a viability of 1.1 x 10^{11} cfu.ml^{-1}. After 12 days of storage at 4°C, all treatments maintained high LAB viability and among the treatments, yoghurt starter culture encapsulated with 4% alginate-1.5% taro starch had the highest viability with 3.4 x 10^{10} cfu.ml^{-1}(Figure 6). According to the International Dairy Federation (1995), the recommended viability of LAB in yoghurt and other fermented dairy products is about 10^{7} cfu/ml. One of the advantages of utilizing taro starch along with alginate for the encapsulation of lactic acid bacteria is the prebiotic potential of the taro which promotes the growth of the bacteria. Moreover, it has the ability to protect the microbial cells from adverse environmental conditions without inhibiting the activity of LAB to degrade fermentable sugars into lactic acid and other compounds.

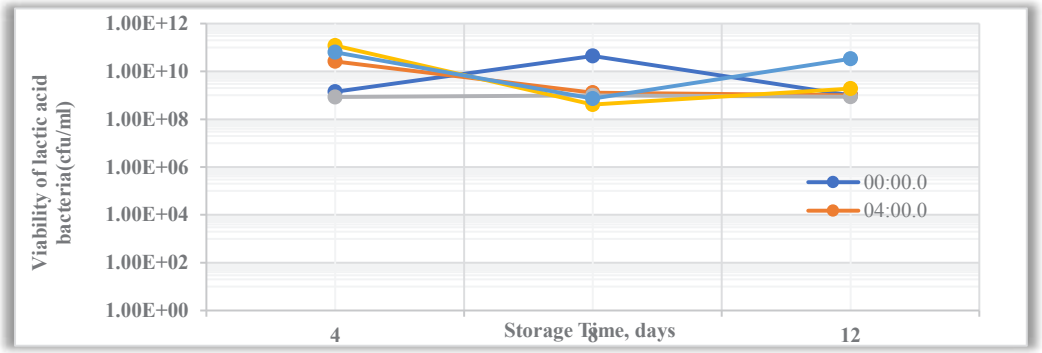


Figure 6: Viability of lactic acid bacteria in different treatments (alginate: taro starch ratio) of encapsulated yoghurt starter culture (cfu/ml) during storage period.

Physicochemical properties

Table 3: pH of fermented reconstituted skim milk as affected by different treatments of encapsulated yoghurt starter culture at given period of time

TREATMENTS (alginate:taro starch)	INCUBATION PERIOD (42°C, HOURS)		STORAGE PERIOD (4°C, DAYS)		
	0	6	4	8	12
0:0.0 (T_1)	6.50	4.60	4.55	4.40	4.50
4:0.0 (T_2)	6.40	4.90	4.65	4.55	4.55
4:0.5 (T_3)	6.45	4.80	4.65	4.55	4.55
4:1.0 (T_4)	6.40	4.75	4.65	4.45	4.50
4:1.5 (T_5)	6.40	4.75	4.60	4.50	4.50

As mentioned on the results above, the optimum pH of yoghurt is 4.5 and a minimum of 0.8% lactic acid. No significant difference was observed among treatments for both the pH and %TTA even after 12 days of storage at 4°C (Tables 3 and 4). This implies that both the non-encapsulated and encapsulated starter cultures have the same effect on the physicochemical properties of reconstituted skim milk. Moreover, further increase in the acidity upon storage can be associated with the residual activity of LAB or post-fermentation acidification which may have caused chemical alterations (Miguel *et. al.* 2004).

Table 4: Percent total titratable acidity (%TTA) of fermented reconstituted skim milk as affected by different treatments of encapsulated yoghurt starter culture at given period of time.

TREATMENTS (alginate:taro starch)	INCUBATION PERIOD (42°C, HOURS)		STORAGE PERIOD (4°C, DAYS)		
	0	6	4	8	12

0:0.0 (T_1)	0.3141	1.0964	1.2449	1.4277	1.0622
4:0.0 (T_2)	0.2741	0.7538	0.7995	1.0508	1.0964
4:0.5 (T_3)	0.2576	0.7538	0.7995	1.0051	1.2221
4:1.0 (T_4)	0.2690	0.8223	0.9137	1.0051	1.2563
4:1.5 (T_5)	0.2913	0.8566	0.8908	0.9822	1.4276

Based on the results shown above, it can be concluded that the incorporation of taro (*Colocasia esculenta* [L.] Schott) in yoghurt is indeed a promising development due to its high starch content and prebiotic potential. It was able to encourage the growth and proliferation of beneficial microorganisms as well as protect the lactic acid bacteria cells inside the alginate-taro starch gel beads without inhibiting the activity to degrade fermentable sugars into lactic acid.

Moreover, to further study the effect of taro in yoghurt, proximate analysis can be conducted to determine its effect on the nutritional quality of yoghurt. On the other hand, simulation in gastric juice and bile-acid tolerance can be conducted to determine the stability of alginate-taro starch gel beads.

4. Acknowledgement

Praise be to Him who caused everything to work together for the good. Praise be to Him who have gone before me and sustained me all throughout the conduct of the study. Praise be to Him whose grace is ever sufficient for me. For the staffs in Philippine Rootcrops Research and Training Center (PhilRootcrops-VSU) and National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB) who have been so accommodating during the conduct of the study, you are God's answered prayer. Thank you for all the assistance and push to move forward when things got rough and tough. For my family, friends and church mates, your prayers and your encouragement have not gone in vain. All glory to Him who have led me into a triumphal procession in Christ and from whom all blessings flow.

References

1) Chen, M. J., Chen, K. N., & Kuo, Y. T. (2007). Optimal thermo-tolerance of Bifidobacterium bifidum in gellan alginate microparticles *Biotech and bioeng*, *98*(2), 411-419.
2) Codex Alimentarius. (2008). Codex standard for fermented milks.
3) Food and Agriculture Organization of the United Nations (FAO). (1997). Production Yearbook 1996. (50) 96.
4) Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition*, *125*(6), 1401-1412.
5) International Dairy Federation (IDF) Bulletin No. 306. (1995). Fermented and non-fermented milk products. Detection and enumeration of *Lactobacillus acidophilus.* Culture media. *Bull. Int. Dairy Fed.* No. 306. 3:3-13.
6) Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation echniques of probiotics for yoghurt. *International Dairy Journal*, *13*(1), 3-13.
7) Lacroix, C., & Yildirim, S. (2007). Fermentation technologies for the production of probiotics with high viability and functionality. *Current Opinion in Biotechnology*, *18*(2), 176-183.
8) Miguel, D. P., Valdez, G. F., & Rossi, E. A. (2008). Sensory and chemical aspects of frozen soy yogurt fermented with Enterococcus faecium and Lactobacillus jugurti. *Alimentos e Nutrição Araraquara*, *15*(3), 197-201.
9) Onwueme IC. (1994*)*. Tropical root and tuber crops production, perspectives and future prospects. FAO Plant Production & Protection Paper 126- FAO, Rome. 228.
10) Romanoswki, P. (2010). Yogurt. How Products are Made. *http://www.madehow.com/Volume4/Yogurt.html.*
11) Sheu, T. Y., & Marshall, R. T. (1993). Microentrapment of lactobacilli in calcium alginate gels. *Journal of Food Science*, *58*(3), 557-561.
12) Tan, J. D.(2010). Production of Taro Wine from taro and black glutinous rice. Progress report of VSU Funded Projects. 2009 2011.
13) Yoo, I. K., Seong, G. H., Chang, H. N., & Park, J. K. (1996). Encapsulation of Lactobacillus casei cells in liquid-core alginate capsules for lactic acid production. *Enzyme and microbial technology*, *19*(6), 428-433.

21. SUBSTITUTING COCNUT WATER WITH BEAN SPROUT IN THE PRODUCTION OF BACTERIAL CELLULOSE (NATADECOCO)

Nguyen Thi Ngan[1], Dinh Thi Dinh[1], Le Thi Thu Thuy[1], and Nguyen Thi Nguyen Thao[2]
[1]Dong Nai University of Technology
[2]Phu Yen University
Corresponding author's Email: nguyenthingan@dntu.edu.vn

Abstract
Bacterial cellulose (Nata de coco) is a chewy, translucent, jelly-like food produced by the fermentation of coconut waterwhich gels through the production of microbial cellulose . Originating in the Philippines, nata de coco is most commonly sweetened as a candy or dessert, and can accompany a variety of foods, including pickles, drinks, ice cream, puddings, and fruit cocktails.The microbial celluloseused is *Acetobacter xylinium*, This study determined the feasibility of using bean sprout to replace coconut water in producing natadecoco Optimal result of coconut water substitution in a 1 litre of the culture included: 10% juice of bean sprout (100g bean sprout), 20% v/v ethanol 98%, 20g sugar, 4 ml acetic acid, ratio of monosunfat / amino phosphate is 2 : 1, and pH = 3.6-4.1 .

Keywords: Bacterial cellulose, Natadecoco, bean sprout, *Acetobacter xylinium*

1. Introduction

Bacterial cellulose (Nata-de-coco) is a chewy, jelly-like food, and quite popular snack in Vietnam and other countries. Nata-de-coco, a bacterial cellulose product, which is usually prepared by *Acetobacter xylinum* grown in mature coconut water. This product is not high nutrient, less energy and high cellulose content.

Polysaccharide-producing microorganisms are simple and capable of constructing a polymer from available raw materials and secondary raw material sources. Products from beets (molasses, sugar syrup, and saccharose), corn (starch, hydrolyzed starch, glucose syrup, and glucose), and potatoes (starch and starch hydrolyzates) can be used for producing such polymers. Inedible substrates such as peat hydrolyzates, wood, dextran production wastes, petrochemical wastes and products, ethanol, methanol, glycerin, and ethylene glycol are often suitable.

Bacterial cellulose maybe made from pineapple juice, but Coconut juice seems to be a better substrate than pineapple juice. In order to be able to produce natade coco from localities where coconut water is not available, we conduct research on the production of nata de coco from coconut water substitute extracted from bean sprouts. This method had advantage of easy and cheap. The antioxidant activity of bean sprouts was studied in many researches. Active ingredients in different lengths of bean sprouts were extracted with water. Concentrations of the main proteins and polyphenols were determined. Germination of mung beans dramatically increased vitamin C content in mung bean sprouts in a time-dependent manner and reached the peak on day 8 of germination up to 285 mg/100 g DW, almost 24 times higher than the initial concentration in mung bean seeds ($p < 0.05$).

2. Materials and methods

Materials

Acetobacter xylinium from Bach Khoa University. The organism was grown in the medium of $(NH_4)_2SO_4$ 8g, $(NH_4)_2HPO_4$ 2g, sugar 20g, coconut water 1000L shake in 24 hours at 30^oC.

Prepare the medium: Juice of 100g bean sprout (boil bean sprout in 20 - 25 minutes, supplement SA/ DAP, sugar/ pasteurize this solution was heated to a temperature of 100 ^oC in 10- 15 minutes and continued cooling to 30^oC / This fermentation is mixed with CH_3COOH 40% to change pH to 3.6 to 4.1 at 28 – 32^oC.

Methods

Samples were collected at regular intervals for 2 weeks of fermentation to quantify cell mass, substrate

consumption, and bacterial cellulose production.

Dry weight of bacteria cellulose (BC)

To determine the dry weight of BC, the purified samples were dried at 45 °C until reaching constant weight and kept for further characterization.

Statistical analysis

The data were analyzed by microsoft excel (version 2007) and Stargraphic was used to test for the least significant different (LSD) with the confident interval of 95%.

3. Results and discussion

Choose the optimal medium to get the good biomass

Table 1: Four Culture Mediums were prepared to experiment choose the optimal medium

Component (g/l)	Medium 1	Medium 2	Medium 3	Medium 4
Coconut water (ml)	1000	0	0	0
Sugar (g)	20	20	20	20
Amonsunfat (g)	8	8	8	8
amonhydrophotphat	2	2	2	2
bean sprout (g)	0	100	100	100
Ethanol 98%	0	0	40	40
CH_3COOH (ml)	0	0	5	5

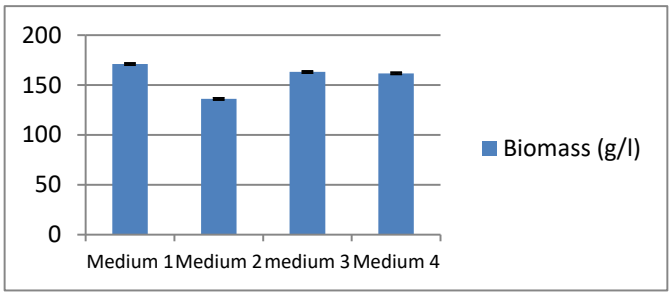


Figure 1: Biomass in the medium.

With Four Culture Mediums were prepared to experiment choose the optimal medium was shown in table 1**, b**ased on the results presented Figure 1, we found bacteria *Acetobacter xylinium* was capable of producing biomass in all four environments. However, in medium (**3**) there was the highest volume and approximately medium 1 equivalent to the biomass of coconut water. Therefore, we choose medium 3 to carry out further experiments, as in medium **(3)** has many advantages over medium 2 and medium 4 and more the material is easy to find, very plentiful and cheap.

Study on effect of pH value to the biomass

Table 2: Influenced of pH on Biomass

CH3COOH (ml)	pH first	Biomass (g/l)	pH after 12 days
3	4.13	$152.43^{b} \pm 1.5$	3.65
4	3.8	$158.38^{a} \pm 1.5$	3.61
5	3.71	$138.98^{c} \pm 1$	3.52
6	3.6	$123.59^{d} \pm 1.5$	3.51
7	3.56	$114.31^{e} \pm 1.5$	3.4
8	3.4	$118.15^{e} \pm 1.5$	3.2

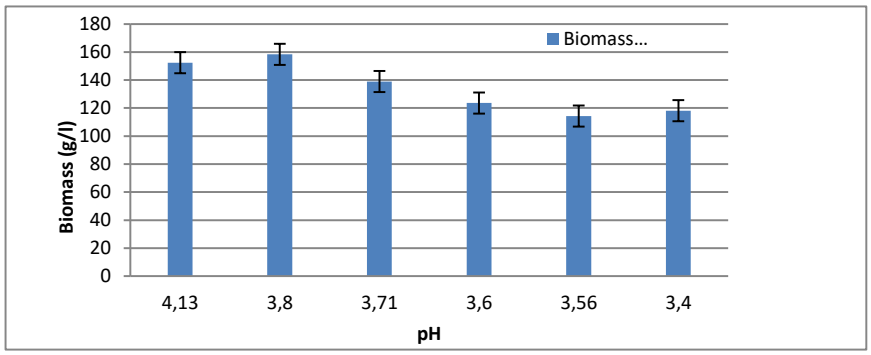


Figure 2: The rate of biomass growth depends on the pH of the medium.

Result in table 2 and Fig 2, we see that pH of culture medium directly influences to producing biomass of *Acetobacter xylinium*. The acetic acid was broken down to carbon dioxide and water which allow more energy generated by bacteria. Thus, leading to more efficient utilization of sugars for cellulose synthesis.

Final pH of culture medium was reduced. Accoding to our results, the optimum pH for the grow of *Acetobacter xylinium* 3.6- 4.13. At pH value 3.8 (volume of CH_3COOH 4ml, the biomass is largest.

There were significant differences in biomass various pH of culture medium (with $p<0.05$ at confidence level 95%). So that we choosed pH 3.8 (4 ml acetic acid).

Study on effecting of bean sprout juice

The medium was choosen the optimum and change bean sprout juice 50g, 100g, 150g, 200g in 1 litre medium. pH = 4. Result was showed in fig 3.

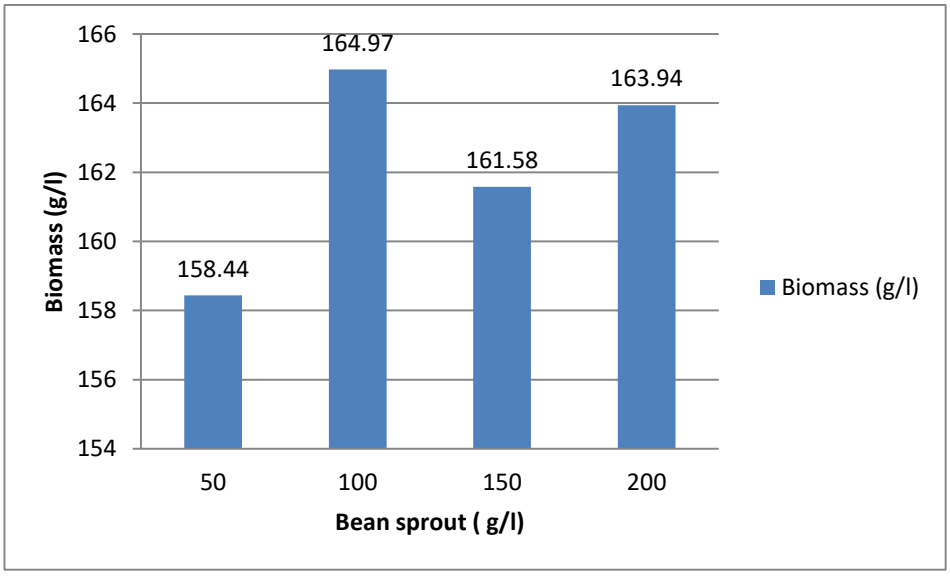


Figure 3: The rate of biomass growth depends on the Bean sprout of the culture medium.

There weren't significant differences in biomass various bean sprout of culture medium (with $p<0.05$ at confidence level 95%). We chose mass of bean sprout 100g/l to avoid squander and the biomass largest.

Effecting of ethanol on biomass

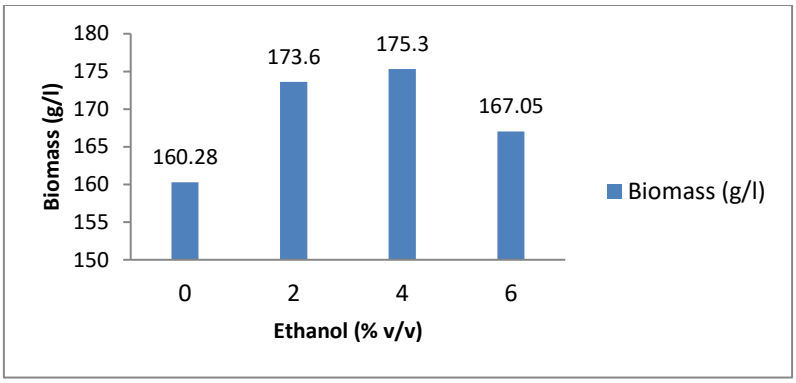


Figure 3: The rate of biomass growth depends on the Ethanol of the culture medium.

Fig 3 shown that supplement ethanol in culture media made increase biomass of *Acetobacter xylinium*. However, ratio of ethanol increases to 6%, biomass begin down. Maybe, inhibition of cell growth by the generated acetate. [4]. Fermention process is optimum at ethanol from 2-4%. There weren't significant differences in biomass various ratio of ethanol of culture medium (with $p<0.05$ at confidence level 95%). We chose ethanol 2% to the next experiment.

Influence of inorganic matter ratio Monosunphat/Aminophotphat (SA/DAP) to medium on biomass

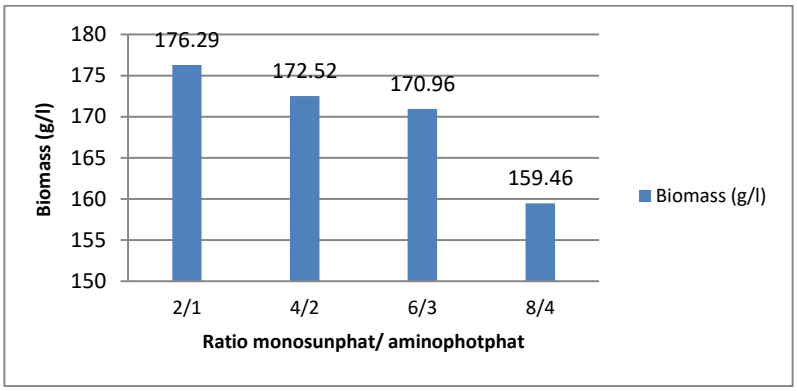


Figure 4: The rate of biomass growth depends on the Ratio monosunphat/ aminophotphat of the culture medium.

Based Fig 3, we see that biomass depend on SA/DAP. When we incerease ratio of SA/DAP to 8/4 biomass down. At 3 ratio 2/1, 4/2 and 6/3 there weren't significant differences in biomass various ratio of SA/DAP of culture medium (with $p<0.05$ at confidence level 95%). We choose optimum ratio SA/DAP 4/2 (4g SA/ l medium and 2g Dap/l medium).

4. Conclusion

Optimal result of coconut water substitution in a 1 litre of the culture included: 10% juice of bean sprout (100g bean sprout), 20% v/v ethanol 98%, 20g sugar, 4 ml acetic acid, ratio of monosunfat / amino phosphate is 2: 1, and pH = 3.6-4.1. Bean sprout juice is suitable to production Bacteria cellulose.

References

1) Dongyan, T., Yinmao, D., Li, L., Yueheng, L., Congfen, H., & Jixiang, L. (2014). Antioxidant activity in mung bean sprouts and safety of extracts for cosmetic use. *Journal of cosmetic science*, *65*(4), 207-216.

2) George, J., & Sabapathi, S. N. (2015). Cellulose nanocrystals: synthesis, functional properties, and applications. *Nanotechnology, science and applications*, *8*, 45.
3) Hsu, G. S. W., Lu, Y. F., Chang, S. H., & Hsu, S. Y. (2011). Antihypertensive effect of mung bean sprout extracts in spontaneously hypertensive rats. *Journal of food biochemistry*, *35*(1), 278-288.
4) Naritomi, T., Kouda, T., Yano, H., & Yoshinaga, F. (1998). Effect of ethanol on bacterial cellulose production from fructose in continuous culture. *Journal of fermentation and bio engineering*, *85*(6), 598-603.
5) Shamolina, I. I. (1997). Prospects for use of microbial raw material for fabrication of fibre and film materials. Review. *Fibre Chemistry*, *29*(1), 1-8.
6) Vandamme, E. J., De Baets, S., Vanbaelen, A., Joris, K., & De Wulf, P. (1998). Improved production of bacterial cellulose and its application potential. *Polymer Degradation and Stability*, *59*(1 3), 93-99.
7) Tua, M. F. A. K. (2017). Effects of agitation conditions on Bacterial Cellulose production by Acetobacter xylinum 0416 in fermentation of matured coconut water medium *Malaysian Journal of Analytical Sciences*, *21*(1), 261-266.

22. INVESTIGATION OF ORGANOCHLORINE PESTICIDE RESIDUES DURING THE PRODUCTION PROCESS OF SUGAR

Wilailak Janjamroon[1], Wiparat Nisapai[2], Pongsert Sriprom[1]*
[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[2]Faculty of Engineering, Chaiyaphum Rajabhat University, Nafai, Mueang Chaiyaphum province, Thailand
Corresponding author's Email: pongsert.sr@kmitl.ac.th

Abstract

Sugarcane is raw material for sugar production. Most sugarcane cultivation has used organochlorine pesticides. Some organochlorine has been banned such as DDT and HCH which were contaminated in crop and environmental. This study investigates organochlorine pesticides residues in sugar production processes. Sugarcane juice, sugarcane bagasse, sugar and molasses are samples which were collected from 4 regions (northern, northeastern, eastern and central) in Thailand. Samples were extracted using Quick Easy Cheap Effective Rugged Safe (QuEChERS) extraction method. Organochlorine pesticides extracted were analyzed by Gas Chromatograph-Mass Spectrometer (GC-MS). The result showed that p, p' DDE and o, p' DDT was the highest OCPs contaminated in bagasse. γ-HCH, β-HCH, heptachlor, aldrin, o, p' DDT, and p, p'-DDE were found in all sample source. However, sugar product and molasses had found low level of organochlorine pesticide was eliminated by high temperature process. Therefore, organochlorine pesticide residues in sugar processing products were investigated which concerned to monitoring pesticide residues in sugarcane cultivated in Thailand and aimed to study the risk assessment for consumer in the future.

Keywords: Organochlorine pesticides, sugar processing products, QuEChERS

1. Introduction

All of sugar production in Thailand based on sugarcane that the farmers used pesticides to control weeds and insects on sugarcane fields. Most widely pesticides that they were used in the past was organochlorine pesticide (OCPs) notably dichlorodiphenyl-trichloroethane (DDTs), hexachlorocyclohexane (HCHs), dieldrin, endrin, and heptachlor. Some OCPs had more ten years haft-life (Zhang et al, 2017). It leads to contamination from environment to sugarcane crop, especially sugar, is the major product was direct and indirect consume and secondary products include bagasse and molasses was used as raw material in feed production. Moreover, molasses is one alternative ingredient in food products such as sauce, soy sauce, bakery and beer (Hickenbottom, 1996)had an opportunity of OCPs contamination.

OCPshad difference substances included DDTs, HCHs, hexachlorobenzene, pentachlorobenzene, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene and chlordecone. The excess OCPs residue can be accumulated in crop and affects the consumer health (Manviri et al, 2017). They were banned entirely worldwide in 2001 (Stockholm Convention on Persistent Organic Pollutants) (UNEP, 2009).Thailand was not approved DDTs in 1983, followed by aldrin, dieldrin and heptachlor in 1988 and HCHs (β-HCH and γ-HCH) in 2001.

Previous studies had reported levels of pesticides in environment and crop. Mace et al (2017) was studied OCPs that contaminated in soil and raw food in rural area of Tajikistan, DDTs and γ-HCH was detected in entire study areas (0.70-25.00 mg/kg). Miensah (2007) had detected Endrin, Heptachlor, and α-endosulfan in the range of 0.0050 - 0.027 mg/kg in Ghana maize samples. Skrbić and Predojević (2008) studied OCPs contaminated in sugar related product from Vojvodina, Serbia, DDTs in sugar, beet pulp and molasseswere 0.132, 0.220 and0.820 mg/g respectively. HCHs in sugar, beet pulp and molasseswere 0.188, 0.073, 0.05 mg/kg. However, there is no study on sugarcane and sugar processing products in Thailand. Thus, the purpose of this study is to investigate organochlorine pesticide residue in sugar processing products include sugar cane juice, bagasse, sugar and molasses.

2. Materials and methods

Chemicals used in this study were pesticide grade and HPLC grade. Acetonitrile (C_2H_3N)and petroleum ether 40-60 EC were purchased from QReC, New Zealand. Magnesiumsulfateanhydrous($MgSO_4$),sodium sulphate anhydrous ($NaSO_4$) and florisil (heat for 3 h at 135 ^oC) were purchased from Merck,Germany. Sodium chloride (NaCl) was purchased from Ajax Finechem, Australia. Primary secondary amine (PSA) (40 lm particle size) was obtained from Varian. Toluene ($C_6H_5CH_3$)andacetone(C_3H_6O) were purchased from Labscan,China.

Sugarcane juice, bagasse, raw sugar, white sugar and molasses were collected from 4 sugar production regions of Thailand (northern, northeastern, eastern and central). Sugarcane was crushed and the juices were stored in glass bottle with lock caps and kept in frozen at -18 ^oC until analysis.

The total of 40 samples were prepared using extraction and cleaned up. Sugarcane juice samples were performed using a modified method of Quick Easy Cheap Effective Rugged and Safe (QuEChERS). The 10 mL of sample was transferred into a centrifuge tube. The 10 mL of acetonitrile were added and the solution was mixed using a vortex mixer for 1 min. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added and the solution was mixed again for 1 min. The tube was centrifuged for 10 min at 1200 rpm. Cleaned-up was performed according to Lehotay (2007). The 4 mL of upper layer solution was transferred to a centrifuge tube containing 200 mg PSA and 600 mg anhydrous $MgSO_4$. The extract was mixed using a vortex for 30 sec and then centrifuged for 3 min at 3500 rpm. 2 mL of the upper layer were transferred into an evaporated flask and evaporated in a water bath at 82°C until total dryness. The extract was diluted in 0.5 mL toluene before GC-MS analysis.

Raw sugar, white sugar, bagasse and molasses were performed using AOAC Official method 970.52 (1996). 20 g of sample were extracted with a 350 mL 35% (v/v) distilled water in acetonitrile, blend 5 min at high speed and suction filtered through a Buchner funnel fitted with Whatman paper no. 40. The filtrate was added in separator funnel and re-extract with petroleum ether 100 mL, shaked 1 min. After that, the sample was added by 10 mL saturated sodium chloride solution, and 600 mL distilled water, shaked 30 sec andremoved the aqueous phase. Then, rinse with 100 mL distilled water for 2 times. After extracted, 15 g anhydrous sodium sulphate was added. The extracted solution was transferred through florisil. After cleaned-up, the solution was concentrated in a evaporator at 60^oC until dry and redissolved in 0.2 ml acetone before GC-MS analysis.

OCPs analysis was carried out on a GC–MS (Model Perkin Elmer Clarus 500, USA) equipped with Elite-5MS capillary column 30m x 0.25mm, 0.25µm (Perkin Elmer, USA) employing the following conditions: Injector temperature was 240°C and 1.0 μL of sample was injected. The oven temperature was started at 80 $^{\circ}C$ (held for 4 min), increased by 30 $^{\circ}Cmin^{-1}$ to 200$^{\circ}C$ (held for 1 min), and finally increased by 2.5 $^{\circ}Cmin^{-1}$ to 240 $^{\circ}C$ (held for 1 min). The carrier gas was helium, 99.999%, at a flow rate of 0.8 $mLmin^{-1}$. The MS was operated in the full scan EI mode. The mass range was 40–550 Da with a 0.5 s/scan with 70 eV generating electrons energy. The data system contains all the software required for calibration, collection of GC/MS spectra and data processing for qualitative and quantitative analysis. Also, it contains a NIST library with more than 62,000 mass spectra for standard compounds.

3. Results and discussion

Organochlorine pesticide residue in sugarcane juice, bagasse, raw sugar, white sugar and molasses samples were investigated shown in fig.1. The percentage of sugarcane juice samples that detected β-HCH, γ-HCH, heptachlor, o,p'-DDT and p,p'-DDE were 50.0%, 100%, 87.5% 87.5% and 50.0% respectively (fig1.a). The percentage of bagasse samples that detected β-HCH, γ-HCH, heptachlor, aldrin, o,p'-DDT, p,p'-DDE and dieldrin were entire 100% (fig1.b). The percentage of raw sugar samples that detected β-HCH, γ-HCH, heptachlor, aldrin, o,p'-DDT, p,p'-DDE and dieldrin were 71.4%, 100.0%, 71.4%, 71.4%, 100% 100% and 71.4% (fig1.c). The percentage of white sugar samples that detected β-HCH, γ-HCH, heptachlor, aldrin, o,p'-DDT, p,p'-DDE and dieldrin were 75.0 %, 100.0%, 87.5%, 100%, 100% 100% and 50.0% (fig1.d). The percentage of molasses samples that detected β-HCH, γ-HCH, heptachlor, aldrin, o,p'-DDT, p,p'-DDE and dieldrin were 75.0 %, 100.0%, 75.0%, 75.0%, 100% 100% and 25.0% (fig1.d). This indicated that prohibited OCPs remain in the cultivated area and reached to sugarcane crop and sugar processing products. Skrbic and Predojevic (2008)

reported that the most frequently determined OCPs residue in crop and related products was identified p, p'-DDT of all samples analyzed at 76.9%, followed by γ-HCH (66.7%), β-HCH (48.7%), and endosulfan II (41.0%).

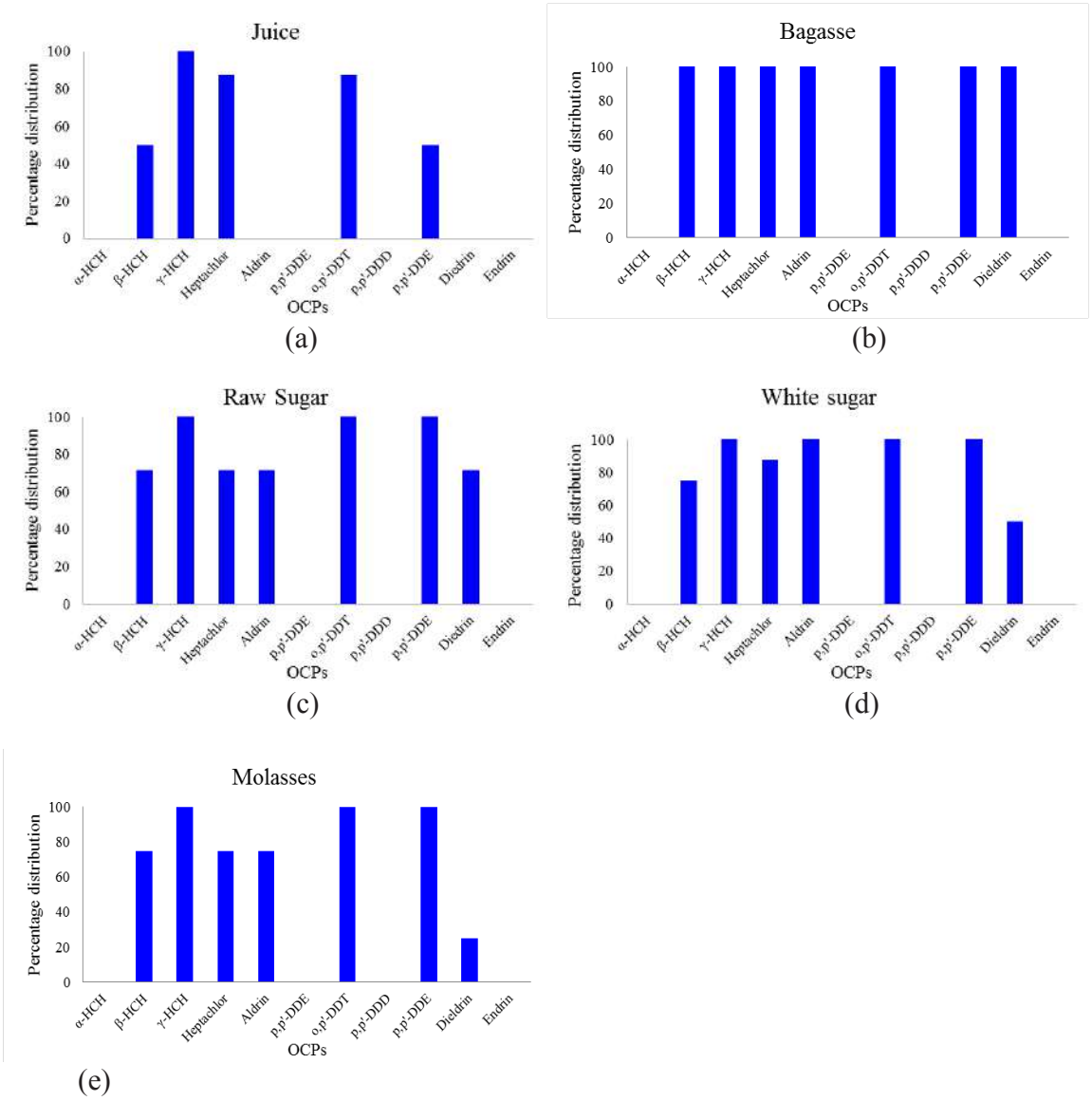


Figure 1: Percentage distribution of OCPs in Sugarcane juice (a), Bagasse (b), raw sugar (c), white sugar (d) and molasses (e).

The concentration of OCPsin sugarcane juice, bagasse, raw sugar, white sugar and molasses samples were detected shown in table 1. DDTs (o,p'-DDT and p,p'-DDE) were highly found in all samples. The mean value of OCPs in sugar bagasse samples was highest in the range of 0.6504 – 5.000 mg/kg, followed by raw sugar, white sugar, sugarcane juice and molasses. This indicated that OCPs residue was decreased from low-temperature processing product to high-temperature processing product excluded sugarcane juice because OCPs are lipophilic and may be accumulated in fatty tissues of sugar wax. The decrease of OCPs comply

with the study of effect of drying temperature processing on pesticide level in milk found that the percentage of p,p'-DDT, lindane, dieldrin and heptachlor decreasing 95%, 63%, 81% and 56% respectively (Stemp, 1966).

Table 1: The concentration of OCPsin sugar processing samples (mg/kg)

	Juice	Bagasse	Raw sugar	White sugar	Molasses
α-HCH	<0.001	<0.001	<0.001	<0.001	<0.001
β-HCH	0.0168 ± 0.011	3.7642 ± 0.495	0.0347 ± 0.056	0.0278 ± 0.028	0.0132 ± 0.008
γ -HCH	0.0691 ± 0.013	0.6504 ± 0.373	0.4014 ± 0.014	0.3271 ± 0.239	0.0347 ± 0.004
Heptachlor	0.0889 ± 0.024	3.8974 ± 0.911	0.0444 ± 0.016	0.6415 ± 0.128	0.0729 ± 0.042
Aldrin	0.0155 ± 0.005	3.9397 ± 0.139	0.0719 ± 0.119	0.0987 ± 0.007	0.0077 ± 0.007
p,p'-DDE	<0.001	<0.001	<0.001	<0.001	<0.001
o,p'-DDT	0.0917 ± 0.034	5.000	0.5764 ± 0.346	0.3186 ± 0.055	0.0692 ± 0.043
p,p'-DDD	<0.001	<0.001	<0.001	<0.001	<0.001
p,p'-DDE	0.0367 ± 0.002	5.000	1.2381 ± 0.119	0.4487 ± 0.521	0.0681 ± 0.009
Dieldrin	<0.001	4.6608 ± 0.001	0.1914 ± 0.432	<0.001	<0.001
Endrin	<0.001	<0.001	<0.001	<0.001	<0.001

4. Conclusion

There were seven OCPs that investigated in this study were β-HCH, γ-HCH, heptachlor, aldrin, o,p'-DDT, p,p'-DDE and dieldrin. The most investigated OCPs was γ-HCH that found in each sample. OCPs contamination. OCPs level were highly contaminated in bagasse, especially DDTs (p, p'DDE and o, p'DDT), followed by raw sugar. While white sugar and molasses had found low level of organochlorine pesticide was eliminated by high temperature process.

References

1) AOAC.(1996). Official Method 970.52 Organoc orine and Organophosphorus Pesticide Residues General Multiresidue. AOAC International, Arlington.
2) Barron, M. G., Ashurova, Z. J., Kukaniev, M. A., Avloev, H. K., Khaidarov, K. K., Jamshedov, J. N., & Manzenyuk, O. (2017). Residues of organochlorine pesticides in surface soil and raw foods from rural areas of the Republic of Tajikistan. *Environmental Pollution*, *224*, 494-502.
3) Hickenbottom, J. (1996). Use of molasses in bakery products. American Institute of Baking Technical Bulletin.
4) Kaushik, G., Satya, S., & Naik, S. N. (2009). Food processing a tool to pesticide residue dissipation–A review. *Food Research International*, *42*(1), 26-40.
5) Lehotay, S. J., Tully, J., Garca, A. V., Contreras, M., Mol, H., Heinke, V., ... & Poulsen, M. E. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *Journal of AOAC International*, *90*(2), 485-520.
6) Rani, M., Shanker, U., & Jassal, V. (2017). Recent strategies for removal and degradation of persistent & toxic organochlorine pesticides using nano-particles: a review. *Journal of environmental management*, *190*, 208-222.
7) Škrbić, B., & Predojević, Z. (2008). Levels of organochlorine pesticides in crops and related products from Vojvodina, Serbia: Estimated dietary intake. *Archives of environmental contamination and toxicology*, *54*(4), 628-636.
8) United Nations Environment Programme. (2009). Stockholm Convention on Persistent Organic Pollutants (POPs) as amended in 2009, United Nation Treaty Collection.
9) Zhang, Q., Xia, Z., Wu, M., Wang, L., & Yang, H. (2017). Human health risk assessment of DDTs and HCHs through dietary exposure in Nanjing, China. *Chemosphere*, *177*, 211-216.

23. IS JAPANESE NUTRITION EDUCATION EFFECTIVE FOR VIETNAMESE MOTHER?

Naomi Sakuma[1], and Ikuko Shimada[2]
[1]Jyumonji University Graduate School,
[2]Ohka Elementary school, University of Kochi
Corresponding author's Email: naomi12077@gmail.com

Abstract
Urban area people having obesity and diabetes have increased in Vietnam which is a very serious problem. This problem will also lead to the increase of children having diabetes. It had been investigated the thinking of snacks and healthy body image, before and after lunch for elementary school students' mother's nutritional education. The subjects were 52 mothers of elementary school students from two international schools in Hanoi, Vietnam. Nutrition education is the same content that Japanese nutritionist teacher lectures for Japanese children and parents. The contents of the questionnaire are body images and whether snacks are necessary before and after lunch. After the lecture, the necessity of taking snacks have decreased from 100% (before participation) to "25.9%" ($P < 0.05$). The most suitable for nutritional supplementation is that 96.4% of mothers chose healthy meals or food with less sugar and oil than sweet bread and junk food. It was suggested to the urban area mothers in Japanese nutrition education method that changed their idea of snacks by the Japanese nutritionist teacher.

Keywords: Diabetes, Vietnam, nutritionist teacher, Shokuiku, nutrition education, school lunch, eating habits, obesity

1. Introduction
Vietnam has a rapid economic growth with GDP of 6.8% in 2017. There are three main provinces involved in medical care: The Ministry of Health, the Ministry of Investment Planning, and the Ministry of Science and Technology. In 2017, 47 dietitians were also born. However, diabetes increases in urban areas of Vietnam, which is a serious problem. Adults with diabetes in 2002 gained 1.8% nationwide average, 5.7% in 2012, 3.1 times in 10 years. In urban areas, the prevalence of diabetes in rural areas is assumed to be 1.2 times, and the obesity which becomes a major factor of diabetes is urban area (Ho Chi Minh) in 2004 and 31.6% of males and 33.6% of females. Childhood obesity is also rapidly increasing mainly in urban areas. 48.9% of boys and 33.8% of girls are said to be obese or overweight in Ho Chi Minh. According to the 2005 survey, Elementary schools in central Hanoi this time are said to have over 20% obese children. Stalls such as confectionery and drinks are also common in the vicinity of elementary school during the lunch break and after school. In the 2000 nutritional survey, oil intake increased from 3.0g in 1990 to 7g, sugar intake from 0.76g to 7.81g. It is said that parents are pampering their children to over eat junk food and having less exercise, which is the main cause of obesity. On the other hand, poor people in rural areas nutritional situation is bad, and "double burden of malnutrition" is also a problem. This time, it is a survey at an international school that offers snacks before and after meal service, and it covers the wealthy. Therefore, we aimed to improve nutritional education for mothers with a large influence on children's diet, and to improve awareness of how to eat diabetes to reduce the risk of diabetes.

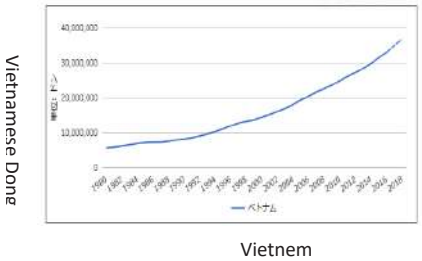


Figure 1: GDP of Vietnam, Vietnam's GDP in the vear 2017 is 6.8%.

2. Materials and Methods
In Japan, the basic law on food education was enacted in 2005, and the nutrition education of the people is determined by law. Elementary school lunch in Japan implementation rate is 99%, with a nutritionally balanced menu, with teaching materials we are promoting food education. The target is nutrition education for children, parents and local people. It is a nutritionist teacher with a nutritionist's

license and a teacher' s license, which is responsible mainly for that work. In this survey, nutrition education teachers are doing for elementary school students and parents in Japan at two international schools in Hanoi, Vietnam.We held two courses with morning and afternoon on October 14, 2017.There are 52 mothers participated and aimed at improving awareness of how to eat. The contents of nutrition education provided four items.

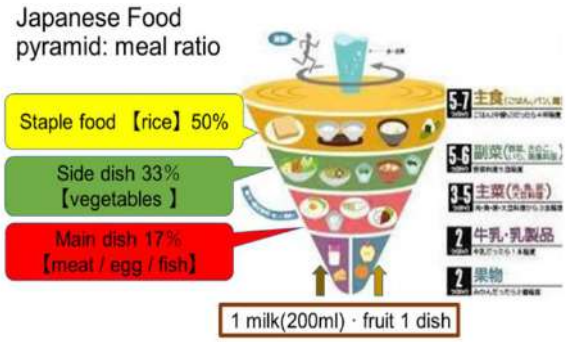


Figure 2: Actual lectured slides

3) Be careful of taking oil and sugar too.

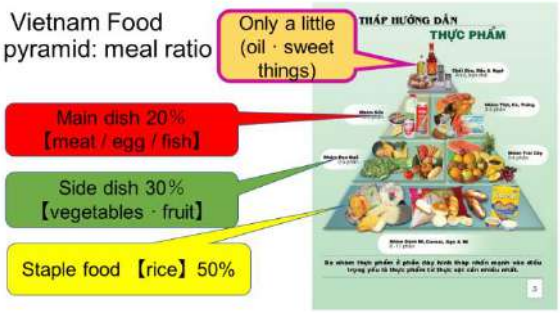


Figure 4: Actual lectured slides.

Also, in elementary schools in Japan where the average age of obesity is 9.2% .[15] snacks before and after lunch have not been issued. Also, I taught that Japanese children are playing outside with break time.

1) Food is roughly divided into three functions.

Figure 3: Actual lectured slides.

2) Vietnam and Japan possess same staple foods of rice, we can think Japanese cuisine and Vietnamese cuisine same dietary patterns and the amount of side dishes should be twice as main dishes

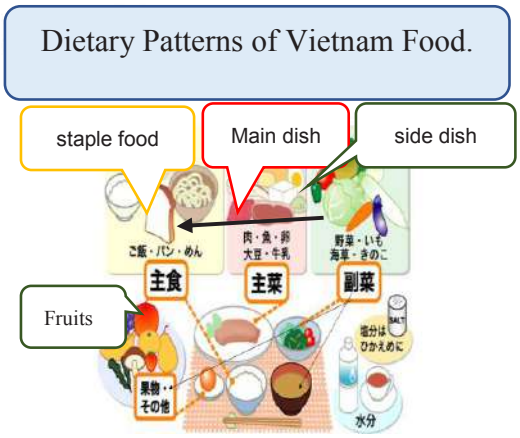


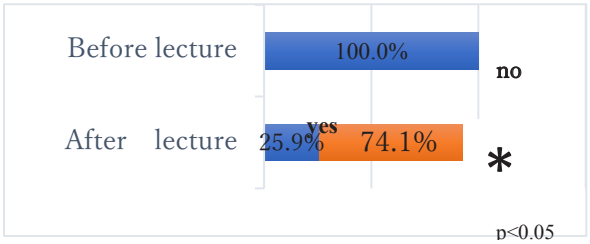
Figure 5: Actual lectured slides.

4) We taught students on the balance between ingestion and consumption of energy. (calorie)

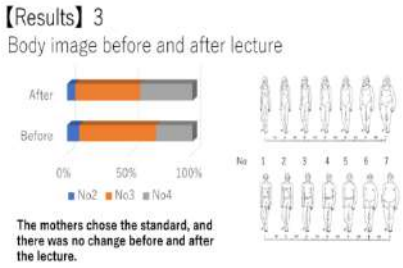
The pictures of children playing at school break time. After the lecture, we took a questionnaire and analyzed 31 respondents who did not have a blank. Statistics used Excel 2016.

3. Result

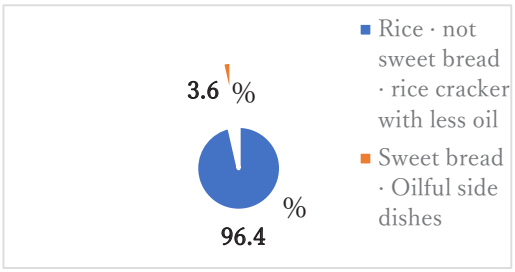
Question 1 "Do you think it is necessary to have a snack before and after lunch?" "What kind of food is good if you give snacks to children?



Question 2 "What kind of things would you prefer if you let the child eat snacks?"



Although 100% it is necessary to eat snacks before and after lunch after lecturing on nutrition education. 96% was "Good prepared with less sweet bread and oil". The snack was not a sweet one or a child's want, and there was a conscious improvement of balanced nutritional supply necessary for growth



Question 3 "Which is a healthy child's body image?"
For healthy body images, 100% of mothers chose "somewhat thinner from standard than before" lectures.

4. Conclusion

It found out in nutrition education in Vietnam, staple

food of the same rice, taking a Japanese diet form as an example, there was a mother who is entrusted with housework "Improving consciousness on how to reduce diabetes risk "One international elementary school in this survey is considering the health of children, and also considering abolishing the provision of snacks before and after lunch. As for the body image, I could not compare the physical weight of the actual child, so I would like to go there in the future. Also, I would like to investigate the relationship between the fact that young women in Vietnam lose weight and they tend to have eating disorders [15] and that the mother's body image sees somewhat slender models as "health" from standard.
I would like to think whether Japan's nutritional education is also suitable for Cambodian mother who staples the same rice in the future. This survey does not show the whole country of Vietnam in the survey of 2 international schools in urban areas.

5. Conflict of interest

All the authors declare that they have no conflict of interest.

References

Co, N. (2015). Confusion of dietary habits of Hanoi college students in Vietnam/10.1186 /s40337015-0054-2

Do Thi Gok Jipp Director. (2013). "Diabetes prevention program 2013 in Ho Chi Minh City" Ho Chi Minh City Nutrition Center.

Ho Chi Minh City Nutrition Center. (2015). Child obesity data site. http://www.vietjo.com/news/social/151224115440.html

Japanese nutritionist teacher system. (2005). MEXT Ministry HP.

http://www.mext.go.jp/a_menu/shotou/eiyou/04111101/003.htm

Khue, N. T. (2015). Diabetes in Vietnam. Annals of global health, 81(6), 870-873.
Kids eating too much, exercising too little. (2005). Vietnam news.
METI. (2016). Promotion of medical technology / service base/ Vietnamese edition/Detailed research report on healthcare market environment in emerging countries. Ministry of Foreign Affairs of Japan. (2017).
Ministry of Health National Institute of Nutrition. (2003). p68-71
National Hanoi Medical University Faculty of Nutrition established. (2013). http://www.hmu.edu.vn/news/default.aspx
Nguyan Lam Hong. (2007). Diabetes Asia Network HP/.http://www.dmnet.co.jp/asia_network/10/10.php
Parents to blame for obese children. (2009). Vietnam news.
School health statistics Heisei 29 Results. (2017). Ministry of Education, Culture, Sports, Science and Technology HP. http://www.mext.go.jp/b_menu/toukei/chusa05/hoken/kekka/k_detail/1399280.htm
Urban children eat too much junk food. (2009). Vietnam news.
Vietnam General Information Site. (2015). VIETJO. https://www.vietjo.com/news/statistics/150908063033.html
Yuko Yoshimoto. (2013). Nutritional Status, Lifestyle and Eating Habit among Preschool Children in Urban Vietnam during Economic Change: The Double Burden of Malnutrition/ Osaka University 39 p175-197

24. MICROBIAL QUALITY ASSESSMENT AND PREVALENCE OF *Escherichia coli* OF STREET-VENDED GRILLED CHICKEN INTESTINES AND PORK BARBEQUE IN LEYETE, PHILIPINES

Karl Michael F. Lumando[1], Inish Chris P. Mesias[1] and Mary Rose M. Maniego[2]
[1]Department of Food Science and Technology, Visayas State University, Visca, Baybay City, Leyte, Philippines
[2]Philippine Root Crop Research and Training Center, Visayas State University, Visca, Baybay City, Leyte, Philippines
Corresponding author's Email: karlmichaellumando1303@gmail.com, inishchris.mesias@vsu.edu.ph, and rosemaniego7@gmail.com

Abstract
In the Philippines, street foods are popular due to its affordability and accessibility. Although the advantages cannot be negated, however, the safety of street-vended foods is a particular problem. These foods are considered among the sources that can transmit pathogenic microorganisms causing foodborne illnesses. Since consumers are continuously exposed to the risk of contracting these diseases, microbiological quality of these types of foods is of great food safety concern. In this study, the microbiological quality of the grilled chicken intestines and pork barbeque sold inone city in Leyte, Philippines was assessed. Results were compared to the local FDA's microbiological guideline. *ComBase*, an online tool for quantitative and predictive microbiology, was used in the study to predict how the *E. coli* survive and grow under a variety of food-related conditions fromproduction to consumption continuum. Results for grilled chicken intestine revealed that 16.67% (n=42) and 90.48% (n=42) exceeded the standard limit for *E. coli* and aerobic plate count (APC) respectively. For pork barbeque,23.81% (n=42) were unsatisfactory for *E. coli* and 90.48%(n=42) for APC. Growthmodel of *E. coli* predicted a maximum growth rate of 0.134 log concentration/hour and a minimum doubling time of 2.264hours. The data obtained in this study can be used as input for microbial risk assessment and in identifying science-based interventions to control the hazards as implicated with the results.

Keywords: Grilled chicken intestines, pork barbeque, *Escherichia coli*, ComBase

1. Introduction

The demand for inexpensive, ready-to-eat and accessible food, such as street foods, have accentuated as number of marginal populations in cities has risen (Elli, 2016). According to Food and Agriculture Organization (FAO) (2007), as cited by Cardoso et al. (2014), an estimated 2.5 billion people consume street foods everyday around the world despite being an unimpressive artisanal business. It has become a main source of livelihood of millions of the urban poor for informal market sector due to the worsening poverty in the previous decades and consequent search for cheap alternative food items thereby making a substantial contribution to the economies of developing countries (Thanh, 2015).

Looking into the other side, there are a number of aspects of street foods which make them promising vehicles for micronutrient fortification (Draper, 1996). Street foods contribute significantly to both nutrition and food security for millions of people along the food chain (Akinyele, 1998). An analysis of some popular meat-based street foods in the Philippines, for instance, scored highest in terms of total energy and protein content, average 193 kcal and 16 g protein per meal (Draper, 1996).

The advantages cannot be negated; however, street food vending often faces precarious working condition with higher risk of contamination as it harbor pathogenic microorganisms – a serious concern for food safety (Lin &Yamao, 2014; Elli, 2016). Street food vendors tend to overlook the importance of safety on the food they serve giving it the potential to cause great havoc through food-related illnesses and jeopardize public health as the food safety aspect of this informal food businessreceive insufficient attention by authority concerned (Nonato et al., 2016). Although the government authority in the Philippines is making initiatives to ensure public health and consumers' safety in street-vended foods, nevertheless, strategies for efficiently improving food safety can only be developed after obtaining science-based studies on local foods, conditions and practices along

the area of production to consumption continuum (Mesias, 2018). There is a growing need for baseline microbiological studies of street-vended foods for a more effective and efficient intervention. Upon being said, major issues that must be addressed for these types of food in the country are: the capacity of the vendors to comply with Good Manufacturing Practices (GMPs) and their knowledge on principles and approaches in safe food processing and vending. GMP is an important factor for processors to produce safe, quality and affordable products.In this paper thereof, the microbiological quality (such as the APC, total coliform and fecal coliform) and prevalence of *Escherichiacoli* in street-vended grilled chicken intestines and pork barbeque in Leyte, Philippines were determined. Grilled chicken intestines and pork barbeque were among the top selling street foods in the locality. Microbiological information of these foods can be used as impacts in formulating a basic guide on proper handling and preparation for the handlers and vendors. In addition, results of this study can be used as basis for policy makers or local governments in identifying strategies for mitigation and in establishing guidelines for food safety programs.

2. Materials and methods

2.1 Sampling and sample collection

Meat-based street food samples were procured from different street food stalls located in Baybay City, Leyte, Philippines. Sampling was done randomly and covered only those stalls available within the city and neighboring barangays. In determining the amount of samples necessary to detect a fraction of positive samples, the binomial distribution described by Evers (2001) and ICMSF (1986), as cited by Mesias (2017), was used. With a probability of 90% and an indicative maximum set fraction of positive samples of 10%, a total of 42 samples were collected. The samples were gathered from afternoon to evening, as this is when most customers come to buy grilled chicken intestines and pork barbeque, over different days during the course of a week. Sampling was done aseptically. Upon collection, the samples were initially kept in an icepack cooled, insulated cool box with crushed ice. Thereafter, samples were transported to the Microbe Culture and Fermentation Laboratory of the PhilRootCrops at Visayas State University for analysis.

2.2 Microbiological and physicochemical assessment

2.2.1 Microbiological analysis

The microbiological quality and safety of the samples were analyzed through determination of the presence and counts of coliform bacteria and pathogenic *E. coli*. Aerobic Plate Count (APC) of the samples was also examined for the presence of general types of microorganisms. The test procedures being followed were based with the requirements of the Bacteriological Analytical Manual (BAM) (Hitchens et al., 2001). After a series of tests, samples that were found positive for *E. coli* were isolated and cultured.

2.2.1.1 Detection and enumeration of E. coli

The most probable number (MPN) technique described in the Bacteriological Analytical Manual was used to detect and enumerate the presence of *E. coli*. Samples that projected positive results were read in the MPN table for 3 tubes at 90% confidence intervals.

2.2.2 Physicochemical analysis

The parameters covered were only those contributory to the growth and proliferation of *E. coli*, such as temperature upon microbial analysis, water activity (A_w) and pH. Samples were triplicated upon examination.

2.3 Assessment of the growth behavior of E. coli

An online tool, *Combase*, was used for the quantitative and predictive microbiology. The tool assisted in studying and predicting how the *E. coli* survived and grew under diverse conditions. It was also used for modeling the level of growth of the *E. coli*after grilling up to a certain period prior to consumption. The data used on the online tool included temperature, pH, A_w and initial concentration of the *E. coli*.

2.4 Data processing and analysis

The prevalence of *E. coli* on the samples was determined using the equation below. Microbiological test results were compared to the available microbiological guidelines for ready-to-eat (RTE) foods since street foods are considered to be part of this category. In this study, the Food and Drug Administration (FDA) microbiological guidelines for RTE foods served as the basis for the evaluation of the microbiological quality of the selected meat-based street foods.

$$\% \text{ Prevalence} = \frac{\text{Number of positive samples}}{\text{Total number of samples examined}} \times 100$$

3. Results and discussion

3.1 Aerobic plate count (APC) of the samples

Table 1 shows the Aerobic Plate Count (APC) and physicochemical properties of grilled chicken intestines and pork barbeque under ambient conditions after grilling. Results of grilled chicken intestines revealed an APC value ranging from 2.3 to 7.3 $\log_{10}$/g with a mean cell concentration of 6.30 $\log_{10}$/g. On the other hand, pork barbeque samples has an APC value ranging from 2.5-7.0 $\log_{10}$/g with a mean cell concentration of 5.28 $\log_{10}$/g. Of these, 90.48% (38 out of 42) on each type of meat-based street food samples exceeded FDA's standard limit (4.0 $\log_{10}$/g). The APC results were anticipated because the inherent values of pH and A_w of the samples were close to the optimum requirements for progressive cell count.

The temperature of most grilled chicken intestines and pork barbeque during microbial analysis remained in the hazard temperature zone for holding foods. The temperature of the test products were between -6.9 to 25.0^{o}C and -4.8 to 25.9^{o}C with mean values of 8.50^{o}C and 6.50^{o}C, respectively. A number of pathogenic and spoilage microorganisms have optimum temperature for survival and proliferation in the said temperature ranges (Eley, 1992).

The pH values of grilled chicken intestines and pork barbeque ranged from 4.99-6.46 and 5.11-6.68, respectively, which were conducive for microbial growth. Though some of the obtained pH values lie outside the optimum level(6.0-8.0) for microbial growth, still microorganisms were observed as the slightly decrease in pH can be attributed to the acids produced by microorganisms as they grow. The excreted acid lowers the pH of the surrounding environment and thus eventually brings bacterial count and growth to a halt but not enough to incapacitate them thereby giving an optimum cell concentration of a certain value. The decrease in pH values of the samples may also be due to the effects of several types of deterioration. Spoilage in meat has been attributed to the activities of enzymes on the fatty acids or other organic acids like lactic acid, the end result of either chemical or microbial deteriorations (Frazier &Westhoff, 1988).

The water activity (A_w) of each sample of grilled chicken intestine and pork barbeque showed arbitrary values which ranged from 0.862-0.925 and 0.830-0.923, respectively. Low A_w values may be attributed to dehydration of the product while on display or during grilling. Skewered pre-cooked chicken intestines were stacked on trays and exposed to the environment during hold-on display. Also, some trays were placed near the grilling areas. The dehydration of the samples was inevitable as a result of exposure to wind and heat over the vending period. High A_w values, on the other hand, are results of improper time-temperature combination during grilling.

Table 1: Aerobic plate count and physicochemical properties of the selected meat-based street food samples

SAMPLE	MEAN AEROBIC PLATE COUNT ($\log_{10}$/g); n=42	LIMIT* ($\log_{10}$/g)	PHYSICOCHEMICAL PROPERTIES		
			Temperature (^{o}C)**	pH**	Water activity (A_w)**
Grilled chicken intestines	6.30	4.0	8.50	5.98	0.886
Pork barbeque	5.28	4.0	6.50	5.89	0.876

**Limits were based on the Revised Guidelines for the Assessment of Microbiological Quality of RTE Foods, FDA (2013)*
***values are means of three trials*

3.2 Coliform bacteria contamination

3.2.1 Grilled chicken intestines

Among the representative grilled chicken intestines from different areas in the test locality, about 64.29% (27 out of 42) and 57.14% (24 out of 42) were found to exceed the acceptable limit (3.0 MPN/g) set by

FDAboth for total and fecalcoliform, respectively (Table 2). Of the 64.29%, the cell count ranged from <3.0 to >1100 MPN/g. In addition, most samples (13 samples) had a cell concentration of <3.0 MPN/g followed by >1100 MPN/g (8 samples) and 43 MPN/g (4 samples). The fecal coliform result also showed a cell count ranging from <3.0 to >1100 MPN/g. The highest number of samples (18 samples) has a cell concentration of <3.0 MPN/g followed by 3.6 MPN/gwith 4 samples while the third most number of samples (3 samples) have different cell concentrations of 460 MPN/g, 1100 MPN/g and >1100 MPN/g.

Table 2: Frequency of the occurrence of coliform bacteria on grilled chicken intestines

TOTAL COLIFORM		FECAL COLIFORM		LIMIT* (MPN/g)
Count (MPN/g)	Frequency (n=42)	Count (MPN/g)	Frequency (n=42)	
<3.0	13	<3.0	18	3.0
3.0	2	3.6	4	3.0
3.6	2	6.1	1	3.0
7.2	2	11	2	3.0
7.4	1	15	1	3.0
9.2	2	23	2	3.0
9.4	1	28	1	3.0
11	1	36	1	3.0
16	1	43	2	3.0
23	2	240	1	3.0
36	1	460	3	3.0
43	4	1100	3	3.0
240	1	>1100	3	3.0
1100	1	-	-	3.0
>1100	8	-	-	3.0

**Limits were based on the Revised Guidelines for the Assessment of Microbiological Quality of RTE Foods, FDA (2013)*

3.2.2 Pork barbeque

From the evaluated pork barbeque samples, about 52.38% (22 out of 42) and 45.24% (19 out of 42) were found to exceed the acceptable limit (3.0 MPN/g) set by FDA both for total and fecal coliform, respectively (Table 3). The cell count of the positive samples still ranged from <3.0 to >1100 MPN/g for both types of coliform. As tested, most samples (19 samples) had a cell concentration of <3.0 MPN/g for total coliform bacteria. However, a number of samples (6 samples) were found to have a cell concentration of 3.6 MPN/g earning second in order while giving cell concentrations 43 MPN/g and >1100 MPN/g to be third as both concentrations had a lump count of 4 samples. For the fecal coliform, the same behavior was observed, <3.0 MPN/g as the concentration with the greatest number of samples (22 samples). Cell concentrations 3.6 MPN/g and >1100 MPN/g comes as second in hierarchal order (with 4 samples) thereby making 9.2 MPN/g and 23 MPN/g as third (with 2 samples).

Table 3: Frequency of the occurrence of coliform bacteria on pork barbeque

TOTALCOLIFORM		FECAL COLIFORM		LIMIT* (MPN/g)
Count (MPN/g)	Frequency (n=42)	Count (MPN/g)	Frequency (n=42)	
<3.0	19	<3.0	22	3.0
3.0	1	3.0	1	3.0
3.6	6	3.6	4	3.0
7.4	1	6.2	1	3.0
11	3	9.2	2	3.0
15	1	11	1	3.0
23	1	16	1	3.0
28	1	23	2	3.0
43	4	36	1	3.0
290	1	43	1	3.0
>1100	4	64	1	3.0
-	-	290	1	3.0
-	-	>1100	4	3.0

**Limits were based on the Revised Guidelines for the Assessment of Microbiological Quality of RTE Foods, FDA (2013)*

Generally, the results in coliform bacteria contaminationfor both types of meat-based street food were in congruent to the poorly implemented good manufacturing practices by different processors and vendors all over the area. Traditional processing, unreliable sources of water, unhygienic environment and materials used in processing, and poor sanitary condition in the market were some of the possible contributory factors toward significant high percentage of coliform bacteria contamination. Prolonged exposure to the surrogate storage of food items without controlled temperature also facilitates growth of microbes and development of toxins. Since the toxins are heat-stable, the incriminated food still has the potential to cause food poisoning even if it is further heat treated. Microbiological contamination can also be traced to insufficient timeand internal temperature during grilling. Pathogenic microorganisms can be eliminated, inhibited or inactivated in grilled chicken intestines at the average internal temperature of 83.0^{o}C. Hence, these areas should be given much attention to eliminate such possible sources of contamination (Azanza&Gedaria, 1998).

3.3 E. coli contamination

3.3.1 Grilled chicken intestines

Results of the *E. coli* test showed that 9 out of 42 samples were positive thereby registering a prevalence of 21.43%. Of the 9 positive samples, 7 (16.67%) were identified to have exceeded the acceptable limit of FDA for RTE foods (Table 4). The cell concentration of *E. coli* isolated from grilled chicken intestines ranged from 3.0 to 93.0 MPN/g with an arbitrary distribution of samples to each concentration.

Table 4: *E. coli* concentration on grilled chicken intestines

Count (MPN/g)	Frequency (n=42)	FDA Standard* (MPN/g)
3.0	2	3.0
3.6	2	3.0
6.2	1	3.0
7.4	1	3.0
9.2	1	3.0

23	1	3.0
93	1	3.0

**FDA Standard was based on the Revised Guidelines for the Assessment of Microbiological Quality of RTE Foods, FDA, 2013*

3.3.2 Pork barbeque

The prevalence of *E. coli* in pork barbeque was computed to be 33.33% (14 out of 42) with cell concentration ranged from 3.0 to 150 MPN/g. Out of 14 positive samples, only 10 samples (23.81%) were identified to have exceeded the acceptable limit of FDA for RTE foods (Table 5) with most samples having a cell concentration of 3.0 MPN/g and 3.6 MPN/g.

Table 5: *E. coli* contamination on pork barbeque

Count (MPN/g)	Frequency (n=42)	FDA Standard* (MPN/g)
3.0	4	3.0
3.6	3	3.0
7.2	1	3.0
9.2	2	3.0
15	1	3.0
23	2	3.0
150	1	3.0

**FDA Standard was based on the Revised Guidelines for the Assessment of Microbiological Quality of RTE Foods, FDA, 2013*

High *E. coli* count in food products reflect the poor sanitation level of food handlers during food preparation and handling. Since street foods are considered as an unimpressive artisanal business, there is a greater exposure of the product to contamination due to contact surfaces (See, 2016). Presence of such microorganism can also be due to the insufficient handwashing of the processors, microflora of the samples and survival of *E. coli*because of insufficient time-temperature combination during grilling (Azanza&Gedaria, 1998).

3.4 Estimated growth rate and doubling time of E. coli

Given the primary data on pH, A_w and initial *E. coli* concentration of the grilled chicken intestines (0.56-1.97 log_{10}/g), the model projected a maximum growth rate of 0.065-0.134 log concentration/hr (Table 6). The doubling time for *E. coli* was found to be within 2.251-4.619 hours. For pork barbeque, the maximum growth rate ranged from 0.071-0.131 log concentration/hrwith an initial cell count ranging from 0.48-2.18 log_{10}/g (Table 7). The doubling time, on the other hand, was within 2.290-4.220 hours.

Table 6: Predictive modelling for the growth of *E. coli* in grilled chicken intestines

SAMPLES	pH	A_w	INITIAL *E. coli* COUNT (log_{10}/g)	MAX. GROWTH RATE OF *E. coli* (log conc./hr)	DOUBLING TIME (hr)
I_2	6.29	0.896	0.56	0.131	2.290
I_4	6.20	0.907	0.87	0.129	2.331
I_8	6.31	0.917	0.48	0.131	2.290
I_{13}	6.52	0.903	1.36	0.134	2.251
I_{15}	5.98	0.898	0.48	0.122	2.459
I_{20}	6.10	0.876	0.79	0.126	2.387
I_{24}	5.04	0.885	0.56	0.065	4.619
I_{26}	4.99	0.895	1.97	0.065	4.619
I_{31}	6.36	0.880	0.96	0.133	2.264

Table 7: Predictive modelling for the growth of *E. coli* in pork barbeque

SAMPLES	pH	A_w	INITIAL *E. coli* COUNT (log_{10}/g)	MAX. GROWTH RATE OF *E. coli* (log conc./hr)	DOUBLING TIME (hr)
P_6	5.49	0.904	0.96	0.096	3.124
P_7	6.34	0.923	0.48	0.131	2.290

P_8	5.55	0.907	0.56	0.096	3.124
P_{12}	5.67	0.912	0.86	0.108	2.788
P_{13}	5.73	0.893	0.48	0.108	2.788
P_{15}	6.33	0.893	1.18	0.131	2.290
P_{22}	5.78	0.860	2.18	0.113	2.657
P_{23}	5.73	0.885	0.96	0.108	2.788
P_{24}	5.73	0.875	1.36	0.108	2.788
P_{26}	5.14	0.884	0.56	0.071	4.220
P_{30}	5.88	0.872	0.48	0.118	2.548
P_{31}	5.79	0.840	0.56	0.113	2.657
P_{33}	5.31	0.885	0.48	0.084	3.588
P_{34}	5.54	0.901	1.36	0.096	3.124

Figure 1 presents the predicted *E. coli* growth on grilled chicken intestines for a period until 10 hours. There was a continuous increase trend of *E. coli* concentration with respect to time. The acceptable limit of *E. coli* is 0.6 $\log_{10}$ cells/g. Initially, four samples (I_2, I_8, I_{15} and I_{24}) were compliant to the acceptable limit with cell concentration ranged from 0.48-0.56 $\log_{10}$ cells/g. However, after 2 hours, two of these samples (I_2 and I_{24}) were already beyond the standard limit while keeping the remaining two samples (I_8 and I_{15}) at bay until 8 hours.

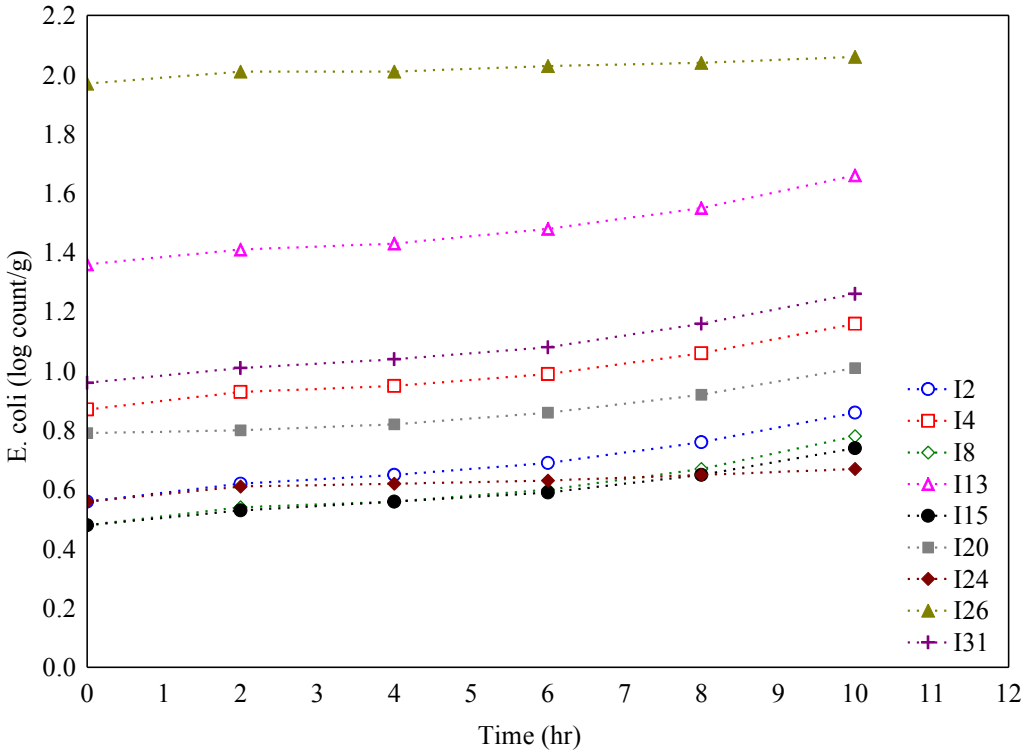


Figure 1: Predicted *E. coli* growth on grilled chicken intestines.

Figure 2 shows the predicted growth of *E. coli* in pork barbeque for a period of 10 hours. The effect of time exposure was distinctly observed in the simulation that there will be a gradual increase in cell concentration with longer exposure time prior to consumption. Initially, 7 samples (P_7, P_8, P_{13}, P_{26}, P_{30}, P_{31} and P_{33}) had *E. coli* concentration in compliant to the acceptable limit. However, two of these samples (P_8 and P_{31}) exceeded the acceptable level after 6 hours.

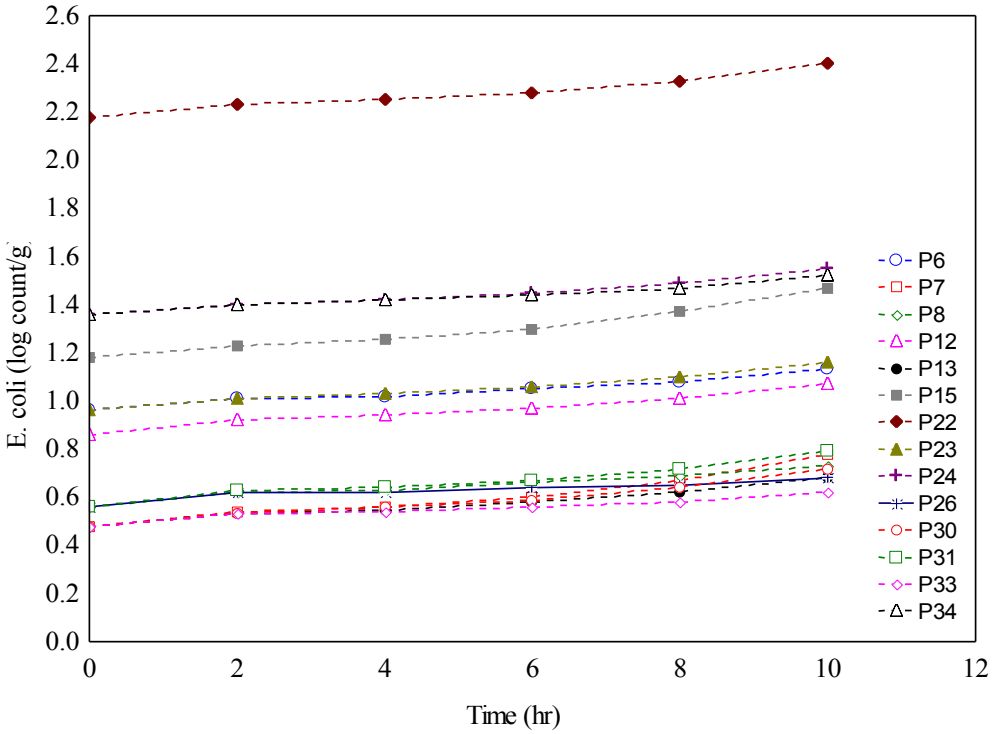


Figure 6: Predicted *E. coli* growth on pork barbeque.

Comparing both results of the predicted growth concentration of *E. coli*for both types of meat-based samples, it was observed that the said microorganism has a more drastic increase in cell concentration over a certain periodin grilled chicken intestines. The growth rate difference between the two can be attributed to the more favorable condition for its growth and proliferation, and also of the natural microflora of coliform bacteria which is 10^3 MPN/g (Wehr, 1982; as cited by Azanza &Gedaria, 1998). This indicates that the growth of *E. coli* in grilled chicken intestines is morepotentially life-threatening if not controlled properly as it keeps on increasing rapidly. Establishing and implementation of the necessary corrective measures should then follow to avoid this pathogenic microorganism from causing outbreaks.

4. Acknowledgment

The authors would like to recognize and extend their outmost gratitude to the Department of Science and Technology (DOST) for their manifold financial assistance in making this research possible.

5. References

1) Akinyele, I. O. (1998). Street foods and their contribution to the food security and nutritional status of Nigerians. *West African Journal of Foods and Nutrition*, *1*(1), 6-20.
2) Azanza, M.P.V. and Gedaria, A.I. (1998). Microbial Hazards of Street-Vended Grilled Chicken Intestine. Science Diliman.10(2):1-11.
3) Cardoso, R., Companion, M. and Marras, S.R. (2014). Street Food: Culture, Economy, Health and Governance. Routledge Taylor and Francis Group, London and New York, 1-5. ISBN 978-1-138 02368-0
4) [CFS] Centre for Food Safety (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items: revised). Food and Environmental Hygiene Department, Queensway, Hong Kong.
5) Draper, A. (1996). Street foods in developing countries: The potential for micronutrient fortification. John Snow, Incorporated, OMNI PROJECT, London, 72-74.

6) Eley, A.R. (1992). Microbial Food Poisoning. Chapman and Hall Press, London, 191-192.
7) Elli, J.D. (2016). Assessment of Street Food in Catbalogan City, Philippines. Journal of Academic Research. 1(1):11-20.
8) Frazier, W.C. and Westhoff, D.C. (1988). Food Microbiology. John Wiley and Sons, New York, 210-240.
9) Hitchins, A.D., Feng, P., Watkins, W.D., Rippey, S.R. and Chandler, L.A. (2001). Bacteriological Analytical Manual: Methods for Specific Pathogens (*Escherichia coli* and the Coliform Bacteria). U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 68-104.
10) [ICMSF] International Commission on Microbiological Specifications for Foods (1986). Microorganisms in Food 2: Sampling for Micro-biological Analysis: Principles and Specific Applications.University of Toronto Press, Toronto. ISBN 0802056938.
11) Lin, W. Y. and Yamao, M. (2014). Street foods safety in Yangon: A case study on street food vendors' socio economics and production aspects. Proceedings of the First Asia-Pacific Conference on Global Business, Economics, Finance and Social Sciences. Singapore. 1-11. ISBN 978-1 941505-15-1.
12) Mesias, I.C.P. (2017). Assessment of the Prevalence and Anitimicrobial Resistance of food borne Pathogens from Meat based Street Foods in Selected Cities in Leyte. Detailed Project proposal, Department of Science and Technology.
13) Mesias, I.C.P. (2018). Quantitative risk assessment of E. coli in street-vended cassava-based delicacies in the Philippines. Institute of Physics Conference Series: Earth and Environmental Science. 102:1-7.
14) Nonato, I.L., De Almeida Minussi, L.O., Pascoal, G.B. and De-Souza, D.A. (2016). Nutritional Issues Concerning Street Foods. Journal of Clinical Nutrition and Dietetics. 2(1):1-9.
15) See, C.J. (2016). Aerobic Bacterial, Coliform, *Escherichia coli*, and *Staphylococcus aureus* Counts of Randomly Selected Street Foods in Kampar, Perak. Department of Biological Science, UniversitiTunku Abdul Rahman, Kampar, Perak.
16) Thanh, T.N.C. (2015). Food safety behavior, attitudes and practices of street food vendors and consumers in Vietnam.Master's Dissertation in Master of Science in Nutrition and Rural Development, Ghent University, Belgium, 77.
17) Wehr, H.M. (1982). Attitudes and Policies of Governmental Agencies on Microbial Criteria for Foods – An update. Food Technology. 36:45-54, 94.

25. TRACK SOURCES OF *ESCHERICHIA COLI* CONTAMINATION IN A COOKED PORK PROCESSING PLANT BY PHYLOGENETIC GROUP TECHNIQUE.

Thitima Kulteeratawat[1], Aphacha Jindaprasert[1,*], Suwimon Keeratipibul[2]
[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand
[2]Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[*]Corresponding author: aphacha.ji@kmitl.ac.th

ABSTRACT

Escherichia coli was often found in meat processing premeses and many products were rejected. The mean of this study was to apply phylogenetic group technique to track source of contamination of *E. coli* in a cooked pork processing plant. A total of 100 heating and freezing products and 560 swab samples from workers hands, equipments, machines and environmental surfaces in post-heating area were screened for the presence of *E. coli*. The positive isolates were selected on Eosin Methylene Blue Agar (EMB) to analyze by using triplex polymerase chain reaction (PCR) and determined *E. coli* into four main phylogenetic groups as A, B1, B2 and D. All *E. coli* isolates were detected from stainless handcart (20%) and floor surface of ingredient mixing room (40%). In addition, *E. coli* strain*s* from both sources were determined into same group as A. This finding suggested that the cleaning of stainless handcart and floor surface of ingredient mixing room should be performed in regular basis to reduce the actual source of contamination of *E. coli* in a cooked pork processing plant.

Keywords: *Escherichia coli*, phylogenetic group, pork processing plant, and contamination

1. Introduction

At present, the issue of food safety is a global priority. It is desirable that the food manufacturers must have controlled the food quality and safety such as microbiological criteria by the regulatory standard of each importing country. However, the microbial contaminations are often found in food processing plants such as pork meat, tracking the essential pathogens to the source of contamination in cooked pork product remains poorly studied in Thailand.

Since *Escherichia coli* was identified in 1885, *it* had become one of the most widely studied bacterial species (Clements et al., 2010). *E. coli* is gram-negative, non-spore forming bacteria, member of Enterobacteriaceae. It is one of fecal coliforms that is found in human feces and warm-blooded animals and indicates the contamination of poor sanitation in the production process (Sanyos and Saccavadit, 2010). Some strain of *E. coli* was foodborne pathogen, such as *E. coli* O157: H7 causes serious harm in human through consumption of contaminated food product. Not only it causes severe diarrhea, but also causes blood poisoning, meningitis, pneumonia, bladder inflammation, kidney failure or death (Waruenee, 2011). In 1996, there were 9,578 patients and 11 deaths in Japan from the consumption of foods contaminated with *E. coli* O157: H7 (WHO, 1996). The regulatory standards of Japan and the Department of Livestock of Thailand for heated meat products require a zero tolerance (must test negative) for *E. coli* (JETRO, 2011; Department of Livestock Development, 2008). However, *E. coli* was still often found in the meat processing area and many products were rejected (interview data from quality assurance (QA) manager of the cooked pork processing meat in Thailand). There are various sources of contamination of *E. coli* which is difficult to prevent contamination to the products. Currently, the analysis of *E. coli* for the food industry such as dry rehydrated film (Association of Official Analytical Chemists, 2002) or enumeration of *Escherichia coli* and the coliform bacteria (Feng et al., 2002) are detected at the species level. Both of the rapid and traditional methods are the techniques for enumeration of occurrence of *E. coli* which cannot

classify *E. coli* at the strain level. It means if *E. coli* is detected in both of products and environmental surfaces, there will be not possible to find the relation of contamination of *E. coli* from the environment to the products or it may be different strains. Therefore, there has been a limitation to find the real source of product contamination.

There are early studies applying the molecular method to track source of contamination of various pathogenic microorganisms and classifying bacteria into the gene level. In each molecular method such as pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), phylogenetic group or multiple-locus variable number tandem repeat (MLVA), refer to the difference of the genetic material of the organism is the same as the individual fingerprints to identify the source of bacterial contamination. Keeratipibul and Techaruwichit (2012) reported that using PCR-RAPD-based DNA fingerprinting to classify and track the source of *Listeria* contamination in a cooked chicken plant. There were the sampling of cooked products, processed goods, and environmental swabs to identify sources of contamination that affect the products. Yang et al. (2017) showed that using MLVA technique to map the source of *E. coli* contamination on beef in the beef packing plant. Pavlickoova et al. (2017) reported that comparison of *E. coli* biofilm formation was isolated from chicken meat and phylogenetic group, *E. coli* could live on the surface of food processing equipment and was resistant to cleaning. They were mostly found in phylogroup A.

The molecular studies of *E. coli* strains in products and environmental surfaces in the food processing can provide serious information to know the actual source of contamination for the development of better control and prevention for foodborne pathogen. However, few studies have report on *E. coli* profile and the control in Thai cooked pork processing plants. There was no report of the molecular typing of *E. coli* to track the source of contamination to implement more understanding and control this microbe in Thailand. Therefore, the purpose of this study was to apply phylogenetic group method to track the source of contamination of *E. coli* in a cooked pork processing plant.

2. Materials and methods

2.1 Processing plant and product manufacturing

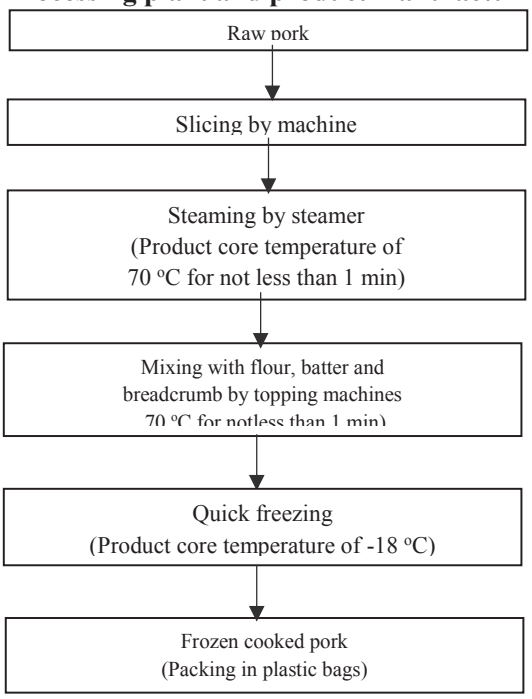


Fig. 1 The flow diagram of the cooked pork processing line.

This study was performed in the cooked pork processing plant in Thailand. The production line had a processing capacity of 12 metric tons per a day, there was only day shift for production, using raw pork achieved from a certified slaughterhouse. The flow diagram of the cooked pork processing line was presented in Fig. 1. The raw meat was sliced and cooked by steamer at the product core temperature up to 70 °C for not less than 1 min. Then the cooked meat was mixed with powder, batter, and breadcrumb by machines at the post-heating area. After that the cooked pork was frozen in air blast freezer until the product core temperature was lower 18 °C and packed in a plastic bag. There were cleaning programs for twice a day (half day and end of day) for machines and areas. The cleaning steps were consisted of pre-cleaning to remove most dirt, washing with detergents, rinsing with water and sanitizing with sanitizers.

2.2 Sampling

The different intermediate stages of pork processing, finished products and surfaces of the processing environment were sampled for 10 weeks between January to March 2018. Sampling was executed one day per week at two times per day. Samples were

collected at the first batch production at 7.30 and 13.00 h. A total of 100 samples of products, and 560 swab samples were collected and analyzed *E. coli*.

Samples of pork products were collected after each process step of cooked pork processing, i.e., after steaming, mixing with flour, mixing with batter, mixing with breadcrumb in machines, freezing and packing. Each sample was collected 300 g, and sealed in a sterile plastic bag and transported to a laboratory. Samples were analyzed for *E. coli* within the sampling date.

Environmental swab samples were collated in the different processing areas. Sampling sites were segregated into three zones, based on the contact and nearness to the product. Zone 1 were the product-contact surfaces such as ingredient sieving machine, mixer, conveyor belts, zone 2 were non-contact surface products, but close to products such as end of pipe tap at ingredient mixing room, weighing, the handcart and zone 3 were the non-product contact surfaces in processing area such as floors and walls that were further away from the products. All sampling sites in the production environment were swabbed with sterile cotton swabs moistened with 0.1% peptone broth (w/v). After sampling, the swabs were soaked in 10 ml of *E. coli* broth and transport to the laboratory. Samples were also analyzed for *E. coli* within the sampling date.

2.3 Microbiological analysis

An additional 450 ml of Butterfield' s buffered phosphate diluent (BPB) was added to a stomacher bag containing 50 g pork samples in each step were homogenized for 1 min, 3 ml. of suspension were transferred to tube containing 17 ml. of *E. coli* broth (2 tubes) and incubated in a water bath at 44.5 + 0.2 ^{o}C for 22 hours. For swab samples, they were soaked in 10 ml of *E. coli* broth, incubated at 44.5 $\pm$ 0.2 ^{o}C for 22 hours as same as pork samples and observed for the presence of gas. After incubation, the positive tubes were producing gas. One loop of all positive samples was streaked on Eosin Methylene Blue Agar (EMB), incubated at 35 $\pm$ 1 ^{o}C for 24 $\pm$ 2 hours. Characteristics of *E. coli* colonies on EMB agar were dark purple colonies. In addition, certain lactose-fermenting bacteria produced flat, dark colonies with a green metallic sheen. This method was required by customer of a cooked pork process plant which used for testing *E. coli* contamination in products and environmental surface at current. Usually, it would report as positive in 0.6 g. if *E. coli* was detected or report as not detected in 0.6 g if *E. coli* was absented. Then the culture plates containing *E. coli* on EMB agar were stored at 4 ^{o}C before transporting to the molecular laboratory.

2.4 *E. coli* identification

The positive isolates, two colonies with morphological characteristics of *E. coli* were selected on Eosin Methylene Blue Agar (EMB) to determine phylogenetic groups by using the triplex polymerase chain reaction (PCR) which used a combination of two genes as *chuA* and *yjaA* and a DNA fragment TSPE4.C2 (Fig. 2).

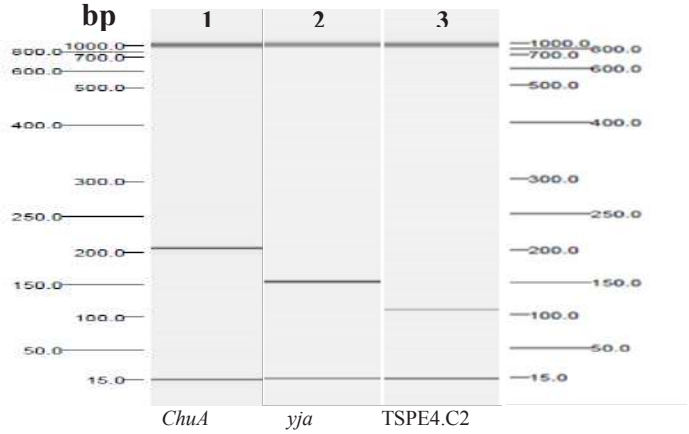


Fig. 2 The sizes of PCR products from primers *chuA*, *yjaA*, and TSPE4.C2

It could divide the strains of *E. coli* into four main phylogenetic groups as A, B1, B2, and D showed in Table 1 (Clermont et al., 2000). The *chuA* gene appears only in strains of *E. coli* in phylogroup B2 and D. Thus, it could be separated *E. coli* strains in phylogroup B2 and D from *E. coli* phylogroup A and B1. In the same way, the isolates of *E. coli* in phylogroup B2 and D were separated because the *yjaA* gene appeared only in *E. coli* phylogroup B2. For the isolation of *E. coli* phylogroup A and B1, a DNA fragment TSPE4.C2 present in *E. coli* phylogroup B1 and absented in *E. coli* phylogroup A. The positive isolates were compared DNA with the reference cultures (control cultures) for phylogenetic groups identification.

Table 1 Identification of *E. coli* phylogroups by using the triplex polymerase chain reaction (PCR) with the combination of two genes as *chuA* and *yjaA* and a DNA fragment TSPE4.C2 (Clermont et al., 2000).

E. coli phylogroups	*chuA*	*yjaA*	TSPE4.C2
A	-	+/-	-
B1	-	+/-	+
B2	+	+	+/-
D	+	-	+/-

2.5 The reference cultures

There were three reference cultures of *E. coli* which were different phylogenetic groups, consisted of *E. coli* ATCC11303 for phylogroup A, *E. coli* ATCC10536 for phylogroup B1 and *E. coli* ATCC43888 (O157:H7) for phylogroup D.

3. Results and discussion

From a total of 100 heating and freezing products, collected from five processing steps, none of the samples were positive for *E. coli.* A total of 220 environmental samples from zone 1 surfaces, 100 samples from zone 2 and 240 samples from zone 3 were swabbed and revealed the prevalence of *E. coli* as 0.00, 4.00 and 3.64 % of the samples, severally (Table 2).

No *E. coli* was found at zone 1. For zone 2, it was detected *E. coli* only the handcart for transferring batter bucket (both of before start production in the morning and after lunchtime). For zone 3, it also was detected *E. coli* only the surface of the floor in ingredient mixing room, it was often found in the morning (detected 6 times in 10 times) but decreased in the afternoon (detected 2 times in 10 times). *E. coli* strains from both sources from the handcart and the surface of the floor in ingredient mixing room were determined into the same group as phylogroup A (Table 3).

Neither of products and the product-contact surfaces (zone 1) was found *E. coli* at both sampling times (the start of production time and after lunch time) indicated that the cleaning and disinfection of the product-contact surfaces at lunch time and after the end of production could effectively remove *E. coli* contamination. Because of *E. coli* was not detected at environmental samples from zone 1 (Table 2). However, *E. coli* still occurred at the handcart for transferring batter bucket after mixing (zone 2), it was in agreement with previous study reported that there was detected *E. coli* when swabbed some equipment such as hanger, carcass cutting tables and transportation cart (in the pig and cattle slaughter house and meat processing plants) (Schlegelová et al., 2010). Moreover, the surface of the floor in ingredient mixing room (zone 3) where was mixed batter also detected *E. coli*. Although the environmental surface of zone 3 was far away and not direct contact to the products, there was some movement of workers and equipments during production such as transferring ingredient from ingredient mixer room to processing area. It could be cross contaminated from zone 3 to zone 2 and would have the risk to contaminate to zone 1 and the products.

Table 2 Prevalence (% positive from 560 swab samples) of *E. coli* on the environment of the cooked pork processing area.

Swab area	Swab areas	Amounts of swab	Prevalence of *E. coli*
Zone 1	Flour, Batter, Bread crumb topping machines	60	0
	Ingredient mixer	20	0
	Ingredient sieving machine	20	0
	Conveyor of bread crumb crushing machine	20	0
	Transfer conveyors	80	0
	Gloves	40	0
	Total	240	0.00
Zone 2	Controller of machines	40	0
	Weighing	20	0
	The handcart	20	20
	End of pipe tap at ingredient mixing room	20	0
	Total	100	4.00
Zone 3	Electric wire	20	0
	Walls of ingredient sieving room,	10	0
	Walls of ingredient mixing room	10	0
	Walls of bread crumb crushing room	10	0
	Walls of processing area	10	0
	Walls of machine-washing room	10	0
	Floor of ingredient sieving room	10	0
Zone 3	Floor of ingredient mixing room,	10	40
	Floor of bread crumb crushing room	10	0
	Floor of processing area	10	0
	Floor of machine-washing room	10	0
	Total	220	3.64
	Grand total	**560**	**2.14**

Table 3 Phylogenic group of *E. coli* strains on the environment of the cooked pork processing area.

Swab surfaces	Zone	Swab samples	Amounts of isolate colonies	Phylogenic groups
the handcart	2	4	8	A
the surface of floor in ingredient mixing room	3	8	16	A
Reference cultures				
E. coli ATCC43888 (O157:H7)		-	-	D
E. coli ATCC11303		-	-	A
E. coli ATCC10536		-	-	B1

For tracking sources of contamination of *E. coli* by phylogenetic group technique, this study showed that both of these sources were determined into the same group as phylogroup A. Therefore, there was possible to cross contaminate between the surface of the floor and the handcart. Usually, the floor of ingredient mixing room was cleaned and sanitized after the end of production in the evening and leave it overnight. The result of swabs in the next day before start production was often found *E. coli* contamination, suggesting that the cleaning and sanitizing at the end of production could not completely eliminate *E. coli*. Thus, the cleaning of the floor in ingredient mixing room was revised, including the frequent cleaning and strictly implemented to reduce the source of contamination of *E. coli*. The hygiene of workers also should be more controlled such as washing hand before carry the batter bucket to supply to topping machine including

reconsider the movement of workers in production area to prevent the risk of cross contamination.

E. coli are both natural flora of humans and important pathogens causing significant morbidness and decease worldwide (Clements et al., 2010). Then the screening of *E. coli* to prevent contamination in food is very important. From this study showed that applying phylogenetic group technique for tracking *E. coli* contamination in processing area, it could support the manufacturing to identify real source of *E. coli* contamination more quickly in order to develop effective sanitation program.

4. Conclusion

For tracking sources of contamination of *E. coli* by phylogenetic group technique, this study showed phylogroup identification of *E. coli* which was detected in each source. There are useful for the manufacturing to better understand the relation of contamination of *E. coli* in processing area and able to improve sanitation program which solve problem directed to the point. Finally, it can be prevented the presence of *E. coli* in products including ensure the safety of foods from pathogenic *E. coli* strains.

5. Acknowledgments

We are obliged to the management and staff of the frozen cooked pork factory and microbiological and molecular laboratory that participated in this study. This work is in part supported the presentation expense by King Mongkut's Institute of Technology Ladkrabang, Bangkok.

6. References

1) Association of Official Analytical Chemists (2002) AOAC official method 991.14 coliform and *Escherichia coli* count in foods dry rehydratable film methods [online].
2) Clements A., J. C. Young, N. Constantinou and G. Frankel (2010) Infection strategies of enteric pathogenic *Escherichia coli*. Gut Microbes 3(2): 71–87.
3) Clermont, O., S. Bonacorsi, and E. Bingen (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. Applied and Environment Microbiology 66: 4555 –4558.
4) Department of Livestock Development (2008) Department of Livestock Microbiological criteria for livestock products for export [online].
5) Feng F., Stephen D. Weagant, Michael A. Grant and W. Burkhardt (2002). Enumeration of *Escherichia coli* and the coliform bacteria [online].
6) Japan External Trade Organization (JETRO) (2011) Specifications and Standards for Foods, Food Additives, etc. Under the Food Sanitation Act 2010 [online].
7) Keeratipibul S. and P. Techaruwichit (2012) Tracking sources of *Listeria* contamination in a cooked chicken meat factory by PCR-RAPD-based DNA finger printing. Food Control 27: 64 - 72**.**
8) Pavlickova S., A. Klancnik, M. Dolezalova, S.M. Mozina, and I. Holko (2017) Antibiotic resistance, virulence factors and biofilm formation ability in *Escherichia coli* strains isolated from chicken meat and wildlife in the Czech Republic. Journal of Environmental Science and Health. 8: 570-576.
9) Sanyos C., and S. Saccavadit (2010) Analysis measurement *E. coli* and Coliform in pork that compared between slaughter house hanging system and on the sieve table system by using test kit. [online].
10) Schlegelova J., V. Babak, M. Holasova, L. Konstantinova, L. Necidova, F. Sisak, H. Vlkova, P. Roubal and Z. Jaglic (2010) Microbial contamination after sanitation of food contact surfaces in daily and meat processing plants. Czech Journal of Food Sciences 28: 450 – 461.
11) Waruenee Preechanuechitkul (2011) *Small things called E coli.* Kasikorn newspaper 84(4): 77 – 82.
12) World Health Organization (WHO). (1996) Enterohaemorrhagic *Escherichia coli* in Japan - Update [online].
13) Yang X., A. He, M. Badoni, F. Tran, and H. Wang (2017) Mapping sources of contamination of *Escherichia coli* on beef in the fabrication facility of a commercial beef packing plant. Food control 75: 153 – 159.

26. The survey of eating habits of elderly people receiving home support

IkukoShimada[1)], Miki Hiroi[2)], Chie Tokuhiro[3)], Keiko Watanabe [4)], Keiko Matsui[2)], Naomi Sakuma[5)]

And Satoshi Numata[1)]

University of Kochi[1)] Hosogi Hospital[2)], Mimasaka University[3)], Kochi Gakuen college[4)]
Jyumonji University Graduate School[5)]

Contact address：Ikuko Shimada

781-8515 2751-1 Ike, Kochi city, Kochi,Japan
Tel: +81-90-2891-1890　Fax +81-88-847-8584
E-mail:piyokichi1964@yahoo.co.jp

Abstract

In this study, we aimed to clarify the actual condition of meals at home of elderly people receiving home support and their problems and lead to an improvement of quality of life.The subjects are elderly people who participate in A hospital home division and care services. We distributed questionnaires and collected them from people with consent. Distribution and collection period was from August 2013 to the end of September 2013.Questionnaires 1 were about nine items about eating habit, physical condition and living status.Questionnaires 2 were asking them whether they intake ten food items (meat, fish and shellfish, eggs, milk, fats and oils, soy and soy products, green yellow vegetables, seaweed, potatoes, fruits)or not. Five men and 35 women got responses. The mean age was 82.6 ± 7.1 years.When eating out or buying food, the best reference was "taste" by 42.5%, followed by "nutritional component" by 27.5%.Among the nutrition labeling, the most helpful reference was "salt equivalent". 39.0% of respondents had trouble of "the amount that can be eaten decreased". In Questionnaire 2, ten food items intake survey results by nutritional status, fruits and green-yellow Vegetables were taken almost every day through all groups of good, normal and low nutrition groups. The one with the lowest intake frequency was seaweed and potatoes.It is suggested that it is important to pay attention to factors related to nutrition such as food intake and chewing for multiple items in elderly health promotion.

Keywords

Home support, eating habit, malnutrition

Introduction

Japan has reached the world of aging society and through the post-war. The number of elderly people who need care is increasing, however, elderly care service such as nursing homes are not enough. Therefore, home support for caring elderly people are becoming ordinary as elderly care. From the research about consciousness of caring elderly people of 2012 by the Cabinet Office, the most comfortable place where elderly would like to have care was at home (Figure1) . Actually, there was a big difference of lifelong between man and woman, especially for man, 9.31 years between healthy life and average life and 12.68 years for woman (Ministry of Health, Labor and Welfare, 2010)(Figure 2).Furthermore, it may be a serious issue that the amount of food intake of fat and meat by elderly who is over seventy years old is decreasing[1)]. It may cause sarcopenia and become long between their average life and healthy life. Research by Shibata and et.al (2007) showed that elderly people have a tendency to avoid meat and fat. It was also suggested that elderly people should have variety of food and it may effect for quality of life. Therefore, in this study, we aimed to clarify the actual condition of meals at home of elderly people receiving home support and their problems and lead to an improvement of quality of life.

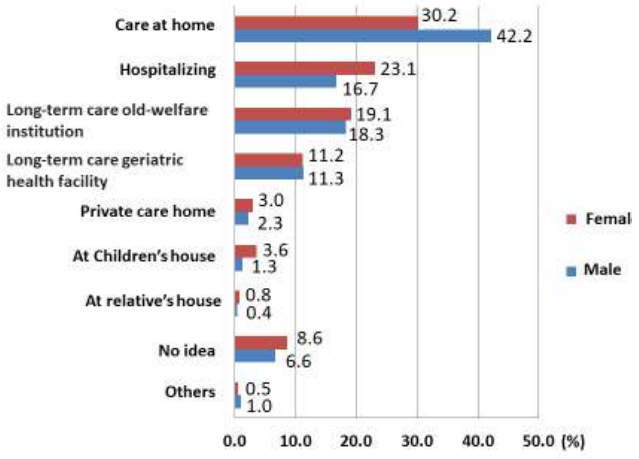


Fig.1 Consciousness of caring elderly people

Fig.2 Difference of between average life and healthy life

Methods

Targets are elderly people who participate in A hospital home division and care services. We distributed questionnaires and collected them from people with consent. Distribution and collection period was from August 2013 to the end of September 2013. Questionnaire 1 are composed 9questions, such as 1) Breakfast 2) Any information when eat outside 3) Chewing 4) Teeth 5) Dietary problems 6) Any food you want to eat 7) Smoking and Drinking Alcohol 8) Body data (Age, Body weight, Degree of care, Blood pressure, Medical history, Nutritional status) 9) Resident type(live with family or live alone). Questionnaire 2 are record for ten items food intake every day. They make a record the frequency of food intake such as meat, seafood, egg, milk, fat, soy and soy bean products, green and yellow vegetable, seaweed, potatoes, and fruits. Record duration is ten days at maximum. Statistical analysis was by Excel 2010.Ethical consideration; Implemented with approval of A hospital ethics review committee.

Results

Forty elderly people cooperated the research and their profile is below (Table1). All of them were over eighty years old. In male group, there was no people who had the necessary care degree 2 or more, and 39.9% of woman had the necessary care degree 2 or more. In the medical history, woman group had hypertension and diabetes mellitus, man group had heart disease, hypertension and diabetes mellitus. Nutritional status was not their diagnosis but their idea for themselves. None of them didn't smoke, but 11.4 of woman had alcohol.

Table.1 Profile

		Male (n=5)	Female (n=35)	Total (n=40)
Average (years)		81.4±4.2	82.8±7.5	82.6±7.1
Average BMI (kg/m²)		22.9±2.6	24.0±9.0	23.1±9.4
Necessary support 1 (%)		20.0	8.6	10.0
Necessary support 2 (%)		20.0	22.9	22.5
Necessary care degree1 (%)		60.0	28.6	32.5
Necessary care degree 2 (%)		0.0	5.7	5.0
Necessary care degree 3 (%)		0.0	17.1	15.0
Necessary care degree 4 (%)		0.0	5.7	5.0
No answer (%)		0.0	11.4	10.0
Systolic blood pressure average (mmHg)		122	127	127
Diastolic blood pressure average (mmHg)		70	68	69
Medical History	Hypertension (%)	20.0	31.4	30.0
	DM (%)	20.0	25.7	25.0
	Kidney disease (%)	0.0	5.7	5.0
	Heart disease (%)	40.0	2	22.5
	No disease (%)	0.0	17.1	15.0
	No answer (%)	20.0	28.6	15.0
Nutritional Status	Good (%)	20.0	22.8	22.5
	Normal (%)	80.0	62.9	65.0
	Malnutrition (%)	0.0	2.9	2.5
	No answer (%)	0.0	11.4	10.0
Smoking	Yes (%)	0.0	0.0	0.0
	No (%)	80.0	82.9	82.5
	No answer (%)	20.0	17.1	17.5
Alcohol	Yes (%)	0.0	11.4	10.0
	No (%)	80.0	68.6	70.0
	No answer (%)	20.0	20.0	20.0

Questionnaire 1; From the Question 1, " Are you usually take Breakfast? " over 80 % of them took breakfast every day. It was seen no significant difference between each group in nutritional status and living status (Figure3). In the Question2, "What is the nutrition label with the top priority?" energy and salt equivalent were most chosen in each group and vitamins came thirdly (Figure4). As other opinion was that I do not know the calories because I make it myself, but consider the vitamins, making the salt less. In the Question3, the most important factor when eating outside or buying was price and taste in both group (Figure5). In the Question7, we asked whether they would have any troubles about eating food, most common troubles were about declining the amount of food intake (Figure6). Questionnaire 2; From the food intake frequency questionnaire by those who live with their family, the average for one day were shown by living status (Table2 and Table3). In the living group, milk, fruits, green-yellow vegetables were taken almost daily. Food with the lowest intake frequency was eggs and potatoes in both groups. No significance was seen in both.

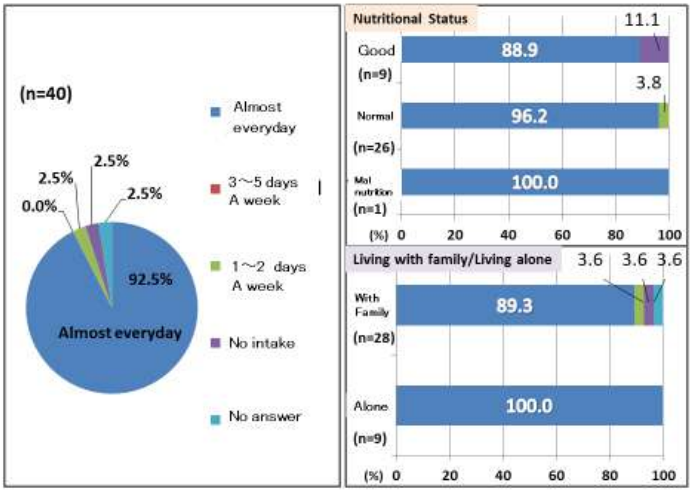


Fig.3 Are you usually taking breakfast?

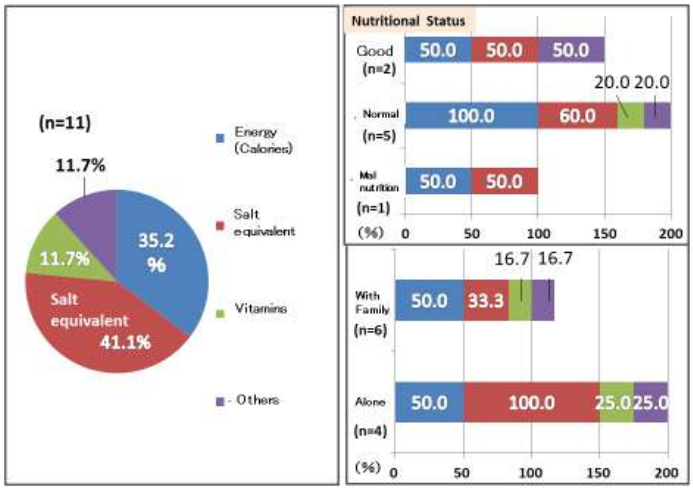


Fig.4 What is the nutrition label with the top priority ?

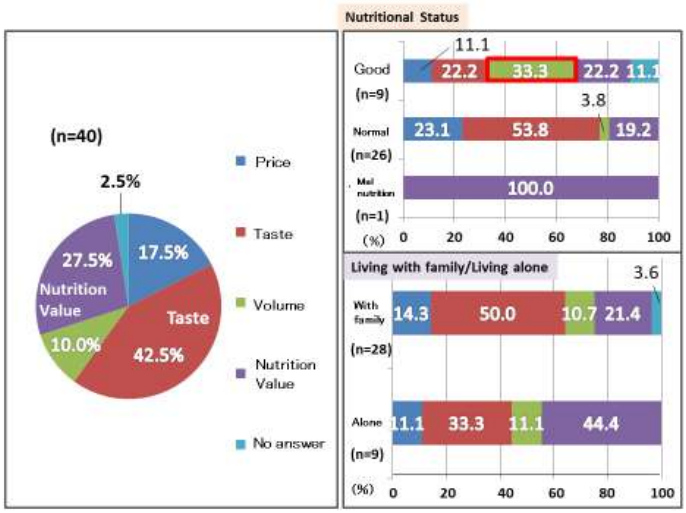


Fig.5 The most important factor when eating outside or buying food

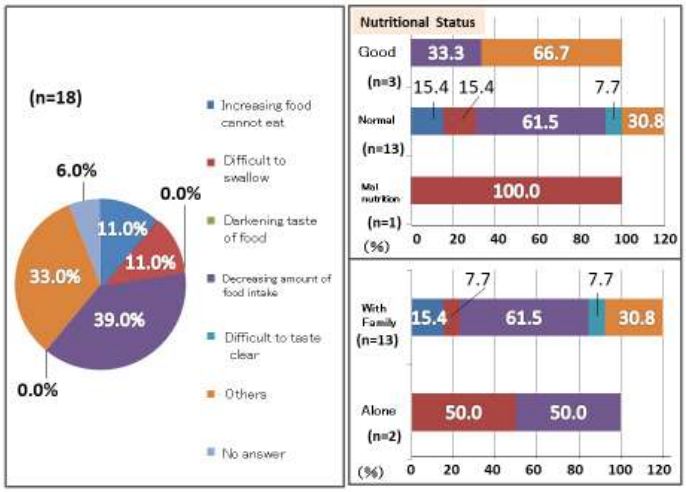


Fig.6 Any troubles about eating food

Conclusions

As the limit of the research, sample size was small and we need further research. However, the research group was seemed to be interested in their health from the results. In the Dietary Reference Intakes (DRIs 2015), estimated average necessary amount of protein was calculated as 0.85g/kg /day, and it was more than one of adults as 0.72g/kg/day. Those who don' have any kidney disease may be possible to take more protein and may lead to prevent sarcopenia[2)]. In the research, man and woman were seemed to be in interested in food intake and maintaining their body. However, other observation for three years among elderly woman showed an increase of risks of frailty because of small amount of protein intake[3)]. The cross-sectional study also suggested the relation between frailty and protein intake[4)]. From the preceding research, ingestion of high-quality protein is important for the elderly, and among them, positive intake of animal protein is encouraged. A group of elderly men who ingested a lot of animal protein, compared with the group with the lowest intake of animal protein, according to a previous study that carried out follow - up for seven years for men and women in their 60s, it is said that the functional deterioration has remained at 39% [5)]. Studies have also shown that intake of animal protein is more effective for prevention of diabetes than intake of vegetable protein [6)] In the research, we asked only the frequency of food intake and could not guess the amount of protein intake. There was no significant difference between the group living with the family and the group living alone, but there was a slight difference in the meal. I think that it is important to make an atmosphere that you can enjoy your meals, such as making opportunities to go out as much as possible. It is an inevitable social phenomenon for Japan now, but thinking that by considering the dietary environment of the elderly it will lead to the extension of healthy life expectancy.

Conflict of interests; The authors of this document confirm there is no conflict of interests.

References

1) Kumagai, et al (2003), Effects of dietary variety declines in high-level functional capacity in elderly people living in a community,Vol.50(12), 1117-1124.
2) Nutrition for elderly (2016 recognized) www.mhlw.go.jp/file/05-Shingikai-10901000.../0000042643.pdf Ministry of Health, Labor and Welfare.
3) Beasley JM, LaCroix AZ, Neuhouser ML, et al.（2010） Protein intake and incident frailty in the Women's Health Initiative observational study. J Am Geriatr Soc **58** ,1063-71.
4) .Kobayashi S, Asakura K, Suga H, et al.(2013) High protein intake is associated with low prevalenceof frailty among old Japanese women : a multicenter cross-sectional study. Nutr J **12,**164.
5) Imai E, Tsubota-Utsugi M, Kikuya M, et al.(2014) Animal protein intake is associated with higher-level functional capacity in elderly adults: the Ohasama study.J Am Geriatr Soc, Mar, 62(3):426-34. doi: 10.1111/jgs.12690.
6) Pounis GD, Tyrovolas S, Antonopoulou M, et al.(2010) Long-term animal-protein consumption is associated with an increased prevalence of diabetes among the elderly: the Mediterranean Islands (MEDIS) study. Diabetes Meteb, Dec;36(6 Pt 1):484-90.