VBFoodNET

VBFoodNet 2017 International Conference

SAFETY AND QUALITY IN THE FOOD CHAIN

PROCEEDINGS BOOK

Sponsors

ARES ACADÉMIE DE RECHERCHE ET D'ENSEIGNEMENT SUPÉRIEUR

EMBASSY OF BELGIUM

NAFOSTED National Foundation for Science & Technology Development

VECO Vietnam

CJ CẦU TRE

nlf Nonglamfood Preserving the value of nature Lưu giữ giá trị thiên nhiên

12-14 NOVEMBER 2017

NONG LAM UNIVERSITY - HO CHI MINH CITY

PROCEEDINGS BOOK

VBFoodNet 2017 International Conference

SAFETY AND QUALITY IN THE FOOD CHAIN

12-14 November 2017

Nong Lam University, Ho Chi Minh City, Vietnam

Organizers

Nong Lam University
Ho Chi Minh City

Food Science and Technology Network
between Vietnam and Belgium

Sponsors

ACADÉMIE DE RECHERCHE ET D'ENSEIGNEMENT SUPÉRIEUR

EMBASSY OF BELGIUM

National Foundation for Science & Technology Development

VECO Vietnam

Preserving the value of nature

Proceedings Book

VBFoodNet 2017 International Conference

SAFETY AND QUALITY IN THE FOOD CHAIN

12-14 November 2017

Nong Lam University, Ho Chi Minh City, Vietnam

Conference chair

Prof. Dr. Nguyen Hay

VBFoodNet chair

Assoc. Prof. Dr. Tran Thi Dinh

WELCOME

Dear Professors, colleagues, and friends,

We are pleased to announce that VBFoodNet (Food Science and Technology Network between Vietnam and Belgium) is organizing its fifth international conference on 12th-14th November 2017, at Nong Lam University – Ho Chi Minh city, Thu Duc District, Ho Chi Minh City, Vietnam.

The topic of this year's conference is 'Safety and Quality in Food Chain'. Participants from broad background related to food will gather to discuss issues and solutions on safety and quality in three food chain categories, including vegetable and fruit products, dairy and meat products and aquaculture products.

All previous conferences welcomed participants from more than ten countries. This year, it is also expected to attract many participants around the world from governmental sector as well as research and education organizations and industry since food chain is now the global focus to tackle with food safety and security.

VBFoodNet chair

Assoc. Prof. Dr. Tran Thi Dinh

(Chairwoman of VBFoodNet)

Conference chair

Prof. Dr. Nguyen Hay

(President of Nong Lam University
Ho Chi Minh City, Vietnam)

VBFOODNET AND PARTNERS

FOOD SCIENCE AND TECHNOLOGY NETWORK BETWEEN VIETNAM AND BELGIUM (VBFoodNet)

History and progress

In March 2009, the first idea about a network between Belgium and Viet Nam in the area of Food Science and Technology was initiated by professor Koen Dewettinck, (Ugent, Belgium), who has been involved in many collaborations with Vietnam. On 19th March 2010, the first network meeting was organized at Nong Lam University, where several partners were brought around the table. On 11th November 2010, the second network meeting was organized again at Nong Lam University. Where participants havecome to a consensus about the principles of the network. In the course of 2011, partners agreed on the network name and a draft of the network agreement: VBFoodNet was born! In Autumn 2011, the official inauguration of the Food Science and Technology Network between Vietnam and Belgium was launched at the second international conference on Food Safety and Food Quality in South-East Asia from 9th to 12th November 2011 on Can Tho city. In March 2012, the network agreement was signed in Ho Chi Minh City in the presence of H.R.M. prince Philippe Belgium, now King of Belgium. The network actually is comprised of 9 Vietnamese and 5 Belgium institutes and hopes to attract more interested institutes.

Objectives

The Food Science and Technology Network between Vietnam and Belgium (VBFoodNet) was established in order to promote effective collaboration among Vietnamese academic and research institutions in the field of food science and technology. It also aims at promoting effective collaboration between Vietnamese and Belgian academic and research institutions in the field of food science and technology. This is done by sharing relevant information and existing resources such as expertise, equipment, scientific and academic materials, by collaborating in training and research and by seeking financial support for training and improving research capacity of all the Network members.

Partner names and websites

Ghent University (UGent)	http://www.ugent.be/
Nong Lam University - Ho Chi Minh City (NLU)	http://www.hcmuaf.edu.vn/
Can Tho University (CTU)	http://www.ctu.edu.vn/
Vietnam National University of Agriculture (VNUA)	http://www.vnua.edu.vn/eng/
Ho Chi Minh City University of Industry (HUI)	http://www.hui.edu.vn/
Hanoi University of Science and Technology (HUST)	http://www.hust.edu.vn/web/vi/home
Hue University (HueUni)	http://hueuni.edu.vn/
Food Industries Research Institute (FIRI)	http://firi.vn/
Université catholique de Louvain (UCL)	http://www.uclouvain.be/
University of Liège (ULg)	http://www.ulg.ac.be/
Catholic University of Leuven (KU Leuven)	http://www.kuleuven.be/
University of Namur (UNamur)	http://www.fundp.ac.be/
Ho Chi Minh City University of Food Industry (HUFI)	http://www.cntp.edu.vn/
Nha Trang University (NTU)	http://www.ntu.edu.vn/
An Giang University (AGU)	http://www.agu.edu.vn/
National Institute of Nutrition (NIN)	http://viendinhduong.vn/
Thai Nguyen University of Agriculture and Forestry (TUAF)	http://tuaf.edu.vn/en-Us

VBFoodNet 2017 International Conference

SAFETY AND QUALITY IN THE FOOD CHAIN

Organizing Committee

Prof. Dr. Nguyen Hay (Chair, Nong Lam University, Vietnam)

Prof. Dr. Ir. Koen Dewettinck (Ghent University, Belgium)

Assoc. Prof. Dr. Phan Tai Huan (Nong Lam University, Vietnam)

Dr. Thien Trung Le (Nong Lam University, Vietnam)

Ms. Katleen Anthierens (Ghent University, Belgium)

Dr. Nguyen Ngoc Thuy (Nong Lam University, Vietnam)

Assoc. Prof. Dr. Tran Thi Dinh (Vietnam National University of Agriculture, Vietnam)

Assoc. Prof. Dr. Ly Nguyen Binh (Can Tho University, Vietnam)

Dr. Kha Chan Tuyen (Nong Lam University, Vietnam)

Dr. Vu Thi Lam An (Nong Lam University, Vietnam)

Dr. Duong Thi Ngoc Diep (Nong Lam University, Vietnam)

Scientific Committee

Prof. Dr. Ir. Koen Dewettinck (Ghent University, Belgium) (Chairman)

Assoc. Prof. Dr. Tran Thi Dinh (Vietnam National University of Agriculture, Vietnam)

Prof. Dr. Ir. Marie-Louise Scippo (Université de Liège, Belgium)

Prof. Dr. David Picha (Louisiana State University, USA)

Prof. Dr. Ir. Patrick Kestemont (University of Namur, Belgium)

Prof. Dr. Ir. Yvan Larondelle (Université Catholique de Louvain, Belgium)

Prof. Dr. Ir. Bart Nicolai (Catholic University of Leuven, Belgium)

Prof. Dr. Ir. Katleen Raes (Ghent University, Belgium)

Prof. Dr. Nguyen Hay (Nong Lam University, Vietnam)

Assoc. Prof. Dr. Ly Nguyen Binh (Can Tho University, Vietnam)

Assoc. Prof. Dr. Phan Tai Huan (Nong Lam University, Vietnam)

Assoc. Prof. Dr. Do Thi Bich Thuy (Hue University, Vietnam)

Dr. Mai Thi Tuyet Nga (Nha Trang University, Vietnam)

Dr. Thien Trung Le (Nong Lam University, Vietnam)

Secretary, information & logistics committee

Dr. Thien Trung Le (Nong Lam University, Vietnam)

Ms. Trinh Thi Thao Ngan (Nong Lam University, Vietnam)

Mr. Nguyen Huu Thien (Nong Lam University, Vietnam)

Mr. Nguyen Manh Cuong (Nong Lam University, Vietnam)

Dr. Duong Thi Ngoc Diep (Nong Lam University, Vietnam)

Ms. Katleen Anthierens (Ghent University, Belgium)

Mr. Nguyen Trung Hau (Nong Lam University, Vietnam)

Dr. Vu Thi Lam An (Nong Lam University, Vietnam)

Dr. Kha Chan Tuyen (Nong Lam University, Vietnam)

Dr. Nguyen Minh Xuan Hong (Nong Lam University, Vietnam)

Mr. Luong Hong Quang (Nong Lam University, Vietnam)

Conference chair

Prof. Dr. Nguyen Hay

(Nong Lam University – Ho Chi Minh City, Vietnam)

VBFoodNet chair

Assoc. Prof. Dr. Tran Thi Dinh

(VNUA, Vietnam).

Scientific committee chair

Prof. Dr. Ir. Koen Dewettinck

(Ghent University, Belgium).

Organizing committee chair

Prof. Dr. Nguyen Hay

(Nong Lam University – Ho Chi Minh City, Vietnam)

VBFoodNet 2017 International Conference

SAFETY AND QUALITY ON THE FOOD CHAIN

November 12-14, 2017

NONG LAM UNIVERSITY – HO CHI MINH CITY

CONFERENCE PROGRAMME

November 11 ▶ 12: **PhD SUMMER SCHOOL**

Resistant starch and low-carb food

Assoc. Prof. Dr. Pham Van Hung

Microencapsulation of bioactive component

Dr. Duong Thi Ngoc Diep

Novel Techniques for Extraction of Bioactive compounds from Plant Material

Dr. Kha Chan Tuyen

Membrane separations for food processing

Dr. Le Trung Thien

Monday November 13 ▶ **OPENING SESSION**

8h00 > 8h30: **Registration**

8h30 > 9h00: **Opening Ceremony:**

Opening speech: **Prof. Dr. Nguyen Hay** (President of Nong Lam University, Vietnam)

Message from **Embassy of Belgium in Vietnam**

Introduction about VBFoodNet: **Assoc. Prof. Dr. Tran Thi Dinh**(Chairwoman of VBFoodNet)

SESSION 1: SAFETY AND QUALITY OF VEGETABLE & FRUIT PRODUCTS

Chairpersons: Prof. Dr. Ir. Katleen Raes (Ghent University, Belgium) and Assoc. Prof. Dr. Tran Thi Dinh (Vietnam National University of Agriculture)

9h00 > 9h30: **Keynote lecture 1:** Nondestructive testing of fruit and vegetable quality for automatic sorting

Prof. Dr. Ir. Bart Nicolai (KU Leuven, Belgium)

9h30 > 9h45 Participatory Guarantee Systems: building trust and safety in vegetable value chains

Mrs. Charlotte Flechet (VECO Vietnam)

9h45 > 10h00: O1: Screening of chemical composition, in vitro antioxidant activity and phenolic profiles of tropical fruits by-products.

Nguyen Nhat Minh Phuong (Gent University)

O2: Quality changes of dragon fruit *(Hylocereus undatus*) stored at different controlled

10h00 > 10h15: atmosphere conditions

Phuc L. Ho (Vietnam National University of Agriculture)

10h15 > 10h45: **Coffee break – Poster session**

10h45 > 11h15: **Keynote lecture 2:** International Market Requirements for Fresh Produce and Export Opportunities for Vietnam

Prof. Dr. David Picha (Louisiana State University, USA)

11h15 > 11h30: O3: Molecular characterization of Theobroma cacao L. cultivars from Vietnam

Prof. Dr. Ir. Kathy Messens (Ghent University, Belgium)

11h30 > 11h45: O4: Light-Emitting Diodes improve Ergothioneine Content and Antioxidative Properties of Selected Mushroom Varieties

Dr. Nguyen The Han (Nha Trang University)

11h45 > 12h00: O5: Intelligent Computer Vision System for vegetables and fruits quality Inspection using Soft Computing Techniques

Assoc. Prof. Dr. Narendra V G (Manipal University)

12h00 > 13h30: Lunch break

SESSION 2: SAFETY AND QUALITY OF DAIRY & MEAT PRODUCTS

Chairpersons: Prof. Dr. Marie-Louise Scippo (University of Liège, Belgium) & Dr. Mai Thi Tuyet Nga (Nha Trang University, Vietnam)

13h30 > 14h15: **Keynote lecture:** Milk fat globule membrane material: challenges and opportunities for the food industry

Prof. Dr. Ir. Koen Dewettinck (Ghent University, Belgium)

14h15 > 14h30: O6: Degradation of opioid peptides and identification of degradation products during yoghurt processing using LC-HRMS

Dr. Duc Doan Nguyen (Vietnam National University of Agriculture)

14h30 > 14h45: **Coffee break – Poster session**

14h45 > 15h15: **Keynote lecture:** Meat and meat products: Nutritional quality versus health concerns

Prof. Dr. Ir. Marie-Louise Scippo (University of Liège, Belgium)

15h15 > 16h00: O7: Nutritive value of third party certified organic feed formulation for organic native swine at DA-NVES

Celerina Miranda (Nueva Vizcaya Experiment Station)

16h00 > 16h15: O8: Incorporation of Gac (*Momordica cochinchinensis*) aril powder as natural colorant and/or carotenoid supplement for sausage product

Dr. Tuyen C. Kha (Nong Lam University)

SESSION 3: SAFETY AND QUALITY OF AQUACULTURE PRODUCTS

Chairpersons: Prof. Dr. Patrick Kestemont (Namur, Belgium) & Assoc. Prof. Dr. Ly Nguyen Binh (Can Tho University, Vietnam)

16h15 > 17h00 **Keynote lecture:** Searching for alternatives to drugs and chemicals in striped catfish *Pangasianodon hypophthalmus* health management - the use of plant extracts as dietary immunostimulants

Prof. Dr. Ir. Patrick Kestemont (University of Namur, Belgium)

18h00 > 22h00: **Gala dinner**

Tuesday November 14

SESSION 3: SAFETY AND QUALITY OF AQUACULTURE PRODUCTS (Cont.)

Chairpersons: Prof. Dr. Patrick Kestemont (Namur, Belgium) & Prof. Dr. Ly Nguyen Binh (Can Tho University, Vietnam)

9h00 > 9h15: O9: Seafood safety compliance with hygiene regulations within Vietnamese domestic distribution chains.

Dr. Hong Phuc Luu (Nha Trang University)

9h15 > 9h30: O10: Microalgae biomass as an excellent natural source for functional foods and nutraceuticals

Dr. Duc Bach Nguyen (Vietnam National University of Agricuture)

9h30 > 10h00: O11: Detection and Quantification of Polycyclic Aromatic Hydrocarbons in Smoked Freshwater Fish in Cambodian markets

Dr. Mith Hasika (Institute of Technology of Cambodia)

10h00 > 10h15: **Coffee break – Poster session**

10h15 > 10h30: O12: Effect of *Phyllanthus amarus* Schum. et Thonn. and *Euphorbia hirta L*. extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during iced storage

Le Anh Dao Nguyen (Can Tho university)

10h30 > 10h45 O13: Effects of processing conditions on quality of Tra (*Pangasianodon hypophthalmus*) catfish fillets and derived products

Dr. Thien Trung Le (Nong Lam University)

11h00 > 11h30: **Best poster award: Assoc. Prof. Dr. Phan Tai Huan**, Dean Faculty of Food Science and Technology, Nong Lam University – Ho Chi Minh City

11h30 > 11h45 **Closing ceremony: Prof. Dr. Ir. Koen Dewettinck** (Ghent University, Belgium)

11h45 > 13h00: Lunch break

13h00 > 14h00: **Network Meeting**

TABLE OF CONTENT

KEYNOTE LECTURE ABSTRACTS

Nondestructive testing of fruit and vegetable quality for automatic sorting

Bart Nicolaï

BIOSYST/MeBioS, KU Leuven, Heverlee, Belgium

The ISO 9000 standard defines quality as the 'degree to which a set of inherent characteristics fulfills requirements of the customer'. For the horticultural industry this requires the availability of nondestructive techniques to inspect quality attributes of fruit and vegetables and to use these techniques for online sorting and grading. While until a decade ago this was limited to color, size and absence of skin defects, novel technologies have appeared that allow to measure important taste attributes such as sugar content and firmness nondestructively at commercial grading speeds. However, internal disorders remain difficult to detect.

In this presentation we will compare different nondestructive techniques for online inspection of fruit and vegetables. We will address the limitations of optical methods and then focus on novel technologies based on magnetic resonance imaging and X-ray radiography and tomography. We will show how recent developments in both hardware and software enable to reduce the acquisition and data processing times that inherently come with them to levels which are becoming compatible with industrial requirements.

Milk fat globule membrane material: challenges and opportunities for the food industry

Koen Dewettinck[1]

[1]*Ghent University, Belgium*

The fat globules in milk consist of a triglyceride core, surrounded by a thin membrane, called the milk fat globule membrane (MFGM). This membrane, about 10–20nm in cross-section, acts as an emulsifier and protects the globules from coalescence and enzymatic degradation. The MFGM has gained a lot of attention the last decade, due to the growing interest in its nutritional and technological properties. The MFGM is highly structured and contains unique polar lipids and membrane-specific proteins. Sphingolipids (highly bioactive molecules, mainly present in polar lipids from animal origin) account for up to one third of the MFGM polar lipid fraction. Scientific evidence on the nutritional benefits of these sphingolipids is accumulating. Moreover, it is assumed that the MFGM proteins also possess specific nutritional and health-promoting properties. As such, due to their origin and structure, MFGM polar lipids and proteins could be used as an emulsifier or stabilizer, combining technological and nutritional functionality. This presentation deals with the composition and structure of the MFGM, MFGM purification techniques on laboratory and industrial scale, the nutritional aspects of MFGM polar lipids and proteins, and finally the technological aspects and applications of MFGM material in dairy and non-dairy foods.

Meat and meat products: Nutritional quality versus health concerns

Prof. Marie-Louise Scippo (University of Liège, Belgium)

This talk will focus on both positive and negative health effects of meat consumption.

Meat is a very interesting source of micro and macronutrients. Its consumption allows to cover parts of our needs in minerals, proteins (and in particular essential amino acids), as well as lipids. About lipids, meat is generally poor in healthy polyunsaturated fatty acids (PUFA), but it is possible to produce meat enriched with PUFA, in particular with omega-3 PUFA. Some examples will be given. In case of consumption of omega 3 fatty acids enriched meat, the consumer will have to be careful with the cooking process, as the PUFA are very sensitive to oxidation, resulting in the formation of toxic compounds.

About the negative effects of meat consumption, a recent report from WHO draw the attention on the positive correlation between red meat (and processed red meat) consumption and colorectal cancer (CRC), a disease that is more common in developed countries. The term “red meat” refers to fresh red meats (mainly beef, pork, sheep or lamb but also other meats except poultry meat). Processed red meat products are defined as ”red meats preserved by salting (mainly with addition of nitrite and/or nitrate) and one or more other treatments”. For fresh meat, polycyclic aromatic hydrocarbons and/or nitroso compounds formed during cooking could explain this carcinogenic effect, while in processed meat, nitrites could be incriminated. Other mechanisms have also been proposed. The conclusion is that the CRC development is not associated with one unique causative agent, but with the presence of different compounds, acting on multiple stages of CRC. To reduce the risk of CRC development, it is recommended to limit the fresh red meat consumption to 300 -500 g per week, and to consume only occasionally processed red meat.

Searching for alternatives to drugs and chemicals in striped catfish *Pangasianodon hypophthalmus* health management - the use of plant extracts as dietary immunostimulants

Kestemont Patrick[1], Truong Quynh Nhu[1,2], Oger Mathilde[1], Le Thi Bach[3], Nguyen Le Anh Dao[2,4], Tran Minh Phu[2], Bui Thi Buu Hue[3], Scippo Marie-Louise[4], Quetin-Leclercq Joelle[5], Do Thi Thanh Huong[2], Nguyen Thanh Phuong[2] and Bui Thi Bich Hang[2]

(1) University of Namur – Research Unit in Environmental and Evolutionary Biology, Belgium,
(2) Can Tho University – College of Aquaculture and Fisheries, Vietnam,
(3) Can Tho University – College of Natural Sciences, Vietnam,
(4) University of Liège – Department of Food Sciences, Belgium,
(5) Université catholique de Louvain – Louvain Drug Research Institute, Belgium.

In the Mekong delta of Vietnam, the striped catfish *Pangasianodon hypophthalmus* has known within two decades an amazing increase of its production, making it an example of the success stories in Asian aquaculture. The fish is mainly produced in earthen ponds under high stocking density, which degrades the water quality and, as a corollary, supports the emergence of diseases due to parasitic and bacterial contamination. In order to cope with disease outbreaks, fish farmers routinely apply chemicals and antibiotics, with potential negative impacts on fish flesh and water quality as well as increased resistance of bacteria to antibiotics. Experiments conducted during the bilateral Vietnam-Belgium project Deltaquasafe (2009-2014) demonstrated the efficiency of immunostimulants *Escherichia coli* lipopolysaccharides (*E. coli* LPS) or levamisole (an antihelmintic compound) as successful alternatives to the use of antibiotics against the contamination by *Edwardsiella ictaluri*, the main bacteria affecting the production of striped catfish. However, the cost of LPS and the restricted use of levamisole limit the application of these compounds as immunostimulants in striped catfish culture practices. Within the AquaBioActive project (2015-2020), extracts from a large set of Vietnamese wild or cultivated plants were tested *in vitro* on striped catfish peripheral blood mononuclear cells or kidney cells in order to select the most efficient ones in stimulating the fish innate and adaptive immunity. Several relevant biomarkers including lyzozyme and complement activities, total immunoglobulins and expression of selected candidate genes were analyzed for evaluating the immune response. Among the 20 plant extracts tested in vitro, three (*Phyllanthus amarus*, *Euphorbia hirta* and *Psidium guajava*) were selected and supplied through the diet to striped catfish juveniles during 2 months in vivo experiment, followed by a bacterial challenge test with *E. ictaluri*. Immune responses and resistance of striped catfish to bacterial infection were assessed in the different treatments and compared with the in vitro responses. The modes of action of these natural compounds are under investigation through the analysis of a set of candidate immune genes as well as gut microbiota in treated fish.

ORAL ABSTRACTS

Session 1: Safety and quality of vegetable & fruit products

Code: O1

Screening of chemical composition, in vitro antioxidant activity and phenolic profiles of tropical fruits by-products

Nguyen Nhat Minh Phuong[a,b,c,d,e], Thien Trung Le[d], Hanne Vissenaekens[a,b,c], Gerard Bryan Gonzales[b], John Van Camp[b], Guy Smagghe[c], Katleen Raes[a]

[a]*Ghent University, Department of Industrial Biological Sciences, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium*

[b]*Ghent University, Department of Food Safety and Food Quality, Coupure Links 653, 9000 Ghent, Belgium*

[c]*Ghent University, Department of Crop Protection, Coupure Links 653, 9000 Ghent, Belgium*

[d]*Nong Lam University, Department of Food Engineering, Faculty of Food Science and Technology, Ho Chi Minh City, Vietnam*

[e]*Can Tho University, Department of Food Technology, College of Agriculture and Applied Biology, Can Tho City, Vietnam*

Various tropical fruits namely red-skinned passion fruit (*Passiflora edulis*), mango (*Mangifera indica L.*), longan (*Dimocarpus longan L.*), rambutan (*Nephelium lappaceum L.*), white-flesh dragon fruit (*Hylocereus undatus*) and red-flesh dragon fruit (*Hylocereus polyrhizus*) were screened for their chemical composition and in vitro antioxidant activity. Especially, the identification of soluble and bound phenolic profiles of all fruit by-products (peel, seed and pulp) was mainly addressed in this frame work. Rambutan peel extract exhibited the highest total phenolic content (TPC) in both of soluble and bound forms (12.68 and 11.70 g GAE/ 100g d.m., respectively). Mango seed and mango seed kernel occupied the second rank of total soluble phenolic content (8-9 g GAE/ 100g d.m.), followed by the others, ranging from 0.5-2 g GAE/ 100g d.m. The same trends were found for antioxidant activities determined by DPPH, ABTS and FRAP assays. The Pearson correlations coefficients (r) between TPC and antioxidant activities obtained from three assays in all samples were high ($0.89 \leq r \leq 0.995$, $P < 0.01$). By using UPLC-MS/MS analysis detailed profiles of soluble and bound phenolic compounds in the different fruit by-products were obtained. For example, the phenolic compounds, ellagic acid, geraniin, quercetin hexoside, gallic and galloyshikimic acid were predominant in rambutan peel, whereas, mangiferin, ellagic acid and galloy(di)glucoside were major compounds in mango seed. In dragon fruit by-products, isorhamnetin derivatives were predominantly present, while kaempherol derivatives were mainly found in longan pulps. These findings have contributed significantly to the database of phenolic profiles of tropical fruits in Southeast-Asia. Herein, by-products of fruit processing like peels and seeds seems promising to be used as ingredients for extending the shelf-life of food products due to their antioxidant and antimicrobial properties; or their extracts can be used as a supplement in the formulation of health care products.

Keywords: *Tropical fruits / antioxidant activity / phenolic profile / UPLC-MS/MS*

Code: O2

Quality changes of dragon fruit *(Hylocereus undatus)* stored at different controlled atmosphere conditions

Phuc L. Ho[a,b], Dinh T. Tran[b], Maarten L.A.T.M. Hertog[a], Quyen T. Nguyen[b], Huong T. Than[b], Paulien Veulemans[a], Nicolaï M. Bart[a,c]

[a] *BIOSYST-MeBioS, Faculty of Bioscience Engineering, KU Leuven, Willem de Croylaan 42, B-3001 Leuven, Belgium.*

[b] *Faculty of Food Science and Technology, Vietnam National University of Agriculture, Hanoi, Vietnam*

[c] *Flanders Centre of Postharvest Technology, Willem de Croylaan 42, B-3001 Leuven, Belgium.*

Dragon fruit (*Hylocereus undatus*) is an exotic fruit with attractive appearance and taste largely grown in Vietnam with shelf life limited to 3 to 5 days. Our previous study has shown that by storing at 6 °C shelf life was extended to 30-35 d. However, this duration is not long enough to export to distant prospective markets such as Europe or America. Controlled atmosphere (CA) is an advanced technology which has been proven useful to extend the shelf life of many fruits.

The purpose of this study was to find an optimal CA condition to maintain the quality and prolong the shelf life of dragon fruit at 6°C. From a preliminary study on the respiration rate of dragon fruit it was found that CO_2 did not have a significant effect on resipration rate and that an O_2 concentration of 2 kPa appeared to be an optimal condition to reduce the metabolic activity since it minimized respiration while keeping fermentation at the lowest level. Based on these findings, several CA conditions were defined to be evaluated in the current study. Instrumental methods were used to analyze quality attributes while a preference test with a 9-points hedonic scale was used to evaluate fruit quality at harvest and after 10 d, 20 d, 30 d, 40 d and 50 d of cold storage. The results showed that fruit stored under 2 kPa O_2 had lower respiration rate and disease development, as compared to those stored at normal air and at 5 kPa O_2. However, the 2 kPa O2 treatment did not improve sensory score of the fruit. Fruit stored at 0.5 kPa O_2 were expected to be undergone fermentation but data showed that their general quality were not significantly affected. The combination of 5 kPa CO_2 and 2 kPa O_2 gave a positive effect on fruit quality because it not only reduced respiration rate, prevented fruit from fermentation but also maintained fruit firmness, acidity and sensory quality.

It can be concluded that storage at 2 kPa O_2 + 5 kPa CO_2 and 6^oC seems to be the optimal CA storage for dragon fruit. Under this condition, the shelf life of the fruit could be extended till 50 d of storage. The current findings open up the possibilities for exporters to start exploring distant markets.

Code: O3

Molecular characterization of Theobroma cacao L. cultivars from Vietnam

Helena everaert[a,d], Hayley Rottiers[a,d], Jocelyn De Wever[a], Kim Hong Tang[c], An Lam Vu[c], Linh Duy Nguyen[c], Jocelyn De Wever[a], Viet Ha Lam Thi[b], Phuoc Pham Hong Duc[c], Phuong Tran Diem[b], Koen Dewettinck[d], Kathy Messens[a]

[a] *Ghent University Department of Applied Biosciences, Ghent, Belgium*

[b] *Ghent University Department of Applied Biosciences, Ghent, Belgium and Ghent University, Department of Food Safety and Food Quality, Ghent, Belgium and Cantho University, Biotechnology Research and Development Institute, Cantho city, Vietnam*

[c] *Nong Lam University, Cocoa Research and Development Project, Ho Chi Minh City, Vietnam*

[d] *Ghent University, Department of Food Safety and Food Quality, Ghent, Belgium*

Increase in global chocolate consumption may cause a future cocoa shortage and subsequently an elevation of cocoa bean price. In order to accommodate the market's demand, cocoa trees were planted in Southern Vietnam. Thanks to the appropriate climate, humidity and soil, Vietnam is currently growing as a cocoa-producing country. The cocoa quality depends on various factors e.g. the genotype/cultivar and post-harvest processing. Hence, to maintain cocoa quality, there is a need for characterization and identification methods to eliminate low quality cocoa genotypes early in the process. The genetic background of Vietnamese cocoa is presumably very diverse; hence few researches have been conducted. In the past, genetic research on cocoa was done mainly using RAPD and RFLP. In this study, microsatellite markers and single nucleotide polymorphisms (SNPs) were used to assay the genetic relationships and to differentiate 75 cocoa cultivars sampled in Vietnam. Descriptive statistics of the used markers were calculated and results from clustering approaches were linked to the cocoa variety. The results provided a strong genetic basis for the Vietnamese cocoa industry in cocoa cultivars selection and valuable genetic material conservation. Further, unlike most current studies which solely based on genetic data, a first investigation was done to link molecular data with technological and sensorial data. The combined information can be linked with cocoa quality and chocolate production to assess the full potential of cocoa in Vietnam. The outcome obtained specifically in Vietnam could also be applied in other countries to selectively access cocoa quality for chocolate industry.

Keywords: *Vietnam / genetic diversity / microsatellite markers / Theobroma Cacao L*

Code: O4
Light-Emitting Diodes improve Ergothioneine Content and Antioxidative Properties of Selected Mushroom Varieties

Nguyen The Han[a], Toshiaki Ohshima[b]

[a] *Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Khanh Hoa, Vietnam*

[b] *Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan 4, Minato-ku, Tokyo 108-8477, Japan.*

Mushrooms are a popular food source containing many bioactive ingredients with health benefits. Here, we studied the influences of maturity stage and light exposure stress induced by light-emitting diode (LED) light sources on the contents of 2-thiol-L-histidine betaine (ergothioneine, ESH) and total phenolics, and the antioxidative capacities of two species of edible mushrooms, Grifola frondosa and Lentinula edodes. Mushroom specimens were harvested at four different developmental stages (S1, S2, S3, and S4). The effects of maturity stage differed between species. For G. frondosa, the contents of ESH and total phenolics changed remarkably during developmental stages. The ESH content steadily declined from 1.76 to 0.41 mg/g dry weight as the fruiting body matured from the S1 to S4 stage. No significant difference in the content of total phenolics was observed at S1 and S2. The concentration of total phenolics decreased significantly from S2 to S3. In regard to L. edodes, the contents of antioxidative compounds as well as the antioxidant capacities of the cap were remarkably higher than those of the stem. The contents of ESH and total phenolics in the cap remained stable between the S1 and S3 stages, before increasing significantly at the fully open cap stage (S4). We also examined the effects of different wavelengths of LED light on the antioxidative components of the G. frondosa fruiting body. The concentrations of ESH and total phenolics as well as antioxidative activities were enhanced significantly upon exposure to blue LED light. These data will assist mushroom cultivators and consumers to identify the optimal harvesting time and mushroom cultivation in order to retain optimal functional and nutritional properties.

Keywords: *Edible mushrooms / Antioxidant / Ergothioneine / Light-emitting diode*

Code: O5

Intelligent Computer Vision System for vegetables and fruits quality Inspection using Soft Computing Techniques

Narendra V G[a] and Srikanth Prabhu[b]

[a] *Associate Professor, Dept. of CSE, Manipal Institute of Technology, Manipal University, Manipal, INDIA-576104 E-mail: narendra.vg@manipal.edu*

[b] *Associate Professor, Dept. of CSE, Manipal Institute of Technology, Manipal University, Manipal, INDIA-576104*

The quality of food products is very important for the human health. The large population and the increased requirements of food products makes it difficult to arrive the desired quality. The quality inspection and sorting tons of fruits and vegetables manually is a slow, costly, and an inaccurate process. In this research a vision-based quality inspection and sorting system is developed, to increase the quality of food products. The quality inspection and sorting process depends on capturing the image of the fruits/vegetables, analyzing captured image to discard defected products in order to identify the good or bad. Four different systems for different food products have been developed namely, Orange, Lemon, Sweet Lime, and Tomato. A dataset of 1200 images is used to train and test the vision systems (300 images for each). The obtained accuracy ranges from 85.00% to 95.00% for Orange, Lemon, Sweet Lime and Tomato used soft-computing techniques such as Back propagation neural network and Probabilistic neural network.

Keywords: Quality Inspection of fruits and Vegetables / Back propagation neural network / Probabilistic neural network / Decision tree

Code: O6
Degradation of opioid peptides and identification of degradation products during yoghurt processing using LC-HRMS

Duc Doan Nguyen[a], Busetti Francesco[b], Stuart Johnson[c], Vicky Solah[c]

[a] *Vietnam National University of Agriculture*

[b] *Edith Cowan University, Australia*

[c] *Curtin Univeristy, Australia*

The degradation of opioid peptides, beta-casomorphin 5 (BCM5) and beta-casomorphin 7 (BCM7) by *Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* ssp. *bulgaricus*, and identification of degradation products during yoghurt processing were investigated by means of liquid chromatography-high resolution mass spectrometry (LC-HRMS). Bovine UHT milk was fermented with: (i) a single strain of *L. delbrueckii* ssp. *bulgaricus*, (ii) a single strain of *S. thermophilus* and (iii) the mixture of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* to pH 4.5 and then stored at 4 °C for 1 and 7 days. Results shown *L. delbrueckii* ssp. *bulgaricus* and/*or S. thermophilus* completely degraded BCM5 and BCM7 upon fermentation to pH 4.5 and degradation products were significantly influenced by bacteria strains and storage time. Four peptides, β-CNf60-61, β-CNf62-63, β-CNf64-66 and β-CNf62-66 were tentatively identified through high resolution MS/MS experiments; however it was not possible to confirm if either milk protein or beta-casomorphins was a source releasing these peptides. Nonetheless, in this study a new peptide β-CNf64-66 released by *L. delbrueckii* ssp. *bulgaricus* was identified for the first time and therefore this peptide now needs to be investigated for any bioactive properties.

Keywords: beta-casomorphinopioid peptides / yoghurt cultures / LC-HRMS /

Session 2: Safety and quality of dairy and meat products

Code: O7
Nutritive value of third party certified organic feed formulation for organic native swine at DA-NVES

Celerina Miranda[a], Charles Fernandez, Jr.[a],
Vincent Jim Palor[a], Erlinda Puducay[a]

[a] *Nueva Vizcaya Experiment Station*

Native pigs can be found in many parts of the country mostly in far-flung villages or barangays. These pigs are known for their ability to grow and reproduce even under adverse conditions, more resistant to parasites and common diseases and require low cost production inputs in terms of housing and feeding. Using organically and locally available resources for feedstuff help in attaining higher profit that is more favorable to farmers.

This project aims to evaluate the nutritive value of formulated feed ration using indigenous feedstuff for organic native pig production and expansion of established feedstuff production area.

Existing three (3) feed formulations for organic native pig was documented. Feed formulation 1 includes 20% Taro, 10% Chayote, 20% Corn, 10% Darak, 5% Salt, 20% Tigi, 10% Banana and 5% Molasses; feed formulation 2 comprises 20 % Taro, 10% Cassava, 10% Chayote, 20% Papaya, 20% Corn, 10% Darak, 5% Salt and 5% Molasses; feed formulation 3 compose 20% Taro, 20% Kangkong, 20% Kamote, 20% Corn, 10% Darak, 5% Salt and 5% Molasses. Samples per formulation were collected and undergone laboratory analysis to document the nutritive value.

Results of the laboratory analysis for sample 1 shows 8.14% Crude Protein, 12.91% Crude Fiber, 2.6% Crude Fat, 9.27% Moisture and 18.80% Ash, while sample 2 gave 7.5% CP, 8.37% CFib, 1.94% CFat, 8.68% Moisture and 16.71% Ash and sample 3 reveals 8.01% CP, 9.37% CFib, 2.2% CFat, 7.97% Moisture and 17.29% Ash. Feed formulation requirements based on Philippine National Standards includes Carbohydrates, Proteins, Fats, Minerals and Vitamins. Based from result, the feedstuff production area shall be expanded to sustain the production of formulated feeds and further enhance in compliance to PNS for Organic Swine Production.

Keywords: Indigenous Feedstuff / Organic Native Swine / Third Party Certified / Nutritive Value

Code: O8

Incorporation of Gac (*Momordica cochinchinensis*) aril powder as natural colorant and/or carotenoid supplement for sausage product

Tuyen Chan Kha[a], Anh Thuy Vu [a]

[a] *Nong Lam University*

In general, colour, appearance and texture are three of the main quality parameters of meat and meat products for consumers who concern about natural and healthy food products. Nitrites are widely used as curing agents in sausage and other cured meat products but it is a toxic additive. Therefore, it is desirable to replace the use of nitrite by natural additives such as carotenoids, which are the consumer preferences.

The results showed that the appropriate formulation for the sausage product was the ratio of pork meat to fat of 2.5 to 1.0. The addition of Gac aril powder was found to be 1.5% under optimal processing conditions of the cooking temperature of 78^{o}C and the cooking time of 40 minutes. According to response surface optimization using central composite design, the content of lycopene and β-carotene was to be optimized as 2912.7 μg/100g and 3682.4 μg/100g, respectively. In addition, color characteristics of optimized sausage product were Lightness of 66, Chroma of 48.3, Hue angle of 75.9 and total colour difference of 25.3. The sausage product added Gac powder had a high stability when stored at -18°C.

In summary, using gac aril powder as a natural coloring additive and/or a carotenoid supplement for sausage product was successfully obtained as the final product showed an attractive color and high content of carotenoids.

Keywords *Gac aril / Sausage / Lycopene / Beta-carotene*

Session 3: Safety and quality of aquaculture products

Code: O9
Seafood safety compliance with hygiene regulations within Vietnamese domestic distribution chains.

Hong Phuc Luu[a], Vuong Van Tran[a]

[a] *Nha Trang University*

In order to improve the safety of seafood in the domestic seafood distribution chains (DSDCs) in Vietnam, a better understanding of current hygiene and practices compliance with government regulations is needed. Infrastructure conditions and documents related to hygiene procedures of trading places, including 6 fishing ports, 32 fish trading establishments, and 12 fish markets in three provinces were evaluated using checklists based on the Vietnamese Government regulations. The hygiene handling practices of 135 fish distributors were also observed by using notational analysis methods. The notational analysis included observation of actions related to microbiological contamination in terms of hand washing or glove changing and the cleaning and sanitising of tools and equipment. Additionally, microbiological quality of 135 samples of raw finfish at points along distribution chains was compared with national standards. The results indicated that all trading places could be classified as non-compliant or seriously non-compliant with the regulations. The practices of fish distributors were also assessed to be at high risk for contamination of raw fish. The findings showed that approximately 42 and 39 percent of samples from fishing ports and fish markets respectively were classified as unacceptable according to the microbiological standards of Vietnam. Taken together, the findings strongly suggest that further effort to sanitise the DSDCs is needed.

Keywords: *seafood safety / safety compliance / Microbiological quality /*

Code: O10
Microalgae biomass as an excellent natural source for functional foods and nutraceuticals

Duc Bach Nguyen[a], Thi Bich Thuy Ly[b]

[a] *Vietnam National University of Agricuture*

[b] *Institute of Biotechnology*

Recently, the global demand of macroalgal and microalgal foods is rapidly growing due to their functional benefits on both human nutrition and health. Biomass of some microalgae species contain significant amounts of protein, lipid, chlorophyll, carotenoids, vitamins, minerals, and pigments. These components have various beneficial effects in the aspects of nutrient, facilitating the digestion, protection and enhance health. Most known microalgae genus have been recognized as Spirulina, Botryococcus, Chlorella, Dunaliella, Haematococcus, and Nostoc containing protein, polyunsaturated fatty acids (PUFA), bioactive compounds such as antioxidant, anti-carcinogen, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. Biomass of Spirulina platensis is an excellent source of protein efficiently absorbed through the gastrointestinal tract. The unique amino acid profile of Spirulina platensis is pefect for human nutrition because all essential amino acids, particularly leucine, valine and isoleucine can be found. Nutritional and toxicological evaluations have demonstrated that microalgal biomass is beneficial as a food supplement or substitute for conventional protein sources, including plant protein. PUFAs from microalgae including arachidonic acid, linolenic acid and eicosapentaenoic acid are pharmacologically important for diet, improving immune system, anti-inflammation, prevention of cardiovascular diseases as well as neuron degradation. The polysaccharides isolated from marine microalgae have antioxidant properties to scavenge reactive oxygen species and anti-tumor. Recently, fucoidan and sulfated polysaccharide has been investigated in the development of new medicines and functional foods. Vitamins with antioxidant properties such as tocopherols and ascorbic acid, water-soluble vitamins or vitamin B-complex, folic acid, pantothenic acid, nicotinic acid, inositol and biotin are important sources to improve health. Major pigments in microalgae such as carotenoids and phycobiliproteins are commonly used in dietary supplements, food colorant, and pharmaceutical products. In addition, the high content of mineral elements in biomass also contributes for the excellent natural source of microalgae as functional foods and nutraceuticals.

Keywords: *microalgae biomass / Spirulina platensis / bioactive compounds / antioxidants*

Code: O11
Detection and Quantification of Polycyclic Aromatic Hydrocarbons in Smoked Freshwater Fish in Cambodian markets

Mith Hasika[a], Marie-Louise Scippo[b], Caroline Douny[b], François Brose[b]

[a] *Department of Chemical Engineering and Food Technology, Institute of Technology of Cambodia, PO. Box 86, Russian Federation Boulevard, Cambodia*

[b] *Department of Food Science, FARAH, University of Liège, Colonster Boulevard, Sart Tilman, 4000B-Liège, Belgium*

Smoking fish has been traditionally processed to give special smoke flavor and crispiness to fish flesh, which contributes in improving household income as well as part of Cambodian economics. However, traditional smoking, one of the oldest food preservation methods, is still widely used in fish processing and it may generate some potential carcinogenic compounds, such as polycyclic aromatic hydrocarbons (PAHs) that can affect consumer's health. Benzo(a)pyrene (BaP), considered as the marker of carcinogenicity among PAHs, is considered as very mutagenic and carcinogenic. Therefore, it was inspiring to carry out a preliminary study of assessment of smoked freshwater fish quality in terms of chemical contaminants (PAHs) in local markets in Phnom Penh capital. Out of nine markets, Oreussey market was a main target for diversification of smoked fish as it contained the highest number of stores (24) commercializing these products as retailers and wholesalers. Five types of smoked fish including Trey Andoeng (Clarias spp.), Trey Kaes (Micronema spp.), Trey Riel (Cirrhinus spp.), Trey Slek Reussey (Paralaubuca typus) and Trey Changvamoul (Rasbora spp.) were commonly available in the market and collected for further investigation. The samples were subjected to analysis by HPLC coupled with fluorescence detection and the quantification of 15 PAHs, including benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene, was realised. The results were compared with maximum acceptable concentrations allowed by European legislation (835/2011/EC).

Keywords: *Smoked fish / Chemical contaminant / Polycyclic aromatic hydrocarbons / HPLC*

Code: O12

Effect of *Phyllanthus amarus* Schum. et Thonn. and *Euphorbia hirta* L. extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during iced storage

Le Anh Dao Nguyen[a], Thi Dung Hoang[b], Minh Phu Tran[a], Kim Dang Pham[b], , Thi Bach Le[a], Quynh Nhu Truong[a], Thi Bich Hang Bui[a], Thi Buu Hue Bui[a], Thi Thanh Huong Do[a], Thanh Phuong Nguyen[a],
Joëlle Quetin-Leclercq[c], Patrick Kestemont[d], Marie-Louise Scippo[e]

[a] *Can Tho university*

[b] *Vietnam National University of Agriculture*

[c] *Université catholique de Louvain*

[d] *University of Namur*

[e] *University of Liège*

Quality changes during iced storage (<4^{o}C) of striped catfish (*Pangasianodon hypophthalmus*) fillets were studied after dip treatment in aqueous solutions of *Phyllanthus amarus* Schum. et Thonn. (0.02% and 0.04%, w/v) or *Euphorbia hirta* L. (0. 06% and 0.02%, w/v). The control (tap water) and the treated fish samples were analyzed periodically for total viable counts or TVC, biochemical parameters (peroxide value, thiobarbituric acid reactive substances, total volatile basic nitrogen or TVB-N), physicochemical parameters (pH, texture, water holding capacity or WHC) and sensory properties. The results indicated that both *P. amarus* and *E. hirta* extract used in dip treatments were able to improve iced storage of striped catfish fillets. *P. amarus* and *E. hirta* extracts obviously inhibited the formation of primary lipid oxidation in striped catfish fillets during iced storage as well as could retain their good quality characteristics in terms of sensory assessment. Both plant extract dip treatments did not affect the TVB-N, texture and WHC of striped catfish fillets compared to the control group. The group of fish fillets dipped 0.04% *P. amarus* displayed lower TVC pH values during the initial storage period whereas *E. hirta* extract did not present those properties. Based on the TVC value and sensory quality, it was shown that the fish fillets dipped in a solution containing 0.04% *P. amarus* or 0.06% *E. hirta* could more effectively maintain their good sensory quality and allowed the shelf life of fillets to be prolonged until 12 days under iced storage.

Keywords: Dip treatments / iced storage / Pangasianodon hypophthalmus / Euphorbia hirta L., Phyllanthus amarus Schum. et Thonn.

Code: O13

Effects of processing conditions on quality of Tra (*Pangasianodon hypophthalmus*) catfish fillets and derived products

Thien Trung Le[b], Thuy Linh Nguyen[a], Thach Bao Tran[b], Thi Thanh Thao Nguyen[b], La Vy Nguyen[b], Ngoc Diem Huynh[b], Anh Trinh Nguyen[b], Trung Hau Nguyen[b], Thi Lam An Vu[b]

[a] *Faculty of Fishery, Nong Lam University - Ho Chi Minh City, Vietnam*

[b] *Faculty of Food Science and Technology, Nong Lam University - Ho Chi Minh City, Vietnam*

Vietnam produces about a million tons of Tra catfish a year. A major part of this production is for processing frozen fillets for exportation. There is not a standardized processing procedure and companies practice differently based on their experience and facility availability. Processing conditions of fish influence quality of the obtained fillets. However, there has not been a thorough research to address this issue for Tra catfish. The objectives of this two-year project are to study the (1) the effects of electrical stimulation on rigor mortis development (the possibility of using electrical stimulation to shorten the rigor mortis development to facilitate post rigor mortis processing), (2) effects of processing conditions (pre-rigor and post-rigor filleting) in combination with electrical stimulation to the quality of the fresh and frozen/thawed fillets as well as (3) to the derived products including fish gel and smoked Tra fish. Parameters including color, pH, texture, water-holding-capacity, cooking loss, drip loss were among other to be analyzed. Quality parameters are influenced differently with processing conditions. The results of the project add to the knowledge about Tra catfish processing and build a background for Tra catfish processing companies to improve their processing practices.

Keywords: *Tra fish (Pangasius) / Electrical stimulation / rigor mortis / Quality*

POSTER ABSTRACTS

Session 1: Safety and quality of vegetable & fruit products

Code: P1

Effect of processing and storage condition on the stability of betacyanin in juice of red-fleshed dragon fruit (*Hylocereus polyrhizus*)

Phan Thi Thanh Que[a], Tong Thi Anh Ngoc[a], Nguyen Thi Thu Thuy[a], Le Duy Nghia[a]

[a] *Can Tho University*

The stability of betacyanin in fruit juice produced from red-fleshed dragon fruit (Hylocereus polyrhizus) was investigated. The objective of the study was to determine the effects of the addition of ascorbic acid, the pasteurization regime as well as different storage conditions [i.e. in brown/transparent glass bottle, stored in light at room temperature (30±2^{o}C) or in dark in cooling chamber (13^{o}C)] for 3 weeks, on the betacyanin content in the final product. UV-visible spectrophotometry at 538nm was used for analyzing the betacyanin content. In addition, to ensure the safety of product, the Pasteurisation Unit (PU) value was calculated. The results showed that the addition of 0.2% ascorbic acid, pH 4.0, and pasteurization at 80^{o}C for 15 min (calculated PU-value, 5.33 min) were selected as the best processing conditions to retain betacyanin in red-fleshed dragon fruit juice. The storage conditions had a significant effect on the betacyanin retention. Storage of red-fleshed dragon juice packed in the brown glass bottles at room temperature with light exposure for 3 weeks, showed a 52% betacyanin retention compared to that of the juice packaged in the transparent glass bottles (20% retention). With stored for 2 weeks in the dark at 13^{o}C, both types of packagings showed an almost similar betacyanin retention (i.e. > 90%).

Keywords: *betacyanin / ascorbic acid / dragon fruit / storage*

Code: P2

Effect of adding extract from carrot skins on the biomass yield and quality of Pleurotus sajor-caju cultivated on sawdust substrate

Hanh Quyen Dang[a], Xuan Duy Nguyen[b], Anh Tuan Nguyen[b]

[a]*Phu Yen Department of Science and Technology*
[b]*Nha Trang University*

Carrot skins (CS) contain a lot of useful nutritional components. However, CS is considered as wastes from processes of carrot products. In this study, the extract from CS was used as additive ingredients toward cultivation medium of edible mushroom. Accordingly, effect of addition of CS extract on biomass yield and quality of fruiting body of *Pleurotus sajor-caju* cultivated on sawdust substrate were investigated. Results showed that addtion of 0.5% CS extract into sawdust substrate medium increased 36.7% biomass yield compared with the control. For quality targets, the color of the fresh fruiting body for the treated samples with 0.5% CS was more grey than the control samples. For chemical component included moisture, ash, protein, lipid and carbohydrate, there was not significant between the treated and the control samples ($p > 0.05$). However, there were various differences in amino acids profile and fatty acids profiles among the samples recorded. Findings in this investigation provides the useful information for applying the extract from carrot skins in the edible mushroom cultivation industry.

Keywords: Carrot skins / biomass yield / quality / Pleurotus sajor-caju

Code: P3

Preliminary study on wood vinegar production from various woods in Vietnam and its application in agricultural area

Xuan Duy Nguyen[a], Duc Vu Luong[a]

[a] *Nha Trang University*

Wood vinegar (WV), also called pyroligneous acid or liquid smoke, is a by-product of charcoal or biochar production. This is a bio-based product that is safe and friendly with environment. The global market of the WV is valued at 3.2 million US dollar in 2015, and it is expected to grow at a rate of 7.1% during 2016 – 2023. Various applications of the WV in agricultural area have been recorded last time. In Vietnam, wood vinegar production is almost not developed widely and it is still a new field. In this study, therefore, we firstly report about wood vinegar production from several different woods. Additionally, quality characteristics of the WV is also measured and compared with the commercial products available from the market. Results indicated that the WV produced from Vietnamese woods had quality targets as similar as the commercial products when comparing based on colour, pH value, and specific weight. Particularly, antibacterial activities and polyphenol contents of the WV is significantly higher than the commercial product ($p < 0.05$). Extraction yield of WV can reach approximately 12%, depending on type of wood. Preliminary applications of WV in preventing disease on dragon fruit and mushroom indicated positive results. Our findings reveal potential of using the wood vinegar in agricultural applications for a sustainable agriculture.

Keywords: *Antibacterial activity / agricultural area / by-product / pyroligneous acid*

Code: P4

Drying condition optimization of tomato waste for lycopene extraction

Lan Thi Hoang Nguyen[a], Thanh Thi Kim Nguyen[a], Nguyen Ngoc Cuong[a], Nguyen Minh Chau[a], Marie-Louise Scippo[b], Dinh Thi Tran[a]

[a]*Faculty of Food Science and Technology, Vietnam National University of Agriculture, Vietnam*
[b]*Department of Food Science, University of Liège, Belgium*

Corresponding author: Dinh Thi Tran, email: ttdinh@vnua.edu.vn

Lycopene is one of 750 carotenoids found in nature and is responsible for the deep-red color of fruits. Lycopene is also known as a potential antioxidant because it is proven to be responsible for protecting cell against oxidative damage, thereby decreasing the risk of chronic diseases. Lycopene represents about 80-90% of total carotenoids in tomato. Tomato processing industry produces large amounts of solid waste which can be utilized to extract lycopene. Before extraction, it is important to prepare the dried tomato waste. In this study, the convective drying condition namely drying temperature and the final moisture content of tomato waste were optimized using response surface methodology. The results showed that the optimal drying temperature and the final moisture content of the dried tomato waste were found to be 65^{o}C and 23% respectively. Using the tomato waste dried under this condition as extraction material, the lycopene content and its antioxidant capacity were obtained to be 8.915mg/g DW and 8.13 µmol trolox equivalent/g dry weight by extracting with ethyl acetate solvent. Lycopene extracted from this dried tomato waste in this study could be used to produce functional foods

Keywords: *Lycopene / Tomato / Drying condition /*

Code: P5

Evaluation of spent brewer's yeast autolysis by using Bacillus subtilis overproducing enzyme ?-glucanase

Vinh Hoang Nguyen[a], Hoang Anh Nguyen[a]

[a] *Vietnam National University of Agriculture*

Spent brewer's yeast cells contain high levels of nutritional value such as vitamins (especially B group vitamins), total protein content (about 40-60%) and amino acids which is a potential resource to produce yeast extract. Yeast cell wall is a gluco-mano-lipo-protein complex, in which glucan accounts for 35-45%. β – glucanase is produced by a large diversity of microorganisms including Bacillus species. Use of Bacillus subtilis overproducing enzyme β-glucanase or β-glucanase extract from these strain to do autolysis spent brewer's yeast is still receiving considerable attention. In this study, two strains of Bacillus spp. (A7.2 and A7.7) producing high amount of enzyme β-glucanase were used to autolysis of spent brewer's yeast. The result showed that with 5% of Bacilus subtillis in optimal conditions after 43 hours of cell autolysis, the highest amount of total protein content were 171.64 mg/g and 193,99 mg/g cell biomass with A7.7 and A7.2, respectively, and total amino acids content were 138.18 mg/g with A7.7 and 152.25 mg/g with A7.2. With 4% of enzyme β-glucanase produced from two Bacilus subtillis strains in optimal conditions after 19 hours of hydrolysis incubation can also give the highest amount of total protein content (132.55 mg/g with A7.7 and 145.42 mg/g with A7.2) and amino acids (126.14 mg/g with A7.7 and 137.23 mg/g with A7.2). Results indicated that Bacillus substilis or enzyme β-glucanase overproduced from them is promising to apply in spent brewer's yeast autolysis to produce valuable yeast extract.

Keywords: *Bacillus subtilis / ?-glucanase / spent brewer's yeast /*

Code: P6

Development and Promotion of Third Party Certified Organic Upland Rice

CELERINA MIRANDA[a], Czarina Nova Pini[a], Lexter Tapec[a]

[a] *Department of Agriculture - Regional Field Office No. 02 Nueva Vizcaya Experiment Station*

The passage of Organic Agriculture Act of 2010 or RA 10068 signals a paradigm shift in agricultural production from conventional practices to organic agriculture. Rice plays a dominant role in crop production for upland areas in the Philippines. Growing upland rice was practice in the marginal areas but not given importance due to low production.

This project aims to harness the potential of upland rice ecosystem as source of rice supply, purify and mass produce identified promising varieties and produce Third Party Certified Organic seeds.

Collection and characterization of Upland Rice was conducted with an area of 0.50 hectare initially established at the Department of Agriculture-Nueva Vizcaya Experiment Station, Tapaya, Villaros, Bagabag, Nueva Vizcaya, Philippines planted with three (3) entries namely Galo from Alfonso Castañeda, Mindoro from Santa Fe, and Palawan from Ambaguio all municipalities of Nueva Vizcaya, Philippines. Organic management practices were implemented and Galo Upland Rice was found best performer among the three entries.

Galo was now Third Party Certified by Organic Certification Center of the Philippines (OCCP) with Organic Certificate No. 019816-2 and Producer Code 16020065.

For sustainability, Galo area expanded into 2.0 hectares and seeds produced were initially distributed to ten (10) farmers at Ambaguio and Dupax del Norte, Nueva Vizcaya, Philippines thru Community Seedbank (CSB) project planted to 5 hectares. This project already served 167 farmer adoptors covering 107.25 hectares in seven (7) municipalities.

Keywords: Collection / Characterization / Third Party Certified / Galo

Code: P7

THE SUPPLY CHAIN ANALYSIS OF PEANUTS: A CASE STUDY IN QUANG NAM PROVINCE OF VIETNAM

Tan Nguyen Huynh[a]

[a] *Dong Nai Technology University*

Peanuts production plays a major role in the daily social-economic activities in Quang Nam province of Vietnam. Nevertheless, peanuts production is facing several daunting challenges, mainly instable price of product. The local authority have not yet understood how supply chain of peanuts works. Based on the above, the main purposes of this research were to (1) address supply chain for peanuts production; (2) describe the key players in the chain and analyze the linkage among them; (3) suggest some useful recommendations to improve the performance of supply chain in the next period. To do so, the study randomly surveyed 170 peanuts cultivators from three districts where have the largest cultivation areas of peanuts in Quang Nam province. Next, the research investigated some agencies that provided inputs and distributed outputs. All data obtained from the investigation was coded, and then imported into SPSS software for analysis. The research results demonstrated that supply chain of peanuts in Quang Nam province was relatively sophisticated, involving many stakeholders: inputs suppliers, peanuts farmers, wholesalers inside and outside province, retailers inside and outside province, and foreign importers. The outcome of this study also indicated that inputs for production were provided from three sources such as 22.3% from inside-province premises, 35.9% from tier 2 agency, 41.8% from tier 1 agency, meanwhile almost volume of peanuts was bought directly to wholesalers (about 82.8%) with payment either on credit or in cash. Furthermore, consumption price varied in huge range, especially for domestic consumption channel, and foreign consumption of peanuts brought profit higher than domestic consumption. Importantly, the paper strongly recommended that the local authority should urge to enact policies to encourage cultivators to access official credit sources and to promote the export performance of peanuts. Lastly, this is the first research in Quang Nam related to the analysis of supply chain of peanuts. Hence, more studies should be undertaken to confirm these results for better policies.

Keywords: *peanuts production / supply chain for peanuts / supply chain analysis / stakeholder*s

Code: P8

Longan (*Dimocarpus longan*) Propagation and Commercialization, Technology Adoption and Utilization in the Philippines

CELERINA MIRANDA[a], Primitivo Jose Santos[b], Salvador Bulda[a], Valeriano Corales[a], Lexter Tapec[a]

[a] *Department of Agriculture - Regional Field Office No. 02 Nueva Vizcaya Experiment Station*

[b] *Institute of Plant Breeding-University of the Philippines Los Baños, Laguna, Philippines*

This project was conducted to benchmark data of existing longan trees in Regions 2, 10 and 11. Identify and characterize longan that flowers naturally to be asexually propagated for establishment of small orchard.

There are 1,526 trees surveyed with 365 flowering trees and 262 fruiting trees in region 2 while in region 11 with six (6) trees (4 years old). Cultivar at Nueva Vizcaya Experiment Station (NVES), Bagabag, Nueva Vizcaya, Philippines was selected for its fruiting ability at higher elevation (651.45 masl) while trees at Claveria, Cagayan, Philippines were noted by its flowering and fruiting characteristics in lowland condition (20-23 masl).

The LRALVIAR Pride Longan of the Nueva Vizcaya Experiment Station was deliberated by the National Seed Industry Council bearing the code NSIC 2016 Lg 01.

Superimposed trial is implemented with the application of Muriate of Potash (0-0-60) 500 g/tree: (AA1 - Control (No application); MP1 - at flower setting; MP2 - at fruit setting). ANOVA reveals significant differences in total soluble solid (%Brix) at 140 days after flower setting which gave the highest TSS (24.79) compared to other treatment trees using LSD.

Propagated cultivars were used to establish four (4) site of small orchard in regions 02, 10 and 11 with 10 trees per site.

Keywords: *Longan / Muriate of Potash / LRALVIAR PRIDE / Small Longan Orchard*

Code: P9

Study on the production of kudzu drink with good sensorial quality and effect of this drink on reduction of sleeping time, alcohol concentration in blood of ethanol treated rats

Thao Yen Linh Dang[a], Thi Thanh Thuy Nguyen[a]

[a] *Vietnam National University of Agriculture*

Kudzu (Pueraria thomsoni Benth) is a kind of perennial vines, which are grown in Vietnam, Laos, China and India. In addition to starch, kudzu roots also have bioactive compounds such as: puerarin, daidzin, daidzein, etc. that plays an important role in reducing the risk of cardiovascular disease, osteoporosis and cancer prevention (Do Tat Loi, 2000). Besides, they also have the effect reduces of alcohol intoxication and reducing the effects of alcohol consumption (Chang et al., 1994). This study aimed to find the effect of extract time and temperature for production of kudzu drink containing bioactive substances and good sensory qualities similar to commercial drinks. This product was evaluated on the effects of reducing sleeping time and blood alcohol concentration of ethanol-treated rats. Dried kudzu root shell was extracted by water with the ratio of material:water at 1:50, extract temperature at 65, 70, 75 and 80^{o}C for 15, 20 and 25 minutes and sterilized at 115^{o}C in 10 minutes. The result showed that, at the treatment of 75^{o}C – 20 minutes, the kudzu drink with puerarin, daidzin and daidzein content at 184,32; 42,46; 20,60 µg/ml respectively and its sensorial quality was similar to the Chineses Gelao beverages. After that, kudzu drink with amount at 0; 2; 2,5; 3.0; 3,5; 4.0 ml/kg body weight was given to two groups of rats (fully-fed rats and 12-hour-fasted rats) to test sleeping time and blood alcohol concentration of ethanol treated rats. The result indicated that the amount of kudzu drink at 3,5 ml/kg body weight for fully-fed rats and 3 ml/ kg body weight for 12-hour-fasted rats were capable to shorten the sleep time. Also, only 2ml/ kg body weight of kudzu drink had the effect of reducing the blood alcohol concentration for both groups of fully-fed rats and 12-hour-fasted rats.

Keywords: *kudzu drink / alcohol concentration / puerarin / daidzin*

Code: P10
CHEMICAL COMPOSITON, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF CITRUS LIMONIA OSBECK

Thanh Le Huu[a], Chi Pham Thi Lan[a], Phi Nguyen Thi Lan[a]

[a] *Bach Khoa University*

Essential oils (EO) with antioxidant and antibacterial activities are recognized as potential sources of natural compounds to improve the shelf life and the safety of food. The study was carried out to determine the chemical composition, antioxidant and antibacterial activities of essential oil extracted from leaves of Citrus limonia Osbeck (Da Lat lime). The essential oil was extracted by hydro-distillation method. A total 53 components were identified from essential oils by GC-MS, in which limonene (9.69%), citronellol (8.34%), citronellal (4.31%), b-pinene (3.33%), b-caryophyllene (3.27%), a-terpineol (2.09%) were found as the major compounds.

The antioxidant activities of Da lat lime essential oils were determined by DPPH scavenging and Ferric thiocyanate (FTC) assays. The results showed that the essential oils had high antioxidant activities. The IC50 values of the EO in DPPH scavenging and FTC assays were 5.8 mg/ml and 74.06 mg/ml, respectively. The antibacterial activities were determined by growth inhibition zone diameter and broth dilution assays against the food-pathogenic bacteria such as S.aureus, B.cereus, P.aeruginosa and S.typhi. The Da Lat lime essential oil possessed the highest inhibition on P.aeruginosa. These results suggest that essential oils extracted from leaves Citrus limonia Osbeck (Da Lat lime) could be used as a way of preventing the growth of common food poisoning.

Keywords: *Chemical composition / Antioxidant / Antibacterial activities / Citrus limonia Óbeck*

Code: P11

Quinoa (*Chenopodium quinoa Willd*.): Nutritional value and Utilization to produce instant calcium- rich nutritional powder

Huong Thi Lan Tran[a], Bao Huy Nguyen[a]

[a] *Vietnam National University of Agriculture*

Quinoa *(Chenopodium quinoa Willd*.) which is considered a pseudocereal or pseudograin, has been recognized as a complete food due to its protein quality. It has remarkable high nutritional value; not only from its protein content (15- 18%) but also from its great amino acid balance. It is an important source of minerals and vitamins, and has also been found to contain compounds like polyphenols, phytosterols, and flavonoids with possible nutraceutical benefits. Besides, it has been considered an oil crop, with an interesting proportion of omega-6 and a notable vitamin E content. Quinoa starch has low glycemix index. Beside of that, Quinoa is a crop that is adaptable to harsh climates: drought-resistant, salt tolerant. In Vietnam, it is a promising alternative cultivar for crop restructuring in the context of climate change. In order to ensure sustainable quinoa production, diversification of quinoa products such as instant calcium- rich nutritional powder needs to be carry. Processing line of the nutritional powder includes following steps: Clean and Dry grains to constant moiture; Extrude; Grind and sieve; Mix and packing, sealing and storage. In whole processing line, our study only focused on identifying the recipes so as to ensure adequate supply of energy, adequate nutrition and good organoleptic qualities. The results showed that the mixture of 35.2- 35.5 % quinoa, 8.5-10% rice; 20% sugar; 24% mild powder, 7.5-8.0% maltodextrin and 0.3%vanilla was the best recipy. In the sensory test 81.7 % of consumers rated as preferred products. Products also meet hygiene and food safety standards and its shelflife last for 24 months.

Keywords: Quinoa / Chenopodium quinoa Willd. / instant calcium- rich nutritional powder / recipy

Code: P12

Determination of maturity stage and effects of some preparations on the quality of Taiwanese papaya fruit during storage

Thi Minh Nguyet Hoang[a]

[a] *Vietnam National University of Agriculture*

Papaya is one tropical fruit which is widely cultivated in many regions of Vietnam. The fruit has tasty flesh and attactive aroma and flavour, and it is very flavourable to customers. However, it is difficult to maintain the fruit 's quality for several days, because it is very sensitive with outside impacts such as physical injuries, micro-organisms, and sencesnery. To maintain best eat-ting quality, the fruit has reached physiological maturity, the yellow area on the fruit pods from 25% to 50% and treatment with aloe vera gel solution, the fruit should be keep at 10-120C for 15-20 days.

Keywords: *Papaya / maturity stage / aloe vera gel solution / storage*

Code: P13

DETERMINATION OF TECHNOLOGICAL CONDITIONS FOR BRANDY PINEAPLE FERMENTTATION

Thi Hien Dinh[a], Van Lam Nguyen[a]

[a] *Vietnam National University Of Agriculture*

Pineaple used for the brandy production was selected pineapple Queen (Bao Son - Bac Giang province). Enzyme Pectinex Ultra SP-L was added into the pineapple juice with the concentration of 0,025% (v/v). Fermentation juice was enriched with sugar to reach the final total sugar concentration of 180 g/l. Fermentation was performed at 16^0C, 18^0C, 20^0C, 22^0C, 24^0C and selected strain *Saccharomyces cerevisiae* C5 was used at different rates of 0,1%, 0,2%, 0,3%, 0,4%, 0,5%, 0,6% (w/w). Results showed that 0,4% (v/v) *Saccharomyces cerevisiae* C5 used for the fermentation at 20°C, during 7 days were the optimum conditions for the brandy production.

Keywords: pineapple juice / fermented juice / yeast / Fermentation

Code: P14

Enzyme-assisted vacuum distillation essential oils from Vietnamese citrus leaves, their chemical compositions, antioxidant and antimicrobial activities

Nguyen Thi Phuong Giau[a], Pham Thi Lan Chi[b],
Pham Van Hung[a], Nguyen Thi Lan Phi[a]

[a] *School of Biotechnology, International University, Vietnam National University in HCMC. Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam.*

[b] *Faculty of Chemical Engineering, Bach Khoa University HoChiMinh City. 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam.*

Essential oils (EOs) are natural compounds containing complicated biologically active substances, and they are being used as flavoring agents, as also preservative agent in food technology. This study was conducted to determine the antioxidant and antimicrobial activities of EOs extracted from leaves of two citrus varities. Leaves of Long An lime (*Citrus aurantifolia* Swingle) and Dao lime (Citrus hybrid – *Citrus spp*.) were extracted by enzyme- assisted vacuum distillation. The chemical components of these EOs were determined by gas chromatography coupled with mass spectrometry (GC-MS). The results showed that limonene and β-pinene are the main compound in two EOs. The antioxidant activities of EO were measured by Ferric Thiocyanate assay. Two kinds of EO showed high antioxidant activities. The antimicrobial activities were tested by diffusion and dilution method against the foodborne microorganisms. The activity of Long An lime leaf EO was stronger than that of Dao lime EO on *S.aureus*, *B.cereus*, *S.typhi*. However, in case of *P.aeruginosa*, the inhibition of Dao lime EOs was stronger than that of Long An lime EO. According to the reults, EOs extract from different leaves of Citrus varieties may have different antimicrobial and antioxidant activities. The results of this study showed that essential oils of citrus leaves could be widely used as flavoring and preservatives in food industry.

Keywords: enzyme- assisted vacuum distillation, essential oil, citrus leaves, chemical compositions, antimicrobial activities, antioxidant activities. /

Code: P15

Effects of *Aloe vera* gel coatings on postharvest quality of honeydew melon fruit (*Cucumis melo* L.)

Nguyen Thi Hanh[a], Nguyen Thi Thu Nga[a]

[a] *Faculty of Food Science and Technology, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Ha Noi*

Honeydew melon (*Cucumis melo* L.) is a highly nutritious fruit but the climacteric respiration property lead to rapid quality degradation, short shelf-life and low marketability. This study aimed to evaluate the effects of coating made from *Aloe vera* gel on weight loss, firmness, respiration and ethylene production rate, total soluble solid and ascorbic acid content in order to extend the shelf-life of honeydew melon stored at ambient temperature of 28 ± 3 ^{0}C and relative humidity of 80 – 90 % for nine days. The applications of coating from *Aloe vera* gel on honeydew melon were shown to be effective in maintaining the quality of the fruits. This coating reduced the weight loss, respiratory and ethylene production rate, retaining the firmness and biochemical component of fruits. The coating solutions with 60 % *Aloe vera* gel give the best results.

Keywords: *Aloe vera / coating / honeydew melon /*

Code: P16
COMPARE THE INFLUENCE OF SEVERAL METHODS ON REDUCING SUGAR AND COLOUR OF HONEY

Nguyen Xuan Nam[a]

[a] *Ho Chi Minh City University of Technology*

The aim of this study was to compare influence of several methods on reducing sugar and colour of honey include thermal processing, microwave, ultrasound, and immobilized enzyme. The results showed that reducing sugar content increased (average 8.22% with thermal, 9.47% with microwave, 4.98% with ultrasonic waves processing), the colour fluctuated but not significantly. White processing with Chitosan-invertase improved 28.89% reducing sugar content and significant improved the colour (?E = 3,9) of honey.

Keywords: thermal processing / microwave processing / ultrasonic wave processing / ultrasonic wave processing

Code: P17
FACTORS AFFECTED ON THE DISCOLORATION OF DEEP FRIED EGGPLANT

Nguyen Huu Thien[a], Huan Phan Tai[a]

[a] *Faculty of Food Science and Technology,Nong Lam University – Ho Chi Minh City*

Eggplant is an ordinary kind of vegetable but it could make several benefits in terms of economy such as high productivity, short-time planting periods, making delicious dishes, etc. Additionally, eggplant also has been proved to nutritionally affect on preventing and treating some diseases. Eggplant normally is consumed in fresh ones or cooked and served at home and restaurant; while the processed eggplant (e.i. canned, frozen, deep-fried ones) that could add value for it has been not popular.

This study was carried out on deep-fried eggplant to examine the factors that caused the discoloration in storage. The treating conditions with CaCl2, including concentrations (0, 1, 2, 3 and 4%) and soaking time (0, 5,10,15 and 20 minutes) were investigated in turn; the colour (L*, a* and b* values) after 48-hour cooled storage period were the indices of assessment. The results showed that the eggplant dipped into CaCl2 solution 2% for 5 minutes had the smallest changes in L*,a* and b* values, which indicated for the lowest discoloration as compared to other treatments. Similarly, the deep-frying conditions, including temperatures (140, 160, 180 and 200^{o}C) and time (30, 60, 120 and180 seconds) were also consecutively studied. The results showed that both frying temperature and frying time affected on the discoloration and the optimum frying conditions were found to be 160^{o}C for 210 seconds.

Keywords: Deep fried / Eggplant / Discoloration /

Code: P18

EFFECT OF THERMAL PROCESSING AND STORAGE ON QUALITY OF CAJUPUT (*MELALEUCA CAJUPUTI*) HONEY FROM BAC LIEU – VIETNAM

Nguyen Xuan Nam[a], Huynh Ngoc Oanh[a], Phan Phuoc Hien[b]

[a] *Ho Chi Minh City University of Technology*

[b] *Southern Cantho University*

The aim of the present work was to find out if thermal processing and storage conditions can affect some of the main honey quality paramenters (reducing sugar (RS), hydroxymethylfurfural (HMF), diastase number (DN), water and colour) of cajuput honey was obtained from Bac Lieu – Vietnam. RS content were analyzed by DNS method, HMF under AOAC 980.23, diastase activity based on AOAC 958.09, water content according to AOAC 969.38B and colour parameters (L*, a*, b*) were established in the CIE system. The results of the analysis showed that the physico-chemical characteristics of fresh honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81. Honey was heated at 50, 60, 70°C for 20, 30, and 40 min then the honey sample was left to room temperature (20-23^{o}C), stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks. The changes in the RS, HMF, DN, water and colour were observed. Thermal processing applied to cajuput honey did not effect on RS, water content and colour, while HMF and DN affected significantly. The storage time and temperature affected significantly on colour.

Keywords: *honey / thermal processing / storage*

Code: P19
EFFECT OF MICROWAVE PROCESSING AND STORAGE ON QUALITY OF CAJUPUT (*MELALEUCA CAJUPUTI)* HONEY FROM BAC LIEU – VIETNAM

Nguyen Xuan Nam[a], Huynh Ngoc Oanh[a], Phan Phuoc Hien[b]

[a] *Ho Chi Minh City University of Technology*

[b] *Southern Cantho University*

The aim of the present work was to find out if microwave processing and storage conditions can affect some of the main honey quality paramenters (reducing sugar (RS), hydroxymethylfurfural (HMF), diastase number (DN), water and colour) of cajuput honey was obtained from Bac Lieu – Vietnam. RS content were analyzed by DNS method, HMF under AOAC 980.23, diastase activity based on AOAC 958.09, water content according to AOAC 969.38B and colour parameters (L*, a*, b*) were established in the CIE system. The results of the analysis showed that the physico-chemical characteristics of fresh honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81. Honey was processed at continues power 30P (480 W), 50P (800 W), 70P (1120 W) for 20, 30, and 40 seconds then the honey sample was left to room temperature (20-23^{o}C), stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks. The changes in the RS, HMF, DN, water and colour were observed. Microwave processing applied to cajuput honey did not effect on colour, while RS, HMF, DN and water content affected significantly. The storage time and temperature affected significantly on RS, DN and colour.

Keywords: *honey / microwave processing / storage*

Code: P20
CHANGES IN ALPHA–GALACTOSIDASE ACTIVITY AND OLIGOSACCHARIDES DURING GERMINATION OF SOYBEAN SEEDS

Thi Phuong Lien Duong[a], Thi Bich Tram Phan[a], Sy Trung Trang[b], Thanh Toan Ha[c]

[a] *College of Agriculture and Applied Biology, Cantho University, Vietnam*

[b] *Faculty of Food Technology, Nha Trang University, Vietnam*

[c] *Biotechnology Research and Development Institute, Cantho University, Vietn*am

The effect of germination process on α–galactosidase activities, oligosaccharides and reducing sugars contents in soybean seeds was investigated. Soaked soybean seeds were germinated at 25^{o}C in dark condition for 72 hours. Samples were collected every 12 hours during germination for analysis the α–galactosidase activities, which was monitored with a synthetic substrate ρ-nitrophenyl-α-D-galactopyranoside (ρNPGal). The freeze-dried samples were prepared for determination of raffinose, stachyose, sucrose by thin layer chromatography (TLC) and reducing sugars were assayed by reaction with Nitro Salicylic acid (DNS). After soaking, the activity of α–galactosidase inceased 1.84 times compared to that in the soybean seeds and reached to maximum value (164.3±2.5 U/100g, db) after 12 hours of germination. The increase in α–galactosidase activity led to the decreasing in raffinose and stachyose contents during soaking and germination. In addition, the degradation of these undesirable components followed the first order exponental equation (R2 = 0.97 – 0.99). Sucrose content remained after soaking and up to 12 hours of germination and reduced significantly after that. Final result of the hydrolysis of raffinose, stachyose and sucrose was significant increase in reducing sugars that can be used as energy-source during germination of soybean seeds.

Keywords: ?–galactosidase / germination / oligosaccharides / soybeans

Code: P21

EFFECT OF POSTHARVEST TREATMENT WITH OXALIC ACID ON THE QUALITY OF PEACH *(Prunus persica L.)*

Vu Thi Kim Oanh[a]

[a] *Vietnam National University of Agriculture*

Browning has been a major problem in fresh peach (*Prunus persica L.)* preservation. The aim of this study is to identify the optimum mode of postharvest treatment with Oxalic acid in order to prevent the browning and prolong storage shelf-life of peach fruit. The two factors, concentration of Oxalic acid and time dipping to Oxalic acid, were taken in to account. The experiment on concentration of Oxalic acid was carried out with 3 concentrations of 1.0; 2.0 and 3.0 mM along in 10 minutes dipping. Then the experiment on time dipping to Oxalic acid was conducted with 3 levels of 5; 10 and 15 minutes along with the best Oxalic acid concentration selected from previous experiment. The results showed that the treatment laid on 2.0mM Oxalic acid in 15 minutes dipping gave highest quality Peach after 42 days storage in low temperature condition.

Keywords: *Peach / Prunus persica L. / Oxalic acid / browning*

Code: P22

IMPACTS OF MAIN FACTORS ON ALCOHOLIC FERMENTATION OF CACAO PULP JUICE BY *SACCHAROMYCES CEREVISIAE* VTCC – Y – 0011

Minh Hien Nguyen, Thi Thanh Van Tran, Tai Huan Phan*

Nong Lam University - Ho Chi Minh City

* *Corresponding author: pthuan@hcmuaf.edu.vn*

Response surface methodology (RSM) was applied to select optimal conditions of varied factors including total soluble solid content, pH and inoculum size for fermentation of cacao pulp juice by *Saccharomyces cerevisiae* VTCC – Y – 0011 to produce ethanol. The experiments were carried out according to the central composite design (CCD) with total soluble solid content ranged from 20 to 24 °Bx, inoculum size from 1 to 5% yeast and pH value from 3 to 5. A quadratic model was respectively developed to correlate the preparation variables to the ethanol yield. The results showed that a production of ethanol from the cacao pulp juice could be achieved up to 10.45% (v/v) after 168 h at optimum conditions of 22 °Bx, 3.5% yeast and pH 4.5. The predicted values for optimization of process variables was found to be in agreement with the experimental values.

Keywords: *fermentation / cacao pulp juice / Saccharomyces cerevisiae / RSM*

Code: P24
Effects of storage conditions and packaging materials on the quality of Vietnamese dark chocolate.

Ngoc Anh Thuy Phan[a], Tuyet Mai Nguyen[a]

[a] *International University - Vietnam National University HCM City*

Storage stability in tropical condition has a great impact on the quality and acceptability of Vietnamese dark chocolate. This research was aimed to investigate the influence of 3 storage conditions and 3 packaging modes on the physicochemical quality of Vietnamese 70% dark chocolate during 10 weeks of storage. The studied conditions were room condition, refrigerator, and freezer. The applied packaging modes were original packing paper of a commercial chocolate, aluminum foil with moisture absorber, and aluminum foil with plastic. Analytical quality parameters included moisture absorption, hardness, bloom formation, peroxide value (PV), and total phenolic content (TPC). After 10 weeks, chocolate samples in all 3 conditions exhibited increases in hardness, moisture absorption and visible signs of blooming. These changes were evidence of degradation in the sensorial physical quality of chocolate. Blooming occurred after only 2 weeks in room condition but appeared after 4 to 6 weeks in fridge and freezer. Whitish bloom completely covered the chocolate surface in room condition after 10 weeks. Original packaging allowed the most moisture uptake. After 10 weeks, PV still lied in the acceptable range, and more than 70% of TPC still remained. Hence, there were significant changes in appearance and physical quality of dark chocolate, but the biochemical quality or nutritional value was not significantly affected, by storage conditions, and packaging for the 10-week period. These results could be used as references by manufacturers in tropical countries to better preserve their products.

Keywords: *Chocolate / Packaging materials / Storage conditions /*

Code: P25

IMPROVING THE HONEY INDUSTRY (*APIS MELLIFERA*) IN BAC LIEU – VIETNAM

Nguyen Xuan Nam[a]

[a] *Ho Chi Minh City University of Technology*

The region of Bac Lieu has a number of features that make it particularly suitable for the development of honey. However, the Bac Lieu honey industry have not developed, the quality of its honey also have not yet met the demands of consumers in the market. Because of primitive processing techniques and a very limited array of products. To improve the industrial of Bac Lieu honey, we must research to rigorous regulation and quality control of honey in all stages, including beekeeping, harvesting, storage and processing.

Keywords: *Apis mellifera / Bac Lieu / honey / industry*

Code: P26

UTILIZATION OF GAC FRUIT (*MOMORDICA COCHINCHINENSIS SPRENG.*) IN MAKING JAPANESE WAXY RICE CAKE (MOCHI)

Le Nhat Minh Ha[a], Quoc Tuan Dang[a]

[a] *International University*

The purpose of this study was to evaluate the potential of using Gac fruit in making mochi. Two types of dried Gac aril (full-fat and defatted) were added in the mochi skin. Meanwhile, the standard filling (red bean sweet paste) was substituted with Gac pulp powder (30 g/300 g red bean) as well as Gac aril oil at three levels (no Gac oil, less Gac oil, and more Gac oil). All formulations were pre-selected by the sensory evaluation. The quality attributes (β-carotene and lycopene contents, physicochemical characteristics, and sensory properties) of six different Gac mochi were compared with the control mochi which was absent in Gac materials. With increasing amount of Gac incorporation, especially Gac oil, the β-carotene and lycopene content of the Gac mochi greatly increased and were significantly higher than those of the control sample. The highest β-carotene and lycopene contents obtained were 0.4270 ± 0.0081 mg and 0.2380 ± 0.0055 mg per 100 g mochi, respectively. The physicochemical properties of rice cake were also affected as the addition of Gac oil and Gac full-fat aril led to an increase in the fat content (by as much as 265%) while the substitution with Gac pulp decreased the content of protein (by up to 15%). The carbohydrate contents of Gac mochi were significantly different ($p<0.05$) and somewhat higher than that of the control sample. Meanwhile, the moisture and ash contents showed no substantial changes. Sensory quality characteristics of mochi were evaluated including appearance, hardness, chewiness, smoothness, ease-to-eat, and flavor. The sensory scores varied over a wide range. The best Gac mochi with high levels of β-carotene and lycopene contents, high content of protein, and high degree of acceptance was discovered.

Keywords: *?-carotene / Gac fruit / lycopene / mochi*

Code: P27

EFFECTS OF THE DIFFERENT EXOGENOUS ETHYLENE FUMIGATION ON QUALITY OF RI 6 DURIAN IN RIPENING

Duong Thi Cam Nhung[a], Nguyen Van Phong[a]

[a] *Department of Postharvest Technology, Southern Horticultural Research Institute (SOFRI), Long Dinh Ward, Chau Thanh District, Tien Giang Province, Vietnam.*

Studies on the effect of exogenous ethylene fumigation approaches on the ripening process of "Ri 6" durian fruit was conducted to find out ripen effectively method. The experiment was arranged completely randomized design with one factor as various treaments (ethephone dip 500ppm, ethylene from ethephone in NaOH, ethylene genergator with conc. 200 ppm-24hrs and cotrol) with five replication, each replication equal to one fruit. Durian fruits with 90% mature uniform size were harvested from durian orchard belonging to Thanh Binh commune, Vung Liem district, Vinh Long province. After harvest, fruits were transported carefully to the postharvest laboratory and treated with ethylene as described above. After treatment, the samples kept in ambient (27-29 oC) and recorded days to ripe edibility. Among three ripening approaches, both fumigations with ethylene gas obtained from ethylene genergator and ethephone reacted with NaOH could be considered as the most suitable for repening Ri-6 durian fruits. The ripening time of Ri-6 durian fruits using the fumigation was shortened down 2 days and fruit gave optimum eating quality.

Keywords: ethephon, ethylene, respiration, Ri 6 durian, ripening.

Code: P28

OPTIMIZATION OF THE POLYPHENOLICS EXTRACTION FROM RED RICE BRAN BY RESPONSE SURFACE METHODOLOGY

Thi Bich Tram Phan[a], Tri Liem Nguyen[b], Thi Phuong Lien Duong[a]

[a] *College of Agriculture and Applied Biology, Cantho University, Vietnam*

[b] *High School of Ngo Van Can, Ben Tre, Vietnam*

This study aims to determine the best extraction conditions and find out the effects of ethanol concentration (20 – 80%), added acetic acid concentration (0 – 20%), extraction time (0 – 6.5 hours), temperature (25 – 100^{o}C), number of extraction cycles (1 – 4) and solid–solvent ratio (1/4 – 1/10 w/v) on total polyphenolic content (TPC) of red rice bran. The three parameters, solvent concentration (48 – 68%), added acetic acid concentration (10 – 15%) and extraction time (120 – 240 min) were able to be optimized using the Box Behnken design (BBD) with a quadratic regression model built by using response surface methodology (RSM). The experiments were carried out according to 30 runs with 3 variables and 3 central points and 1 replicate design for the optimization the TPC extraction. The extracts were analyzed the TPC spectrophotometrically according to the Folin-Ciocalteu colorimetric assay. The optimal extraction conditions were determined as follows: ethanol concentration 54.5%, added acetic acid concentration 13.1%, extraction time 210 min, temperature 40^{o}C, 3 cycles of extraction and solid–solvent ratio 1/6 w/v. Using these extraction conditions, the experimental yield of TPC was 2391.1±5.9 mg GAE/100 g DW that were in close significant agreement with predicted value ($p < 0.05$). The experimental result were fitted to a second order quadratic polynomial model and they have shown a good fit to the proposed model ($R2 = 0.99$). With these conditions, the antioxidant capacity assayed by 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical–scavenging activity in term of IC50 value of extract was 108.1±2.9 μg DW/mL. The study result indicates the suitability of the developed model and the success of RSM in optimizing the extraction conditions.

Keywords: *antioxidant activity / extraction / phenolic compounds / red rice bran*

Code: P29

AFFECTS OF HARVESTING MATURITY ON QUALITY OF RI 6 DURIAN FRUITS IN NATURALLY RIPENING

Duong Thi Cam Nhung[a], Nguyen Van Phong[a]

[a] *Department of Postharvest Technology, Southern Horticultural Research Institute (SOFRI), Long Dinh Ward, Chau Thanh District, Tien Giang Province, Vietnam.*

To determine proper harvesting maturity indices and naturally ripening of durian fruit cv. "Ri 6", the study was conducted on durian orchards at Thanh Binh commune, Vung Liem district, Vinh Long province. Hanging up the cards was done on the bunches of durian trees, which were being flowering and the fruits were harvested at five various stages 85, 90, 95, 100, 105 days after anthesis. Fruits after harvesting were brought to the lab to evaluate the physico-chemical properties then kept at ambient (27-29^{o}C) and recorded days to ripe edibility. Sensory and physico-chemical characteristics were assessed. Results of the study indicated that, Ri-6 durian fruits harvested at 95-100 days after anthesis had an appearance of peel in greenish-yellow color with dark brown grooves and possessed 13-16% starch in the flesh and this stage was recorded as the optimum maturity for harvesting. Moreover, the naturally ripening duration of fruit under ambient condition were from 5-6 days with best eating quality.

Keywords: maturity, naturally ripening, respiration, Ri 6 durian

Code: P30

Intelligent system to estimate the geometric and surface color properties to discriminate almonds' varieties using Soft Computing Techniques

Narendra VG[a] Srikanth Prabhu[a]

[a] *Manipal University*

Image processing techniques are increasingly applied in quality evaluation and sorting applications of agricultural and food products. This work has accessed the use of image processing for inspecting surface color of almonds varieties. A computer vision system is developed and experiments are conducted to determine color change in almonds varieties. Conversion of RGB to YCbCr values is done via image processing and prediction models are developed to estimate color parameters from computer vision system (CVS) data. Compared to the calculated color values hue angle and chroma, a yellowness index computed from intermediate YCbCr values is found to be much more adept accurately predicting almonds varieties color from CVS data. Therefore, it is shown that the CVS was capable of producing accurate morphological and color values for the almonds varieties investigated. The findings of this study can be incorporated for development of a robust system for quality prediction and establishment of a CVS for automatic grading and sorting of almonds.

Keywords: Geometric and surface color / Almonds varieties / Computer vision system / Intelligent system

Code: P31

Ultrasound-assisted extraction of polyphenols from mango (*Mangifera indica L.*) seed kernels using response surface methodology

Nguyen Nhat Minh Phuong[abc], Thi Hong Loan Pham[b], Van Camp John[a], Trung Thien Le[b], Raes Katleen[a]

[a] *Gent University*

[b] *Nong Lam University*

[c] *Can Tho University*

Phenolic compounds from mango seed kernel (MSK) were extracted by ultrasound-assisted extraction technology. Various parameters such as extraction steps, material-to-solvent ratio, sonication time, a number of ultrasound-assisted treatments and extraction temperature were optimized by single-factor or multiple-factor experiments. The experiments were also carried out according to three-level, three-variable Box-Behnken design. The best combinations of these variables were obtained for the maximum extraction of phenolic compounds, free radical scavenging activity (DPPH and ABTS assays) by using response surface methodology. The results showed that the maximum concentration of phenolic content in MSK, which was 169.80 ± 1.2 mg GAE/g d.m., could be obtained at 16 min of sonication time under power of 200 W (applying for the first extraction step) with extraction temperature of 52 °C, the material-to-solvent ratio of 1:12 and two-time extraction cycle. Moreover, the antioxidant activity assayed by DPPH and ABTS agents was 310.73 ± 2.32 and 313.13 ± 1.95 µM AAE/g d.m., respectively. The total phenolic content extracted by the ultrasound-assisted method was 9.3 % higher than that of conventional extraction (no ultrasound application).

Keywords: Ultrasound-assisted extraction / mango seed kernel / response surface methodology / antioxidant activity

Code: P32
DEVELOPMENT OF FERMENTED JUICE FROM RED FLESHY DRAGON FRUIT

Nguyen Thi Quyen[1], Dinh Thi Hien[1], Maarten Hertog[3], Bart M Nicolaï[2,3], Dinh T Tran[1]

[1]Faculty of Food Science and Technology, Vietnam National University of Agriculture, Trauquy, Gialam, Hanoi, Vietnam.

[2]Flanders Centre of Postharvest Technology, Willem de Croylaan 42, B-3001 Leuven, Belgium.
[3]BIOSYST-MeBioS, Faculty of Bioscience Engineering, KU Leuven, Willem de Croylaan 42, B-3001 Leuven, Belgium.

Corresponding author: Dinh Thi Tran. Tel: +84 626 177 18; fax: +84 43 8276 554.

E-mail address: ttdinh@vnua.edu.vn

Dragon fruit *(Hylocereus costaricensis)* is a subtropical fruit, which is widely grown in many regions in Vietnam. It has high commercial value in the domestic and international markets as it has an appealing natural red fleshy color, high nutritional value, and pleasant flavour. The fruit is mainly consumed in fresh while huge quantity is harvested in short period in summer. Consequently, large amount of rotten fruit goes to waste. Therefore, development of technologies to process it into different products is in high demand. The current study aimed to develop a new product, the fermented juice from red fleshy dragon fruit. To reach this objective, several main factors influencing on quality of fermented juice were studied. The experiments were designed using the JMP sofware. The instrumental methods were used to measure the quality of juice during fermentation while preference test using 9-point Hedonic scale was performed to evaluate the consumer acceptance of the product. The results showed that the best parameters for making fermented juice were the mixing ratio of dragon fruit juice and assam apple juice of 9:1, the initial soluble solids content of 24.7 ^{o}Bx, and the ethanol content of 3 - 4% v/v. This product opens up the possibility to diversify the processed foods from dragon fruit in the market.

Keywords: *fermented juice / red dragon fruit /assam apple*

Code: P33

SALT REDUCTION IN FRIED POTATO STICKS WITH THE ADDITION OF SZECHUAN PEPPER

Le Bao Tram Hong[a], Yaowapa Lorjaroenphon[a]

[a] *Kasetsart University, Department of Food Science and Technology*

There is strong evidence for the link between high dietary sodium and increased risk of cardiovascular disease. However, salt reduction often negatively affects sensory quality followed by the economic profit loss due to the important functions of salt in various food aspects. The addition of some herbs and spices has been reported to compensate these limitations due to their exotic flavors. In this study, Szechuan pepper (*Zanthoxylum simulans*) was applied into fried potato sticks to determine whether it could support the reduction of salt in term of maintaining saltiness. The salty taste boosting property of Szechuan pepper and its capacity to apply in fried potato sticks were evaluated by sensory panel. In the first test, saltiness of the three filtered solutions (1% salt, 1% grounded Szechuan pepper, and the combination of 1% salt and 1% pepper (by weight)) and water were ranked (n=34). The result shown that the solution contained both salt and pepper was ranked at the most salty followed by salt solution, pepper solution, and water. Next, 10, 20, and 30% salt reduction of fried potato sticks were prepared by seasoning with 1% of grounded Szechuan pepper plus 0.9, 0.8, or 0.7% of salt, respectively. The saltiness of salt-reduced potato sticks were then compared to those of control sample (1% salt) by different-from-control test (n=26). The saltiness perception of 30% salt reduction sample was significantly different from those of control ($p \leq 0.05$). This result indicated that the application of 1% Szechuan pepper was successful in at least 20% salt reduction to maintain the saltiness perception of fried potato sticks. Szechuan pepper, thus, could be considered as useful agent for lowering salt content of food product.

Keywords: Szechuan pepper (Zanthoxylum simulans) / salt reduction / sensory evaluation / fried potato stick

Code: P34

EFFECT OF ENZYME TREATMENT ON QUALITY OF WHITE MULBERRY *(MORUS ALBA L.)* FRUIT JUICE EXTRACT

Hoang Thao Ly Nguyen[a], Si Ngoc Le[a], Minh Xuan Hong Nguyen[b]

[a] *Research and Development Center for High Technology Agriculture, Ho Chi Minh city, Vietnam*

[b] *Nong Lam University, Ho Chi Minh city, Vietnam*

The effect of enzyme Pectinex (Novozyme, Denmark) treatment on the extraction efficiency and the quality of white mulberry (*Morus alba* L.) fruit juice extract was investigated. White mulberry purée was treated with enzyme at different concentration (0; 0.2; 0.4; 0.6; 0.8%) and incubated at 45^{o}C in different time period (1, 2, 3 hours). After that, the extraction efficiency and the quality of white mulberry fruit juice extract were evaluated and compared with the untreated one. The results showed that treatment with Pectinex enzyme at 0.6% concentration for 2 hours gave higher extraction efficiency (86.26%) than untreated one (57.11%). It was due to the catalytic activity of Pectinex enzyme to breakdown of the cell wall of white mulberry. The quality of extracted juice was also better with higher total sugar content (9.59%) and reducing sugar content (1.77%). The higher total polyphenol content (65.59 mg GAE/ g) proved the good quality of the fruit juice extract.

.Keywords Pectinex / extraction / Morus alba L. / polyphenol

Code: P35

Factors influencing production of wine from dragon fruit

Than Thi Huong[a], Pham Thi Le Huong[a], Manfred Goessinger[b], Klaus Dürrschmid[c], Dietmar Haltrich[c], Bart M Nicolaï[d], Dinh T Tran[a]

[a]*Faculty of Food Science and Technology, Vietnam National University of Agriculture, Trauquy, Gialam, Hanoi, Vietnam.*

[b]*HBLA und Bundesamt für Wein-und Obstbau, Wiener Strasse 74, A-3400 Klosterneuburg, Austria.*

[c]*Institute of Food Science, University of Natural Resources and Life Sciences, Austria*

[d]*BIOSYST-MeBioS, Faculty of Bioscience Engineering, KU Leuven, Willem de Croylaan 42, B-3001 Leuven, Belgium.*

Corresponding author: Dinh Thi Tran. Tel: +84 626 177 18; fax: +84 43 8276 554.

E-mail address: ttdinh@vnua.edu.vn

Dragon fruit *(Hylocereus undatus)* is a subtropical fruit, which has recently been grown in many provinces in Vietnam. It is one of the most valuable fruit because of its delicious taste and excellent nutritional properties. At present, the fruit is mainly consumed in fresh while huge quantity is harvested in short period, making its postharvest losses huge. Therefore, development of technologies to process it into different products is urgently needed. The current research aimed to develop the wine making process from dragon fruit. To reach this objective, several main factors influencing wine quality such as yeast strain, mixing ratio of dragon fruit juice and assam apple juice, and final sugar content of wine were studied. The instrumental methods were used to measure the changes of the soluble solids content, sugar content, titratable acidity, alcohol content, total phenolic content and antioxidant capacity of wine during fermentation while a spontaneous liking test was used for sensory evaluation of the final products with naive consumers. The results showed that the combination of the yeast strain of *Oenoferm Freddo*, the mixing ratio of 80 % dragon fruit juice and 20 % assam apple juice, and the final sugar content of 3 % gave the best wine quality. The further research will focus on maturation stage of wine to improve its quality before entering the market.

Keywords: Dragon fruit/ Hylocereus undatus/ fermentation/ wine

Code: P36

THE IMPACTS OF PASTEURIZATION AND STORAGE CONDITIONS ON THE VOLATILE COMPOUNDS IN RHODOMYRTUS TOMENTOSA JUICE

Van Thanh Dao[a], Tan Bao Le[b], Binh Ly Nguyen[b]

[a] *An Giang University*

[b] *Can Tho University*

The study was to analyse volatile compounds of Sim juice, using the method of static headspace combined with gas chromatography mass spectrometry (GC-MS). This study aimed at identifying the impacts of pasteurization process (85^{o}C, 90^{o}C and 95°C) and storage conditions (ambient temperature and 4°C) on the volatile compounds of Sim juice. The results showed that there were differences in the volatile compounds of Sim juice at different pasteurization temperatures and times. There were disappearance of the volatile compounds or formation of the new one depending on pasteurization temperature and time. At the same temperature, longer time of pasteurization resulted in more volatile compounds but destroyed several compounds in Sim juice. At different pasteurization temperatures and times, 32 volatile compounds including alcohols, esters, hydrocarbons, phenols, furans, aldehydes, acids groups are identified in total.

Storage conditions affected the stability of the volatile compounds of Sim juice. The volatile compounds of Sim juice samples stored at ambient temperature had more changes than ones stored at 4^{o}C. The volatile compounds with more changes were erythro-2-ethyl-3-ol-ethoxybutan-1, 4,5-Octanediol, α-pinene, Phenylacetone ketoxime, 3,4-epoxy-eryhtro-2- octanol and 3-Methylpyridine.

Keywords: Sim juice / gas chromatography mass spectrometry (GC-MS) / pasteurization temperature / headspace

Code: P37

Effects of chocolate types and storage temperatures on changes of total phenolic content and concentrations of vitamin C of acerola jam filled-chocolates during storage

Linh K. Vo[a], Mai T. Nguyen[a], Katleen Raes[b], Thien T. Le[c*]

[a] *Department of Food Technology, School of Biotechnology, InternationalUniversity – Vietnam National University HCMC. Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam.*

[b] *Department of Industrial Biological Science, Faculty of Bioscience Engineering, Ghent University, Kortrijk, Belgium*

[c] *Faculty of Food Science and Technology, Nong Lam University – Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam*

**Correspondence: Thien T.Le, Faculty of Food Science and Technology, Nong Lam University – Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam*

Email: le.trungthien@hcmuaf.edu.vn

This study was conducted to investigate the effects of types of chocolate shell (milk and dark chocolate), storage time and storage temperatures (7^{o}C and 25^{o}C) on the changes of phenolic compounds and vitamin C of acerola jam filled–chocolates. For the acerola jam filling, vitamin C, total phenolic content (TPC), and pH were analyzed. For the chocolate shell, TPC was determined. It was found that storage time and storage temperatures significantly influenced the changes of the antioxidants (total phenolic content and vitamin C) and pH of the filling and the shell. Storing at 7oC could preserve the antioxidants of the filling better than storing at 25^{o}C. Total phenolic content in the chocolate shell was higher at 25^{o}C than at 7^{o}C. Regarding the types of chocolate shell, there was no significant difference between milk and dark chocolate shells in preserving vitamin C and phenolic compounds of the filled acerola jam. pH of acerola jam filling in dark and milk chocolate shells stored at 25^{o}C increased significantly after 6 weeks. However, pH of those stored at 7^{o}C was mostly unchanged.

Keywords: *antioxidants / acerola jam / filled-chocolate / storage conditions*

Code: P38

Dietary supplementation of Phyllanthus amarus, Psidium guajava and Euphorbia hirta on immunological role and disease resistance in Pangasianodon hypophthalmus against Edwardsiella ictaluri

Truong Quynh Nhu[1,2], Bui Thi Bich Hang[2], Le Thi Bach[3], Nguyen Le Anh Dao[2,4], Bui Thi Buu Hue[3], Scippo Marie-Louise[4], Quetin-Leclercq Joelle[5],Tran Minh Phu[2], Do Thi Thanh Huong[2], Nguyen Thanh Phuong[2] and Kestemont Patrick[1]

(1) University of Namur – Research Unit in Environmental and Evolutionary Biology, Belgium,

(2) Can Tho University – College of Aquaculture and Fisheries, Vietnam,

(3) Can Tho University – College of Natural Sciences, Vietnam,

(4) University of Liège – Department of Food Sciences, Belgium,

(5) Université catholique de Louvain – Louvain Drug Research Institute, Belgium.

Bacillary necrosis of Pangasius (BNP) caused by *Edwardsiella* (*E*.) *ictaluri* which is a major bacterial pathogen, inflicts serious economic losses in fingerlings and juvenile stripped catfish (*Pangasianodon hypophthalmus*) production. With an aim to eliminate the adverse effects of chemical measures, the present study determines the effects of three different plant extracts (*Phyllanthus (P.) amarus*, *Psidium (P.) guajava* and *Euphorbia (E.) hirta*) on the immune response of *Pangasianodon hypophthalmus* and its susceptibility to *E. ictaluri* infection. Fish (15-20 gram) were divided into 7 groups fed diets containing 0.2% and 1% of *P. amarus,* 0.2% and 1% *P. Guajava*, 0.4%, 2% of *E. hirta* and basa diet (control) for 8 weeks. Various immune parameters were examined at 4th week (W4) and 8th week (W8) post-feeding. Challenge test with *E. ictaluri* was observed at the end of the trial, and mortalities were recorded over 15 days post-infection. The results showed that the red blood cells were not affected by plant extracts. In contracts, total white blood cells, lymphocytes, monocytes and neutrophils were increased in fish fed with plant extracts incorporated diets on W4 and W8. Serum lysozyme activity was increased significantly in W8 with diets containing 2% *E. hirta*, 0.2 % *P. amarus* and 0.2 % *P. guajava* compared to control group ($p<0.05$); while fish were fed with 0.4% *P. amarus* and 0.2 % *P. guajava* showed highest skin muscosal lysozyme among treatments at W8. Dietary administration of *E. hirta*, *P. amarus* and *P. guajava L.* enhanced the resistance of *E.ictaluri* infection. Specifically, the lowest mortality was recorded in fish fed with 0.2% and 1% *P. guajava L.* (52.8% of both) compared to control (92.6%); followed by dietary administration with 0.2 % of *P. amarus* (55.6%) and 2% of *E. hirta* (63.9%) ($p<0.05$). Herbal extracts therefore represent as dietary immunostimulants - a promising feed additive for striped catfish in commercial culture.

Code: P39

SELECTION AND IDENTIFICATION OF BACILLUS PRODUCING ENZYME THERMOSTABLE ?- GALACTOSIDASE

Thi Na Tran[a], Hoang Anh Nguyen[a], Tien Thanh Nguyen[b]

[a] *Vietnam National University of Agriculture*

[b] *Hanoi University of Science and Technology*

In this study, 160 strains of Bacillus isolated from cow rumens, Chilli sources, fermented meats, dairy industry effluent, fermented milk, pig small intestine in diferent areas of Vietnam were used to screen strains of interest producing b- galactosidase on agar plate supplemented Xgal. The data indicated that 24/160 isolated Bacillus spp produced b- galactosidase, in which 6 strains (NT2.7, NT2.8, DC1, DC2, TO1.1, TO1.8) with heavy blue colour were used to determine enzyme acitivity. b-galactosidase from NT2.8 with highest activity 42.4 U/l was tested thermostability at 50ºC, 55ºC và 60ºC. Results showed that β-galactosidase was very stable at this three temperatures, after 50 hours of 60ºC incubation, enzyme activity was still remained 50.6%. Strain NT2.8 was identified to be Bacillus flexus based on the comparison of sequence encoding for 16rRNA gene

Keywords *Bacillus spp / ?-galactosidase / /*

Code: P40

Beneficial combination of ?–glucan with dietary lipid sources on growth, immune response, fatty acid profile and expression of genes involved in immunology, lipid biosynthesis and eicosanoid process in carp (*Cyprinus carpio)*

Thi Mai Nguyen
Vietnam National University of Agriculture

In recent years, the influence of nutrition on fish immunology is of increasing interest; however, data on common carp (Cyprinus carpio) are still rather limited. This study aims to evaluate the effects of a combination of an immunostimulant compound (β–glucan) with different dietary lipid sources on the immune response, fatty acid profile and their interactions in common carp juveniles (16.3±0.6g body weight). Six isoproteic (39.1%) and isolipidic (10%) diets containing three different lipid sources (cod liver oil – CLO as fish oil, linseed oil – LO and sunflower oil – SFO, as plant oils) were formulated with (CLO+, LO+, SFO+) or without (CLO, LO, SFO) β–glucan supplementation. Fish were fed a daily ration of 4% body weight during 9 weeks (growth trial) and then challenged with Aeromonas hydrophyla for 10 days to determine their resistance to bacterial contamination. Husbandry parameters were recorded during the growth trial. At the end of the experiment and at the second day of the bacterial challenge test, blood plasma was sampled for lysozyme and complement (ACH50) activity assays; liver and muscle were used for fatty acid analyses; kidney and liver were also collected and used for gene expression analyses. No significant differences of final body weight (FBW), specific growth rate (SGR), daily weight gain (DWG), feed conversion rate (FCR) and survival rate (SR) ($P>0.05$) were observed between diets. We observed an influence of lipid source and β–glucan supplementation on lysozyme activity ($P<0.05$) but no effects were found on plasma ACH50 neither at the end of the growth trial, nor after the challenge test. We also found a diminution of these immune parameters in the challenge test compared with those assayed at the end of the growth trial ($P<0.05$). Fatty acid profiles in fish liver and muscle reflected those of the diets. The difference was observed in all studied criteria (satured fatty acid – SFA; monounsatured fatty acid – MUFA; polyunsatured fatty acid – PUFA and long chain polyunsatured fatty acid – LC-PUFA ($P<0.05$). The supplementation of dietary β-glucan did not influence the fatty acid composition in carp liver and muscle. Cod liver oil was the best dietary lipid source in terms of essential fatty acid content (ARA, EPA and DHA) and n3/n6 ratio ($P<0.05$) in fish tissues, but linseed oil appeared as a suitable alternative plant oil in common carp. The expression of several genes in kidney (nk, lyz, il-8) and liver (elovl5, fads2; pla2a2, ara1) involved in immune function, lipid biosynthesis and eicosanoid process respectively was also investigated but did not display any difference between treatments ($P>0.05$). In conclusion, the supplementation of β-glucan at the dose of 0.25g.kg diet-1 increased the lyzozyme activity but not the growth and fatty acid composition in common carp.

Keywords: *Fatty acid profile / Immunostimulant suplementation / ?–glucan /*

Session 2: Safety and quality of dairy and meat products

Code: P41

OPTIMIZING THE LOIN PORK HYDROLYSIS BY ALCALASE TO PRODUCE PEPTID AND AMINO ACID FOR SONDE FEEDING FOOD

Quynh Hoa Nguyen Thi[a], Anh Dao Dong Thi[a]

[a] *Hochiminh City University of Food Industry*

In this research, we conducted a survey of the nutritional ingredients of raw pork and optimized the proteolytic conditions by Alcalase enzyme to produce meat protein powder used as food for feeding patients through catheters. In particular we examine and select suitable conditions for hydrolysis process. Alcalase is chosen and the favorable hydrolysis conditions are: ratio of substrate/water (w/w) is 1/6; the rate of enzyme content and raw materials (E/S) 3.0% (w/w); pH7.5; hydrolysis temperature 55^{o}C at 180 min. Hydrolysis with optimal conditions achieved the lowest viscosity 2.240 ± 0.092 cP and the degree of hydrolysis (DH) is 31.390 ± 0.138%. The peptide product of the hydrolysis process was analysed by electrophoresis, obtained the major molecular weight of 8kDa. The final product will be examined as part of the nutrition, amino acid concentrations, as well as biological indicators.

Keywords: *Alcalase / loin pork hydrolysis / protein powder / spray drying*

Code: P42

Visible and near infrared spectroscopy for rapid detection and monitoring of cleaning water contaminant in egg products

Nghia Nguyen-Do-Trong[a], Seppe Driesen[a], Michelle Hallemans[a], Ivana Mikušová[a], Floor Vanelderen[a], Bach Trong Nguyen[a,b,*], Bart De Ketelaere[a], Wouter Saeys[a]

[a] *MeBioS Biophotonics Lab, KU Leuven Department of Biosystems, Kasteelpark Arenberg 30, 3001 Leuven, Belgium*

[b] *Faculty of Food Technology, Nha Trang Univeristy, Nha Trang City, Vietnam*

**Email: ntbachnt@ntu.edu.vn*

The aim of this research is to investigate if it is possible to quickly detect water contamination in commercial egg products (egg yolk, egg white or whole egg) using visible and near infrared (Vis-NIR) spectroscopy in lab conditions. For each type of product, 63 samples, having 7 levels of water contamination (%w/w): 0, 1, 2, 5, 10, 20, 50 % were prepared by adding different amounts of de-ionized water into the commercial liquid products. Each contamination level contained 9 replicate samples prepared from 9 different cartons to capture inter-package variation for the measured sample set. Transflectance spectra of the contaminated egg yolk and whole egg samples and the transmittance spectra of the contaminated egg white samples were measured with a spectrophotometer covering two ranges: 300-1100 nm (S1) and 900-1750 nm (S2). The obtained spectroscopic data were divided into two sets: a calibration and a validation set containing respectively 2/3 and 1/3 of the number of spectra of each contamination level. Partial Least Squares (PLS) Regression was employed in combination with different spectral pre-processing strategies to train a multivariate statistical model to predict the water contamination concentration using the calibration dataset. The obtained best calibration models were then applied on the validation dataset, which had not been used for model training, to validate the prediction performances of the models. The obtained results indicated high prediction performances on the validation set: egg yolk using S1: RMSEP = 1,216 %w/w, R^2_P = 0,995; whole egg using S1: RMSEP =2,328 %w/w, R^2_P = 0,906; egg white using S2: RMSEP = 1,736 %w/w, R^2_P = 0,992. These research outcomes clearly confirmed the potential of using Vis-NIR spectroscopy for rapid detection and monitoring of cleaning water contamination in egg products. Further research in more realistic conditions in the factory should be implemented in the future to develop a new sensing system for determining automatically if the production batch contains cleaning agent.

Keywords: *visible and near infrared spectroscopy / egg product / PLS regression* /

Code: P43

MALDI-TOF MS AND $(GTG)_5$-PCR FINGERPRINTING FOR CLASSIFICATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM SOME TRADITIONAL VIETNAMESE FERMENTED FOOD

Thi Lam Doan Nguyen[a], Margo Cnockaert[b], Koenraad Van Hoorde[b], Maarten Aert[b], Peter Vandamme[b]

[a] *Vietnam Natural University of Agriculture*

[b] *Ghent University, Belgium*

The objective of the present study was to evaluate the use of MALDI-TOF MS as an alternative to $(GTG)_5$-PCR fingerprinting for the identification of LAB associated with fermented food samples. A total of 119 strains of LAB from fermented meat (nem chua) were analyzed with both MALDI-TOF MS and $(GTG)_5$-PCR fingerprinting. Cluster analysis of the profiles revealed five species represent by a single isolate were clustered separately both in $(GTG)_5$-PCR and in MALDI-TOF MS; five species grouped alike for $(GTG)_5$-PCR and for MALDI-TOF MS, however, differences in minimal similarity between the delineated $(GTG)_5$-PCR and MALDI-TOF MS clusters could be observed; three species showed more heterogeneity in their MALDI-TOF MS profiles compared to their $(GTG)_5$-PCR profiles; two species, each represented by a single MALDI-TOF clusters were subdivided in (GTG)5-PCR dendrogram. As proof of efficacy of the MALDI-TOF MS, LAB diversity from one fermented mustard sample was analyzed using MALDI- TOF MS. PheS gene sequencing was used for validation.

Keywords: MALDI-TOF MS / $(GTG)_5$-PCR / lactic acid bacteria / fermented food

Code: P44

Food safety knowledge, attitudes and practices of street food vendors in Hau Giang city, Vietnam

Anh Ngoc Tong Thi[a], Vinh Hoa Le[b], Thi Thanh Que Phan[a], Van Lanh Nguyen[c]

[a] *Can Tho University*

[b] *Food safety department of Hau Giang*

[c] *Preventive Healthcare Center of Hau Giang*

The aim of this study is to evaluate the food safety knowledge, attitudes and practices of vendors of street food in Hau Giang city, Vietnam. There were 400 vendors from eight districts participated in this study. The results showed that the large majority of the vendors were women (80%) and without food safety training (65.5%). Food safety knowledge of street food vendors depended on districts, level of education and food safety training ($p < 0.05$). In general, vendors exhibited average food safety knowledge and attitudes levels. The majority of vendors did not know that *Salmonella* spp. (78,8%), *Staphylococcus aureus* (80,5%) and *Escherichia coli* (78,8%) are foodborne pathogens. During observing food handling habits of vendors, there were 91.7% of the vendors handled food with bare hands. Higher than 95% of the vendors handled money while serving food but only 1.3% washed their hands thereafter. These findings highlighted that vendors in Hau Giang city generally have poor food handling practices and personal hygiene. These results of this study should be used to generate part of proper strategies for improving the safety of street food.

Keywords: *food safety / vendor / street food / Hau Giang*

Code: P45
Detection and quantification of Auramine O contamination level in Vietnamese foods

Thi Ngoc Trang Nguyen[a], Quoc Tuan Dang[a]

[a] *International University*

This study was aimed to examine the presence of Auramine O (AO) in Vietnamese foods in order to evaluate the situation of food quality and food safety issues in Vietnam. The work contained both detection and quantification of AO in suspected foods sold in street markets and supermarkets in Ho Chi Minh City. Thin layer chromatography (TLC) was applied for quick detection of AO based on its fluorescence characteristic under UV light. To quantify the level of AO in foods, ultra-violet visible spectrophotometer at 339 nm of wavelength was used. Among 120 food samples collected randomly, there was 25±5.6% of total samples contaminated with AO, all of which belonged to a category of small-scale food products purchased from local or street markets. None of food samples from supermarkets showed the presence of AO. Among the positive samples, solid foods took the largest portion which was 15.3% while powder samples made 8.3% among total food samples. AO is an industrial dye banned in food all over the world, its level of contamination in this study, however, ranged from 3 to 19 ppm, which was hundred to thousand times higher than the data reported recently from the investigation in Vietnamese foods.

Keywords: *auramine O / TLC / UV-vis / vietnamese foods*

Code: P46
DETERMINATION OF GAMMA-AMINOBUTYRIC ACID (GABA) CONTENT IN VIETNAMESE TRADITIONAL FERMENTED FOODS

Phuong Hong LE[a&b], Liesa VERSCHEURE[a], Thien Trung LE[c], Yannick VERHEUST[a], Katleen RAES[a]

[a] *Department of Industrial Biological Sciences, Ghent University (UGent), Kortrijk Campus, Belgium*
[b] *Department of Chemical Engineering, Nong Lam University (NLU), Ho Chi Minh City, Vietnam*
[c] *Faculty of Food Science and Technology, Nong Lam University (NLU), Ho Chi Minh City, Vietnam*

Email: Katleen.Raes@UGent.be; PhuongHong.Le@UGent.be

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that is widely present in nature from microorganisms to plants and animals. It is well known that GABA is a major neurotransmitter in animals and it has several other important physiological functions, such as induction of hypotension, tranquilizer effects, preventing diabetic conditions. In foodstuffs, GABA can be found mostly in germinated seeds and fermented foods.

The aim of this study was to analyze the GABA content in different Vietnamese traditional fermented foods (dairy, fish, meat and plant-based) using two methods: a spectrophotometric and a HPLC-DAD/Fluorescence method. In a first step, the GABA extraction from the food matrix was optimized. Optimally, 0.5 gram of sample was extracted with 5ml of 75% ethanol (one step extraction for liquid food matrix and two steps extraction for solid food matrix) for 15 min at 22^{o}C. For HPLC analysis, a derivatization with o-phthaldialdehyde was needed, followed by injection on a Nucleodur C18 column. Detection was performed at 338 nm with DAD and at an excitation and emission wavelength of 340nm and 455nm, respectively, with fluorescence detection. The obtained LOD and LOQ was 0.51ppm and 1.02ppm, respectively. For spectrophotometric analysis, the reaction with phenol and hypochlorite to form indophenol was first carried out and then the absorbance was recorded at 630nm. For the different fermented foods, the GABA content ranged between 0.08 and 16.29 mg/g DM and 2.3 and 127 mg/g DM after HPLC and spectrophotometric analysis, respectively. The spectrophotometric method gave a false picture of the amount of GABA in samples due to excess of interference factors. It was not possible to find a correlation between the HPLC and spectrophotometric method. HPLC analysis showed that fermented anchovy and fish sauce had the highest amount of GABA content (6.21 and 16.29 mg/g DM, respectively). These samples are potential matrices to isolate the GABA-producing microorganisms and to investigate the kinetics of the GAD enzyme to produce GABA.

Keywords: *GABA / Fermented foods / Extraction / Determination method*

Code: P47
Detection of Salmonella in pork meat purchased in markets in Ho Chi Minh City using real-time PCR

Thi Anh Thu Ha[a], Thi Kieu Giang Huynh[a], Tieu Lam Tran[a], Phan Thu Hien Truong[a]
Thi Lam An Vu[a], Oberheitmann Boris[b]

[a] *Nong Lam University, Ho Chi Minh city*

[b] *Q-Bioanalytic GmbH, Bremerhaven, Germany*

Corresponding author: laman.vu@gmail.com

Salmonella is one of the most significant pathogens causing food and feed safety concerns. Pork has been identified as an important source for human salmonellosis. The lack of proper cooling facilities increases the risk of the foodborne disease.

The aim of the study was to evaluate the Salmonella contamination at street counters.

In this study 141 pork samples were analyzed for the presence of Salmonella applying the OneCup real time PCR test kit supplied from Q-Bioanalytic GmbH, Germany. The pork samples were purchased from retail counters on streets in Thu Duc District, Ho Chi Minh City, where there is no refrigeration during the time the meat samples are on sale. The real-time PCR procedure followed DIN EN ISO 20837 and 20838 including a pre-enrichment step in buffered peptone water. After that, DNA extraction and purification was applied using QuickBlue DNA Extraction and Purification Kit of Q-Bioanalytic GmbH. The kit uses paramagnetic nano-particles for purification. The results showed that out of 141 pork samples 22% were positive.

The current results indicate that food safety at street counters should be improved by implementing appropriate hygienic measures.

Keywords: *Key words: Salmonella, street counters, real-time PCR, food hygiene, food safety /*

Code: P48
RESEARCH ON SPRAY DRYING CONDITIONS FOR PROTEIN HYDROLYSATE OF CROCODILE MEAT

Vo Cam Hong Phung[a], Hoang Tan Dang[a], Tang Thanh Thanh Pham[b], Dinh Don Le[a], Thien Trung Le[a]

[a] *Nong Lam University*

[b] *Vietnam National University in HCMC*

Conversion of the protein hydrolysate liquid into a powder will enhance its shelf-life, reduce the storage and transportation costs and increase its application possibilities. One of the suitable approaches for this is spray drying because of its ability to maintain nutritional value of the obtained powder. The spray drying efficiency is strongly influenced by spray drying parameters. Therefore, the objective of this study is to screen the effects of several parameters (drying aid concentration, inlet air temperatures, and feed flow rates) on spray drying of crocodile meat protein hydrolysate. The results from screening experiments showed that experimented parameters had significant effects on recovery of dry matter, recovery of proteins and the obtained powder properties (total protein content, moisture content and antioxidant capacity). Besides, there were significant effects of inlet air temperatures and drying aid concentrations on response variables. The conditions for maximum recovery of dry matter, maximum recovery of proteins, and minimum moisture content of the spray-dried powder were found at maltodextrin concentration of 19.47% (w/w), inlet air temperature of 142.71^{o}C, input feed flow rate of 10 ml.min-1. The maximum recovery of dry matter is 69.51%, protein recovery yield reached 76.94% and the powder quality indicators such as humidity, activity antioxidation are respectively 5.33% and 1.87 mg.ml-1.

Keywords: *Antioxidant capacity / crocodile meat / protein hydrolysate / spray drying*

Code: P49

Incorporation of Gac (*Momordica cochinchinensis*) aril powder as natural colorant and/or carotenoid supplement for sausage product

Tuyen Chan Kha[a], Anh Thuy Vu[a]

[a] *Nong Lam University*

In general, colour, appearance and texture are three of the main quality parameters of meat and meat products for consumers who concern about natural and healthy food products. Nitrites are widely used as curing agents in sausage and other cured meat products but it is a toxic additive. Therefore, it is desirable to replace the use of nitrite by natural additives such as carotenoids, which are the consumer preferences.

The results showed that the appropriate formulation for the sausage product was the ratio of pork meat to fat of 2.5 to 1.0. The addition of Gac aril powder was found to be 1.5% under optimal processing conditions of the cooking temperature of 78^{o}C and the cooking time of 40 minutes. According to response surface optimization using central composite design, the content of lycopene and β-carotene was to be optimized as 2912.7 μg/100g and 3682.4 μg/100g, respectively. In addition, color characteristics of optimized sausage product were Lightness of 66, Chroma of 48.3, Hue angle of 75.9 and total colour difference of 25.3. The sausage product added Gac powder had a high stability when stored at -18°C.

In summary, using gac aril powder as a natural coloring additive and/or a carotenoid supplement for sausage product was successfully obtained as the final product showed an attractive color and high content of carotenoids.

Keywords: *Gac aril / Sausage / Lycopene / Beta-carotene*

Code: P50
SURVEY OF FOOD SAFETY MANAGEMENT SYSTEMS USED IN CATERS IN THE REGION OF HANOI AND BACNINH

Duy Tham BUI[a], Viet Phu TU[a]

[a] Vietnam National University of Agriculture

In recent years, the situation of food poisoning has no sign of decreasing in number which is even more complicated. Legislation has been enacted to ensure the food safety in restaurants, hotels and cafeterias. Some quality management systems such as HACCP or ISO 22000 only show effective in food production at industrial scale. However, smaller scale like restaurants, hotels and cafeterias with complex and large number of raw materials, processes and outputs, are lacking appropriated food safety management systems and practice guidelines. This study was carried out in order to evaluate the food safety management systems used in caters in the region of Hanoi and BacNinh. In addition, the feedback from food safety manager of these caters in conducting food safety measure has also been researched. The study surveyed over 280 food businesses in Hanoi and BacNinh. These caters are from different scales based on the number of meals served during the day, from small businesses like street venders to restaurants, hotels and cafeterias. The contents of survey included: General awareness about the requirements of law; safety practices; training; and difficulties in implementing food safety measure. Survey results showed significant differences in each survey content between cater of different scales, particularly in food safety practices. Survey results also indicated the difficulties of different groups of caters in fulfilling the requirements of the law. This problem has multiple causes, including the inner causes and also the reasons which come from the lack of detailed guidance or a not good communication with authorities.

Keywords *survey / food safety / management system / caters*

Code: P51

Changes of encapsulated polyphenols supplemented to yogurt making

Ho Thi Phu My[a], Ha Thi Mai Trang[a], Hoang Quang Binh[a]

[a] *Nong Lam University – Ho Chi Minh City*

Polyphenols (PP) are natural antioxidants in plant materials. These components possess many health beneficial properties. In this study, a polyphenolic extract from mango kernel was encapsulated using coacervation method. The capsules were then added to yogurt making. The changes of PP during pasteurization, fermentation, and storage of the yogurt were evaluated.

The results of the study showed that the pasteurization stage caused the highest reduction in concentrations of PP in the capsules. Concentrations of PP in the milk (not including the capsules) had a tendency of slight increase, which might indicate some release of PP from the capsules. Total PP (in the capsule and in the milk) reduced significantly after pasteurization. Pasteurization at 85 oC (reduced about 35 % of PP in the capsules) was better than at 90 oC in preservation of PP. Fermentation to reach pH of 4.6 at 43 oC reduced further 20% of PP, whereas, at 37 oC it reduced about 5%. Storage of the yogurt for a month did not reduced significantly PPs both at chilling as well as freezing condition. It seemed the sensitive polyphenol species had been destroyed during previous pasteurization and fermentation. Sensorial evaluation did not find significant difference in scores of PP supplemented yogurt and control yogurt. In conclusion, supplement of encapsulated PP may give some good image for yogurt, however, these components are destroyed significantly during processing, especially during the heat treatment.

Keywords: *encapsulated polyphenols / yogurt / pasteurization / fermentation*

Code: P52

Study on some physicochemical characterizations of exopolysaccharides produced by *Lactobacillus fermentum* TC13 and *Lactobacillus plantarum* T10

Tran Thi Ai Luyen[1,2], Tran Bao Khanh[1,3], Do Thi Bich Thuy[3],

[1]*Faculty of Chemistry, Hue University of Science,*

[2]*Hue Tousism College*

[3]*Faculty of Engineering and Food Technology, Hue University of Agriculture and Forestry*

ABSTRACT: Some physicochemical characterization and antioxidant activities in vitro of exopolysaccharides (EPS) produced by *Lactobacillus fermentum* TC13 (EPS-TC13) and *Lactobacillus plantarum* T10 (EPS-T10) were investigated. The antioxidant activity of EPSs was evaluated with the *in vitro* scavenging abilities on hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals with different concentrations. The results indicated that EPS-TC13 and EPS-W12 showed good scavenging activities on hydroxyl radicals. The scavenging abilities of both EPSs on inhibition of the DPPH radical were related to the concentration of the samples. The higher concentration, the higher level of scavenging ability was found for all samples used in the test. The concentrations required to scavenge 50% of the initial radical for $DPPH^{\bullet}$ by EPS-TC13 and EPS-TC13 were 2.85 and 2.14 mg/mL, respectively. Physical characteristics (such as solubility, holding capacity and good oil binding capacity) of EPS-TC13 and EPS-T10 were also tested in this study. The results from present study are given physical characteristics of both of EPSs were in fair good. The water soluble water holding capacity and good oil binding capacity of EPS-TC13 were 73.33%, 140.55% and 590.78% respectively. Thereby, with EPS-T10, some physicochemical characterizations were 89.20% for solubility, 186.55% for water holding and 579.65% for oil binding capacity respectively. With antioxidant activity and physical characteristics from these EPS, they can become potential applications in food industry.

Key words: scavenging ability, solubility, exopolysaccharide, Lactobacillus

Corresponding author: Tran Thi Ai Luyen
Email: luyenqxqb@gmail.com.

Code: P53

Effect of acid treatment in combination with chitosan coating on quality changes of 'Khoai Chau' longan during low temperature storage

Nguyen Thi Bich Thuy, Nguyen Thi Hanh

Vietnam National University of Agriculture

Corresponding author: ntbthuy@vnua.edu.vn

Longan (Dimocarpus longan) fruit production and global exports are rapidly expanding. Consumer acceptance of this high value crop requires that fruit arrive in excellent condition. Pericarp browning and fungal diseases are the main postharvest problems for longans. This research was conducted to investigate the effects of different acids in combination with chitosan coating on quality changes of 'Khoai Chau' longan during low temperature storag. Longan fruits cv "Khoai Chau" were harvested at 90% maturity. Then fruits were treated by dipping in 3% citric acid in 5 min, 2 mM oxalic acid in 5 min 0r 0.75N HCL in 30 second, then coated by 2% chitosan solution. After drying, fruits were packed in 1% perforated LDPE, and stored at 5^{o}C. It was reported that treatment of longan fruits with different kinds of acid not only improve fruit skin color for 4 weeks, but also reduce the rate of fruit rot. It also maintain the quality of fruits including TSS, TA and vitamin C content at higher level. Among treatments, fruits treated by 3% citric acid in 5 min or 0.75N HCL in 30 second, then coated by 2% chitosan showed the best results in reducing browning rate, decay and maintenance of fruit quality.

Key words: Longan, "Khoai Chau", acid treatment, chitosan coating, low temperature storage.

Code: P54

The study on production process of brown rice milk bottled

Huynh Thanh Tra, Vo Thi Thu Thao,
Bui Truong Dat, Nguyen Huynh Bach Son Long*

Faculty Of Chemical And Environmental Engineering

Lac Hong University, Dong Nai, Viet Nam

No 10 Huynh Van Nghe Street - Buu Long Ward – Bien Hoa City – Dong Nai Province

**Corresponding author: nguyenhuynhbslong@yahoo.com*

Nowadays, the nutritious drinks from natural ingredients are very diverse on the market. Although Vietnam has an abundant supply of rice source, the products made by ingredients of rice are still not popular. We conduct this study to investigate and formulate a production process of brown rice milk with a reasonable prices for consumers popularly.

Keywords: Brownrice, temperature, cooking time, sensory property

Code: P55

The study on processing of black-bone silky fowl with herbals soup product

Nguyen Minh Chanh, Tran Thi Thuy Trang,
Bui Truong Dat, Nguyen Huynh Bach Son Long*

Faculty of Chemical and Enviromental Engineering, Lac Hong University, Dong Nai, Viet Nam

No 10 Huynh Van Nghe Street– Buu Long Ward– Bien Hoa City– Dong Nai Provine

**Corresponding author email: nguyenhuynhbslong@yahoo.com*

The research was carried out on raw black-bone silky fowl and herbals. This research was to finded suitable parameters for production process black-bone silky fowl and herbals soup product. This product intended for everybody, who need nutritional supplements, health recovery. Black-bone silky fowl at week 4, protein content 22.88% (w/w), lipid content 2.19% (w/w) and iron content 18.2 mg /100g were selected as raw materials for processing black-bone silky fowl and herbals soup product. The formula with eight medicinal herbs were used in this study. The result showed that the total polyphenol content and sensory evaluation were used to determine the volume of water and cooking- time. In addition, volume of water 1300ml, 350g raw black-bone silky fowl and 100g herbals cooked for 30 minutes at 84.6oC, pressure 90kPa was also suitable for processing black-bone silky fowl with herbals soup product.

Keywords: black-bone silky fowl, herbals, protein, polyphenols contents

Code: P56

The research on the process of manufacturering soft milk candy and yougurt with probiotic from fresh milk in DOMILK company

Nguyen Minh Tri, Phan Thi Van Anh, Nguyen Thi Ngọc Anh, Nguyen Thi Ngoc Han, Nguyen Thai Thanh Truc, Nguyen Huynh Bach Son Long*

Faculty Of Chemical And Environmental Engineering - Lac Hong University

No 10 Huynh Van Nghe Street - Buu Long Ward – Bien Hoa City – Dong Nai Province

Corresponding author: nguyenhuynhbslong@yahoo.com

In the market, there are many types of soft milk candy and yogurt with many different products. However, The main materials for production are milk powder, reconstituted milk, sugar and additives. The airm of this study was conducted to develop a process for producing soft milk candy and and yougurt with probiotic from fresh milk.

Keyword: fresh milk, cooking time, condensed milk, fermemtion.

Code: P57
Research on producing pharmaceutical tea bags from *piper lolot c.* and *morinda citrifolia l.*

Vu Thanh Nam, Nguyen Duc Manh, Bui Truong Dat,
Nguyen Huynh Bach Son Long*.

Faculty Of Chemical And Environmental Engineering - Lac Hong University, Viet Nam

No 10 Huynh Van Nghe Street - Buu Long Ward – Bien Hoa City – Dong Nai Province

Corresponding author email: nguyenhuynhbslong@yahoo.com

Piper lolot and Morinda citrifolia are plants which exist for a very long time and are well-know for their different characteristcs and uses. By mixing two kinds of these leaves with typical use is to treat osteoarthritis. Through the survey, this kind of pharmaceutical tea bag has not been on the shelf yet. For this reason, we are carrying out to research this topic in order to improve the process, suvery and select appropriate parameter to meet consumers' demand and increase the sensory value for tea bag product from Piper lolot and Morinda citrifolia.

Key words: Piper lolot, Morinda citrifolia, polyphenol, tea bag.

Code: P58
Study on the process of manufacturer tea bags from *monordica charentia l.*

Vo Dinh Nhi, Nguyen Tien Thanh,
Bui Truong Dat, Nguyen Huynh Bach Son Long*

Faculty Of Chemmical And Environmental Engineering

Lac Hong University, Dong Nai, Viet Nam

No 10 Huynh Van Nghe Street – Buu Long Ward – Bien Hoa City – Dong Nai Province

Correspoding author: nguyenhuynhbslong@yahoo.com

Bitter melon (*Monordica Charenria L.*) known as a drug that can prevent and treat a number of diseases such as acne, fever, dry mouth, sore throat and some chronic diseases. Although the market has the tea bag from Bitter melon, but this study is undertake to improve the process, at the same time the survey and selection of appropriate parameters to meet the needs of consumers and adds value to the product sensory tea bag from Bitter melon.

Keywords Monordica Charentia L., Stevia, Blanching time, Blanching temperature, Drying.

Code: P59

Response surface optimization of ultrasound-assisted extraction for Carotenoids from Gac Peel

Hoa Tuan Tran[a], Phuong Lam Nguyen[b], Danh Cong Pham[a], Tuyen Chan Kha[a]*,

[a]*Faculty of Food Science and Technology, Nong Lam University, Ho Chi Minh City*

[b]*American International School, Ho Chi Minh City*

**Corresponding author at email: khachantuyen@hcmuaf.edu.vn*

Gac (Momordica cochinchinensis) peel, which contains relatively high content of beta-carotene and lycopene as compared with many plant foods, is discarded. The study aimed to optimize ultrasound-assisted extraction conditions of Gac peel for maximizing beta-carotene and lycopene contents, using response surface methodology (RSM). The results showed that the data were adequately fitted into second-order polynomial models for beta-carotene and lycopene with R2 values of 0.93 and 0.92, respectively. It was predicted that the optimal extraction conditions within experimental ranges would be ratio of Gac peel to ethanol of 1:30 (w/w), the extraction temperature of 57oC and the extraction time of 40 min. Under such parameters, the maximum content of beta-carotene of 242 mg per 100g and lycopene of 35 mg per 100g were achieved as predicted.

Key words: Gac fruit, Gac peel, carotenoids, lycopene, beta-carotene

Session 3: Safety and quality of aquaculture products

Code: P60
Enzymatical hydrolysis of Tra fish (*Pangasius*) scraps

Bui Thanh Thuy, Hoang Quang Binh, Trinh Ngoc Thao Ngan[1],
Nguyen Anh Trinh[2], Le Trung Thien[1]

[1] *Department of Food Engineering, Faculty of Food Science and Technology, Nong Lam University - Ho Chi Minh City*

[2] *Department of Food Product Development, Faculty of Food Science and Technology, Nong Lam University - Ho Chi Minh City*

Corresponding author: le.trungthien@hcmuaf.edu.vn

In recent years, *Pangasius* (called "Tra" fish in Vietnamese) industry has been more expanding in size and productivity that results in a lot of by-products with fish scraps are the majority. The utilization of fish scraps certainly develops economic benefits and minimizes the number of by-products in and after processing, leading to improving the production efficiency. The fish scraps utilized as the substrate for hydrolysis to produce protein hydrolysates in food application is a potentially advanced approach. The study was carried out to compare the hydrolysis efficiency between two methods: using acid and using enzyme and then to find out the optimum conditions for the selected hydrolysis method. The results showed that the enzyme hydrolysis had the lower protein recovery but superiorly prevailed in protein/dry matter ratio, as well as in terms of sensory evaluation, nutrition, and safety. Based on the results of protein recovery, hydrolysis degree and gel electrophoresis of Alcalase, Papain, Protamex and Neutrase on fish scraps, Alcalase enzyme showed the best hydrolysis performance with its maximum efficiency at pH 8.0 and 55°C with 1.0% of enzyme/material ratio. With Alcalase enzyme, the protein recovery yield was over 70%, while the hydrolysis degree was over 18%. From the results, this research is potentially capable of producing proteins that could be then added into supplemented-protein food.

Keywords: Pangasius/tra fish; protein hydrolysis, enzyme

Code: P61

Effects of False daisy (*Eclipta alba Hassk*) extract on immune response and disease resistance in striped catfish (*Pangasianodon hypophthalmus)*

Hang Bui Thi Bich[a], Quynh Nhu Truong[a], Thi Thanh Huong Do[a], Thanh Phuong Nguyen[a], Kestemont Patrick[b]

[a] *College of Aquaculture and Fisheries*

[b] *Research Unit in Environmental and Evolutionary Biology, University of Namur*

This study aimed to examine the effects of False daisy extract (*Eclipta alba Hassk*) on the immune response of striped catfish (*Pangasianodon hypophthalmus*). The study included 2 main experiments, first experiment was conducted to evaluate the effect of False daisy extract on the immune response of striped catfish leukocyte. The experiments were set up with 5 treatments, including 0, 10, 25, 50, 75, 100 µg /mL of False daisy extract, which were added to medium of leukocyte culture for 24 hours. The results showed that the supplemented treatments were significant higher activity of lysozyme and total Ig ($p < 0.05$) when compared with control treatment. Besides that, False daisy extract also stimulated complement activity of leukocyte but they were not significance among treatments. Experiment 2 aimed to evaluate the effect of False daisy extract on several immune variables of striped catfish and against Edwardsiella ictaluri which cause enteric septicemia of striped catfish. Striped catfish (20 g) were fed with supplemented False daisy extract diets in different concentrations 0; 0.5; 1; 2 and 4% for 4 weeks. Fish blood was collected after 2 and 4 weeks of feeding supplemented False daisy extract diets for hematological analysis, lysozyme activity, complement activity and total of Ig. Results showed that red blood cells, white blood cells, lysozyme activity, complement activity and total of antibody were increased slightly after 2 weeks of experiment and continued to increase after 4 weeks. In particular, fish of treatment 1% False daisy extract for 4 weeks showed highest level of red blood cell, total white blood cell count, lymphocyte, neutrophils, monocytes, lysozyme activity, complement activity and total of Ig. After challenge with Edwardsiella ictaluri, results showed that treatments of 1 and 2% False daisy extract gave higher survival rate than control treatments. Based on these results, it is possible to conclude that supplemented False daisy extract in striped catfish diets at dose of 1-2% to stimulate immunological parameters and against bacteria to protect striped catfish from bacterial disease.

Keywords: False daisy / immune response / striped catfish /

Code: P62
Shelf-life evaluation of fresh catfish (*Pangasius hypophthamuls)* fillets at different storage temperatures

Anh Ngoc Tong Thi[a], Binh Ly Nguyen[a]

[a] *Can Tho University*

The freshness of fish is a big concern to fishery stakeholders. To improve the quality of fresh fish, it is important to evaluate microbiological, sensory, and chemical changes for further determination of shelf-life of fish during storage. Therefore, the fillets of catfish (Pangasius hypophthamuls) stored at 0, 4, 8, 12^{o}C were assessed at the regular time intervals. The changes of quality were strongly dependent on the storage temperatures. The shelf-life of catfish fillets stored at 0, 4, 8, 12^{o}C was therefore prolonged up to 21, 11, 7 and 3 days, respectively. In addition, total volatile basic nitrogen correlated well (r = 0.953) with the total microbial counts while the total microbial counts also correlated well (r = 0.905) with lactic acid bacteria. However, the weak correlation was shown between the total volatile basic nitrogen and the lactic acid bacteria (r = 0.887). These results obtained can be used as a reference tool to improve fishery quality management and to minimize the economic losses as well.

Keywords: *Pangasius hypophthalmus / quality changes / shelf–life / temperature*

Code: P63

Survey on fish consumption situation of ho chi minh city residents

Hong M. X. Nguyen[a]

[a] *Nong Lam University, Ho Chi Minh City*

Fish is an excellent source of nutrients, favoured for its high levels of protein and omega-3 essential fatty acids. However, fish can cause allergic reactions to consumers. Therefore, this survey studied the fish consumption situation of consumers in Ho Chi Minh city in order to understand eating habits and their relationship to fish allergy.

The survey was conducted on 300 people who live, study or work in Ho Chi Minh City by face-to-face interview or via Internet.

The results showed that most people in Ho Chi Minh City like eating fish (82,4%) with the frequency of 1 to 2 times per week (54.2%). 77.6% of consumers usually buy fresh fish for processing rather than frozen fish or processed fish. About 12% of consumers recognized strange symtoms after eating fish, however only 43.6% of them went to see a doctor. The survey also found that catfish is the most consumed fish, while tuna was considered as the highest allergenic fish. In addition, eating uncooked or semi-uncooked fish can cause an allergenic effect on consumers.

From the results of this preliminary survey, it was found that the allergy ratio of fish consumption in Ho Chi Minh City is quite serious, and further researches are needed to ensure the safety for consumers.

Keywords: *survey / fish allergy / fish consumption / Ho Chi Minh City*

Code: P64
Fish paste production from marine fish exploited from Camau province, vietnam

Minh Phu Tran[a], Thi Hong Tuoi Nguyen[a]

[a] *Can Tho University*

Fish paste production from marine fish exploited in Ca Mau province, Vietnam was studied in order to enhance the efficient use of marine resources, contribute to improve the economic value of fisheries. The study included two contents such as (i) evaluate the possibility and quality of marine fish used for fish paste production, (ii) fish paste production from different marine fish sources. Eleven species in nine different orders were identified. Three most popular fish were goatee croaker, tuna and threadfin bream. Nine common species were selected for fish paste production. TVB - N value of marine fish was less than 50 mg/100g, indicated that fish were still fresh and can be used for fish paste production. High meat yield was found in marine fish which is appropriate for fish paste production (58.3% to 61.1%). Marine fish from Cai Doi Vam performed the better fish paste sensory properties (17.3) compared to fish from Ca Mau port (15.9) and Song Doc port (15.2). The highest gel property of fish paste was found in samples collected in Ca Mau port (1213 g.cm). Goatee croaker, pickhandle barracuda, threadfin bream, the bigeye scad, Priacanthidae, lizardfish and bartail flathead are potential species for making fish paste with high sensory property. Thus, marine fish exploited in Ca Mau sea region can be used for fish paste production.

Keywords: *marine fish / fish paste / Ca Mau / Vietnam*

Code: P65
Factors affecting food safety practices of seafood distributors within Vietnamese domestic distribution chains.

Hong Phuc Luu[a]

[a] *Nha Trang University*

In order to improve the safety of seafood in domestic distribution chains in Vietnam, a better understanding of factors affecting the practices of seafood distributors is necessary. The objective of this research was to identify the factors affecting the food safety practices among distributors in three major sites. A mixed methods design including qualitative and quantitative methods was used. Questionnaires were completed by 180 workers at various points of the seafood distribution chain. The survey revealed poor knowledge of food safety and hygiene amongst distributors and ineffective use of food safety management practices throughout the domestic seafood distribution chains. There was generally a low level of compliance with food safety regulations. One potentially positive outcome is that seafood distributors are concerned about critical feedbacks and complaints from consumers. Therefore, improving consumer knowledge may have a positive impact on food safety practices in the domestic seafood distribution chains.

Keywords: *seafood safety / food safety practice / fish contamination /*

Code: P66
Contamination mechanism in major domestic fish distribution chains in vietnam

Hong Phuc Luu[a], Thi To Uyen Dang[a]

[a] *Nha Trang University*

This study has described the principles, operations of the distribution chains, as well as assembling and analysing the contaminant factor for raw fish of domestic fish distribution chains (DFDCs) in Vietnam. The research process was to collect secondary data from management boards of fishing ports and fish markets, authorities' offices and institutes. The primary data were collected by semi-structured interviews with authorities, managers, and fish distributors. The research also observed and monitored time/temperature of fish batches to cross check data obtained from secondary data and interviews at points along distribution chains.The result identified that the fish raw materials have to proceed through many stages. The temperature abuse occurred at all of the stages along the distribution chain. The potential contaminant sources and factors affecting fish safety within the DFDCs were identified and discussed. It can be concluded that the food hygiene and safety of the DFDCs was unsafe and unhygienic handling practices. Therefore, more concern and attention should be paid to these issues by both the government and seafood industry in Vietnam.

Keywords: *seafood safety / distribution chain / fish contamination /*

Code: P67

Preparation of water soluble chitosan in solid state using hydrochloride gas

Cong Minh Nguyen[a], Ngoc Cuong Hoang[b], Van Hoa Nguyen[a]

[a] *Nha Trang University*

[b] *Binh Duong University*

Chitosan powder was adsorbed with hydrochloride gas at different conditions such as reaction temperature and time, size and density of chitosan. The soluble chitosan products was obtained by washing with ethanol and freeze drying. The solubility and viscosity of the resulting chitosan in water were 98.2% and 70 mPa.s, respectively, which is slightly lower than that of original chitosan in 1% acid acetic solution (99.5%; 95 mPa.s). Moreover, the solubility of chitosan treated with hydrochloride gas in solid state was mostly not changed when it was kept for several months. The chemical structure and crystalline properties of products were confirmed by FT-IR and XRD measurements. This method is a facile and efficient method to prepare the water soluble chitosan.

Keywords *Soluble chitosan / Solid state reaction / Solubility / Hydrochloride gas*

Code: P68
EFFECT OF THE PACKAGING AND THE PRESERVATION TEMPERATURE TO QUALITY AND SHELF-LIFE OF FRESH SEA GRAPES (*CAULERPA LENTILLIFERA J.AGARDH, 1837*)

Tuong Le Thi[a], Vu Ngoc Boi[a], Nguyen Thi Thanh Thuy[b]

[a] *Nha Trang University, Viet Nam*

[b] *Vietnam National University of Agriculture*

Fresh sea grapes are succulent, soft, loose and easily perishable by environmental factors, especially the temperarure. The purpose of this study was to determine suitable temperature and the type of packaging which is suitable for preserving fresh sea grapes. The results showed that while the shelf-life of sea grapes preserved in PVC was only 2 days, that in PA, PP were up to 10 days. However, the weight loss, the rate of damage and total aerobic microorganisms of grape seaweeds preserved in PA were lower than that in PP. On the other hand, sea grapes are preserved at 200C and 320C in 6 days and 12 days, respectively, their sensory quality reduces below average level. While sea grapes are preserved at from 230C to 290C, preservation time is up to 18 days. In particular, after 18 preservation days at 230C, the sensory quality of sea grapes is good, their spoilage rate is 5.1%; sea grapes weight decreases 13.4 % and aerobic micro-organisms increase in a safe limitation (<106cfu/g); at the other temperatures, these criteria are higher. This research will help the companies that preservation and processing of sea grapes grows better in the future.

Keywords *Sea grapes / Shelf-life / The preservation temperature / Packaging*

Code: P69
Fabrication and characteristics of fish cake from seabass by-product and fusilier fish

Pham Thi Dan Phuong,[1,*] Le Huyen Tram,[1]
Pham Thi Hien,[1] Nguyen Thi Hang,[1]

[1]*Faculty of Food Technology, Nha Trang University*

Seabass by-product consists of many nutrients with a high content such as protein (22.3 wt.%), lipid (1.4 wt.%) and ash (1.8 wt.%). However, it contents large amount of lipid, which affects on the gel strength of prepared fish cake. In this study, seabass by-product was combined with fusilier fish to produce fish cake at ratio of 1:2.5. Some additives were 6 wt.% of wheat flour and 2 wt.% of gluten. The mixing and the cooling time were 2 and 120 minutes, respectively. The obtained cake showed an enhanced quality including the gel strength (1673 g/cm) and bending moment (AA level). Product contained high protein content (27 wt.%) and it was safety food.

Keywords *Seabass by-products / fusilier fish / fish cakes /*

Email: danphuong@ntu.edu.vn

Code: P70

Quality characteristics and consumer acceptance of a ready-to-eat product developed from yellow fin tuna head and frame meat

Xuan Duy Nguyen[a], Trong Son Do[a]

[a] *Nha Trang University*

The remaining materials from processes at tuna processing factories comprised of a high ratio compared with other wastes, particularly in head and frame parts that reached 15%. Currently, these parts are still not used efficiently at tuna factories in Vietnam. Therefore, utilization of edible meat parts from tuna head and frame attract more attention by tuna producers. In a previous research, we reported that a ready-to-eat product from tuna head and frame meat has been developed successfully. In this study, as a continuing part, the information about quality characteristics and consumer acceptance of a ready-to-eat product developed from yellow fin tuna head and frame were reported. Accordingly, the ready-to-eat product contained moisture, ash, protein, lipid and carbohydrate were 13.6%, 6.7%, 66.3%, 3.8% and 9.6%. Amino acid profiles included fully essential amino acids with a ratio of 46.3%. The product met the food safety requirements for human consumption. Additionally, the consumer acceptance evaluation showed that the flavor and total acceptance scores of the developed product was higher than a similar product from the market ($p < 0.05$). The results obtained in the study revealed that the developed product exhibited a potential of considering to develop at industrial scale.

Keywords *Consumer acceptance / ready-to-eat product / quality charactristics / yellow fin tuna*

Code: P71
Changes of *Pseudomonas* spp. And total viable count in nile tilapia fillets during low temperature storage

Thi Kieu Diem Nguyen[a], Thuy Van Duyen Nguyen[a],
Nguyen Binh Ly[b], Thi Tuyet Nga Mai[a]

[a] *Nha Trang University*

[b] *Can Tho University*

The study aimed to investigate the changes of *Pseudomonas* spp. and total viable count (TVC) in Nile tilapia fillets during low temperature storage at 5 stable temperature regimes (1, 4, 9, 15, and 19 ± 1°C) and 2 dynamic ones, simulating the end of supply chain conditions. Results showed that the microbial counts increased with time and faster at higher temperatures. TVC in tilapia fillets exceeded the allowable limit of 106 CFU/g after 144 h at 1 ± 1 oC (1,1. 107 CFU/g), 48 h at 4 ± 1 oC (2,4. 106 CFU/g), 24 h at 9 ± 1 oC (1,47. 106 CFU/g), 24 h at 15 ± 1 oC (1,83. 107 CFU/g), and 20 h at 19 ± 1 oC (1,28. 108 CFU/g). Meanwhile the counts of Pseudomonas spp. after 144 h at 1 ± 1 oC; 48 h at 4 ± 1 oC; 24 h at 9 ± 1 oC; 24 h at 15 ± 1 oC and 20 h at 19 ± 1 oC were 3,62.104; 2,17.105; 4,43.104; 2,68.105; 1,55.105 CFU/g, respectively. At the 2 dynamic temperature regimes, TVC and Pseudomonas spp. counts raised sharply when there was an increase in storage temperature, revealing the negative effect of temperature abuse to the aquatic product quality.

Keywords *Pseudomonas spp. / total viable count / tilapia fillet / low temperature storage*

Code: P72

Allergen detection by monoclonal and polyclonal antibodies to allergen display in Black Tiger Prawn (*Penaeus monodon*), Blue Swimmer Crab (*Portunus pelagicus*), Pacific Oyster (*Crassotrea gigas*)

Manh Cuong NGUYEN, Sandip D. KAMATH, Shruti S. KAMATH, Roni NUGRAHA, Thimo RUETHERS, Thi Kieu Thu LE, Minh Xuan Hong NGUYEN, Andreas L.LOPATA

Molecular Immunology Group, Centre for Biodiscovery and Molecular Development of Therapeutics , James Cook University, Queensland, Australia.

Allergic diseases have been noticeable increased in most major industrialized countries in recent years. The occurrence of these hypersensitive reactions has steadily increased over the last few decades with asthma and anaphylaxis has become the leading causes of death as reported by the emergency services. High percentage of allergy phenomenon derives from seafood containing the major heat-stable allergen, tropomyosin. The muscle protein tropomyosin (TM) is the most essential pan-allergen responsible for allergic reactions to shellfish, which include crustacean and molluscs.

This research clarifies main differences between polyclonal and monoclonal antibodies which applied in three specimens Black Tiger Prawn (Penaeus monodon), Blue Swimmer Crab (Portunus pelagicus), Pacific Oyster (Crassotrea gigas) to re-inspect the allergen in these species. The extraction was carried out and divided into two groups: raw samples and cooked samples (heated in phosphate buffered saline). The protein estimation of these specimens was conducted by Bradford assay and absorbed at 595 nm. After that, these samples were run in sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot (using polyclonal and monoclonal antibodies), respectively. SDS-PAGE was performed at 160V in 1 hour to separate proteins after their molecular weights. In immunoblot, the separated protein bands were transferred to a carrier membrane (PVDF) which was called blotting step. The proteins adhered to the membrane in the same pattern as they have been separated due to interactions of charges. The proteins on this immunoblot were then accessible for antibody binding for detection.

The protein amounts of SDS-PAGE and immunoblot were appreciated at 10 µg and 7 µg respectively for each well. Furthermore, with the polyclonal antibodies, the dilution for the first and secondary antibodies were determined at 1:20,000 and 1:30,000 respectively while with the monoclonal the dilution is 1:5,000 and 1:20,000 orderly in the first and secondary antibodies.

Code: P73

Retardation of lipid oxidation in dried roud scad fillets by natural antioxidant-containing extract from Voi (*cleistocalyx operculatus)* leaf

Xuan Duy Nguyen[a], Tuan Anh Nguyen[a]

[a]*Nha Trang University*

Lipid oxidation is one of the most important deterioration in lipid-rich food. Marine fish is rich in lipid with a lot of polyunsaturated fatty acids. These fatty acids are very susceptible to oxidation process. Therefore, deteriorations related to lipid oxidation are one of the major sources that occur during production, storage, distribution and consumption. One of the most effective ways of retarding lipid oxidation in foods is to incorporate. In this study, we investigated to apply a natural antioxidant-containing extract extracted from Voi's leaves grown in Vietnam to retard lipid oxidation in dried round scad fillets. Accordingly, the Voi's leaves were extracted at an optimal condition to obtain maximum polyphenol content, including a temperature of 70^{o}C, time of 100 mins, a solvent extract of ethanol 60%, a ratio of solvent and material of 60/1 (v/w). Polyphenol-containing extract was partly purified using a solvent system with an increasing polarity. The highest polyphenol content was found in the water fraction. This polyphenol-rich extract was evaluated antioxidant activity using free radical scavenging ability (ABTS) and reducing power ability (RPA), retardation of lipid oxidation in oil-in-water model system. Finally, the polyphenol-rich extract was applied in preventing lipid oxidation in dried round scad fillets.

Results showed that the polyphenol-rich extract from the Voi's leaves exhibited antioxidant activity in all of tests in vitro. A value of IC50 of ABTS and RPA were 2.97 µg/ml and 5.01 µg/ml. In oil-in-water model system, the polyphenol-rich extract at 200 ppm prevented effectively lipid oxidation compared with the control sample. Dried round scad fillets were treated by the polyphenol-rich extract indicated a significant reduction of lipid oxidation level as shown by conjugated diene index, peroxide value, and thiobarbituric acid reaction substances. These values of the extract-treated fillets were significantly lower than those of the control samples ($p < 0.05$).

Keywords: Cleistocalyx operculatu / natural antioxidant-containing extract / lipid oxidation / round scad fillets

EXTENDED ABSTRACTS

Session 1: Safety and quality of vegetable & fruit products

EFFECT OF MICROWAVE PROCESSING AND STORAGE ON QUALITY OF CAJUPUT (*MELALEUCA CAJUPUTI*) HONEY FROM BAC LIEU – VIETNAM

Nguyen Xuan Nam[1], Huynh Ngoc Oanh[1], Phan Phuoc Hien[2]

[1] *Ho Chi Minh City University of Technology*

[2] *Southern Cantho University*

Corresponding author: nx.nam@hutech.edu.vn

ABSTRACT

The aim of the present work was to find out if microwave processing and storage conditions can affect some of the main honey quality paramenters (reducing sugar (RS), hydroxymethylfurfural (HMF), diastase number (DN), water and colour) of cajuput honey was obtained from Bac Lieu – Vietnam. RS content were analyzed by DNS method, HMF under AOAC 980.23, diastase activity based on AOAC 958.09, water content according to AOAC 969.38B and colour parameters (L*, a*, b*) were established in the CIE system. The results of the analysis showed that the physico-chemical characteristics of fresh honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81. Honey was processed at continues power 30P (480 W), 50P (800 W), 70P (1120 W) for 20, 30, and 40 seconds then the honey sample was left to room temperature (20-23^{o}C), stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks. The changes in the RS, HMF, DN, water and colour were observed. Microwave processing applied to cajuput honey did not effect on colour, while RS, HMF, DN and water content affected significantly. The storage time and temperature affected significantly on RS, DN and colour.

Keywords: honey, microwave processing, storage, reducing sugar, hydroxymethylfurfural, diastase number, water, colour

1. INTRODUCTION

Honey is natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants which the bees collect and transform by combining it with specific substances of their own, dehydrate, store and leave in honeycombs to mature and ripen (Bogdanov, 2002).

It is well known that honey as a natural product may be processed by means of heat processing for two main reasons: first of all, to destroy the micro-organisms that may contaminate it and to modify its tendency to crystallisation or delay the appearance of monosaccharide crystals (Tosi et al., 2002). During crystallisation that leads to phase separation, both liquid and non-liquid phases may coexist. Simultaneously, water activity of the remaining liquid phase begins to increase. This is the result of water release during crystallisation and subsequent decrease of carbohydrates concentration in the liquid phase. This phenomenon makes the honey suitable for the growth of microorganisms like yeasts and fungi and leads to sensory properties modifications and quality damage (Tosi et al., 2004).

Microwave processing may be the solution for honey liquefaction without the loss of bioactivity. Microwaves as an example of volumetric heating will influence the state of aggregation and due to the microwave/matter interaction may be applied for a short time. It results in reduction of quality losses in honey (Kowalski et al., 2012). On the other hand it is well known that microwave processing can cause some damage to bioactive food components (especially enzymes) (Günes et al., 1993; Matsui et al., 2007).

Therefore, in this work, we aimed to evaluate the effect of microwave processing and storage on the honey quality.

2. MATERIAL AND METHODS

Cajuput honey were obtained from Bac Lieu – Vietnam.

Microwave processing of the honey samples (50 g) was performed without stirring in a microwave reactor operating (power level input 1600W = 32W/g = 100P) with continues power 30P (9.6W/g), 50P (16W/g), 70P (22.4W/g), for 20, 30, and 40 seconds.

After all treatments and cooling of the samples to room temperature, RS, HMF, DN, water content and colour were determined as a function of time.

Beside that, honey was stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks, analyzed the main honey quality.

RS can be investigated by the 3,5-dinitrosalicylic acid (DNS) method employing glucose as the standard at 540 nm (Miller, 1959). HMF content is based on AOAC 980.23. DN is based on AOAC 958.09. Water content is based on AOAC 969.38B. Colour parameters (L*, a*, b*) were established in the CIE system using a Minolta Chroma-meter (CR-200 Series, Osaka, Japan).

The data obtained in the study were analyzed statistically using analysis of variance (ANOVA) with statistically significant ($P<0.05$).

3. RESULTS AND DISCUSSION

Table 1. Physicochemical composition of cajuput honey

Physicochemical composition	Contents
Water (%)	23.18±0.72
RS (mg/g)	717.42±8.34
HMF (mg/kg)	4.24±0.31
DN (mg/kg)	4.85±0.24
L*	39.51±0.14
a*	10.51±0.06
b*	31.81±0.23

The composition of honey depends on produced season, origin of nectar and climatic conditions (Fallico et al., 2003). The most important factor affecting honey composition is plant origin (Serrano et al., 2004). The chemical components of the fresh honey sample are shown in Table 1. Water content of cajuput honey (23.18%) higher than Codex standard 12-1981 (< 20%). Water content is highly important for the shelf-life of the honey during storage because a high water content causes honey to ferment and spoil (Perez et al., 1994). RS content (717.42 mg/g, > 600 mg/g), HMF content (4.24 mg/kg, < 40 mg/kg) and DN content (4.85 mg/kg, ≥ 3 mg/kg) were satisfied Codex standard. DN and HMF are considered as the main parameters for evaluating: the freshness, the heat and storage history of honey (Sancho et al., 1992). The color of honey is one of the most factors used to assess the quality of honey. The results showed that color of cajuput honey so dark (L* = 39.51).

Table 2. Effect of microwave processing on honey quality

Processing conditions (Power - Time)	RS (mg/g)	HMF (mg/kg)	DN (mg/kg)	Water content (%)	ΔE
Before treatment	717.42^{a*}±8.34	4.24^{a}±0.31	4.85^{bc}±0.24	23.18^{a}±0.72	0.00
30P-20 sec	715.04^{a}±19.35	5.04^{b}±0.43	4.63^{c}±0.38	22.85^{a}±0.88	0.07
30P-30 sec	724.54^{ab}±4.94	5.49^{b}±0.46	5.54^{ab}±0.74	21.36^{b}±0.19	0.13
30P-40 sec	759.38^{cd}±11.88	6.44^{c}±0.40	6.05^{a}±0.97	21.22^{b}±0.23	0.26
50P-20 sec	747.51^{bc}±12.34	6.99^{cd}±0.17	3.46^{d}±0.15	19.88^{c}±1.32	0.19
50P-30 sec	779.97^{de}±9.89	7.49^{d}±0.25	3.64^{d}±0.24	18.67^{cd}±0.43	0.59
50P-40 sec	816.78^{fg}±12.59	8.13^{e}±0.31	5.66^{ab}±0.73	18.87^{cd}±1.41	0.39

70P-20 sec	831.09^{g}±16.67	8.08^{e}±0.30	3.46^{d}±0.06	17.64^{d}±0.63	0.62
70P-30 sec	795.42^{ef}±7.96	8.93^{f}±0.31	4.63^{c}±0.44	18.31^{de}±0.39	0.59
70P-40 sec	777.86^{de}±25.13	8.38^{ef}±0.40	3.62^{d}±0.46	16.87^{e}±0.24	0.64

* Values in columns with the same letter do not differ significantly at α = 0.05

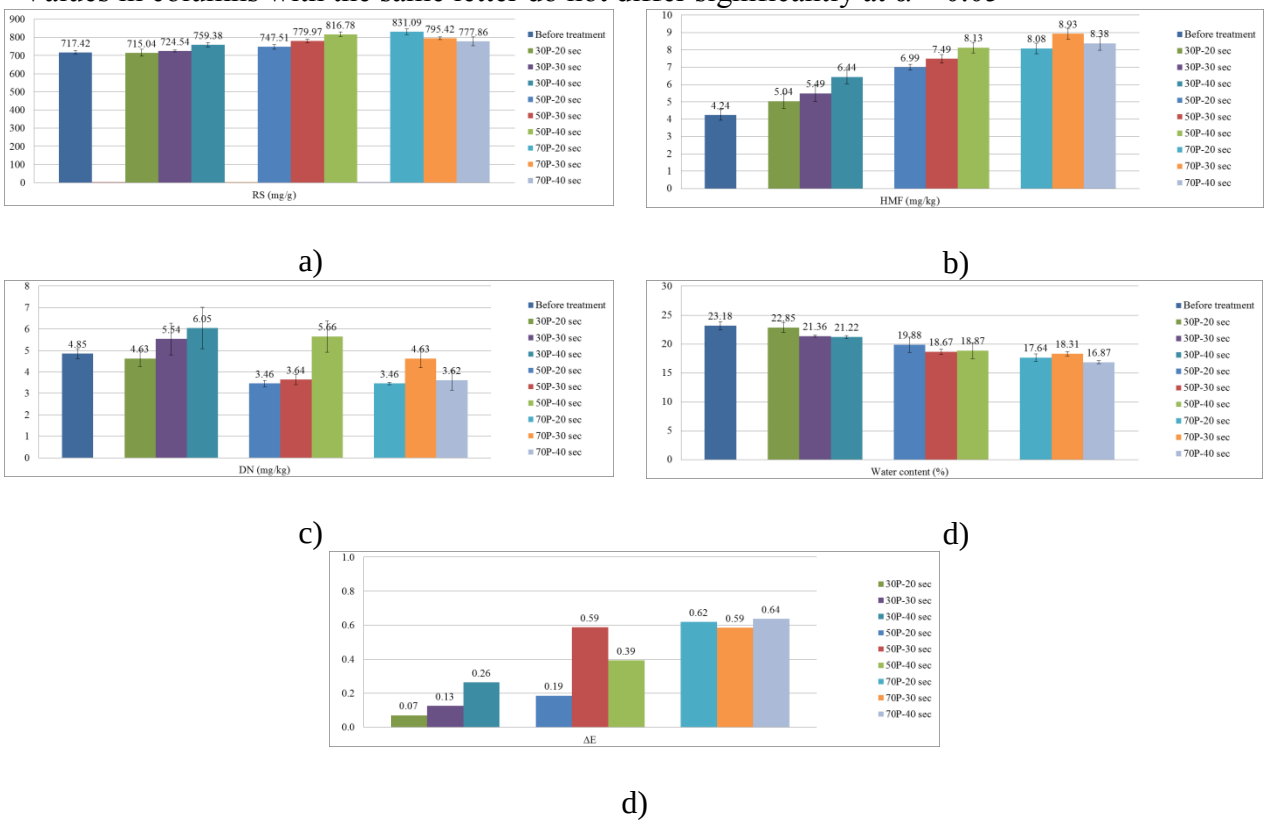


Figure 1. Effect of microwave processing on honey quality

The values of RS content in different power are summarized in Table 2 and Figure 1a. The changes of RS content in the honey sample according to 9 treatments had fluctuated but not significantly until 50P-30 sec (779.97 mg/g). However, the RS did not less than 600 mg/g (Codex Standard 12-1981).

The initial content of HMF was low in the honey and the processing to which honey sample was increased depend on the processing conditions. According to 9 treatments, HMF raised continuously, although according to different ratio are summarized in Table 2 and Figure 1b. At 30P in 20 – 40 sec, HMF from 5.04 – 6.44 mg/kg. At 50P in 20 – 40 sec, HMF from 6.99 – 8.13 mg/kg. At 70P, HMF increased 49.52% (compare with fresh honey). However, the HMF did not more than 40 mg/kg (Codex Standard 12-1981).

The values of DN in different power and time are summarized in Table 2 and Figure 1c. DN had fluctuated according to treatments (from 4.63 – 6.05 mg/kg at 30P, from 3.46 – 5.66 mg/kg at 50P and from 3.46 – 3.62 mg/kg at 70P) but not less than 3 mg/kg (Codex Standard 12-1981).

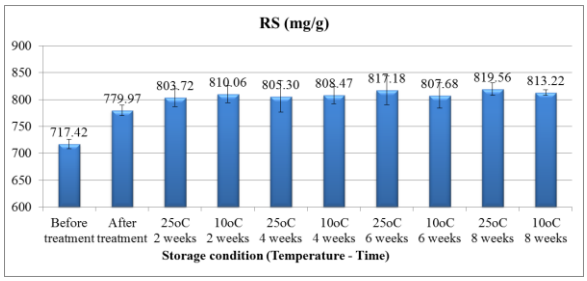
The results showed that as the power and time of microwave processing increased, the water content decreased. At 30P, treatment to 30 seconds to made different from the original water. When the power rises to 50P and 70P in 20 seconds, water content was significantly reduced. However, under conditions of 70P – 40 seconds, the water content (16.87%) was reached to preservative conditions (17 – 18%).

The colour of honey is one of the factors determining its price on the world market, and also its acceptability by the consumer. Light honeys are usually mild in flavor and of a higher commercial value than dark coloured honey (Wootton et al., 1976; White, 1978). According to 9 treatments, colour of honey had fluctuated but the observator does not feel the difference (ΔE < 1).

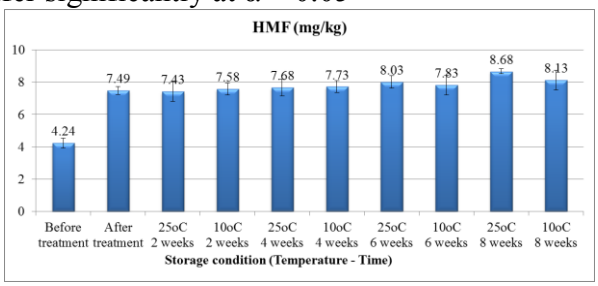
Table 3. Effect of storage on honey quality after microwave processing at 50P – 30 sec

Time (week)	Temperature (°C)	RS (mg/g)	HMF (mg/kg)	DN (mg/kg)	Water content (%)	ΔE
Before treatment		717.42^{a*}±8.34	4.24^{a}±0.31	4.85^{bc}±0.24	23.18^{a}±0.72	0.00
After treatment		779.97^{b}±9.89	7.49^{b}±0.25	3.64^{a}±0.24	18.67^{bc}±0.43	0.59
2	**25°C**	803.72^{bc}±16.68	7.43^{b}±0.62	5.52^{de}±0.49	19.23^{b}±0.96	2.08
	10°C	810.06^{bc}±16.17	7.58^{b}±0.37	5.22^{cd}±0.48	18.60^{bc}±0.41	2.10
4	**25°C**	805.30^{bc}±29.03	7.68^{b}±0.52	5.81^{e}±0.43	19.60^{b}±0.67	3.71
	10°C	808.47^{bc}±15.81	7.73^{b}±0.37	5.16^{cd}±0.21	18.92^{b}±0.68	3.00
6	**25°C**	817.18^{c}±26.70	8.03^{bc}±0.38	5.38^{cde}±0.44	19.51^{b}±0.51	5.11
	10°C	807.68^{bc}±23.91	7.83^{b}±0.62	4.80^{bc}±0.20	17.61^{c}±0.37	3.38
8	**25°C**	819.56^{c}±11.23	8.68^{c}±0.15	4.46^{b}±0.34	19.13^{b}±1.25	7.85
	10°C	813.22^{c}±4.94	8.13^{bc}±0.60	4.37^{b}±0.23	19.28^{b}±1.01	5.50

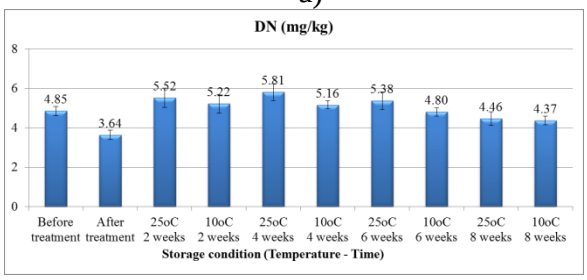
* Values in columns with the same letter do not differ significantly at α = 0.05



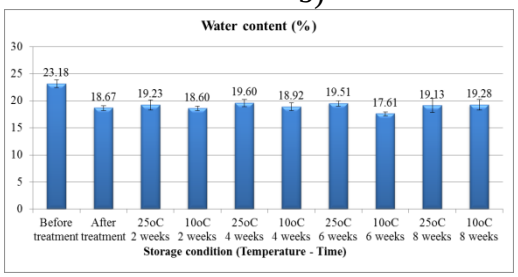
a)



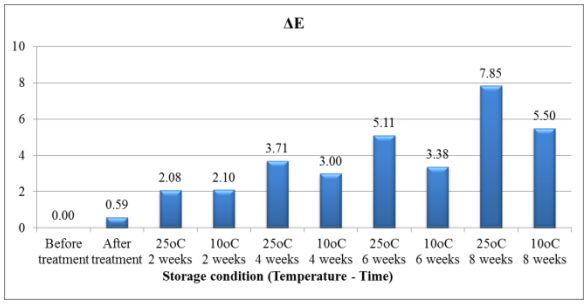
b)



c)



d)



e)

Figure 2. Effect of storage condition after microwave processing on honey quality

After processing at 50P – 30 sec, honey sample was stored at room (<25°C) and cool (<10°C) temperature for eight weeks. The values of RS in different storage conditions are showed in Table 3 and Figure 2a. During the storage, there was no significant (P<0.05) difference among the temperature and time on RS (from 803.72 to 819.56 mg/g).

About HMF content, according to 8 weeks in 25^{o}C and 10^{o}C, HMF had increased continously but there was no significant (P<0.05) difference (except at 8 weeks in 25^{o}C). However, the HMF did not more than 40 mg/kg (Codex Standard 12-1981). The increase of the HMF concentration might be to the diminution of the fructose content (Cervantes et a1., 2000), temperature and time of heating (Karabournioti et al., 2001; Fallico et a1., 2003 and White et a1., 1964), storage conditions, use of metallic containers (Fallico et a1., 2003) and chemical properties of honey, which are attributed to the floral source from where the honey has been extracted. However, no information on the correlation between chemical characteristics and HMF level of honey is available (Fallico et a1., 2003).

At 25^{o}C, during the first 4 weeks, DN increased (5.81 mg/kg). However, DN diminished (4.46 mg/kg) at 8 weeks but not less than 3 mg/kg (Codex Standard 12-1981).

At 10^{o}C, during the first 2 weeks, DN increased (5.22 mg/kg). However, DN diminished (4.37 mg/kg) at 8 weeks but not less than 3 mg/kg (Codex Standard 12-1981).

Similar trend was noticed by White et al., (1964). Sancho et al., (1992) reported depletion in diastase number after studied the effect of storage for two years at 20°C. Cervantes et al., (2000) also reported decrease in diastase number after heating the honey at 55°C and then storing it for three and half months at 26°C. Castro-Vazquez et al., (2008) also reported that diastase number was out of the legal limit in citrus honey stored for 12 months at 40ºC.

At temperature 10^{o}C and 25^{o}C, during 8 weeks, there was no significant (P<0.05) difference in water content.

During 8 weeks storage, as shown in Figure 2e for the color function ΔE after an initial induction period, it followed a period of increase of color with time (2.08, 3.71, 5.11, 7.85 at 25^{o}C and 2.10, 3.00, 3.38, 5.50 at 10^{o}C). This behavior was observed in many products subjected to non-enzymatic browning (Song et al., 1966; Labuza et al., 1980). During shipping to far countries and/or dring storage, darkening of honey may occur, and paralellel changes in its organoleptic properties have detrimental effects on its quality, masking its original aroma, which promotes loss of competitiveness in the world market (Milum, 1939).

4. CONCLUSION

The results showed that the physico-chemical characteristics of cajuput honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81.

Microwave processing applied to cajuput honey did not effect on colour, while RS, HMF, DN and water content affected significantly but still satisfied the limits of Codex Standard 12-1981. The storage time and temperature affected significantly on RS, DN and colour (ΔE > 3, large difference).

5. REFERENCES

1. Bogdanov S. and Martin P. *Swiss Bee Reaserch Centre* 2002; 1-20.
2. Tosi E., Ciappini M., Ré E. and Lucero H. *Food Chemistry* 2002; 77: 71-74.
3. Tosi E., Re E., Lucero H. and Bulacio L. *LWT – Food Science and Technology* 2004; 37(6): 669-678.
4. Kowalski S., Lukasiewicz M., Bednarz S. and Panus M. *Czech Journal of Food Sciences* 2012; 30(1): 21-26.
5. Günes B., and Bayindirli A. *Lebensmittel-Wissenschaft und-Technologie* 1993; 26(5): 406-410.
6. Matsui K. N., Granado L. M., De Oliveira P. V. and Tadini C. C. *LWT – Food Science and Technology* 2007; 40(5): 852-859.
7. Miller. *Analytical Chemistry* 1959; 31(3).
8. Fallico B., Zappala M., Arena E. and Verzea A. *Food Chem.* 2003; 85: 305-313.
9. Serrano S., Villarejo M., Espejo R. and Jordal M. *Food Chem.* 2004; 87: 619-625.
10. Codex Standard for honey 12-1981

11. Perez C., Conchello P., Arino A., Juan T. and Herresa A. *Food Chem.* 1994; 51: 207-210.
12. Sancho M. T., Muniategui S., Huidobro J. F. and Lozano S. *J. Agric. Food chem.* 1992; 4: 134-138.
13. Wootten M., Edwards R. A., Faraji-Haremi R. and Johnson A. T. *Journal of Apicultural Research* 1976; 15: 23-28.
14. White J. W. *Advances in Food Research* 1978; 24: 287-371.
15. Karabournioti S. and Zervalaki P. *Apiacta* 2001; 36(4): 177-181.
16. White J. W., Kushnir I. and Subers M. H. *Food Technology* 1964; 18(4): 153-156.
17. Cervantes M. A. R., Novelo-Gonzalez S. A. and Duch-Sauri E. *Apiacta* 2000; 35: 162-170.
18. Castro-Vazquez L., Diaz-Maroto M. C., Gonzalez-Vinas M. A., De La Fuente E. and Perez-Coello M. S. *J. Agric. Food Chem.* 2008; 6: 1999-2006.
20. Labuza T. P. and Saltmarch M. *In L.B. Rockland, and G.F. Steward, Properties of water related to food quality and stability, Academic Press: San Francisco.* 1980; 605-650.
21. Milum V. G. *American Bee Journal* 1939; 17: 390-392.

EFFECT OF THERMAL PROCESSING AND STORAGE ON QUALITY OF CAJUPUT (*MELALEUCA CAJUPUTI*) HONEY FROM BAC LIEU – VIETNAM

Nguyen Xuan Nam[1], Huynh Ngoc Oanh[1], Phan Phuoc Hien[2]

[1] *Ho Chi Minh City University of Technology*

[2] *Southern Cantho University*

Corresponding author: nx.nam@hutech.edu.vn

ABSTRACT

The aim of the present work was to find out if thermal processing and storage conditions can affect some of the main honey quality paramenters (reducing sugar (RS), hydroxymethylfurfural (HMF), diastase number (DN), water and colour) of cajuput honey was obtained from Bac Lieu – Vietnam. RS content were analyzed by DNS method, HMF under AOAC 980.23, diastase activity based on AOAC 958.09, water content according to AOAC 969.38B and colour parameters (L*, a*, b*) were established in the CIE system. The results of the analysis showed that the physico-chemical characteristics of fresh honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81. Honey was heated at 50, 60, 70°C for 20, 30, and 40 min then the honey sample was left to room temperature (20-23^{o}C), stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks. The changes in the RS, HMF, DN, water and colour were observed. Thermal processing applied to cajuput honey did not effect on RS, water content and colour, while HMF and DN affected significantly. The storage time and temperature affected significantly on colour.

Keywords: honey, thermal processing, storage, reducing sugar, hydroxymethylfurfural, diastase number, water, colour

1. INTRODUCTION

Honey is natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants which the bees collect and transform by combining it with specific substances of their own, dehydrate, store and leave in honeycombs to mature and ripen (Bogdanov, 2002).

It is well known that honey as a natural product may be processed by means of thermal processing for two main reasons: first of all, to destroy the micro-organisms that may contaminate it and to modify its tendency to crystallisation or delay the appearance of monosaccharide crystals (Tosi et al., 2002). During crystallisation that leads to phase separation, both liquid and non-liquid phases may coexist. Simultaneously, water activity of the remaining liquid phase begins to increase. This is the result of water release during crystallisation and subsequent decrease of carbohydrates concentration in the liquid phase. This phenomenon makes the honey suitable for the growth of microorganisms like yeasts and fungi and leads to sensory properties modifications and quality damage (Tosi et al., 2004).

Honey quality is significantly influenced by storage time and heating. Thermal processing of honey is a critical issue for preserving honey integrity during manufacture. Thus honey pasteurization can be safely used to get rid of fermentation (Kretavhičius et al., 2010). Honey should not be heated or treated thermally that lead to a change in its basic formulation or affects its quality, and chemical or biochemical treatments must not be performed to effect on the crystallized honey (Codex Standard 12-1981). It was observed that it can improve the appearance and texture of honey and stop its crystallization (Thrasyvoulou et al., 1994). And, honey nutrients such as sugars, proteins...etc. are prone to thermal decomposition in which much of its nutritional value is lost (Spano et al., 2006).

Therefore, in this work, we aimed to evaluate the effect of thermal processing and storage on the honey quality.

2. MATERIAL AND METHODS

Cajuput honey was obtained from Bac Lieu – Vietnam.

Thermal processing of the honey sample (50 g) was conducted in a water bath (BS-11, Korean) at 50, 60, 70°C for 20, 30, and 40 min (isothermal heating) without stirring.

After all treatments and cooling of the samples to room temperature, RS, HMF, DN, water content and colour were determined as a function of time.

Beside that, honey was stored at room (<25^{o}C) and cool (<10^{o}C) temperature for eight weeks, analyzed the main honey quality.

RS can be investigated by the 3,5-dinitrosalicylic acid (DNS) method employing glucose as the standard at 540 nm (Miller, 1959). HMF content is based on AOAC 980.23. DN is based on AOAC 958.09. Water content is based on AOAC 969.38B. Colour parameters (L*, a*, b*) were established in the CIE system using a Minolta Chroma-meter (CR-200 Series, Osaka, Japan).

The data obtained in the study were analyzed statistically using analysis of variance (ANOVA) with statistically significant ($P<0.05$).

3. RESULTS AND DISCUSSION

Table 1. Physicochemical composition of cajuput honey

Physicochemical composition	Contents
Water (%)	23.18±0.72
RS (mg/g)	717.42±8.34
HMF (mg/kg)	4.24±0.31
DN (mg/kg)	4.85±0.24
L*	39.51±0.14
a*	10.51±0.06
b*	31.81±0.23

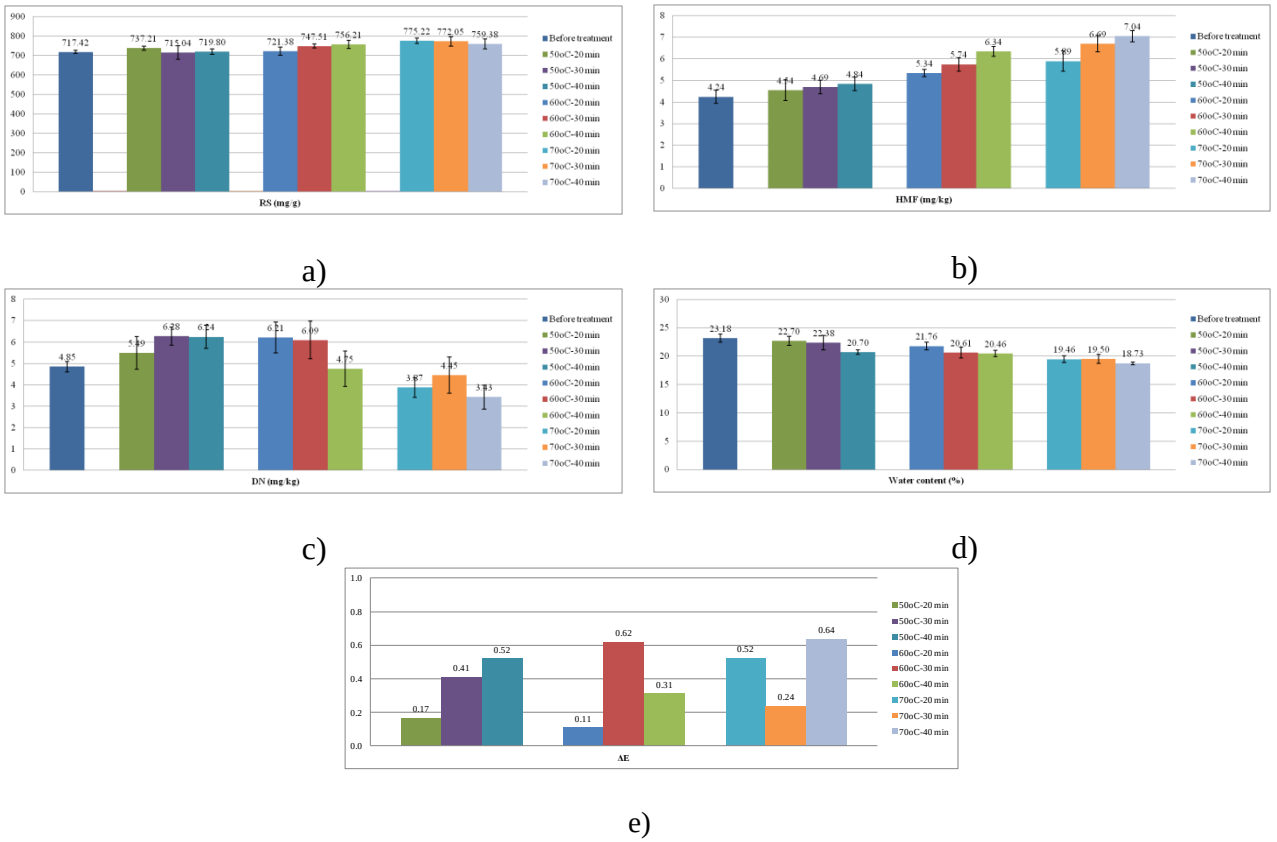
The composition of honey depends on produced season, origin of nectar and climatic conditions (Fallico et al., 2003). The most important factor affecting honey composition is plant origin (Serrano et al., 2004). The chemical components of the fresh honey sample are shown in Table 1. Water content of cajuput honey (23.18%) higher than Codex standard 12-1981 (< 20%). Water content is highly important for the shelf-life of the honey during storage because a high water content causes honey to ferment and spoil (Perez et al., 1994). RS content (717.42 mg/g, > 600 mg/g), HMF content (4.24 mg/kg, < 40 mg/kg) and DN content (4.85 mg/kg, ≥ 3 mg/kg) were satisfied Codex standard. DN and HMF are considered as the main parameters for evaluating: the freshness, the heat and storage history of honey (Sancho et al., 1992). The color of honey is one of the most factors used to assess the quality of honey. The results showed that color of cajuput honey so dark (L* = 39.51).

Table 2. Effect of thermal processing on honey quality

Processing conditions (Temperature - Time)	RS (mg/g)	HMF (mg/kg)	DN (mg/kg)	Water content (%)	ΔE
Before treatment	717.42^{a*}±8.34	4.24^{a}±0.31	4.85^{bc}±0.24	23.18^{a}±0.72	0.00
50^{o}C-20 min	737.21^{ab}±9.89	4.54^{ab}±0.48	5.49^{cd}±0.76	22.7^{ab}±0.81	0.17
50^{o}C-30 min	715.04^{a}±34.62	4.69^{ab}±0.31	6.28^{d}±0.43	22.38^{ab}±1.26	0.41
50^{o}C-40 min	719.80^{a}±13.71	4.84^{bc}±0.31	6.24^{d}±0.54	20.70^{cd}±0.39	0.52
60^{o}C-20 min	721.38^{a}±20.29	5.34^{cd}±0.17	6.21^{d}±0.72	21.76^{bc}±0.69	0.11
60^{o}C-30 min	747.51^{abc}±10.35	5.74^{d}±0.31	6.09^{d}±0.88	20.61^{cd}±0.97	0.62

60°C-40 min	756.21^{bc}±20.48	6.34^{ef}±0.23	4.75^{bc}±0.83	20.46^{d}±0.59	0.31
70°C-20 min	775.22^{c}±13.92	5.89^{de}±0.46	3.87^{ab}±0.45	19.46^{de}±0.58	0.52
70°C-30 min	772.05^{c}±24.72	6.69^{fg}±0.38	4.45^{abc}±0.85	19.50^{de}±0.80	0.24
70°C-40 min	759.38^{bc}±25.02	7.04^{g}±0.26	3.43^{a}±0.56	18.73^{e}±0.24	0.64

* Values in columns with the same letter do not differ significantly at α = 0.05



a) b) c) d) e)

Figure 1. Effect of thermal processing on RS

The values of RS content in different temperatures are summarized in Tables 2 and Figure 1a. The changes of RS content in the honey sample according to 9 treatments had fluctuated but not significantly until 60°C-40 min (756.21 mg/g). However, the RS did not less than 600 mg/g (Codex Standard 12-1981).

The initial content of HMF was low in the honey and the heating to which honey sample was increased depend on the processing conditions. According to 9 treatments, HMF raised continuously, although according to different ratio are summarized in Table 2 and Figure 1b. However, the HMF did not more than 40 mg/kg (Codex Standard 12-1981).

The values of DN in different temperatures and time are summarized in Table 2 and Figure 1c. DN had increased according to treatments from 50°C-20 min to 60°C-30 min. From 60°C-40 min to 70°C-40 min, DN had diminished but not less than 3 mg/kg (Codex Standard 12-1981).

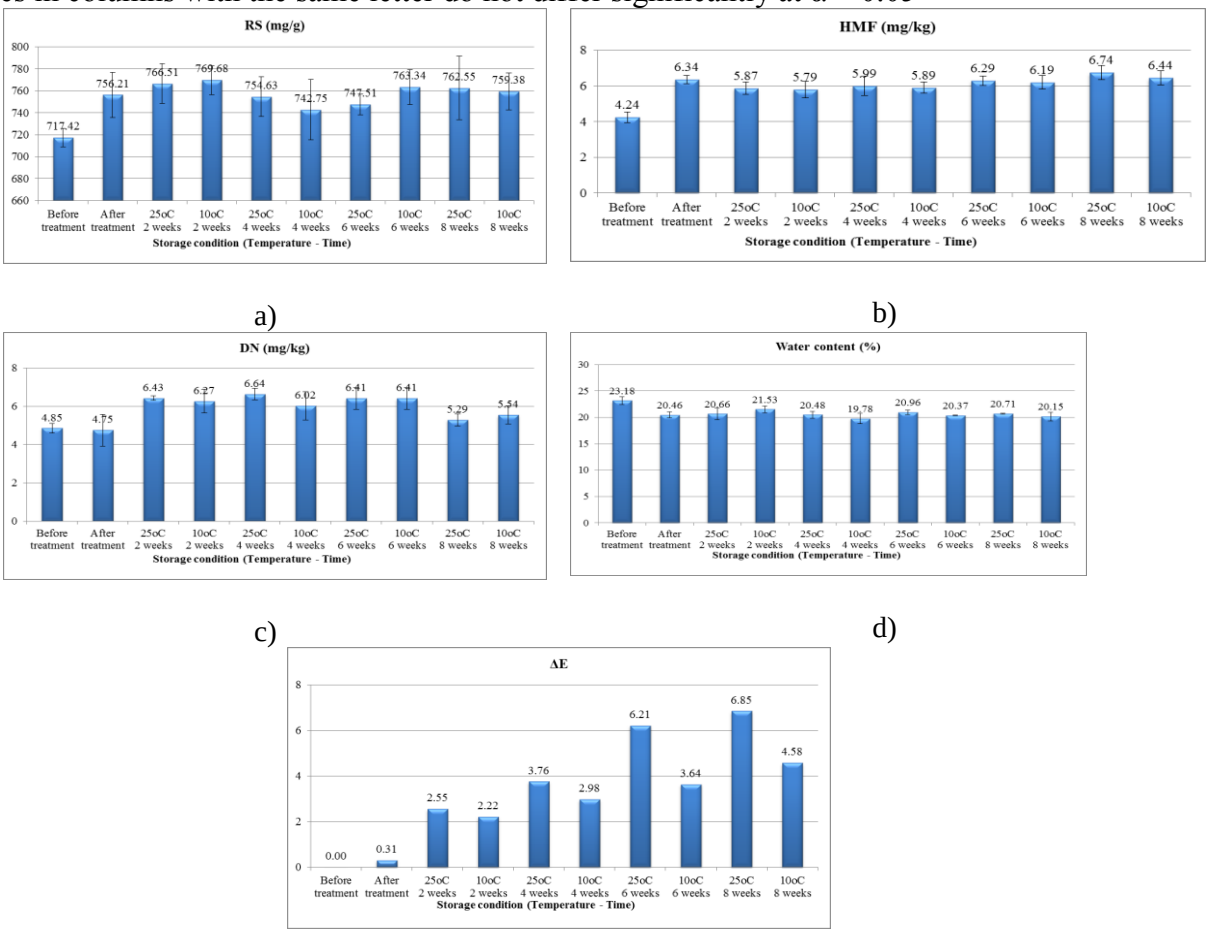
The results showed that the water content depends on the temperature and duration heating. After 40 minutes of heating at 50°C, we would see the different from the original water content. Rises to 60°C to 70°C in 20 minutes, water content was significantly reduced. However, even at 70°C – 40 minutes, the water content (18.73%) was still higher than the ideal storage conditions (17 – 18%).

The colour of honey is one of the factors determining its price on the world market, and also its acceptability by the consumer. Light honeys are usually mild in flavor and of a higher commercial value than dark coloured honey (Wootton et al., 1976; White, 1978). According to 9 treatments, colour of honey had fluctuated but the observator does not feel the difference (ΔE < 1).

Table 3. Effect of storage on honey quality after thermal processing at 60°C - 40 min

Time (week)	Temperature (°C)	RS (mg/g)	HMF (mg/kg)	DN (mg/kg)	Water content (%)	ΔE
Before treatment		717.42^{a*}±8.34	4.24^{a}±0.31	4.85^{a}±0.24	23.18^{abc}±0.72	0.00
After treatment		756.21^{b}±20.48	6.34b^{cd}±0.23	4.75^{a}±0.83	20.46^{d}±0.59	0.31
2	**25°C**	766.51^{b}±17.93	5.87^{bc}±0.34	6.43^{cd}±0.13	20.66^{abc}±1.06	2.55
	10°C	769.68^{b}±13.08	5.79^{b}±0.46	6.27^{cd}±0.62	21.53^{c}±0.65	2.22
4	**25°C**	754.63^{b}±17.93	5.99^{bc}±0.54	6.64^{d}±0.30	20.48^{abc}±0.69	3.76
	10°C	742.75^{ab}±27.39	5.89^{bc}±0.31	6.02^{bcd}±0.74	19.78^{a}±0.97	2.98
6	**25°C**	747.51^{ab}±9.51	6.29b^{cd}±0.26	6.41^{cd}±0.56	20.96^{bc}±0.45	6.21
	10°C	763.34^{b}±15.82	6.19b^{cd}±0.38	6.41^{cd}±0.57	20.37^{abc}±0.06	3.64
8	**25°C**	762.55^{b}±29.03	6.74^{d}±0.40	5.29^{ab}±0.31	20.71^{abc}±0.07	6.85
	10°C	759.38^{b}±16.63	6.44^{cd}±0.40	5.54^{abc}±0.48	20.15^{ab}±0.83	4.58

* Values in columns with the same letter do not differ significantly at α = 0.05



a) b) c) d) e)

Figure 2. Effect of storage condition after thermal processing on RS

After processing at 60°C - 40 min, honey sample was stored at room (<25°C) and cool (<10°C) temperature for eight weeks. The values of RS in different storage conditions are showed in Table 3 and Figure 2a. During the storage, there was no significant (P<0.05) difference among the temperature and time on RS (from 742.75 to 769.68 mg/g).

About HMF content, according to 8 weeks in 25°C and 10°C, HMF had increased continously but there was no significant (P<0.05) difference. However, the HMF did not more than 40 mg/kg (Codex

Standard 12-1981). The increase of the HMF concentration might be to the diminution of the fructose content (Cervantes et a1., 2000), temperature and time of heating (Karabournioti and Zervalaki, 2001; Fallico et a1., 2003 and White et a1., 1964), storage conditions, use of metallic containers (Fallico et a1., 2003) and chemical properties of honey, which are attributed to the floral source from where the honey has been extracted. However, no information on the correlation between chemical characteristics and HMF level of honey is available (Fallico et a1., 2003).

At temperature 10^oC and 25^oC, during the first 6 weeks, DN increased but there was no significant ($P<0.05$) difference from 2 weeks (6.27 mg/kg, 6.43 mg/kg) to 6 weeks (6.41 mg/kg, 6.41 mg/kg). It decreased (5.54 mg/kg, 5.29 mg/kg) after storage for 8 weeks. However, the DN did not below the Codex Standard 12-1981 (not less than 3 G). Similar trend was noticed by White et al., (1964). Sancho et al., (1992) reported depletion in diastase number after studied the effect of storage for two years at 20°C. Cervantes et al., (2000) also reported decrease in diastase number after heating the honey at 55°C and then storing it for three and half months at 26°C. Castro-Vazquez et al., (2008) also reported that diastase number was out of the legal limit in citrus honey stored for 12 months at 40ºC.

At temperature 10^oC and 25^oC, during 8 weeks, there was no significant ($P<0.05$) difference in water content.

During 8 weeks storage, as shown in Figure 2e for the color function ΔE after an initial induction period, it followed a period of increase of color with time (2.55, 3.76, 6.21, 6.85 at 25^oC and 2.22, 2.98, 3.64, 4.58 at 10^oC). This behavior was observed in many products subjected to non-enzymatic browning (Song et al., 1966; Labuza et al., 1980). During shipping to far countries and/or dring storage, darkening of honey may occur, and paralellel changes in its organoleptic properties have detrimental effects on its quality, masking its original aroma, which promotes loss of competitiveness in the world market (Milum, 1939).

4. CONCLUSION

The results showed that the physico-chemical characteristics of cajuput honey was as follows: water content 23.18%, RS 717.42 mg/g, HMF 4.24 mg/kg, DN 4.85 mg/kg, colour parameters L*a*b* 39.51-10.51-31.81.

Thermal processing applied to cajuput honey did not effect on RS, water content and colour, while HMF and DN affected significantly but still satisfied the limits of Codex Standard 12-1981. The storage time and temperature affected significantly on colour ($\Delta E > 3$, large difference).

5. REFERENCES

1. Bogdanov S. and Martin P. *Swiss Bee Reaserch Centre* 2002; 1-20.
2. Tosi E., Ciappini M., Ré E. and Lucero H. *Food Chemistry* 2002; 77: 71-74.
3. Tosi E., Re E., Lucero H. and Bulacio L. *LWT – Food Science and Technology* 2004; 37(6): 669-678.
4. Kretavhičius J., Kurtinaitiene B., Racys J. and Ceksteryit V. *Žemdirbyste Agriculture* 2010; 97: 115-122.
5. Codex Standard for honey 12-1981
6. Thrasyvoulou A., Manikis J. and Tselios D. *Apidologie* 1994; 25: 297-302.
7. Spano N., Casula L., Panzanelli A., Pilo M. I., Piu P. C. and Scanu R. *Italy* 2006; 68: 1390-1395.
8. Miller. *Analytical Chemistry* 1959; 31(3).
9. Fallico B., Zappala M., Arena E. and Verzea A. *Food Chem.* 2003; 85: 305-313.
10. Serrano S., Villarejo M., Espejo R. and Jordal M. *Food Chem.* 2004; 87: 619-625.
11. Perez C., Conchello P., Arino A., Juan T. and Herresa A. *Food Chem.* 1994; 51: 207-210.
12. Sancho M. T., Muniategui S., Huidobro J. F. and Lozano S. *J. Agric. Food chem.* 1992; 4: 134-138.
13. Wootten M., Edwards R. A., Faraji-Haremi R. and Johnson A. T. *Journal of Apicultural Research* 1976; 15: 23-28.

14. White J. W. *Advances in Food Research* 1978; 24: 287-371.
15. Cervantes M. A. R., Novelo-Gonzalez S. A. and Duch-Sauri E. *Apiacta* 2000; 35: 162-170.
16. Karabournioti S. and Zervalaki P. *Apiacta* 2001; 36(4): 177-181.
17. White J. W., Kushnir I. and Subers M. H. *Food Technology* 1964; 18(4): 153-156.
18. Castro-Vazquez L., Diaz-Maroto M. C., Gonzalez-Vinas M. A., De La Fuente E. and Perez-Coello M. S. *J. Agric. Food Chem.* 2008; 6: 1999-2006.
19. Song P. S. and Chichester C. O. *Journal of Food Sciences* 1966; 31: 914-919.
20. Labuza T. P. and Saltmarch M. *In L.B. Rockland, and G.F. Steward, Properties of water related to food quality and stability, Academic Press: San Francisco*. 1980; 605-650.
21. Milum V. G. *American Bee Journal* 1939; 17: 390-392.

SALT REDUCTION IN FRIED POTATO STICKS BY THE ADDITION OF SZECHUAN PEPPER

Tram Le Bao Hong [a] *and Yaowapa Lorjaroenphon*[a,*]
[a]Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University
Bangkok, 10900, Thailand
Email address: **tramhonglebao@gmail.com** and *fagiypl@ku.ac.th (corresponding author)

Introduction

The overconsumption of salt has been globally considered as a cause of hypertension and cardiovascular diseases[1,2,3]. However, salt reduction often negatively affects sensory quality followed by the economic profit loss due to the important functions of salt in various food aspects. The addition of some herbs and spices has been reported to compensate these limitations because of their exotic flavors. Szechuan pepper (*Zanthoxylum simulans*) (Figure 1) is well-known for its sensational and medicinal characteristics described as tingling parethesia, numbing, pungent or mild electric shock[4,5,6]. It is commonly added into entrées and soups for seasoning as dried powder, sauce or pickle and cognitively thought to make foods taste saltier. Rubemamine, rubescenamide and zanthosinamide in *Zanthoxylum* family were patented to potentiate tastes in the mixture of umami, kokumi and salty[7]. The combination between rubemamine and monosodium glutamate also resulted in the higher saltiness intensity of beef extract[8]. However, evaluationof saltiness contribution of Szechuan pepper in food medium is scant. In this study, the salty taste boosting property of Szechuan pepper and its capacity to apply in fried potato sticks were evaluated by sensory panel through ranking and different-from-control tests. It would reveal whether Szechuan pepper could support the reduction of salt in term of maintaining saltiness.

Figure 1. Szechuan pepper (seeds, stems and hulks)

Materials and Methods

Szechuan pepper was purchased from local markets (Bangkok, Thailand) and grounded. Frozen potato sticks were obtained from supermarket,deep-fried in vegetable oil and seasoned with salt and/or Szechuan pepper. Candidates participated in the sensory screening test including taste identification and ranking intensity of 5 basic tastes with different concentrations followed the method reported by Meilgaard *et al.* (2007)[9]. The solutions were sugar, salt (NaCl), caffeine, citric acid, and monosodium glutamate. Thirty four panelists (14 males and 20 females, between 22 to 34 years old) were chosen based on their health, availability and ability to identify the taste and rank same taste solutions according to their intensity.

In the actual testing process, panel attended a ranking test[9]. Grounded Szechuan pepper was added in drinking water (1 g/100 mL). This mixture was passed through filter paper to collect the homogeneous

clear solution of 1% Szechuan pepper. The same manner was applied with salt (1 g/100 mL) and the combination between salt and pepper (1 g each/100 mL). Then, these 3 filtered solutions (1% grounded Szechuan pepper, 1% salt, and the combination of 1% salt and 1% pepper and water) were served to the panel in 2-oz capped white disposable plastic cups labelled with 3-digit randomized codes. The panel worked in individual booths and was asked to use the nose clip to prevent the physicochemical interaction between taste and aroma[10]. Panel applied the palate cleaning process including first with drinking water, white bread and then drinking water again before starting the evaluation and between the samples. They ranked the saltiness intensity of these samples in which lowest intensity among samples was rank 1 and the next lowest was rank 2. There was a 2-min forced break between samples.

There was 28 panelists (12 males and 16 females, between 22 to 34 years old) taking the different-from-control test[9,11,12]. Combination between 1% grounded pepper and 1% salt is the common recipe recommended in well-known cook book[13]. Therefore, the 10, 20, and 30% salt reduction of fried potato sticks were prepared by seasoning with 1% grounded Szechuan pepper plus 0.9, 0.8, or 0.7% salt, respectively. The control was fried potato sticks with 1% salt. Blind control, which was the same as control, was added to measure panel variance during testing. Ten grams of these 5 samples were prepared in 6-oz capped white disposable plastic cup. The control was indicated while 3 salt-reduced samples and blind control were 3-digit randomized coded. Each panel was presented a control plus a test sample or blind control in a section assuring that after 4 sections they could taste all the test samples. They were also informed that they might meet the 2 controls in the same time. Panel was asked to rate the size of the saltiness intensity difference between the control and the other ones in a section using a 10-point scale (0 = none, 9 = extreme difference). The same manner of cleaning and testing procedures as mentioned before were applied. The analysis was carried out using SPSS program (IBM, version 16).

Results and discussion

There was a statistically significant difference in saltiness intensities among samples tested. The result (Table 1) showed that the intensity of the solution contained both salt and pepper was ranked at the most salty followed by salt solution, pepper solution, and water. Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $p<0.0125$. There was significant differences between saltiness intensity of pepper solution and water; and the combination salt-pepper and salt solution ($p<0.0125$). It might imply the effect of Szechuan pepper on the increase of saltiness intdensity.

Solution	Mean	Rank
Water	1.26 ± 0.51^{a}	2
Szechuan pepper	1.91 ± 0.62^{b}	1
Salt	3.03 ± 0.63^{c}	3
Salt and Szechuan pepper	3.79 ± 0.41^{d}	4

*Mean ± standard deviation followed by different letter was significantly different ($p<0.0125$).

Table 1. *Ranking of water, filtered solutions of Szechuan pepper, salt and combination between salt and Szechuan pepper for saltiness intensity.*

To evaluate the effectiveness of pepper addition for salt reduction on food media, potato sticks were used for the next experiment. The difference-from-control test provided a time-saving and cost-effective way of making 4 comparisons in one set of samples and also the estimation of the possible discrepancy. The statistical results were shown in Table 2. A Dunnett's test for multiple comparison with a control revealed that the saltiness perception of 30% salt reduced potato sticks was significantly different from those of control ($p<0.05$). Panel was unable to detect the changes in the 10 and 20 % salt reduction. This result indicated that the application of 1% Szechuan pepper was successful in at least 20% salt reduction to maintain the saltiness perception of fried potato sticks. It was also in agreement with reports about

successful salt reduction in other food media at 20-25% level[14]. The attempt to achieve 30% salt reduction usually has to combine with the other salt replacers to cover the loss of salt appetite[15]. The mean different between the control and blind control samples (5.71) probably resulted from panel who probably was unable to recognize the blind control and tried to give a numerical difference. Panels assumed that they had to provide different scores since it was a difference test, even though there was no actual difference existed.

Sample	blind control	10% salt reduction	20% salt reduction	30% salt reduction
Mean response	5.71 ± 0.98^{a}	5.07 ± 1.51^{a}	5.11 ± 1.71^{a}	4.79 ± 1.37^{b}

*Using a 10-point scale (0 = none, 9 = extreme difference) (n = 28).
**Mean ± standard deviation followed by different letter within a row was significantly different ($p<0.05$).
Table 2. *Mean level of difference from control test for saltiness intensity of potato sticks with Szechuan pepper and different levels of salt reduction.*

Although there was no reported compound in Szechuan pepper that interacted with saltiness receptor to alter taste signal, the addition of pepper contributed a great impact on saltiness. This incident propably was explained by several reasons. First, there were reports about umami capacity to enhance the saltiness in salt-reduction dishes[16,17,18]. The umami tasting components then should be put into consideration as their presence were detected[19] in Szechuan pepper family. Moreover, flavor intensities of food samples were reported to be significantly increased with the addition of Szechuan powder indicating Szechuan pepper possesses the flavor enhancement characteristics[20]. Since flavor is the combination between taste, smell and trigeminal sensation[21], saltiness perception might be positively affected by flavor increase.

Conclusion

Szechuan pepper in the combination with salt as seasoning agent could provide a higher saltiness intensity of fried potato sticks in comparison with the common ones. The 20% of salt reduction in potato sticks could be achieved in term of saltiness perception by the addition of Szechuan pepper. Szechuan pepper, thus, could be considered as useful for lowering salt added in food product. This outcome may be used to reduce salt consumption resulting in a lower risk of sodium-induced diseases.

Acknowledgement

This research was supported by the Kasetsart University Research and Development Institute (KURDI) under grant number 70.59 and by the Graduate Program Scholarship from the Graduate School, Kasetsart University. The first author also received the scholarship from the Department of Food Science and Technology, the Faculty of Agro-Industry.

References

[1] de Wardener, H. E., He, F. J., MacGregor, G. A. *Kidney International* 2004; 66(6): 2454-2466.

[2] Desmond, E. *Meat Science* 2006; 74(1): 188-196.

[3] Ruusunen, M., Puolanne, E. *Meat Science* 2005; 70(3): 531-541.

[4] Bautista, D. M., Sigal, Y. M., Milstein, A. D., Garrison, J. L., Zorn, J. A., Tsuruda, P. R., Nicoll, R. A., Julius, D. *Nature Neuroscience* 2008; 11(7): 772-779.

[5] Bryant, B. P., Mezine, I. *Brain Research* 1999; 842(2): 452-460.

[6] Sugai, E., Morimitsu, Y., Iwasaki, Y., Morita, A., Watanabe, T., Kubota, K. *Bioscience, Biotechnology, and Biochemistry* 2005; 69(10): 1951-1957.

[7] Ley, J. P., Reichelt, K., Paetz, S., Backes, M., Obst, K. *U.S. Patent 20120308703*; 2012.

[8] Backes, M., Obst, K., Bojahr, J., Thorhauer, A., Roudnitzky, N., Paetz, S., Reichelt, K. V., Krammer, G. E., Meyerhof, W., Ley, J. P. *Journal of Agricultural and Food Chemistry* 2015; 63(39): 8694-8704.

[9] Meilgaard, M. C., Carr, B. T. & G.V. Civille. Sensory evaluation techniques. (4th ed). Florida: Taylor and Francis Group 2007.

[10] Rozin, P. *Perception and Psychophysics* 1982, 31(4): 397-401.

[11] Muñoz, A. M, Civille, G. V. & Carr, B. T. Sensory evaluation in quality control. New York: Van Nostrand Reinhold 1992.

[12] Stone, H. & Sidel, J. L.. Sensory evaluation practices. (2nd ed). California: Academic Press 1993.

[13] Dunlop, F. Every grain of rice: simple Chinese home cooking. (1st ed). Bloomsbury: W. W. Norton & Company 2013.

[14] Poinot, P., Arvisenet, G., Texier, F., Lethuaut, L., Mehinagic, E., Vigneau, E., Prost, C. *Food Quality and Preference* 2011, 22(6): 573-580.

[15] Roininen, K., Lähteenmäki, L., Tuorilla, H. *Physiology & Behavior* 1996, 60(3): 953-958.

[16] Lundahl, D. S., Lukes, B. K., McDaniel, M. R., Henderson, L. A. *Journal of Sensory Studies* 1986, 1(3-4): 291-306.

[17] Mojet, J., Heidema J., Christ-Hazelhof, E. *Chemical Senses* 2004, 29(8): 671-681.

[18] Kremer, S., Mojet, J., Shimojo, R. *Journal of Food Science* 2009, 74(6): S255-S262.

[19] Frerot, E., Neirynck, N., Cayeux, I., Yuan, Y. H. J., Yuan Y.M. *Journal of Agricultural and Food Chemistry* 2015, 63(32): 7161-7168.

[20] Hong, T. L. B and Y. Lorjaroenphon. 2016. *In* Proceedings of the 2016 International Conference on Food Properties. 31st May – 2nd June, 2016, Bangkok, Thailand.

[21] Keast, R.S.J., Dalton, P.H. & Breslin, P. A. S. Flavour Perception (1st eds.). Oxford: Blackwell Publishing Ltd. 2004, (Chapter 8).

Changes in alpha–galactosidase activity and oligosaccharides during germination of soybean seeds

***Duong Thi Phuong Lien*[1]**, Phan Thi Bich Tram[1], Trang Sy Trung[2] and Ha Thanh Toan[3]

[1]College of Agriculture and Applied Biology, Cantho University, Vietnam
[2]Faculty of Food Technology, Nha Trang University, Vietnam
[3]Biotechnology Research and Development Institute, Cantho University, Vietnam

1. Introduction

Soybean (*Glycine Max.* (L.) Merril) is an excellent source of proteins for human consumption. However, direct utilization of soybeans is undesirable due to its undigestible oligosaccharides content which may induce flatulence in humanity[1-3]. The undigestible oligosaccharides in soybean seeds are a group of soluble low molecular weight, mainly raffinose [α–D–galactopyranosyl–(1,6)–α–D–glucopyranosyl–β–D–fructofuranoside] and stachyose [α–D–galactopyranosyl–(1,6)–α–D–galactopyranosyl–(1,6)–α–D–glucopyranosyl–β–D–fructofuranoside][2,4]. Since humans gastrointestinal tract lack the enzyme α–galactosidases (E.C. 3.2.1.22, α–D–galactoside galactohydrolase) necessary for hydrolysis of the α–1,6 linkages present in oligosaccharides[1], these components pass intact into the large intestine where the fermentation of anaerobic microorganisms occurs then causing flatulence[4].

Various processing methods can reduce the flatulence of soybeans induced seed germination[3,5], cooking[6,7], ethanol and aqueous extraction[8,9] and fermentation[10,11]. Among them, germination is a relatively simple and unexpensive one that produces a natural type of food[12]. Germination causes marked metabolic changes in the seeds resulted in the susceptibly degratation of carbohydrates[13,14]. The α–galactosidases were suspected to play an important role in the early stages of germination by hydrolyzing galactose-containing oligosaccharides to provide metabolites for the developing seedling[15,16], and thereby the oligosaccharides content was reduced.

This research studied the changes in α–galactosidase activity and oligosaccharide content during the germination of soybean seeds to provide practical recommendations for reducing the flatus activity in soybeans.

2. Materials and methods

2.1 Chemicals

The standard raffinose, stachyose and sucrose were purchased from Merck Company (Germany). The synthetic substrate ρNPGal was from Sigma-Aldrich.

2.2 Soybeans and germination process

Soybeans (*Glycine max* L., MTD 760 variety) were supplied from Department of Genetics and Plant Breeding, College of Agricultural and Applied Biology, Cantho University. Soybeans were cleaned and rinsed with cleaned water before being soaked for 12 hours to reach the equilibrium moisture content at ambient temperature. Soaking process was carried out in drinkable water with the ratio of soybean seeds and water as 1: 5 and the concentration of gibberellic acid in soaking water as 1 mg/L. The soaked beans were drained, rinsed and placed in a germination chamber in dark condition. Watering the seeds was set up two minutes for every 4 hours with cleaned water automatically. The germination process was carried out at the 25^{o}C for 0, 12, 24, 36, 48, 60 and 72 hours. α–galactosidases activities were determined in fresh germinated soybeans and the freeze dried samples were used for analyzing the oligosaccharides and reducing sugar contents.

2.3 Determination of oligosaccharides by thin – layer chromatography (TLC)

Extraction of oligosaccharides: One gram of sample was extracted with 10mL of 70% aqueous ethanol and kept on orbital shaker at 130 rpm for 16 hr. The contents of the flask were filtered through Whatman No.1 filter paper and the residue was further washed with 5mL of 70% ethanol. The filtrate was evaporated in a rotary vacuum evaporator at 45ºC. The concentrated sugar syrup was dissolved in 2 mL of distilled water[17].

TLC of oligosaccharides: Assay of oligosaccharides by TLC was carried out following the method of Tanaka (1975)[18] Tajoddin (2010)[17], with some modifications. A 6 µL of the syrup was applied on chromatographic plates (20 cm × 20 cm) coated with microcrystalline cellulose powder. Spotting of the sugar samples was done by using capillary tubes. Each sample was spotted thrice separately (2 µL for each spotting) and dried. Plates were developed using solvent system n-propanol:ethylacetate:water (6:1:3). The developed plates were sprayed with 1% α-naphthol in alcohol 95% to locate the sugars spots. For quantitative determination the amounts of sucrose, raffinose and stachyose, a sugar spot on a chromatogram was scraped off and the sugar was extracted with 4 ml of distilled water in a test tube overnight at room temperature. One ml of eluent was mixed with 1 ml of 0.02M thiobarbituric acid and 1 ml of concentrated hydrochloric acid. The mixture was heated in a boiling water bath for exactly 6 min, then cooled under running water[19]. The yellow color produced was read at 432.5 nm. The concentration of sugar was calculated from working standards.

2.4 Determination of reducing sugar

The estimation of the reducing water was carried out by DNS reagent (2% NaOH and 20% sodium potassium tartrate). In the estimation, the sample (1 g) was extracted with hot water (70–80^{o}C), filtering and mixing with DNS reagent in a boiling water bath for 10 min. Color intensity was measured at 540 nm and using a standard curve of glucose[20]. The results were expressed as percent in dry weight (%, db).

2.5 Analysis of α–galactosidases activity

A modified procedure of Guimarães (2001) was used to determine α–galactosidase using ρNPGal as substrate. The sample (0.1 g) with 1 mL of citrate buffer 0.1M (pH 5.5) was shaken for one hour, at 20ºC. After centrifugation with the rate of 13,000 rpm, at 4^{o}C for 40 min, the supernatant were used directly for enzyme assay[4]. The enzyme was assayed using a reaction system (1 ml final volume) containing 350µl of 0.1 M sodium acetate buffer (pH 5), 100µl of enzyme preparation and 250µl of 2mM ρNPGal in phosphate butter (pH 4.5). Reaction was conducted for 15 min at 37^{o}C and stopped by the addition of 1 ml of 0.5 M sodium carbonate. The yellow color produced was measured at wavelength of 420nm. Control sample involved adding enzyme extracts after the stop solution had been added. The control was used as the zero calibration reading. The molar extinction coefficient for ρ–nitrophenol was taken as 18,400[21] to calculate the amount of ρ–nitrophenol released. A unit of enzyme activity (U) was defined as the amount of α-galactosidase which liberates 1 µmol of ρ–nitrophenol per min under the given assay conditions[22]. The results were as U/100 g sample on dry weight.

2.6 Data analysis

All data were performed thrice and were submitted to analysis of variance (ANOVA) by Portable Statgraphics Centurion 15.2.11.0. The regression analysis was carried out by Microsoft Excel 2007.

3. Results and discussion

Figure 1 showed the TLC results depicting the separation pattern of standard sugars and oligosaccharides of soybean seed and germinated soybeans. The identity of sucrose, raffinose and stachyose in soybean samples was confirmed by spots of the corresponding standard sugars (Figure 1.A). The spots in Figure 1.B are the oligosaccharides of soybean samples. The change of the contents of raffinose, stachyose, sucrose and reducing sugars of soybeans after soaking and during germination were shown in Table 1. There is little doubt that α–galactosidase in seeds involved in the hydrolysis galactose-containing

oligosaccharides leading to the change in oligosaccharide content[15,16]. So, the activity of α–galactosidase in soybean seeds and germinated soybeans were determined and also shown in Table 1.

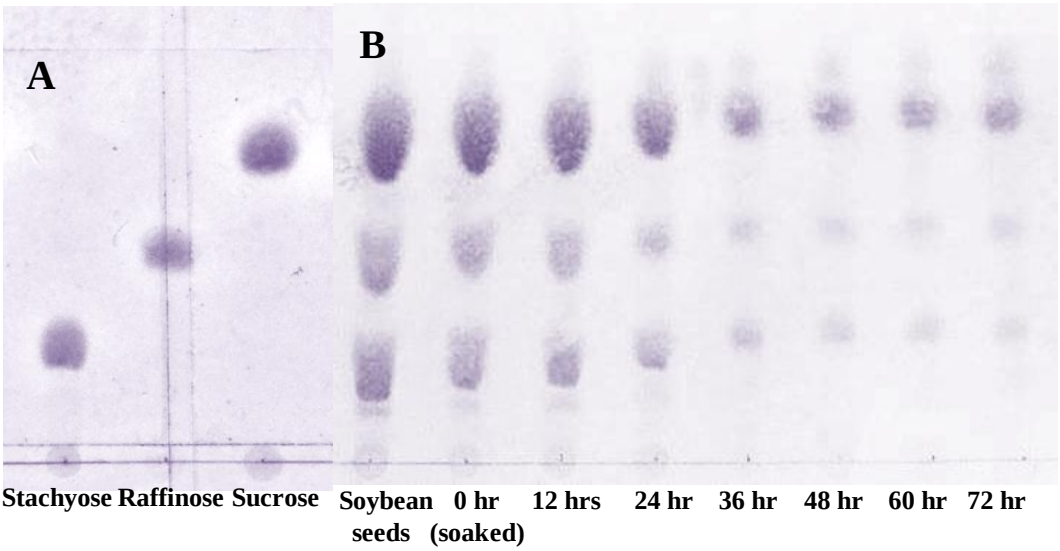


Figure 1. TLC analysis of standard oligosaccharides (A) and soybean samples (B)

Table 1 showed the raffinose and stachyose level in the soybeans were 1.05±0.07 and 4.75±0.09 %, respectively. This is in agreement with the previous study which reported the raffinose and stachyose contents were 0.99 and 4.87% in soybean seeds[23]. The sucrose content of soybean seeds was 5.96±0.08% (Table 1) and was in range of 2.69–8.63% that resulted from the study on the sucrose content of 14 soybean genotypes[24]. Other authors found the ranges of raffinose, stachyose and sucrose of three soybean cultivars were 0.75–1.16, 3.28–4.09 and 3.97–4.81%, respectively[25].

Table 1. Changes in sugars contents and α-galactosidase activity during germination of soybeans

Germination time (hours)	Raffinose (%)	Stachyose (%)	Sucrose (%)	Reducing sugar (%)	α-galactosidase activity (U/100 g)
Soybeans	1.05±0.07^{a}	4.75±0.09^{a}	5.96±0.08^{a}	2.50±0.18^{f}	47.77±1.1^{f}
0 (soaked)	0.95±0.02^{b}	4.45±0.09^{b}	5.79±0.08^{a}	2.41±0.18^{f}	87.92±1.1^{bc}
12	0.86±0.02^{c}	3.78±0.04^{c}	5.77±0.06^{a}	3.14±0.09^{e}	164.29±2.5^{a}
24	0.66±0.02^{d}	3.14±0.03^{d}	5.16±0.07^{b}	3.45±0.10^{e}	93.84±7.1^{b}
36	0.44±0.02^{e}	2.53±0.02^{e}	2.67±0.09^{c}	5.93±0.17^{c}	84.17±2.1^{cd}
48	0.34±0.02^{f}	1.99±0.01^{f}	2.47±0.06^{c}	6.32±0.13^{b}	85.19±6.3^{cd}
60	0.27±0.01^{g}	1.55±0.04^{g}	2.35±0.07^{c}	7.88±0.14^{a}	74.49±4.4^{e}
72	0.21±0.01^{g}	1.15±0.02^{h}	2.34±0.04^{c}	5.22±0.14^{d}	78.09±2.8^{de}

Values represent the means ± standard deviation, (with n=3). Values in a column with different superscripts were significantly different ($p < 0.05$).

The activity of α-galactosidase was detected in the seeds even before imbibition, suggesting that this enzyme is pre-existing and present in the ripe seeds[26,27]. In present study, the α-galactosidase activity was 47.77±1.1 U/100g. The α-galactosidase activity was found 36 U/100g in soybean seeds[28]. Soaking may activate α–galactosidase, leading to the breakdown of the oligosaccharides[29]. Indeed, after soaking α–galactosidase activity increased 1.84 times compared to that in the soybean seeds (Table 1). From the spots on TLC, it is evident the raffinose and stachyose contents decreased after soaking. The percent loss

of raffinose and stachyose after soaking were 9.2 and 6.4%, respectively. The losses of raffinose and stachyose in the soaking water were represented 3.32 and 0.37% of these contents in starting soybeans[2]. However, according to other result, up to 56.3% of oligosaccharides in soybeans was removed by soaking in water for 12 hours[30]. Significant reduction of oligosaccharides by soaking has also been reported in cowpea, mung bean, chickpeas, yellow peas, green peas and soybeans [31,32,30]. Another reason for the reduction in oligosaccharide content after soaking of soybeans is probably due to leaching of oligosaccharides into soaking water. When soybean is soaked, the water is absorbed into the bean, the oligosaccharides dissolved and leach into the surrounding water [30,31].

The statistical results from Table 1 and the spots in Figure 1B demonstrated that raffinose and stachyose contents of soybeans significantly decreased ($p < 0.05$) during germination. As the period of germination was prolonged, significant and successive reduction in oligosaccharides was observed. Highest loss of raffinose and stachyose were observed in soybeans respectively after 72 hours of germination, which were corresponding to 79.59 and 75.83%. Diminishing effect of germination on raffinose and stachyose have been noticed for black gram, mung bean, cowpeas and other legume seeds[1,17,3,7]. During germination, raffinose and stachyose levels decreased in all three soybean cultivars, and it was possible to measure small amounts of these oligosaccharides after six day of germination[25]. In other studies conducted soybean germination, raffinose was concluded to have disappeared on the fourth[33] and on the third day[34], and stachyose was found to have disappeared on the sixth[33] and on the fourth day[34]. The wide variance in findings may be due to differences in type of bean, conditions of germination or activity of enzymes, particularly α-galactosidase[23]. The increasing in α-galactosidase activity in soybeans during early stage of germination in the present study was able to explain the degradation of undigestible oligosaccharides. The highest activity of α-galactosidase in germinated soybeans was 164.3±2.5 U/100g corresponding to the 12 hour germination. After that, it reduced significantly, however, the activity of α-galactosidase in soybeans after 72 hours of germination was still significant higher than that of soybean seeds (Table 1). The observed rule of α-galactosidase activity change during germination is similar to that of cowpeas[31], rosewood seeds[35] and black gram seeds[1]. The α-galactosidase catalyzed the hydrolytic removal of α–1,6–linked–galactose residues from simple oligosaccharides including stachyose and raffinose forming sucrose that can be further hydrolyzed to obtain glucose and fructose[36]. The breakdown of oligosaccharides by active α-galactosidases in seeds took place during germination and even soaking stage before germination[37,17]. The above mechanism of reaction and the rule of α-galactosidases activity change explained why the sucrose content remained high and unchanged after soaking and in the early stages of germination (12 hours from beginning), after that the sucrose content decreased significantly (Table 1 and Figure 1B). Sucrose content in barley was unchanged during first 2 day of germination, but it diminishes markedly after 3 day[38]. Some authors reported an increase in sucrose content during the first stage of germination in black gram[1] and mung beans[17]. Alani *et al.* (1990) stated that sucrose content in cowpeas declined during the first 6 hours of germination, then increased after 24 hours of germination[3]. The alteration in sucrose content during germination was the result from the following events: the hydrolysis of raffinose and stachyose forming sucrose by α-galactosidase enzyme; the hydrolysis further of sucrose forming glucose and fructose used as energy-source for the projection of the radicles and promote development of seedlings[39]. In the first stage, the α–galactosidase activity and content of oligosaccharides were high, so the hydrolysis of oligosaccharides to form sucrose predominated that resulted in high content of sucrose. After this stage, oligosaccharides content decreased the hydrolysis of sucrose to form glucose and fructose became remarkable. The hydrolysis of oligosaccharides as well as sucrose led to increase in reducing sugars during germination. The reducing sugars content in soybeans was highest after 72 hours of germination, which increased 2.1 times compared to that of soybean seeds (Table 1). This was similar to results reported for fenugreek seeds during 96 hours of germination[40].

Germination reduced undigestible oligosaccharides contents in soybeans and there is a positive correlation between the decline in oligosaccharides contents and the depression of seed longevity[41]. Evaluation the

degradation efficiency of undigestible oligosaccharides during germination is important not only the extent of the changes their contents, but also the rate at which they occur. These changes can be described by the application of the following zero order (Eq. I), first order (Eq. II) or second order (Eq. III) equations[42].

$$C = C_o - kt \quad (I)$$

$$C = C_o \exp(-kt) \quad (II)$$

$$1/C = 1/C_o + kt \quad (III)$$

where: C_o– initial content; t – germination time (min); k – reaction rate constant (per min) and C – calculated content.

Table 2 showed the correlation coefficients (R^2) calculated using all equations. In case of raffinose, the best approximation (R^2 = 0.97) were achieved using Eq. II, and the fitted models used for estimation stachyose content were Eq. 1 and Eq. 2 (R^2 = 0.99). The changes in total oligosaccharides content with germination time for mung bean and lentil were simulated by Eq. I (R^2 = 0.96 and 0.95, respectively) and for chickpea by Eq. II (R^2 = 0.97)[42]. In the present study, there was not any model could be stimulated the changes in sucrose and reducing sugar contents as well as α–galactosidase activity with germination time. Beside, the was not relationship between α–galactosidase activity and oligosaccharides contents.

Table 2. Correlation coefficients calculated for the equations applied to oligosaccharides degradation during soybean germination

Raffinose			Stachyose		
Eq.	Models	R^2	Eq.	Models	R^2
(I)	C = 0.93434 – 0.01123 × t	0.95	(I)	C = 4.312 – 0.0462 × t	0.99
(II)	C = 1.04 exp(–0.02284 × t)	0.97	(II)	C = 4.74 exp(–0.0188 × t)	0.99
(III)	1/C = 0.585 + 0.0543 × t	0.93	(III)	1/C = 0.151 + 0.0086 × t	0.92

4. Conclusion

Germination causes significant decreases in undigestible oligosaccharides. This desirable change is mostly due to the action of α–galactosidase in soybean seeds which was activated during soaking and germinating. The hydrolysis of undigestible oligosaccharides leading to increase in reducing sugars that was used as energy-source for the development of seedlings. Reduction of these undesirable components by germination promises promising methods for reducing both flatus-causing factors and antinutrients in soybeans. Germination is one of the most promising methods for reducing flatus-causing factors and improving nutritional value for soybeans.

Acknowledgements

We are thankful to VLIR – NETWORK Project for the partly financial support to this research.

References

[1]. Reddy N., Salunkhe D. *Cereal Chemistry* 1980, 57: 356-360.
[2]. Wang Q., Ke L., Yang D., Bao B., Jiang J., Ying T. *Asia Pacific journal of clinical nutrition* 2007, 16: 89-94.
[3]. Alani S., Smith D., Markakis P. *Food Chemistry* 1990, 38: 153-158.

[4]. Guimarães V. M., de Rezende S. T., Moreira M. A., de Barros E. G., Felix C. R. *Phytochemistry* 2001, 58: 67-73.
[5]. Markakis P. *Journal of Food Science* 1987, 52: 1736-1737.
[6]. Ramadan E. *Food and Public Health* 2012, 2: 11-15.
[7]. Oboh H., Muzquiz M., Burbano C., Cuadrado C., Pedrosa M., Ayet G., Osagie A. *Plant Foods for Human Nutrition* 2000, 55: 97-110.
[8]. Rackis J. J., Honig D., Sessa D. J., Steggerda F. R. *Journal of Agricultural and Food Chemistry* 1970.
[9]. Omosaiye O., Cheryan M., Matthews M. *Journal of Food Science* 1978, 43: 354-360.
[10]. Egounlety M., Aworh O. *Journal of Food Engineering* 2003, 56: 249-254.
[11]. Akinyele I., Akinlosotu A. *Food Chemistry* 1991, 41: 43-53.
[12]. Shah S. A., Zeb A., Masood T., Noreen N., Abbas S. J., Samiullah M., Alim M. A., Muhammad A. *African Journal of Agricultural Research* 2011, 6: 5091-5098.
[13]. Nnanna I., Phillips R. *Journal of Food Science* 1988, 53: 1782-1786.
[14]. Rackis J. *Journal of the American Oil Chemists' Society* 1981, 58: 503-509.
[15]. Dey P. M., Naik S., Pridham J. B. *Phytochemistry* 1986, 25: 1035-1041.
[16]. Dey P. M., Campillo E. D. *Advances in Enzymology and Related Areas of Molecular Biology* 2006, 56: 141-249.
[17]. Tajoddin M., Shinde M., Junna L. *The Bioscar* 2010, 5: 343-346.
[18]. Tanaka M., Thananunkul D., Lee T. C., Chichester C. *Journal of Food Science* 1975, 40: 1087-1088.
[19]. Percheron F. *Comptes Rendus de l'Academie des Science* 1962, 255: 2521-2522.
[20]. Mao C., He G., Du X., Cui M., Gao S. *Advance Journal of Food Science and Technology* 2013, 5: 144-147.
[21]. Reid J. G., Meier H. *Planta* 1973, 112: 301-308.
[22]. Rezende S. T. d., Guimarães V. M., Rodrigues M. d. C., Felix C. R. *Brazilian Archives of Biology and Technology* 2005, 48: 195-202.
[23]. Abdullah A., Baldwin R. E., Minor H. *Journal of Food Protection* 1984, 47: 441-444.
[24]. Teixeira A. I., Ribeiro L. F., Rezende S. T., Barros E. G., Moreira M. A. *Food Chemistry* 2012, 130: 1134-1136.
[25]. Silva H. C., Braga G. L., Bianchi M. d. L. P., Rossi E. A. *Alimentos e Nutrição Araraquara* 1990, 2: 13-19.
[26]. Ataíde G. d. M., Gonçalves J. F. d. C., Guimarães V. M., Bicalho E. M., Flores A. V. *Journal of Seed Science* 2013, 35: 92-98.
[27]. Lahuta L. B., Górecki R. J., Michalczyk D., Piotrowicz-Cieślak A. I. *Electron Journal Polish Agriculture University* 2000, 3: 1-7.
[28]. McCleary B., Matheson N. *Phytochemistry* 1974, 13: 1747-1757.
[29]. Rakshit M., Sharma A., Saha J., Sarkar P. K. *LWT-Food Science and Technology* 2015, 63: 814-820.
[30]. Han I. H., Baik B.-K. *Cereal Chemistry* 2006, 83: 428-433.
[31]. Agbenorhevi J., Oduro I., Ellis W., Abodakpi V., Eleblu S. *Nigerian Food Journal* 2007, 25: 88-94.
[32]. Tajoddin M., Shinde M., Junna L. *The Bioscar* 2010, 5: 343-346.
[33]. East J. W., Nakayama T., Parkman S. *Crop Science* 1972, 12: 7-9.
[34]. Chen A., Luh B. *Food Science* 1976, 3: 13-25.
[35]. Carrijo L. C., Borges E. E. d. L., Pontes C. A., Lopes M. R., Brune A. *Cerne* 2010, 16: 283-289.
[36]. Zhang W., Du F., Wang L., Zhao L., Wang H., Ng T. B. *International Journal of Molecular Sciences* 2015, 16: 29226-29235.
[37]. Blöchl A., Peterbauer T., Hofmann J., Richter A. *Planta* 2008, 228: 99-110.
[38]. MacLeod A. M., Travis D., Wreay D. *Journal of the Institute of Brewing* 1953, 59: 154-165.

[39]. Sett R. *International Journal of Plant & Soil Science* 2016, 12: 1-15.
[40]. El-Mahdy A. R., El-Sebaiy L. A. *Journal of the Science of Food and Agriculture* 1983, 34: 951-956.
[41]. Górecki R., Fordonski G., Halmajan H., Horbowicz M., Jones R., Lahuta L. Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics 2001, (Chapter 5).
[42]. Kadlec P., Dostalova J., Bernaskova J., Skulinova M. *Czech Journal of Food Sciences* 2008, 26: 99.

Optimization of THE POLYPhENOLICS Extraction from RED RICE BRAN by Response Surface Methodology

Phan Thi Bich Tram[1], Nguyen Tri Liem[2] and ***Duong Thi Phuong Lien[1]***

[1]College of Agriculture and Applied Biology, Cantho University, Vietnam
[2]High School of Ngo van Can, Ben Tre,Vietnam

1. Introduction

In many Asian countries, red rice (*Oryza sativa* L.) was considered to be a traditional staple crop[1]. Red rice has been considered as a nutritious food for weak people in traditional Chinese medicine[2] and was also gaining popularity as a functional crop owing to its high polyphenols content[3], anthocyanins[4] and other nutrition components[5]. Many authors found that the total phenolic content was four times higher in pigmented rice than that in non-pigmented varieties[6,7]. Red rice varieties showed higher total phenolics, flavonoids and antioxidant activity than those of white and brown varieties[8,9].

The 'colored rice' is related to the accumulation of phytochemical compounds that are pigment containing and generally accumulate in perdicarp, testa or bran of the rice kernel[10,11]. For this reason, rice bran is considered a rich source of phytochemicals including phenolic compounds which can be used as free radical scavengers[12,13].

For extraction of phenolic compounds in rice bran, the most commonly used solvents are methanol, ethanol and water acidified with acetic acid[14,9]. Ethanol and organic acids are expected because they are less toxic than methanol[15]. Other factors such as concentration and volume of solvent, numbers of extraction cycles, solid–liquid ratio, temperature and time have significant influence on the extraction process. The combination of these factors and the determination of optimal conditions are important in order to obtain a maximum yield of phenolics. From a practical standpoint, research using random single factor has been conducted[16] to extract phenolic compounds from materials. Factors that have significant influence on the yield should be optimized using of mathematical models that describe accurately the isolated and combination effects of different factors. Response surface methodology (RSM) is a powerful mathematical technique based on regression analysis used to develop and optimize products and processes that have two or more factors that influence the response[17-19]. It can be used to determine the best conditions for the extraction of chemical compounds from natural products[20,21]. Thus, this study was aimed at extracting and modeling, through RSM, the total phenolic content, of red rice and to determine antioxidant activity of red rice bran corresponding to the optimum extraction conditions.

2. Materials and methods

2.1 Red rice bran

Red rice (Huyet rong) variety was supplied from Vinh Hung district, Long An province, Vietnam. The crude rice bran was collected from Long An Rice processing factory after milling process. Crude rice bran was blown through the sieve for impurities and shred husks removing. Fine rice bran was dried at 45–50^{o}C in oder to reduce the bran moisture down to 10–12%. The rice bran oil was extracted using petroleum ether by the Soxhlet extraction. Defatted red rice bran samples were kept in PE bag and stored at 0–2^{o}C for analysis.

2.2 Experimental designs

Single factors of extration process such as ethanol concentration (20–80%), added acetic acid concentration (0–20%), time (0–6.5 hours), temperature (25–100^{o}C), number of extraction cycles (1–4) and solid– solvent ratio (1/4–1/10 w/v) were studied seriatim on TPC of red rice bran extracts. Three parameters, solvent concentration (48–68%), added acetic acid concentration (10–15%) and extraction time (120–240 min), were able to be optimized using the Boxe Behnken design (BBD) with a quadratic

regression model built by using RSM. The experiments were carried out according to 30 runs with 3 variables and 3 central points for the optimization the TPC extraction.

2.3 Determination of TPC

The TPC was estimated by Folin-Ciocalteu method[13]. The TPC of samples was expressed as milligrams garlic acid equivalents per 100 gram of dry matter (mg GAE/100g).

2.4 Determination of antioxidant activity

Antioxidant activity of the phytochemicals extracted from rice bran was assessed by measuring their radical scavenging activity that was measured by the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent. The DPPH radical scavenging activity was evaluated from the difference in peak area decrease of the DPPH radical detected at 517 nm between a blank and a sample[13]. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract (µg/ml) to obtain IC50 value in mg/ml.

2.5 Data analysis

The response variables were fitted to a general form of quadratic polynomial model that was showed in equation (1):

$$Y = \beta_o + \sum\beta_i x_i + \sum\beta_{ii} x_i^2 + \sum\beta_{ij} x_i x_j \qquad (1)$$

Where Y represents the predicted responses; β_o, β_i, β_{ii} and β_{ij} are the coefficients of intercept, linear, squared and interaction; x_i and x_j are the independent variables. Portable Statgraphics Centurion software was used to estimate the response of each set of experimental design and optimized conditions. The fit of the models was inspected by the regression coefficient R^2.

3. Results and discussion

3.1 Effect of single factors from extrection process on TPC content

Effect of ethanol concentration on TPC content of the extracts

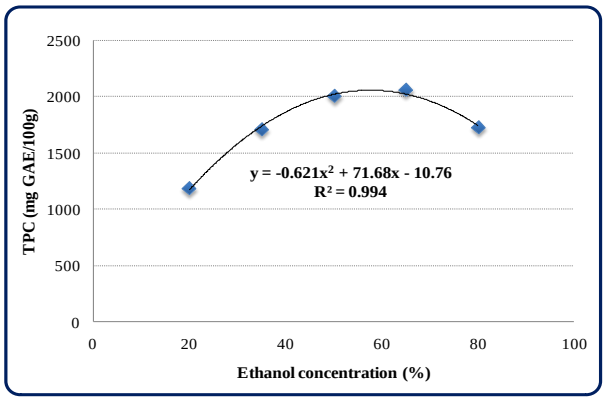


Figure 1: Effect of ethanol concentration on TPC

The relationship between ethanol concentration and TPC in the rice bran extracts was shown in Figure 1 and obeyed the quadratic equation (R^2 = 0.99) as following:

$$TPC = -0.621\ (EtOH)^2 + 71.68\ (EtOH) - 10.76$$

Where, (EtOH) is ethanol concentration (%).

The estimated maximum value of TPC was 2,061 mg GAE/100g corresponding to ethanol concentration of 57.8%. The effective range of ethanol concentration on TPC of the extract was 48–68%.

Effect of acetic acid concentration on TPC of the extracts

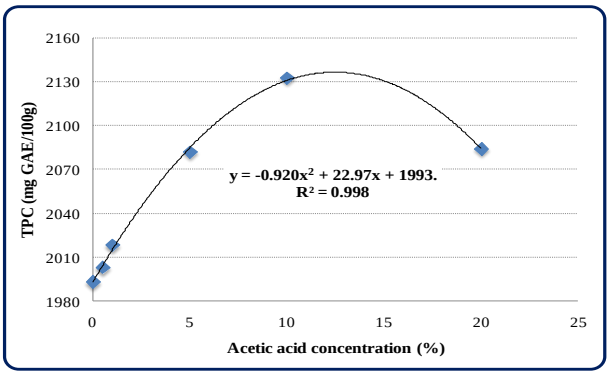


Figure 2: Effect of acetic acid concentration on TPC

Many authors reported that the pH variation could have a positive or a negative effect on extraction of flavonoids and polyphenols, depending on the interaction of the polyphenols with other constituents of each plant[22-24]. Acetic acid added to ethanol solution increased TPC of rice bran extract. The correlation of two variables was displayed in Figure 2 and followed quadratic equation (R^2=0.99):

$$TPC = -0.92\ (Ac.A)^2 + 22.97\ (Ac.A) + 1993$$

Where, (Ac.A) is acetic acid concentration (%).

The estimated maximum value of TPC was 2,136.4 mg GAE/100g corresponding to acetic acid concentration of 12.5%.

Effect of extraction time on TPC of the extracts

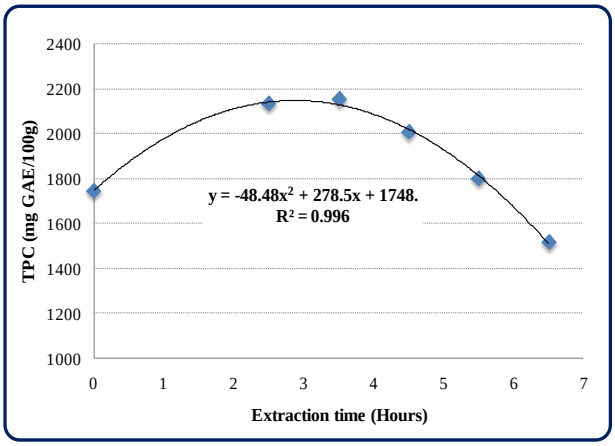


Figure 3: Effect of extraction time on TPC

Extraction time is one of important factors of extraction process[16]. The extraction time effected markedly on TPC of rice bran extracts and the correlation of two variables was shown in Figure 3.

The regression (R^2=0.99) represented dependence of TPC on temperature is shown as:

$$TPC = -48.48\ (\tau)^2 + 278.5\ (\tau) + 1748$$

Where, (τ) is extraction time (hours).

The estimated maximum value of TPC was 2,148 mg GAE/100g corresponding to extraction time of 2.87 hours.

Effect of temperature on TPC of the extracts

Along with the time, temperature is also an important factor of the process of extraction[16]. The change in TPC with the variation of extraction temperature was displayed in Table 1.

Table 1: Effect of extraction temperature on TPC and TAC

Temperature (^{o}C)	TPC (mg GAE/100g)	TAC (mg CGE/100g)
25	1784.3±9.0	114.7±1.4
35	1932.0±12.3	118.1±1.5
45	2130.8±9.0	123.9±1.4
65	2155.1±5.9	105.1±1.1
75	2175.0±9.1	94.7±1.3
85	2190.8±9.0	65.5±0.3
100	2487.7±3.4	33.4±0.4

Values represent the means ± standard deviation, (n=3).

Results indicated that a significant increase in the extraction of total phenolics when increasing the temperature from 25 to 100°C. According to Abad-García *et al.* (2007), denaturation of membranes and a

possible degradation of polyphenolic compounds caused by hydrolysis, internal redox reactions and polymerizations which are detrimental to the extraction yield may happen and influence quantification of bioactive compounds[25]. Cacace and Mazza (2003) reported that flavonoid families, mainly anthocyanin are heat sensible, hence an upper limit must be respected to avoid degradation of the thermo-sensitive phenolic compounds[26]. For these reasons, TAC of extracts was determined to find out the suitable temperature for extraction process of rice bran. The data were shown in Table 4. The regression equation expressed the relation of extraction temperature and TAC is following:

$TAC = –0.0257 (T)^2 + 2.0949 (T) + 78.361$

Where, T is extraction temperature (^oC).

The estimated maximum value of TAC was 121.1 mg CGE/100g corresponding to extraction temperature of 40.7^{o}C. Abad-García *et al.* (2007) reported that temperatures above 40◦C produced an TAC extraction yield decrease[25]. The temperature for TPC extraction from red rice bran was selected as 40^{o}C and the factor can not be optimized using of mathematical models.

Effect of extraction cycle numbers on TPC of rice bran extract

Multiple–step extraction is an important method to improve the extraction yield of polyphenols[27]. The statistical data of TPC corresponding to different numbers of extraction cycles were shown in Table 2. Three cycles extraction showed significant ($p < 0.05$) higher yield of TPC than that of using two cycles of extraction. However, four cycles of extraction could not improve the yield of TPC. Similarly, Duong *et al.* (2015) showed three cycles extraction are suitable for extraction TPC and TFC from soybean[28].

Table 2: Effect of extraction cycle numbers on TPC

Extraction cycle numbers	TPC (mg GAE/100g)
1	1772.4±3.5^c
2	2138.8±6.0^b
3	2342.8±5.9^a
4	2344.8±3.4^a

Values represent the means ± standard deviation, (n=3). Values in a column with different superscripts were significantly different ($p < 0.05$).

Effect of solid– solvent ratio on TPC of rice bran extracts

Solid–solvent ratio may also influenced to the extraction of phenolic compounds from plant materials[29]. In this study, this influence was displayed in Table 3. Solid–solvent ratio of 1:6 (w/v) showed high amount of TPC. A further increase in solid–solvent ratio from 1:6 to 1:10 did not significantly ($p>0.05$) increase TPC. According Tan *et al.* (2011), a high solid to solvent ratio was found to be favorable in extraction of phenolic compounds[30]. It increases concentration gradient, resulting in an increase of diffusion rate that allows greater extraction of solids by solvent[31]. However, active component yields will not continue to increase once equilibrium is reached[32]. Duong *et al.* (2015) obtained a maximum TPC using solid-solvent ratio (1:6, w/v) for soybean extraction[28].

Table 3: Effect of solid– solvent ratio on TPC

Solid– solvent ratio	TPC (mg GAE/100g)
1:4	1568.7±3.8^c
1:5	1770.1±6.6^b
1:6	2344.6±5.9^a
1:10	2350.7±7.6^a

Values represent the means ± standard deviation, (n=3). Values in a column with different superscripts were significantly different ($p < 0.05$).

3.2 Optimization of extraction conditions

Three parameters: X_1, ethanol concentration (48–68%); X_2, added acetic acid concentration (10–15%) and X_3, extraction time (120–240 min), were able to be optimized using the BBD. The experimental design presented thirty combinations (Table 4), including 3 replicates of the central point in order to estimate pure error and to assess the lack of fit of the proposed models. The multiple regression analysis showed that the model (2) was significant ($p < 0.001$) and adequately adjusted the experimental data, presenting p lack of fit = 0.22 and $R^2 = 0.99$, which means that the proposed multiple regression model was able and suitable to explain 99% of the variance.

$$TPC = -7802.67 + 210.059^{***}X_1 + 575.763^{***}X_2 + 6.77677^{***}X_3 - 1.53312^{***}X_1^2 - 2.5125^{***}X_1X_2 - 0.0480208^{***}X_1X_3 - 16.728^{***}X_2^2 - 0.00975^{***}X_3^2 \quad (2)$$

*(*** Significant at $p < 0.001$)*

Table 4: The BBD with experimental data for the extraction of TPC from red rice bran

X_1	X_2	X_3	Y (TPC)	
			Block 1	Block 2
58	15	120	2231.5	2219.7
48	15	180	2296.6	2302.5
48	10	180	2107.4	2107.4
58	12.5	180	2367.5	2385.2
48	12.5	240	2308.4	2302.5
68	12.5	120	2131.0	2125.1
58	12.5	180	2385.2	2379.3
48	12.5	120	2213.8	2213.8
58	10	120	2184.2	2178.3
58	10	240	2243.4	2255.2
58	15	240	2284.7	2296.6
58	12.5	180	2373.4	2367.5
68	10	180	2066.0	2060.1
68	15	180	2012.8	1995.1
68	12.5	240	2101.5	2107.4

The linear and quadratic terms of all variables significantly influenced the polynomial model for TPC assay (Eq. 2). The high values of regression coefficient β_2 indicated that the acetic acid concentration had the major influence or importance in the model. The positive values in the model indicate that an increase of factors tends to increase the response values; on the other hand, negative values indicate that an increase tends to decrease the responses[33]. The significance of quadratic term for all variables, which showed negative value, indicated that an increase in these variables for values beyond the studied range tends to decrease the responses or TPC in the extract.

The response surfaces for variables ethanol concentration and acetic acid concentration versus the extraction time were shown in Figure 4. According to the results, all surface responses showed a concave characteristic, which is in agreement with the significance of quadratic. Additionally, it can be observed from Figure 4 that maximum values of responses were found between the ethanol concentration of 52–56%, acetic acid concentraion of 12,5–3,5% and extraction time of 200–220 min. This probably occurred due to solubility of polyphenol compounds in the mixture solution of water and ethanol; since the antioxidant extraction depends of the solvent polarity[34]. These results agree with results reported by Chooklin (2014), which evaluated the extraction of phenolic compounds from brown rice extract from Sung Yod Phatthalung, using mixture solution of water and ethanol with pH adjustment as solvent[35].

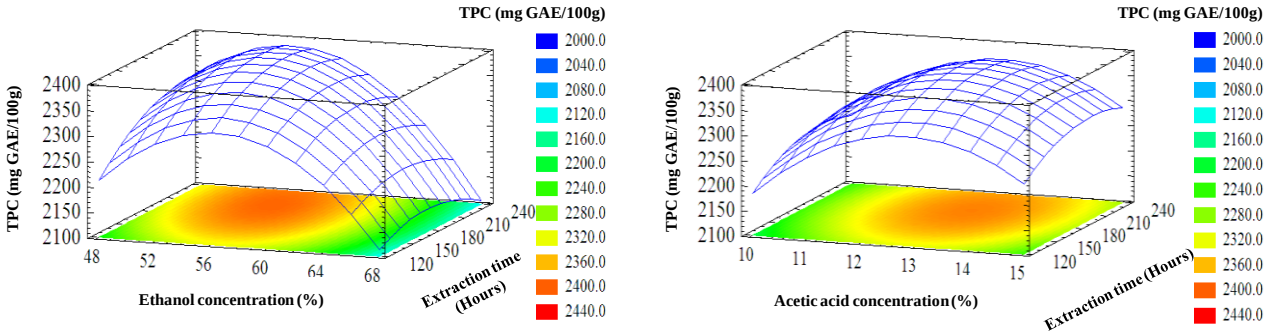


Figure 4: Response surface plot of ethanol concentratio and acetic acid concentration versus the extraction time on the TPC in the extract.

The optimum level of TPC was showed in Table 5. The extraction conditions were as follows: ethanol concentration 54.5%, added acetic acid concentration 13% and extraction time 210 min. A triplicate experiment was set up to validate the optimized conditions. As shown in Table 5, the experimental TPC of red rice bran was 2,391.1±5.9 mg GAE/100g and was in good agreement with the predicted value. Goffman and Bergman (2004) analysed TPC of rice bran from 10 red rice varieties and reported that the TPC were in ranges of 2600–4390 mg GAE/100 g[36]. The verification value for TPC obtained are not significant different predicted values which clearly showed that the model fitted the experimental data very well and therefore optimized the phenolic extraction efficiently within the specified range of process parameters. The antioxidant activity of extract was also determined and displayed in Table 5.

Table 5: Optimum conditions and the value of TPC

Variables	Optimum conditions
Ethanol concentration (%)	54.5
Acetic acid concentration (%)	13.1
Extraction time (min)	210
TPC (Predicted) (mg GAE/100g)	2399.6[a]
TPC (Experiment[1])	2391.1[a]±5.9
IC50 value (Experiment[1]) (µg/mL)	108.1±2.9

(1): Data are presented as mean from triplicate of experimental run ± SD. The same letters within row indicate not significant difference by t' test from hypothesis for mean at 5 % level.

4. Conclusion

Base on the experiment that had been done, the experimental value agreed with the predicted one. The optimal TPC was determined at ethanol with concentration 54.5%, added acetic acid concentration 13.1%, extraction time 210 min at 40°C, the solid–solvent ratio 1:6 (w/v) and three cycles of extraction. Optimization of the extraction procedure using RSM showed data adjusted to the statistic models. The extract of red rice bran exhibited high TPC and antioxidant activity, so is might act as a potential natural antioxidant source.

References

[1]. Chen Q., Fu M., Yue F., Cheng Y. *Food and Nutrition Sciences* 2015, 6: 1277.
[2]. Huang Y.-P., Lai H.-M. *Journal of Food and Drug Analysis* 2016, 24: 564-574.
[3]. Itani T., Ogawa M. *Japanese Journal of Crop Science* 2004, 73: 137-147.
[4]. Oki T., Masuda M., Kobayashi M., Nishiba Y., Furuta S., Suda I., Sato T. *Journal of Agricultural and Food Chemistry* 2002, 50: 7524-7529.

[5]. Yoshida H., Tomiyama Y., Mizushina Y. *Food Chemistry* 2010, 123: 210-215.
[6]. Yawadio R., Tanimori S., Morita N. *Food Chemistry* 2007, 101: 1616-1625.
[7]. de Mira N. V. M., Massaretto I. L., Pascual C. d. S. C. I., Marquez U. M. L. *Journal of Food Composition and Analysis* 2009, 22: 405-409.
[8]. Min B., Gu L., McClung A. M., Bergman C. J., Chen M.-H. *Food Chemistry* 2012, 133: 715-722.
[9]. Shao Y., Xu F., Sun X., Bao J., Beta T. *Journal of Cereal Science* 2014, 59: 211-218.
[10]. Muntana N., Prasong S. *Pakistan Journal of Biological Sciences* 2010, 13: 170.
[11]. Lee J. H. *Food Science and Biotechnology* 2010, 19: 391-397.
[12]. Chen M.-H., Bergman C. *Journal of Food Composition and Analysis* 2005, 18: 319-331.
[13]. Rao A. S., Reddy S. G., Babu P. P., Reddy A. R. *BMC Complementary and Alternative Medicine* 2010, 10: 4.
[14]. Pereira-Caro G., Watanabe S., Crozier A., Fujimura T., Yokota T., Ashihara H. *Food Chemistry* 2013, 141: 2821-2827.
[15]. Escribano-Bailón M. T., Santos-Buelga C., Rivas-Gonzalo J. C. *Journal of Chromatography A* 2004, 1054: 129-141.
[16]. Pedro A. C., Granato D., Rosso N. D. *Food Chemistry* 2016, 191: 12-20.
[17]. Baş D., Boyacı İ. H. *Journal of Food Engineering* 2007, 78: 836-845.
[18]. Granato D., De Castro I. A., Ellendersen L., Masson M. L. *Journal of Food Science* 2010, 75.
[19]. Bassani D. C., Nunes D. S., Granato D. *Anais da Academia Brasileira de Ciências* 2014, 86: 923-934.
[20]. Granato D., Grevink R., Zielinski A. c. A., Nunes D. S., van Ruth S. M. *Journal of Agricultural and Food Chemistry* 2014, 62: 10283-10296.
[21]. Granato D., de Araújo Calado V. M., Jarvis B. *Food Research International* 2014, 55: 137-149.
[22]. Michiels J. A., Kevers C., Pincemail J., Defraigne J. O., Dommes J. *Food Chemistry* 2012, 130: 986-993.
[23]. Boeing J. S., Barizão É. O., e Silva B. C., Montanher P. F., de Cinque Almeida V., Visentainer J. V. *Chemistry Central Journal* 2014, 8: 1-9.
[24]. Pinelo M., Rubilar M., Sineiro J., Nunez M. *Food Chemistry* 2004, 85: 267-273.
[25]. Abad-García B., Berrueta L., López-Márquez D., Crespo-Ferrer I., Gallo B., Vicente F. *Journal of Chromatography A* 2007, 1154: 87-96.
[26]. Cacace J., Mazza G. *Journal of Food Science* 2003, 68: 240-248.
[27]. Chen X., Wu X., Chai W., Feng H., Shi Y., Zhou H., Chen Q. *Journal of Zhejiang University Science B* 2013, 14: 903-915.
[28]. Duong T. P. L., Phan T. B. T., Ha T. T. *Journal of Food and Nutrition Sciences* 2015, 3: 33-38.
[29]. Dai J., Mumper R. J. *Molecules* 2010, 15: 7313-7352.
[30]. Tan P., Tan C., Ho C. *International Food Research Journal* 2011, 18: 557-562.
[31]. Cacace J., Mazza G. *Journal of Food Engineering* 2003, 59: 379-389.
[32]. Herodež Š. S., Hadolin M., Škerget M., Knez Ž. *Food Chemistry* 2003, 80: 275-282.
[33]. Montgomery D. C., 2001. Design and analysis of experiments, John Wiley & Sons

[34]. Tan M. C., Tan C. P., Ho C. *International Food Research Journal* 2013, 20: 3117-3123.
[35]. Chooklin S. *International Food Research Journal* 2014, 21: 291-296.
[36]. Goffman F., Bergman C. *Journal of the Science of Food and Agriculture* 2004, 84: 1235-1240.

IMPACTS OF MAIN FACTORS ON ALCOHOLIC FERMENTATION OF CACAO PULP JUICE BY *SACCHAROMYCES CEREVISIAE* VTCC – Y – 0011

Minh Hien Nguyen, Thi Thanh Van Tran, Tai Huan Phan*
Nong Lam University - Ho Chi Minh City
* *Corresponding author: pthuan@hcmuaf.edu.vn*

Response surface methodology (RSM) was applied to select optimal conditions of varied factors including total soluble solid content, pH and inoculum size for fermentation of cacao pulp juice by *Saccharomyces cerevisiae* VTCC – Y – 0011 to produce ethanol. The experiments were carried out according to the central composite design (CCD) with total soluble solid content ranged from 20 to 24 °Bx, inoculum size from 1 to 5% yeast and pH value from 3 to 5. A quadratic model was respectively developed to correlate the preparation variables to the ethanol yield. The results showed that a production of ethanol from the cacao pulp juice could be achieved up to 10.45% (v/v) after 168 h at optimum conditions of 22 °Bx, 3.5% yeast and pH 4.5. The predicted values for optimization of process variables was found to be in agreement with the experimental values.

Keywords: fermentation / cacao pulp juice / Saccharomyces cerevisiae / RSM

Introduction

In Vietnam, cocoa growing has significantly increased and stared to enter global market in recent years. There are 50,000 ha area under cocoa, and the average yield will be 1.19 tons/ha with about 45,700 tons fermented cocoa bean by 2020. The quality of fermented cocoa bean is currently evaluated by International Cocoa Council resulting in a good taste. A part of the mucilage will be pressed and removed in the cocoa fermentation to reduce the acidity of fermented cocoa bean. According to Anvoh et al. (2009), the cocoa pulp juice was rich in glucose (214.2±6.2g/L), importance mineral such as potassium (950±16.32mg/L), calcium (171.5±34.1mg/L); ascorbic acid (18.3±7.5mg/L), citric acid (9.1±0.6mg/L), malic acid (3.6±0.5mg/L) and acetic acid (2.28±0.7mg/L). However, it was less in fumaric acid, oxalic and lactic acid as well as sodium, magnesium and phosphorus (Anvoh et al., 2009). There were many investigations applying cocoa juice in food and beverage processing such as wine (Dias et al., 2007), beer (Cassiane, 2017), marmalade, jelly, jam, vinegar or soft drink (Adomako, 2006). The aim of this work was to study and optimize the fermentation process of cocoa bean mucilage by the selected strain *Saccharomyces cerevisiae* VTCC–Y– 0011 to produce ethanol.

Materials and Methods

Materials

The cocoa pulp juice: cocoa juice was collected in Cam My – Dong Nai province. After the pods were opened, seeds were pressed by the specific machine, and the juice was collected from mucilage of cocoa beans. The juice was 10% of bean weight with pH value of 3.75±0.007; TSS of 23±2.7 ^{o}Bx; the total sugar of 18.3±2.08% and reducing sugar of 12.7±2.03 g/100 mL.

Yeast: *S. cerevisiae* VTCC-Y- 0011 was supplied by VTCC, IBMT, National University, Ha Noi, Vietnam.

Media culture: Sabouraund broth (Hi Media, India) including 40 g dextrose and 10 g peptone dissolved in 1 L H_2O, pH 5.6, then sterilized for 15 min at 121^{o}C.

Methods

Inoculum preparation: Seed culture was prepared in 250 mL Erlenmeyer flasks containing 50 mL of Sabouraund broth and inoculated by a loop full of the yeast strain from the slant, then incubated at 37^{o}C with the agitation rate of 190 rpm for duration of 24 h.

Effect of the selected S. cerevisiae strains on primary fermentation:
Batch fermentations were carried out in 500 mL Erlenmeyer flasks containing 450 mL of the cocoa pulp juice which was diluted 1.5 times by water and adjusted sugar concentration to 22 ^{o}Bx. Adjusting pH value to 4.5 was done by adding $NaHCO_3$. Sulphur dioxide was also added up to 110 mg of $K_2S_2O_5$ per lit to inhibit bacteria proliferation. After that, *S. cerevisiae* (3% v/v, 1.2 x 10^7 cell/ml) was added into the cultural fermentation and incubated for 168 h at room temperature under anaerobic condition. Sugar consumption was measured by Atago refractometers 0 - 32. Alcohol degree was determined by distillation technique.

Optimization processing parameters for fermentation: A central composite design (CCD) was employed to the experimental data. In this study, three independent process variables were pH (X_1), total soluble solids (TSS) (^{o}Bx; X_2) and inoculum size (%; X_3). The selected response variable was alcohol content (v/v) (Y_1). JMP version 10 was used to fit the quadratic response surface model to the experimental data.

Statistical data processing: Stagraphics version 7.0 was used for data analysis.

Result and discussion

Effect of formation time on sugar consumption and ethanolic production

The selected yeast strain was evaluated in terms of conversion rate of sugar into alcohol. It was found that the selected yeast strain significantly affected various parameters of alcoholic fermentation (Table 1).

Table 1. Changes in Brix (^{o}Bx) and alcohol value during fermentation by *S. cerevisiae* VTCC-Y-0011.

Time (hours)	Changes in TSS value during fermentation (^{o}Bx)	Changes in alcohol value during fermentation (% v/v)
0	22 ± 0.00	0.00 ± 0.00
24	15 ± 0.15	3.57 ± 0.09
48	10 ± 0.15	6.02 ± 0.10

72	8 ± 0.10	7.00 ± 0.14
96	6 ± 0.10	8.15 ± 0.03
120	5 ± 0.11	8.64 ± 0.05
144	4 ± 0.05	9.2 ± 0.01
168	3 ± 0.10	10.08 ± 0.00

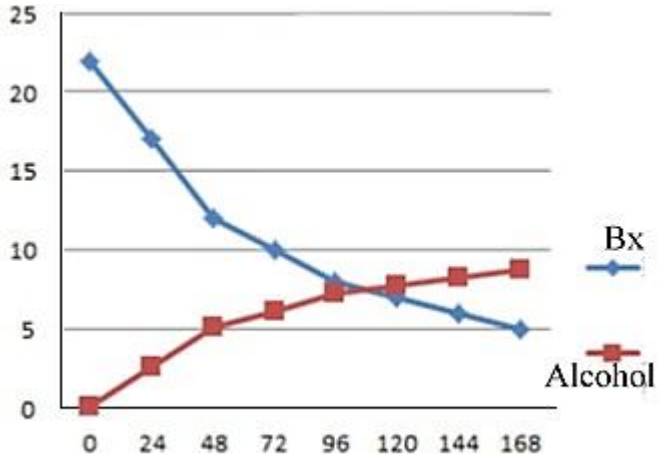


Figure 1. Effect of the strains on fermentation kinetics

Figure 1 shows that the high speed of consumption sugar was in the first 24 h then slowly decreased. The more sugar was consumed, the more alcohol was produced. After 168 h, *S. cerevisiae* VTCC-Y-0011 gave significant effect on sugar consumption and alcohol yield.

Optimization processing parameters for fermentation:
TSS, inoculum and pH play an important role in the fermentation process. Effects of processing parameters to the alcohol content were showed in Table 2.

Table 2. Effecting of TSS, inoculum, pH on alcohol production

Exp	Code	TSS (X_1)	Inoculum size (%) (X_2)	pH (X_3)	Alcohol content (%v/v) (Y_1)
1	a00	18.6	3	4.5	8.28
2	−−−	20	2	4	7
3	−−+	20	2	5	6.9

4	−+−	20	4	4	8.85
5	−++	20	4	5	8.82
6	0a0	22	1.3	4.5	5.6
7	00a	22	3	3.6	10
8	000	22	3	4.5	10.08
9	000	22	3	4.5	10.06
10	000	22	3	4.5	10.08
11	000	22	3	4.5	10.07
12	000	22	3	4.5	10.07
13	000	22	3	4.5	10.08
14	00A	22	3	5.3	9.98
15	0A0	22	4.6	4.5	9.73
16	+−−	24	2	4	7.7
17	+−+	24	2	5	7.68
18	++−	24	4	4	9.57
19	+++	24	4	5	9.5
20	A00	25.3	3	4.5	9.9

Quadratic equation showing effects of factors on alcohol content was obtained as in response of Equation 1 (Eq. 1).

$$Y_1 = 10.09 + 0.41X_1 + 1.05X_2 – 0.48X_1^2 – 0.98X_2^2 \text{ (Eq.1)}$$

where Y is the response (alcohol); X_1, X_2, X_3 were TSS (^oBx), inoculum size (%), pH value, respectively.

The result represented that TSS and inoculum size gave an impact on the fermentation process.

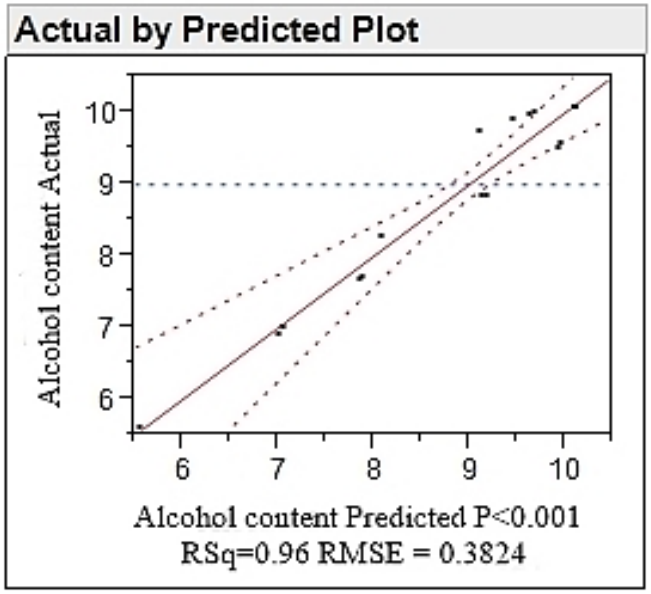


Figure 2. Predicted vs. experimental values of different response alcohol

The goodness of fit of the model was checked by the determination coefficient (R^2). The result (R^2 = 0.96) indicated a high significance of the model and a good consistency between the experimental and predicted values of alcohol yield (Figure 2). The value of $p < 0.0005$ showed TSS (^oBx), inoculum size (%) and pH value had a significant effect on the response value. The contour plots of response surface (Figure 3) can be used to explore the changes of alcohol content with the changes of process parameters.

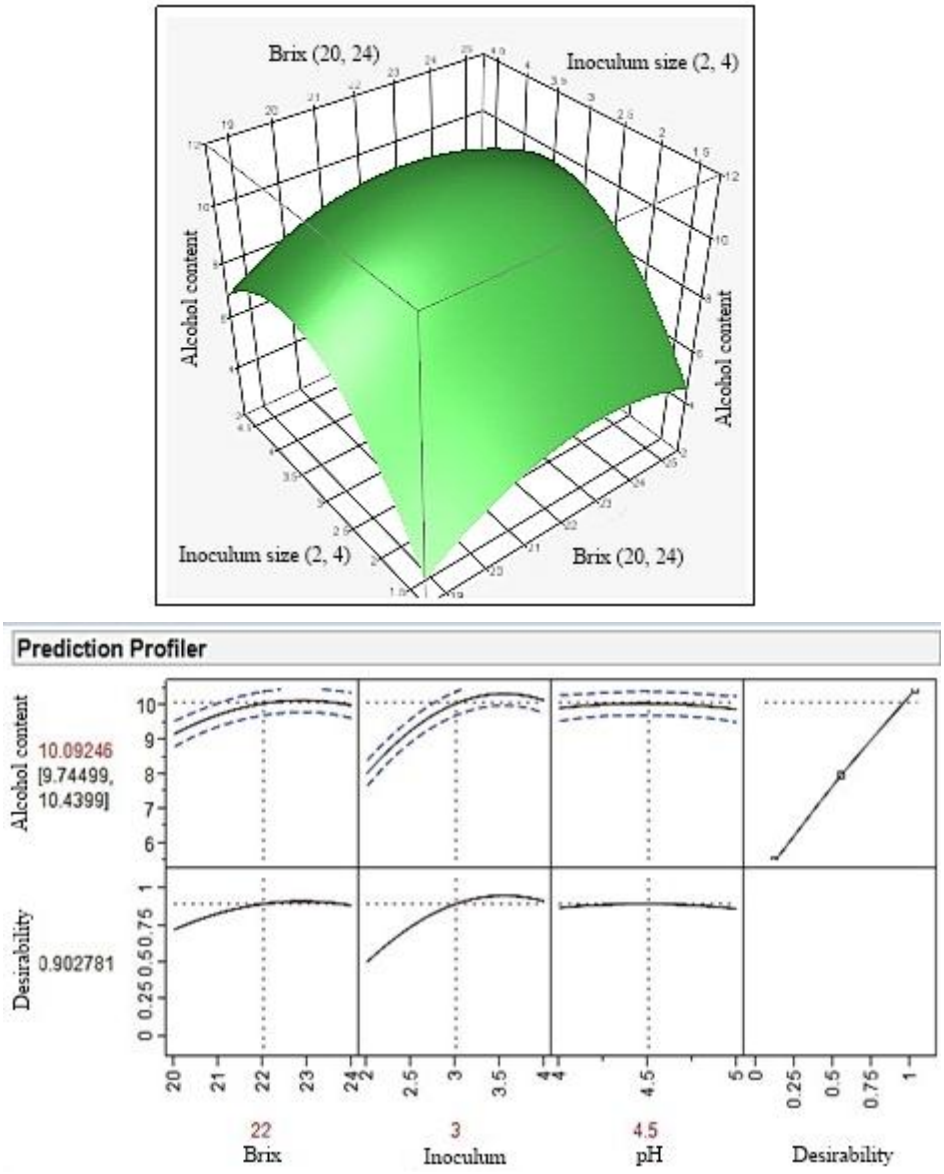


Figure 3. Response surface of ethanol production

Table 3 shows the value of alcohol content which was predicted at optimum conditions of process parameters and compared it with the actual obtained value by carrying out the experiment.

Table 3. Predicted value at the optimum conditions and actually experimental value

Parameters	Predicted value at the optimum conditions	Actual Value
TSS (^{o}Bx)	22.8	23
Inoculum size (%)	3.5	3.5
pH value	4.5	4.5
Alcohol content (% v/v)	10.46	10.45

It can be seen that, with the optimum levels of varied factors, the experimental value of alcohol content of 10.45 (% v/v) was obtained which was very close to its predicted value by CCD. Thus, the processing parameters consisting of 23 ^{o}Bx; 3.5% inoculum size and pH 4.5 were finally selected for the fermentation cacao pulp juice by *S. cerevisiae* VTCC-Y- 0011.

Conclusion

S. cerevisiae VTCC-Y- 0011 was selected because of its impacts on sugar consumption and alcohol production of fermentation process. The optimized conditions for primary fermentation were established as 23 ^{o}Bx; 3.5% yeast and pH 4.5. Under these conditions, alcohol content of 10.45 % v/v was achieved. Further research should be taken into account for second fermentation of cocoa bean mucilage.

Acknowledgement: this work was supported by Department of Science and Technology - Dong Nai Province.

Reference

Disney Ribeiro Dias, Rosane Freitas Schwan, Evandro Sena Freire & Roge rio dos Santos, 2007, *Elaboration of a fruit wine from cocoa (Theobroma cocoa L.) pulp*. International Journal of Food Science and Technology, vol 42, pp 319 – 329.

Anvoh KYB, Zoro BA, Gnakri D, 2009, *Production and characterization of juice from mucilage cocoa beans and its transformation into marmalade*, Pak Journal Nutr. 2009; 8 (2), pp 129 – 133.

Cassiane da Silva Oliveira Nunes, Giovani Brandao Mafra de Carvalho, 2017, *Cocoa pulp in beer production: applicability and fermentative process performance*, Journal of Plot one, published April 18, 2017 in Proceedings of the 12th international Cocoa research conference

Adomako, 2006, *Project on pilot plants to process cocoa by products*. Summary report on a pilot project in Ghana.

EFFECT OF ENZYME TREATMENT ON THE QUALITY OF WHITE MULBERRY (*MORUS ALBA* L.) FRUIT JUICE EXTRACT

Nguyen Hoang Thao Ly[1,2], Le Si Ngoc[1], Nguyen Minh Xuan Hong[2]
[1] Research and Development Center for High Technology Agriculture - Ho Chi Minh city
[2] Nong Lam University - Ho Chi Minh city

1. Introduction

Mulberry belongs to the genus *Morus* of the family Moraceae. It is widely distributed in Asia, Europe, North America, South America, and Africa. While mulberry leaves have been historically used as feed for silkworms, mulberry fruits are nutritional foodstuff. It is a favorite fruit due to its delicious, succulent, juicy but low-calory (43 calories/ 100 g) properties. [3].

According to the World Health Organization (WHO, 2004), mulberry fruit is a source of soluble fiber (inulin, fructo-oligosaccharide) and galacto-oligosaccharide, which helps increase intestinal microflora, digestive support.

In the past few years, polyphenol and flavonoid extracted from natural materials are highly interested in the prevention of cardiovascular disease and some cancers. Mulberry fruit contains a lot of polyphenol (25.7 mg GAE/ g) and flavonoid (65.4 mg/ g). The composition of polyphenol in mulberry is umbelliferone (179.1 mg/ g), chlorogenic acid (226.9 mg/ g), kaempferol (5.8 mg/ g), quercetin (15.2mg/ g), caffeic acid (17.2 mg/ g). They have a significant effect on antioxidant activity, decrease low-density lipoprotein cholesterol, delay the aging process and enhance skin beauty [1].

Currently, mulberry fruit is processed mainly in the form of wine or jam. Meanwhile, the mulberry powder production is limited due to difficulty in extracting fruit juice. In addition to conventional pressing methods, enzyme treatment has been shown to increase the extraction efficiency of some fruits. Therefore, this study examined a number of technical parameters in the use of enzyme to extract the juice from mulberry fruit.

2. Materials and methods

2.1. Raw material

Mulberry fruits were purchased from Da Lat (Lam Dong) at normal ripen stage, dark purple on the fruit surface. The fruits were sorted, de-stemmed, removed the damaged part and rinsed well before being stored in zipper bags (1 kg/ bag) at -4°C (± 1°C) throughout the study to ensure the uniformity and quality of input materials.

Enzyme Pectinex (Novozyme, Denmark), which is in liquid form and brown colour, works well at pH 2 – 5 and temperature 30 – 50^{o}C. Pectinex is a complex enzyme consisting of pectin-transeliminase, polygalacturonase, pectin-esterase, hemicellulose, cellulase, …

2.2. Analytical method

The experiment was arranged in the completely randomized design (CRD) with two factors, including 18 treatments and 3 replications. Factor 1 was the enzyme concentration with 6 treatments: 0; 0.2; 0.4; 0.6; 0.8; and 1%. Factor 2 was the time of enzyme treatment with 3 treatments: 1, 2, and 3 hours.

The raw materials were weighed 1kg per sample and smoothed with a blender. Mulberry purée was treated with Pectinex and incubated at 45^{o}C with different enzyme concentrations in the specified time intervals. The fruit juice extract was retrieved by vacuum filtration with a filter pore size of 20-25 μm. Extraction efficiency, total sugar content, reducing sugar content and total polyphenols content were determined, compared and selected the best enzyme treatment parameters.

The extraction efficiency (%) was calculated by taking the total volume of the fruit juice extract after filtration divide the weigh of initial materials and multiphy with100.

The total sugar content was determined by phenol method (Dubois et al., 1956).

The reducing sugar content was determined by Miller method (Miller, 1959).

The total polyphenol content was determined by Folin – Cioalteu method (Folin and Denis, 1915).

The collected data were statistical analyzed using excel software and JMP 10.0; the significance of differences between the means was determined at $P \leq 0.05$.

3. Results and Discussions

3.1. Characterization of mulberry fruit composition

Composition of mulberry fruit was investigated and presented in Table 1.

Table 1. *Composition of mulberry fruit*

Moisture (%)	89.90 ± 0.10
Total dissolved solids (^{o}Brix)	9.67 ± 0.12
Total ash (%)	3.45 ± 0.07
Total protein (%)	0.45 ± 0.05
Total sugar (%)	5.37 ± 0.03
Reducing sugar (%)	0.51 ± 0.04
Total polyphenol (mg GAE/ g)	24.30 ± 0.06

The results showed that the mulberry fruit was succulent with high moisture content (89.9%). The ash content of 3.45% indicated that the mulberry fruit contains mineral content for good health. Akbulut et al. (2016) identified high mineral content of mulberry fruit, including K, Ca, P, Mg, S, Na and Fe. The total sugar content was 5.37%, lower than the results from Muhammad et al. (2013), 7.55%. The protein content (0.45%) was similar to that of Sighal et al. (2009) 0.5 – 1.4%. The total polyphenol content (24.30 mg GAE / g) was higher than that of Liu et al. (2009) (0.103 mg GAE/ g) and similar to the study of Bea and Suh (2007) (25.70 mg GAE / g).

The quality of mulberry fruit varies depending on many factors such as breed, environmental conditions, climatic conditions, harvesting maturity and so on. In general, mulberry fruit has high total polyphenol content – the antioxidants which are good for health. As a result, mulberry fruit is a suitable source for the powder production, a convenient and healthful product. However, the sugar content in the fruit is low; therefore, it is necessary to add sugar to create a harmonic sweet and sour taste for the product.

3.2. Effect of enzyme treatment on extraction efficiency

Table 2. *Extraction efficiency (%) after enzyme treatment*

Enzyme concentration (%) (Factor A)	**The time of enzyme treatment (hours) (Factor B)**		
	1	**2**	**3**
0	$40.61^{i} \pm 1.47$	$57.11^{f} \pm 1.26$	$57.15^{f} \pm 0.53$
0.2	$47.02^{h} \pm 1.56$	$65.08^{d} \pm 1.33$	$66.49^{d} \pm 0.49$
0.4	$53.39^{g} \pm 2.28$	$73.54^{c} \pm 1.47$	$74.52^{c} \pm 1.22$
0.6	$60.12^{e} \pm 2.64$	**$86.26^{b} \pm 1.92$**	$88.15^{ab} \pm 1.87$
0.8	$66.49^{d} \pm 1.77$	$87.08^{b} \pm 2.42$	$89.77^{a} \pm 0.99$
1	$72.71^{c} \pm 1.50$	$89.92^{a} \pm 1.28$	$90.82^{a} \pm 1.38$
P_A	***		
P_B	***		
P_A*P_B	***		

*Note: The data followed by the same characters are not statistically significant. *** very significant differences (P <0.001).*

Table 2 showed that the extraction efficiency increased proportional to the enzyme concentration and the time of treatment. Mulberry purée treated with Pectinex at 0.6% concentration for 2 hours gave the best extraction efficiency of 86.26%. Enzyme concentration and time of hydrolysis had a simultaneous and statistically significant effect on extractability (P_A*P_B <0.001). When the enzyme concentration increased, the contact between enzyme and substrate increased, resulting in higher extraction efficiency. However, when the enzyme concentration was raised too high, the efficiency did not increased significantly and was not economically beneficial due to the saturated combination between enzyme and substrate [6]. The

enzyme-catalyzed hydrolysis requires a minimum of time for the enzyme to function. Extending the time for enzymatic hydrolysis is necessary to produce a large amount of extracted fruit juice. The short hydrolysis time with 1-hour treatment was not enough to complete hydrolysis; the extraction efficiency was low, ranging from 40.61 to 72.71%. However, the long hydrolysis time (3 hours) did not extract more fluid; it was even more time-consuming and in the risk of microbial contamination.Therefore, the appropriate comdition of pectinase enzyme to treat the fruit was 0,6% concentration for a period of 2 hours to obtain the highest extraction efficiency.

3.3. Effect of enzyme treatment on total sugar content

Table 3 showed that the total sugar content of the fruit juice extract increased proportional to the enzyme concentration and the time of enzyme treatment.

Table 3. *Total sugar content (%) after enzyme treatment*

Enzyme concentration (%) (Factor A)	**The time of enzyme treatment (hours) (Factor B)**		
	1	**2**	**3**
0	$5.49^{r} \pm 1.39$	$5.63^{q} \pm 1.83$	$5.78^{p} \pm 1.83$
0.2	$5.92^{o} \pm 1.17$	$6.07^{n} \pm 2.35$	$6.22^{m} \pm 1.19$
0.4	$6.84^{l} \pm 2.13$	$7.40^{k} \pm 1.62$	$8.38^{j} \pm 1.99$
0.6	$9.09^{i} \pm 1.45$	**$9.59^{f} \pm 1.92$**	$9.81^{e} \pm 1.23$
0.8	$9.20^{h} \pm 1.76$	$9.48^{g} \pm 1.37$	$9.86^{d} \pm 0.51$
1	$10.02^{c} \pm 1.39$	$10.14^{b} \pm 2.07$	$10.20^{a} \pm 1.10$
P_A	***		
P_B	***		
P_A*P_B	***		

*Note: The data followed by the same characters are not statistically significant. *** very significant differences (P <0.001).*

The total sugar content increased when the Pectinex concentration increased from 0.2% to 1% during the treatment period of 1-3 hours. It is due to the catalytic activity of Pectinex to breakdown of the cell wall, breakdown of the structure and freeing up internal components such as water, color compounds, and biological compounds [6]. In addition, the enzyme will attack and break the bonds such as the ester linkage between the phenol and the polymer on the cell wall, increasing the drainage efflux to the external environment [7]. When the enzyme concentration increases, the cell wall destruction is increased, so the fruit juice extract and other components are more abundant. The results showed that the treatment of mulberry purée at 0.6% enzyme concentration for 2 hours for total sugar content (9.59%) achieved the best economic and significant data in terms of statistics with P <0.001.

3.4. Effect of enzyme treatment on reducing sugar content

Reducing sugar is an important component that affects the sensory value and metabolism of nutrients during food processing. The results showed that the reducing sugar content of fruit juice extract was increased accordingly as the increase of enzyme concentration and the time of treatment (Table 4).

Table 4. *Reducing sugar content (%) after enzyme treatment*

Enzyme concentration (%) (Factor A)	**The time of enzyme treatment (hours) (Factor B)**		
	1	**2**	**3**
0	$0.79^{g} \pm 0.26$	$1.03^{f} \pm 1.38$	$1.03^{f} \pm 0.11$
0.2	$1.12^{ef} \pm 0.22$	$1.21^{e} \pm 0.20$	$1.38^{d} \pm 0.15$
0.4	$1.19^{e} \pm 0.49$	$1.48^{cd} \pm 0.71$	$1.64^{b} \pm 0.99$
0.6	$1.46^{d} \pm 0.54$	**$1.77^{a} \pm 0.44$**	$1.77^{a} \pm 0.94$
0.8	$1.58^{bc} \pm 1.21$	$1.76^{a} \pm 0.47$	$1.83^{a} \pm 0.38$
1	$1.61^{b} \pm 0.21$	$1.75^{a} \pm 0.38$	$1.85^{a} \pm 0.69$
P_A	***		

P_B	***
P_A*P_B	*

*Note: The data followed by the same characters are not statistically significant. * significant differences (P <0.05), *** very significant differences (P <0.001).*

Reducing sugar content increased together with enzyme concentrations from 0.4 to 1% over an incubation period of 1 to 3 hours due to the resolution of polysaccharides and oligosaccharides in the mulberry fruit during treament. This result is similar to that of Fang et al. (1986). During enzymatic treatment, long-chain molecules are breaked by increasing monosaccharides and oligosaccharides, so the reducing sugar content in the fruit juice extract is increased [5]. Pectinex consists of complex enzymes, which can act on a lot of substrates and produce many reducing sugars. The results showed that enzyme treatment at 0.6% concentration for 2 hours obtained the best reducing sugar (1.77%) and was statistically significant. The reducing sugar did not change much if the time of enzyme treatment continue to increase.

3.4. Effect of enzyme treatment on total polyphenol content

The total polyphenol content varies when the mulberry purée was treated with different enzyme concentrations during different incubation times (Table 5).

Table 5. *Total polyphenol content (mg GAE/ g) after enzyme treatment*

Enzyme concentration (%) (Factor A)	**The time of enzyme treatment (hours) (Factor B)**		
	1	**2**	**3**
0	$29.99^{h} \pm 1.54$	$30.35^{h} \pm 1.04$	$31.90^{gh} \pm 0.37$
0.2	$32.90^{fg} \pm 1.11$	$33.12^{fg} \pm 1.42$	$34.77^{f} \pm 1.37$
0.4	$41.71^{e} \pm 1.31$	$44.79^{cd} \pm 1.69$	$46.58^{c} \pm 1.50$
0.6	$43.25^{de} \pm 1.58$	**$65.59^{b} \pm 1.56$**	$66.87^{ab} \pm 2.20$
0.8	$44.42^{cd} \pm 0.98$	$66.92^{ab} \pm 0.84$	$67.74^{ab} \pm 0.84$
1	$45.19^{cd} \pm 1.16$	$67.20^{ab} \pm 1.61$	$68.85^{a} \pm 1.10$
P_A	***		
P_B	***		
P_A*P_B	***		

*Note: The data followed by the same characters are not statistically significant. *** very significant differences (P <0.001).*

The results showed that mulberry purée treated with enzyme at 0.6% concentration for 2 hours achieved the best the total polyphenol content (65.59 mg GAE/ g) compared with the untreated (30.35 mg GAE/ g). Pectinex disintegrates pectins that are concentrated mainly in the cell wall and between the flesh cell, which releases the polyphenol compounds in the cytoplasm [2]. Thus, total polyphenol extraction from the mulberry purée was improved. When the time of enzyme treatment was increased up to 2 hours, the total polyphenol content was 65.59 mg GAE/ g and statistically significant (P_B<0.001). If the time of enzyme treatment was increased, the total polyphenol content did not change much.

Therefore, the appropriate enzyme concentration of treatment was 0.6% for a period of 2 hours to obtain the highest total polyphenol content.

4. Conclusions

Enzyme treatment had significant effect on the extraction efficiency and the quality of mulberry fruit juice. The treatment with Pectinex enzyme at 0.6% concentration for 2 hours gave higher extraction efficiency (86.26%), total sugar content (9.59%), reducing sugar content (1.77%) and total polyphenol content (65.59 mg GAE/ g) than untreated one. This extracted fruit juice has good properties that can be used directly or further processed to produce more convenient products such as instant fruit powder or concentrated fruit syrup.

References

[1]. Bea, S.H., and Sun, H.J. Antioxidant activity of five different mulberry cultivars in Korea, *Food Science Technology* 2007, 40: 955 – 962.

[2]. Chen, X., Xu, F., Qin, W., Ma, L., and Zheng, Y. Optimization of enzymatic clarification of green asparagus juice using response surface methodology, *Institute of Food Technologists* 2012, 77 (6).
[3]. Ercisli, S., and Orhan, E. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*M. nigra*) mulberry fruits, *Food Chemistry* 2007, 103 (4): 1380 – 1384.
[4]. Fang, T., Chen, H.E., and Chiou, L.M.J. Effects of heat treatment and subsequent storage on the quality of passion fruit (Passiflora edulis) juice, *Paper presented on 19th Symposium of International Federation of Fruit Juice Producers*, Den Haag, Netherlands 1986.
[5]. Landbo, A.K., Kaack, K., and Meyer, A.S. Statistically designed two step respone surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice, *Innovative Food Science Emerging Technology* 2007, 8: 135 – 142.
[6]. Lê Ngọc Tú, La Văn Chứ, Đặng Thị Thu, Nguyễn Thị Thịnh, Bùi Đức Hợi và Lê Doãn Diên, *Hóa sinh công nghiệp*, Nhà xuất bản khoa học và kỹ thuật Hà Nội 2004.
[7]. Manuel, P., Beatriz, Z., and Meyer, A.S. Selective release of phenol from apple skin: mass transfer kinetics during olvent and enzyme assisted extraction, *Separation and Purification Technology* 2008, 63: 620 – 627.
[8]. Singhal, B.K., Dhar, A., Khan, M.A., Bindroo, B.B., and Fotedar, R.K. Potential economic additions by mulberry fruits in sericulture industry, *Plant Horti Tech* 2009, 9: 47 – 51.

Session 2: Safety and quality of dairy and meat products

EFFECTS OF STORAGE CONDITIONS AND PACKAGING MATERIALS ON THE QUALITY OF VIETNAMESE DARK CHOCOLATE.

***Thuy N A. Phan*[1]**, *Mai T. Nguyen*[2]
[1] Department of Food Technology, School of Biotechnology, International University – Vietnam National University in HCMC. Email: thuypna95@gmail.com
[2] Department of Food Technology, School of Biotechnology, International University – Vietnam National University in HCMC.

Introduction

Chocolate is very heat and humidity sensitive, and preferably kept in cool, dark place at 18^{o}C, and 50% humidity [1]. On the other hand, Vietnam possesses a tropical climate of monthly average maximum temperatures ranging from 30 to 35 ^{o}C, as well as a temperature difference between day and night being up to 10 ^{o}C. Moreover, the humidity ranges from 60% to above 80% in many regions. This climate favors the cocoa tree development but greatly challenges the preservation of chocolate products. The two most encountered problems during storage of chocolates are melting and bloom formation. Melting happens at high temperatures from 29 to 34 ^{o}C [2]. Triglyceride composition in the chocolate matrix can be rearranged during temperature fluctuation, which may lead to fat bloom [3]. On the other hand, the moisture from the humid environment can condense into sweats or directly migrate the chocolate surface due to rapid change from low to high temperature. These water droplets dissolve the sugar content, then leaving the sugar crystals on the chocolate surface when they later evaporate, which is referred to as sugar bloom [4]. Bloom, as well as softening or melting will greatly decrease the acceptability of the product.

Currently, on the national market, chocolate products are mostly packaged in paper-based materials. This material enables easy wrapping, printing and is relatively inexpensive. Other commercialized chocolate brands have their products packaged in plastic, and heat sealed laminate packaging. Aluminum foil is also a good choice for chocolate cover, as it provides a good gas, aroma and vapor barrier. It also can protect chocolate from moderate temperature change [4]. Generally, there is a lack of report on the effectiveness of these packaging materials particularly for the chocolate storage in Vietnam.

If the above storage problems can be sufficiently improved, the lengthen shelf-life of the product can facilitate higher production, as well as greater availability in the market. Thus, it is hypothesized that storage conditions (freezer, refrigerator, simulated room condition) and packaging materials would significantly affect the physical quality (bloom formation, condensation) and physiochemical properties (phenolic content, peroxide value, hardness) of the commercial dark chocolate products.

This research investigates the effect of storage conditions, such as room condition, refrigerator and freezer on the physicochemical properties of Vietnamese chocolate. Furthermore, different forms of packaging shall be tested for the impact on quality of these products. Chocolate manufacturers may use information in this study as references for improving the storage conditions of chocolate produced in tropical countries.

Materials and methods

Materials: Commercial dark chocolate (70% cocoa) were collected from the workship in Tien Giang province, on the date of production. The chocolate samples were similar in size (5±1g), packaged into three different types of packaging and stored in three different climates.

Packaging modes:

+ Package A: original paper foil
+ Package W: aluminum foil – moisture absorber
+ Package P: aluminum foil – plastic.

Storage conditions:

-Freezer (F) compartment in the lab (-20 to -18^{o}C).
-Refrigerator (C) (4 to 7^{o}C) in the lab.
-Room condition (R), (26 to 30^{o}C) was the third storage condition.
Hardness were measured every two-week in triplicate using Texture analyzer (FR 5120, Lutron), at room temperature
Degree of blooming was evaluated on a scale of 5 [5] every 2-week.
Moisture Absorption was measured every 2 weeks by monitoring the weight differences
Extraction: after every 2-week, each sample was submitted to defatting process using n-Hexane
Peroxid Value (PV) of extracted cocoa butter was determined by titration with 0.01N $Na_2S_2O_3$ according to AOCS Method Procedure (2006).
Total Phenolic Content (TPC) of defatted chocolate was measured according to [6].

Results

Hardness Analysis

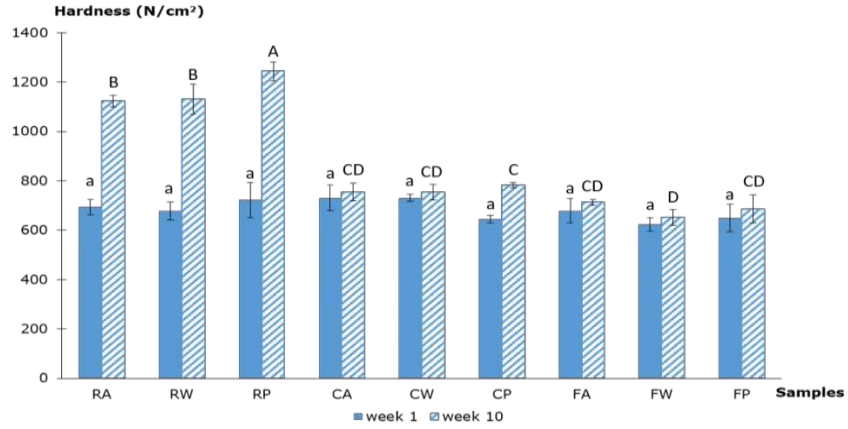


Figure 1: Hardness of dark chocolate stored in room, fridge and freezer condition at week 1 and week 10.
Columns with different lowercase letters are significantly different (P≤0.05).

Figure 1 showed that the hardness of the samples stored for 10 weeks in cooler conditions (fridge and freezer) were relatively higher than the samples stored for one week. However, the hardness of the chocolate samples was highest after 10 weeks of storage under room condition (161%, 167% and 171% increase for RA, RW and RP respectively).
The temperature changed in a wide range in room conditions, as there was air-conditioner during the day (T= 26-28 ^{o}C) but not during the night (T= 28 ^{o}C -30 ^{o}C). This thermal cycle had a strong influence on the hardness of chocolate, known as thermocyclic hardening [3]
The influence of packaging types on the change of chocolate hardness in different storage condition in the 10-week period was not significantly different (P>0.05).

Visual bloom assessment

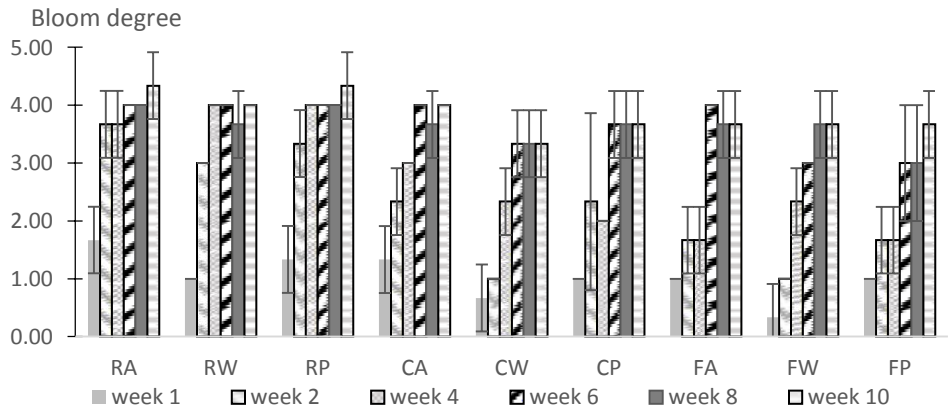


Figure 2: Visual assessment of fat and sugar bloom of dark chocolate stored in room condition, refrigerator and freezer.

The blooming assessment of chocolate in different storage conditions, under the effects of time, was illustrated in Figure 2. Among the three storage conditions, room condition was the one facilitated the bloom formation the most. Groups of chocolate samples stored at room condition started to become dull by a grey film (level 3)

from the second week of storage. It took 4 to 6 weeks for chocolate samples in the fridge to start blooming. The chocolate stored in the freezer had been detected with blooming signs since week 6. On the other hand, from week 6, white spots were visible on the chocolate surface stored at room condition and those spots became more severe in the following weeks.

At the end of the 10-week duration, all chocolate samples stored at room condition were seen at least at level 4, with clear signs of grey and white film. Whereas, for chocolate stored in fridge and freezer, the degree of bloom was still at the early stage, with just a few samples having clear bloom spots.

Previous researches had confirmed a relationship between the increase in instrumental hardness and fat bloom formation [7]. The results of fat bloom and hardness of chocolate after 10 weeks in this projects showed a similar trend.

Moisture absorption

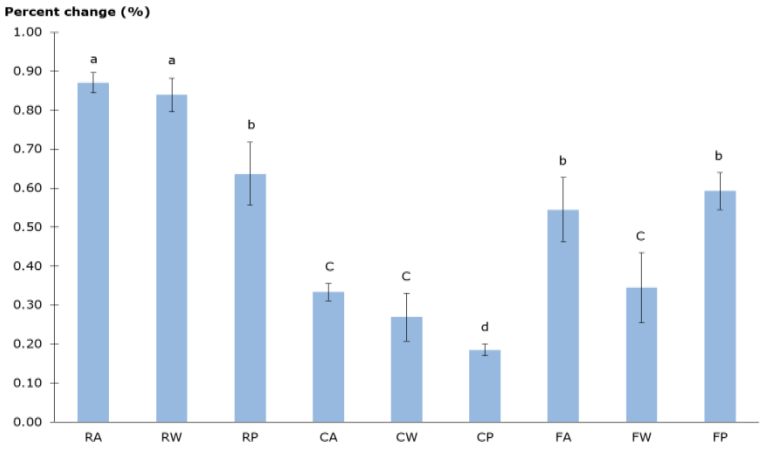


Figure 3:Percent change of absorbed moisture of dark chocolate stored in room, fridge and freezer at week 10.
Columns with different lowercase letters are significantly different (P≤0.05).

After 10 weeks of observation, there was a significant difference in the absorbed moisture among the condition of the room, refrigerator and freezer storage (P≤0.05). Figure 3 illustrated that the samples stored in room condition absorbed moisture the most, while samples stored in the fridge absorbed the least among the three storage conditions. Regarding packaging types, in room condition and refrigerator, package P was the one absorbed less moisture than the other two. On the other hand, in the freezer, there was no significant difference in the amount of absorbed

moisture between A and P packaging, while package W absorbed much less moisture.
The relative humidity of room condition ranged from 43 to 75 %RH (EXTECH SD800) explained the high moisture uptake. The moisture may have penetrated the packaging to the samples. Additionally, room temperature ranged from 26 to 30 ^{o}C, which demonstrated why sugar bloom was inevitably installed in the samples in room condition, during storage [4].

Peroxide value analysis

Table 1:Peroxide value (mEq/kg) of dark chocolate under different storage conditions, after 10 weeks

	Storage time						
	week 0	week 1	week 2	week 4	week 6	week 8	week 10
Room condition (R)							
Original (A)	0.31 ± 0.05^{aX}	0.32 ± 0.03^{aX}	0.3 ± 0.06^{aX}	0.32 ± 0.08^{aX}	0.29 ± 0.03^{aX}	0.36 ± 0.04^{aX}	0.33 ± 0.0^{aX}
Plastic (P)	0.31 ± 0.05^{aX}	0.27 ± 0.04^{aX}	0.29 ± 0.04^{aX}	0.3 ± 0.00^{aX}	0.28 ± 0.03^{aX}	0.32 ± 0.02^{aX}	0.31 ± 0.01^{aXY}
Fridge (C)							
Original (A)	0.31 ± 0.05^{aX}	0.35 ± 0.07^{aX}	0.35 ± 0.04^{aX}	0.33 ± 0.03^{aX}	0.35 ± 0.05^{aX}	0.37 ± 0.05^{aX}	0.31 ± 0.05^{aXY}
Plastic (P)	0.31 ± 0.05^{aX}	0.3 ± 0.00^{aX}	0.32 ± 0.00^{aX}	0.28 ± 0.06^{aX}	0.29 ± 0.04^{aX}	0.32 ± 0.03^{aX}	0.29 ± 0.03^{aXY}
Freezer (F)							
Original (A)	0.31 ± 0.05^{aX}	0.27 ± 0.04^{aX}	0.26 ± 0.08^{aX}	0.29 ± 0.06^{aX}	0.24 ± 0.00^{aX}	0.33 ± 0.05^{aX}	0.27 ± 0.01^{aXY}
Plastic (P)	0.31 ± 0.05^{aX}	0.27 ± 0.01^{aX}	0.3 ± 0.03^{aX}	0.25 ± 0.02^{aX}	0.28 ± 0.03^{aX}	0.26 ± 0.00^{aX}	0.26 ± 0.00^{aY}

Data expressed as mean ± SD, n=3 (three independent samples)
Mean values within treatment followed by different lowercase letters are significantly different at $p \leq 0.05$. Mean values within storage day followed by different uppercase letters are significantly different at $p \leq 0.05$.

Table 1 shows the mean PV of dark chocolate stored under different experimental conditions, within 10 - week period. The PV of all treatments in this study did not go above the standard PV (10 mEq/kg) recommended for edible oil [8]. Hence, there might be the beginning of primary oxidation of the oil, but not quantitatively significant.

Total phenolic content analysis

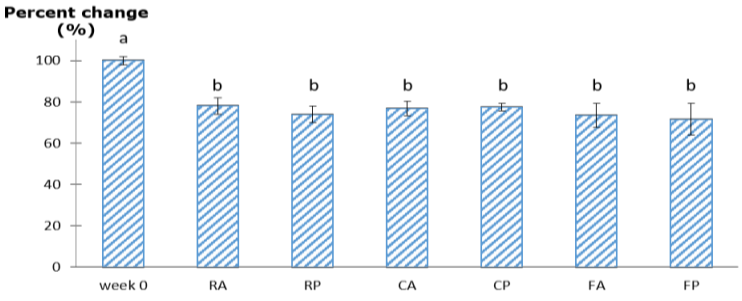


Figure 4 illustrated the percent change of TPC of dark chocolate at week 0 and 10 of the experiment. There was a significant decrease in TPC after 10 weeks ($P \leq 0.05$), but the differences among the storage conditions and packaging modes were not significant ($P > 0.05$). Approximately 70-78% of the TPC remained, after 10 weeks.

Figure 4: Percent change of TPC of dark chocolate under different experimental conditions
Columns with different lowercase letters are significantly different (P≤0.05).

Conclusions

The impact of storage conditions (room condition, refrigerator and freezer) and packaging materials (original paper of chocolate manufacturer, moisture absorber paper with aluminum foil, plastic with aluminum foil) on physicochemical properties of Vietnamese dark chocolate were investigated, during storage of 10 weeks. For physical quality, the chocolate experienced signs of blooming after 2 weeks at room condition and 4-6 weeks at the other conditions. Also, the chocolate samples in all three conditions exhibited an increase in hardness, moisture absorption and visible signs of blooming. Additionally, due to the rough inner surface of the original packaging material (A), the chocolate samples lost the shiny gloss easily at the very first week.

Investigation on biochemical properties of dark chocolate for 10 weeks of different experimental storage also showed noticeable changes, but the peroxide values still lied within an acceptable range and more than 70% of the initial TPC still remained. Hence, there was a significant compromise of appearance and physical quality of dark chocolate after 10 weeks, but the biochemical quality or nutritional value was not significantly affected by storage conditions and packaging materials. Refrigerator and freezer were good conditions for storing chocolate as well as for prolonging visual and textural integrity and could be applied by manufacturers as favorable storage.

References

[1] Biczó, V., Fekete, A., Scherer, R. Progress in Agricultural Engineering Sciences 2013, 9(1): 55-73.
[2] Van Malssen, K., Van Langevelde, A., Peschar, R. & Schenk, H. Journal of the American Oil Chemists Society 1999, 76 (6).
[3] Andrae- Nightingale, l. M., Lee, S. Y., Engeseth, N. J. Journal of Texture Studies 2009, 40(4): 427-444.
[4] Afoakwa, E. O. Chocolate science and technology. John Wiley & Sons 2016.
[5] De Graef, V., Foubert, I., Agache, E., Bernaert, H., Landuyt, A., Vanrolleghem, P. A., & Dewettinck, K. European journal of lipid science and technology 2005, 107(5): 297-306.
[6] Wollgast, J., Anklam, A. Food Research International 2000, 33: 423–447.
[7] Afowaka, E. O., Paterson, A., Fowler, M., Vieira, J. Journal of Food Engineering 2008, 89, 128- 136.
[8] Codex Alimentarius. Codex Standard for Named Vegetable Oils 1999, 210.

STRATEGY FOR DR-CALUX DIOXIN SCREENING IN FEED UNDER EC REGULATION

Scippo M-L[1], Rybertt MS[1], Focant J-F[2], Eppe G[2], Massart A-C[2], De Pauw E[1] and Maghuin-Rogister G[2]
[1]CART Laboratory of Analysis of Foodstuffs of Animal Origin, University of Liège, Boulevard de Colonster B-43b Sart-Tilman, B-4000 Liège, BELGIUM
[2]CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, BELGIUM

Introduction

Since 2001, the European Commission set a legislation defining maximum levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in feed (and food) for 17 congeners[1]. Maximum level values for 12 additional congeners of dioxin-like polychlorinated biphenyls (DL-PCBs) have recently been added to the list and will enter in force soon (4th November 2006)[2].

The reference gas chromatography-isotope dilution high resolution mass spectrometry (GC-IDHRMS) methodology routinely allows the individual identification and quantification of these 29 congeners at the ultra-trace level. Congener-specific data can be used as such for source tracking and identification or can be converted in toxic equivalent quantities (TEQs) based on the use of toxic equivalent factors (TEFs) to assess the overall toxicity[3]. Because the high quality GC-IDHRMS monitoring of dioxins in the food chain is time and resource consuming, alternative sample screening methods are needed. The DR-CALUX (Dioxin Response-Chemically Activated LUciferase gene eXpression) cell-based assay has widely been proposed to screen dioxin and dioxin-like compounds in food and feed samples. Compared to congener-specific GC- IDHRMS data, the CALUX AhR (Aryl hydrocarbon receptor)-activation mediated response directly yield to a TEQ estimation based on a correlation with 2,3,7,8-TeCDD induction of the assay.

A comparison between GC-IDHRMS and DR- CALUX results often shows discrepancies, partly due to differences between the WHO-TEF values and the CALUX REP (relative equivalent potency) values[4,5] measured for each of the PCDD, PCDF and DL-PCB congeners. In addition, although analyte recovery rates are taken into account for calculations in GC-IDHRMS, no correction for analytes loss based on internal standards is possible in a cell based assay.

Such differences make difficult the strict decision of compliance or suspicion of non compliance for samples submitted to biological screening. Another difficulty of the screening stage is to determine a limit of decision, allowing "to select those samples with levels of dioxins and DL-PCBs that are less than 30-40% below or exceed the level of interest"[6] but yielding to a rate of false negative decision lower than 1%[6]. Furthermore, the rate of false positive decision should be very low to ensure profitable use of the screening procedure (all samples suspected to be positive at the screening stage have to be tested by a confirmatory method, i.e., the GC-IDHRMS). We show and evaluate here a DR-CALUX screening strategy for dioxin monitoring in feed samples, allowing correction of DR-CALUX raw data and a rate of false negative les than 1%. The level of contamination with PCDD/F and DL-PCB congeners of a large number of feed samples analyzed routinely by GC-IDHRMS is also presented.

Materials and methods

Samples are unknown samples issued from the routine monitoring activity (with GC-IDHRMS) of the laboratory of mass spectrometry. QC samples and method blanks (BCs) were regularly run for QA/QC purposes. **Extraction:** For GC-IDHRMS analyses of PCDD/Fs and dioxin-like PCBs, samples were extracted and cleaned-up as already described[7]. For DR-CALUX analyses, samples were liquid-liquid (LLE) extracted and cleaned-up according to the method proposed by the manufacturer.

Analysis : GC- IDHRMS analysis of PCDD/Fs and DL-PCBs were performed as already described[7]. **DR-CALUX** originates from BioDetection System (BDS, NL). Briefly, samples extracts were cleaned-up manually using liquid chromatography on acidic silica columns. Final extracts were concentrated in DMSO prior to analysis. All details are available elsewhere[4].

Results are expressed as pg total TEQ/g, because no separation between PCDD/Fs and DL-PCBs is performed.

Results

Dioxin monitoring of feed samples using GC-IDHRMS

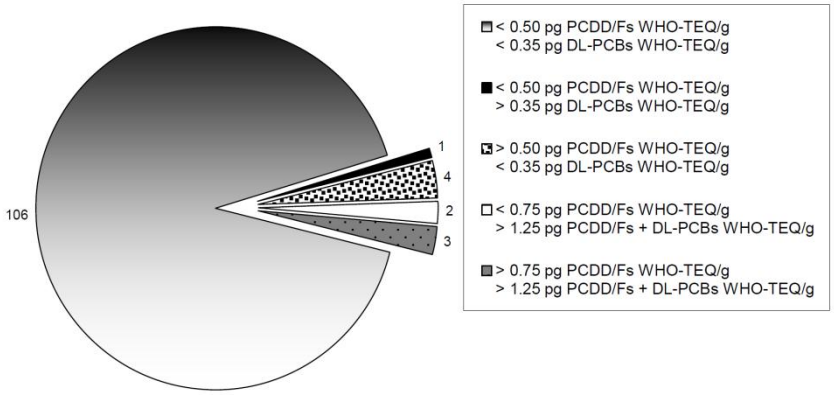


Figure 1 *: Distribution of results obtained from the monitoring of 116 feed samples (GC-IDHRMS method).*

Figure 1 shows results of dioxin (17 PCDD/Fs and 12 DL-PCBs) monitoring of 116 feed samples classified as "Feed materials of plant origin with the exception of vegetable oils and their by products"[1,2]. For these samples, PCDD/Fs and total (PCDD/Fs + DL-PCBs) WHO-TEQ maximal levels are respectively 0.75 and 1.25 pg/g, while separate action levels exist for PCDD/Fs and DL-PCBs [2], which are respectively 0.5 and 0.35 pg WHO-TEQ/g.

113 samples (97%) were below the maximum level for dioxins and furans, from which only two displayed levels above the maximum level for the sum of dioxins, furans and DL-PCBs.

From these 113 samples, 4 are above the PCDD/Fs action level and one is below the PCDD/Fs action level and slightly above the DL-PCBs action level. Only 3 samples (3%) were above both dioxin/furans and total maximum limits, but with a DL-PCBs contamination close to or below their action level.

DR-CALUX screening strategy

Correction of raw data

Usually, when comparing total TEQ levels (dioxins, furans and DL-PCBs) obtained for the same samples, CALUX measurements are lower than GC-IDHRMS. The main reason of this is that REP for DL-PCBs are lower than WHO-TEF[4,5]. Other causes such as loss of analytes during extraction and purification and antagonistic effects of some PCBs congeners can be mentioned.

As shown in a previous project (DIFFERENCE)[8], the use of reference samples to correct DR-CALUX results show a good improvement in the comparison of GC-IDHRMS and DR-CALUX measurement but, unfortunately, these kind of samples are not commercially available.

Compounds	WHO-TEF	DR-CALUX REP[4]	pg/g	pg WHO-TEQ / g	pg DR-CALUX-TEQ / g
2,3,7,8-TCDD	1	1	0.011	0.0110	0.0110
1,2,3,7,8-PeCDD	1	0.5	0.04	0.0400	0.0200
1,2,3,4,7,8-HxCDD	0.1	0.1	0.16	0.0160	0.0160
1,2,3,6,7,8-HxCDD	0.1	0.06	1.985	0.1985	0.1191
1,2,3,7,8,9-HxCDD	0.1	0.05	0.779	0.0779	0.0390
1,2,3,4,6,7,8-HpCDD	0.01	0.03	146.286	1.4629	4.3886
1,2,3,4,6,7,8,9-OCDD	0.0001	0.0005	742.237	0.0742	0.3711
2,3,7,8-TCDF	0.1	0.4	0.092	0.0092	0.0368
1,2,3,7,8-PeCDF	0.05	0.1	0.029	0.0015	0.0029
2,3,4,7,8-PeCDF	0.5	0.4	0.051	0.0255	0.0204
1,2,3,4,7,8-HxCDF	0.1	0.07	0.073	0.0073	0.0051
1,2,3,6,7,8-HxCDF	0.1	0.08	0.044	0.0044	0.0035
2,3,4,6,7,8-HxCDF	0.1	0.1	0.049	0.0049	0.0049
1,2,3,7,8,9-HxCDF	0.1	0.1		0.0000	0.0000
1,2,3,4,6,7,8-HpCDF	0.01	0.01	1.582	0.0158	0.0158
1,2,3,4,7,8,9-HpCDF	0.01	0.04	0.195	0.0020	0.0078
1,2,3,4,6,7,8,9-OCDF	0.0001	0.004	13.129	0.0013	0.0525
TOTAL PCDD/Fs			**907**	**1.95**	**5.11**
PCB 77	0.0001	0.0004	7.33	0.0007	0.0029
PCB 126	0.1	0.04	0.45	0.0446	0.0178
PCB 169	0.01	0.0008	0.08	0.0008	0.0001
PCB 81	0.0001	0.002	0.43	0.0000	0.0009
PCB 105	0.0001	0	28.64	0.0029	0.0000
PCB 114	0.0005	0.00002	2.36	0.0012	0.0000
PCB 118	0.0001	0	93.68	0.0094	0.0000
PCB 123	0.0001	0	2.72	0.0003	0.0000
PCB 156	0.0005	0.00002	12.51	0.0063	0.0003
PCB 157	0.0005	0	2.16	0.0011	0.0000
PCB 167	0.00001	0	6.76	0.0001	0.0000
PCB 189	0.0001	0	1.42	0.0001	0.0000
TOTAL dioxin-like PCBs			**159**	**0.1**	**0.02**
TOTAL			**1065**	**2.0**	**5.14**

Table I *: GC-IDHRMS results of the "home made" feed QC sample.*

We propose here to correct DR-CALUX data using a "home made" quality control (QC) sample. That QC sample, which is a real contaminated feed material, previously used in an interlaboratory study[10], is incorporated in the series of unknown samples and analyzed accordingly. The GC-IDHRMS analysis of this samples showed a very high contamination with the 1,2,3,4,6,7,8-HpCDD congener, which displays a DR-CALUX REP 3 fold higher than its WHO-TEF (0.03 and 0.01 respectively) (table 1). The DR-CALUX measurement of this sample shows a level of 3.7 pg total TEQ/g, which is higher than the GC-IDHRMS measurement (2.0 pg total TEQ/g). This difference comes from the high contribution of the 1,2,3,4,6,7,8-HpCDD congener.

When using DR-CALUX REP instead of WHO-TEF to calculate the TEQ content, we find a calculated concentration of 5.1 pg calculated "DR-CALUX TEQ"/g (last column of Table 1).
This concentration is our reference concentration for the DR-CALUX measurement. The average DR-CALUX response found during the validation of the method was 72% of that DR-CALUX reference concentration (3.7 pg TEQ/g measured with DR-CALUX versus 5.1 pg "REP-calculated" TEQ/g). The resulting average multiplicative correction factor is 1.4. Because this QC contains very low levels of DL-PCBs, it allows correction of unknowns for recovery (analyte loss during extraction and purification steps) assuming that it is roughly the same for all congeners. Practically, the DR-CALUX result found for the unknown is multiplied by the correction factor, calculated from the result obtained for the QC sample analyzed in the same series than the unknown.

Limit of decision at the DR-CALUX screening stage

To calculate a CALUX decision limit allowing meeting the criteria of less than 1% of false negative set for screening techniques[7], we used a statistical approach. To determine the rate of false negative samples (true positive samples declared negative at the screening stage), we have first to define the limit at which a sample is declared positive. The non-compliance of a sample (true positive sample) is only declared at the confirmatory step (GC-IDHRMS) if its concentration is above the maximal limit taking into account the measurement uncertainty[10] (what we call here the GC-IDHRMS decision limit). Until now, regulatory limits exist for the TEQ concentration of PCDD/F congeners only (no DL-PCBs included). By November 4th 2006, regulatory limits for total (PCDD/Fs and DL-PCBs) TEQ concentrations will enter into force, but PCDD/Fs TEQ maximum limits will still be applicable until 31 December 2008[2].

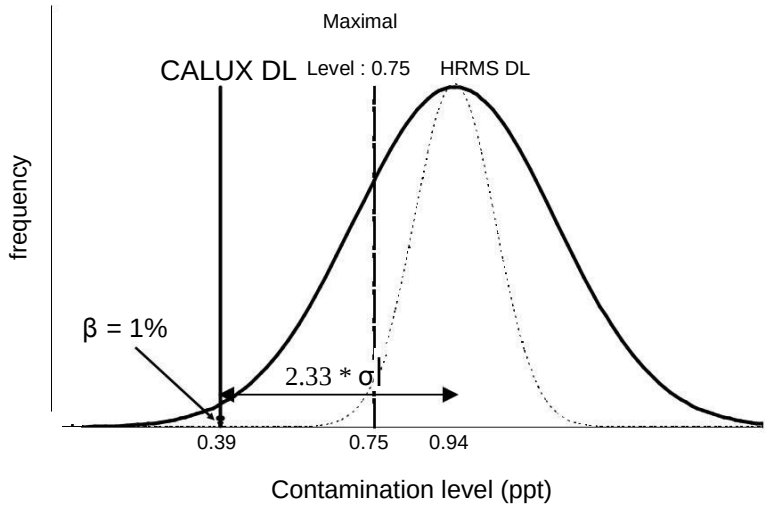


Figure 2 : *Distribution of the contamination measurement of a population of positives samples containing a PCDD/F WHO-TEQ corresponding to the GC-IDHRMS decision limit (i.e. the PCDD/F WHO-TEQ maximal level + the measurement uncertainity). DL : Decision limit.*
σ *: standard deviation of the mean of the CALUX measurements calculated with a CV of 25%. β : beta error, percentage of positive samples below the CALUX decision limit (rate of false negative samples).*

For that reason, and to reduce the false negative rate as much as possible, we choose to calculate a CALUX decision limit that takes into account the lowest maximum limit (e.g. which is the PCDD/Fs TEQ maximum limit). In Figure 2, the dotted distribution corresponds to the expected GC-IDHRMS results for a population of positive samples, contaminated with a PCDD/F WHO-TEQ corresponding to the maximum legal limit plus the expanded uncertainty (we consider that an average expanded uncertainty of 20% is associated to a GC-IDHRMS measurement). The plain line distribution represents the expected DR-CALUX measurements for the same samples. In order to obtain less than 1% of false negative decision (which corresponds to the beta error) at the screening stage, the DR-CALUX decision limit is calculated as the inferior limit of the 99% unilateral confidence interval of a population of results characterized by a mean being equal to the GC-IDHRMS decision limit and a coefficient of variation (CV) of 25%, which is an average reproducibility CV of the DR-CALUX analysis (Figure 2) . This CALUX decision limit has been calculated assuming that the recovery of the DR-CALUX method is 100 % (after correction).

Evaluation of the DR-CALUX screening

Table 3 shows results obtained with both DR-CALUX and GC-IDHRMS methods when analyzing real feed samples. From the 26 samples analyzed, only 3 are above the GC-IDHRMS decision limit for the PCDD/Fs TEQ content. As mentioned in the European Legislation, the screening has to detect samples 30% to 40% below the maximal level, so we evaluated the CALUX screening decision by comparing the number of samples above the DR-CALUX decision limit to the number of samples above 60% of the regulatory limit. Ten samples are above 60% of both the PCDD/Fs and the total (PCDD/Fs + DL-PCBs) WHO-TEQ maximum limits, all detected with the DR-CALUX screening, and 2 samples are above the total TEQ maximal limit only, also detected with the DR-CALUX screening. From the 15 remaining negative samples, four are detected as suspicious after the DR-CALUX screening and are thus false positive.

	pg DR-CALUX-TEQ/g product	DR-CALUX SCREENING Conclusion	pg PCDD/F WHO-TEQ/g product (HRMS)	pg PCDD/F + PCB WHO-TEQ/g product (HRMS)	PCDD/F WHO-TEQ (HRMS) > maximal level - 40% ?	PCDD/F + PCB WHO-TEQ (HRMS) > maximal level - 40% ?	DR-CALUX SCREENING EVALUATION
1	0.19	-	0.11	0.23	-	-	TRUE
2	0.19	-	0.11	0.23	-	-	TRUE
3	0.19	-	0.11	0.22	-	-	TRUE
4	0.19	-	0.11	0.22	-	-	TRUE
5	0.19	-	0.11	0.22	-	-	TRUE
6	0.19	-	0.11	0.22	-	-	TRUE
7	0.19	-	0.11	0.28	-	-	TRUE
8	0.19	-	0.11	0.22	-	-	TRUE
9	0.19	-	0.11	0.23	-	-	TRUE
10	0.50	+	0.11	0.23	-	-	FALSE
11	0.19	-	0.11	0.23	-	-	TRUE
12	0.75	+	0.12	0.25	-	-	FALSE
13	0.19	-	0.13	0.23	-	-	TRUE
14	0.46	+	0.20	0.36	-	-	FALSE
15	1.42	+	0.21	0.40	-	-	FALSE
16	0.51	+	0.37	0.91	-	+	TRUE
17	0.94	+	0.44	0.86	-	+	TRUE
18	1.67	+	0.46	1.23	+	+	TRUE
19	0.44	+	0.64	0.64	+	-	TRUE
20	2.24	+	0.67	0.79	+	+	TRUE
21	1.73	+	0.70	1.42	+	+	TRUE
22	0.65	+	0.71	0.84	+	+	TRUE
23	1.05	+	0.72	0.84	+	+	TRUE
24	2.45	+	0.95	1.07	+	+	TRUE
25	13.34	+	5.64	5.76	+	+	TRUE
26	5.10	+	6.42	6.55	+	+	TRUE

Table 3 *: DR-CALUX (pg DR-CALUX TEQ/g product, QC corrected) and GC-IDHRMS (HRMS) results of the analysis of 26 feed samples. The DR-CALUX screening conclusion is + if the DR-CALUX measured level is above the DR-CALUX decision limit.*

Conclusions

We have developed a strategy to screen feed samples with the DR-CALUX cell-based assay, with a rate of less than 1% of false negative decisions and a "home made" QC sample to correct for recovery. Even if this QC sample does not allow a correction for the difference between REP and TEF, it seems to work well to detect those samples containing a high level of DL-PCBs (samples 16 to 26 of Table 3).

We can conclude that the strategy shown here is good to screen samples in a situation of routine monitoring where a high rate of samples (more than 90%) are negative (compliant with both PCDD/Fs and total (PCDD/Fs + DL-PCBs) WHO-TEQ maximum levels) and a very low rate are non compliant to the total WHO-TEQ maximum level while compliant to the PCDD/Fs one. The congener profile found in the compliant samples corresponds to the background contamination.

On the contrary, in a crisis situation, the congener pattern found in samples depends of the origin of the contamination (such as for example in the Belgian dioxin crisis in feed at the begin of 2006, where feed was contaminated with specific congeners). It would be then interesting to prepare a dedicated reference sample displaying the same congener profile as the one expected in contaminated samples to be used for DR-CALUX correction.

References

[1] Commission Directive 2003/57/EC amending Directive 2002/32/EC.

[2] Commission Directive 2006/13/EC amending Annexes I and II to Directive 2002/32/EC.

[3] Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Shrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. *Environ. Health Perspect* 1998 ; 106 : 775.

[4] Scippo M-L, Eppe G, De Pauw E, Maghuin-Rogister G. *Talanta* 2004; 63: 1193.

[5] Brown DJ, Van Overmeire I, Goeyens L, Chu MD, Denison MS, Clark GC. *Organohalogen compounds* 2002;58: 401.
[6] COMMISSION DIRECTIVE 2002/70/EC.
[7] Focant J-F, Eppe G, Pirard C, De Pauw E. *J Chromatogr A* 2001; 925: 207.
[8] Focant J-F, Eppe G, Scippo M-L, Massart A-C, Pirard C, Maghuin-Rogister G, De Pauw E. *J. Chromatogr. A* 2005 ; 1086 : 45-60.
[9] Eppe G, Cofino W., De Pauw E. *Analytica Chimica Acta* 2004 ;519 :231.
[10] Commission Directive 2005/7/EC of 27 january 2005, amending Directive 2002/70/EC.

Session 3: Safety and quality of aquaculture products

CONTAMINATION MECHANISM IN MAJOR DOMESTIC FISH DISTRIBUTION CHAINS IN VIETNAM.

Lưu Hồng Phúc, Thi To Uyen Dang – Nha Trang University, email: phuclh@ntu.edu.vn, :

1. INTRODUCTION

The government and Vietnamese people are demanding fish with guaranteed food safety and higher quality standards, and these demands are being passed along each stage of the fish distribution chain[1,2,3,4]. This poses new challenges for the seafood industry and government, because only little information has been published so far on the domestic distribution chain in terms of operations and processing issues related to seafood safety.

The lack of understanding of the process and operations within the DFDCs has affected efforts to improve quality[2,5]. What is needed is a clear understanding of the industry as a whole, and therefore it is important to have a study that describes the background, the principles, and the operations of the distribution chains as well as assembling and analysing the contaminant factors of raw fish at every step of fish distribution chains in Vietnam. There was, therefore, this study provides the required information about the distribution chains by presenting process flow charts, describing the process, and monitoring time/temperatures of fish batches of raw fish products at points along distribution chains, and analysing fish contamination mechanisms to raw fish products within the DFDCs in selected areas in Vietnam.

2. MATERIALS AND METHODS

The study of DFDCs was based on both primary and secondary data. The research process was as follows: *Step 1*: Collected secondary data from management boards of fishing ports and fish markets, authorities' offices and institutes. *Step 2*: Conducted semi-structured interviews with authorities, managers, and fish distributors. *Step* 3: Observed fish batches to cross check data obtained from Step 2 and monitor time/temperature of fish batches at points along distribution chains (Steps 2 and 3 provide primary data). The semi-structured interviews were conducted with 53 fish traders (41 middlemen traders and 12 retailers who bought the fish in fishing ports or establishments and then sold fish in retail markets) using guided questions about numbers of workers in the distribution-line, places and times of supply events, fish species for trading, flow diagrams of the DFDCs, methods of preservation, fish handling activities, whether or not their business was registered with the local government, and their relationships with other traders.

A total of 75 random fish batches from six fishing ports were observed with respect to the handling procedures they underwent and researchers accompanied them in their journey to their local retail markets. During the observation, all activities that involved the handling of the fish batches were recorded in order to confirm the operations as described by the traders who were interviewed. The proportion of fish batches that might be divided into smaller amounts to supply each chain was also recorded during observation.

The structures of the DFDCs as described by traders who were interviewed were confirmed by the researcher accompanying each fish batch from fishing ports to retail markets.

Time and temperature measurement

Fifteen batches of fish in each distribution chain were observed for time and temperature. Therefore, a total of 60 batches of fish were measured for temperatures and the durations of their travel, storage, and display at each stage along the chains were recorded. 3,600 samples in 60 fish batches were measured for temperature The temperature measurement was taken as the internal temperature of each fish. A random

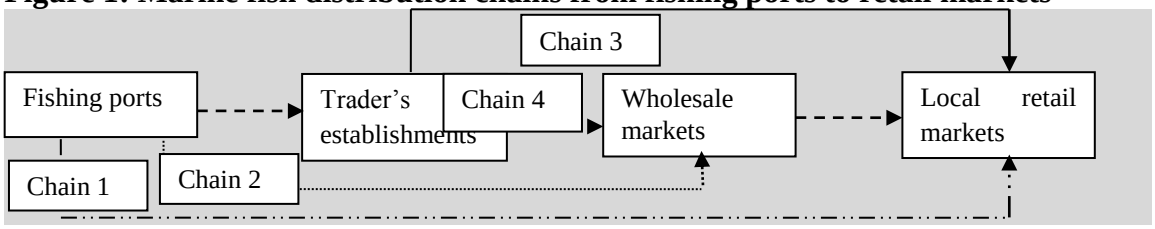
sampling strategy was applied for choosing fish samples for the measuring of the internal temperatures of fish.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the domestic fish distribution chains

The Vietnamese fish distribution chain was examined from ship offloads to retail local markets (Fig 1). This included fishing ports or water's edge, middle trading places (establishments), wholesale markets, and retail markets. The main activities of the fish handlers include sorting, transporting, unloading and loading. Middle traders or fish traders played a role as owners or fish handlers, depending on the size of their business. They might organise their business at a family household level or as an enterprise or as a group of traders. Their jobs were trading and moving fish from the ship offload areas to the final stages of the marketing chains. They did not sell the fish directly to the final consumers. The fish traders might operate at various levels. Retailers were the last in the distribution chains. They worked at local retail markets and sold the fish directly to consumers. Their activities in local retail markets might include: fish handling, fish scaling, and gutting.

Figure 1: Marine fish distribution chains from fishing ports to retail markets



The distribution chain for processing and sale of raw fish involves various levels of handling from fish landing at fishing ports to middle traders to retailers. Once fish are unloaded from ships, they are sold to the first level traders and then proceed through a number of middle trading levels before reaching retailers. The first level traders in turn sell their fish to the second level traders and so on, with each level of trader handling regressively smaller volumes. In some cases, the retailers buy the fish from different middle traders and mix the fish together to sell. Some of the first level traders or the retailers can buy fish without brand name on the fish companies or the fish suppliers with no way of knowing from which fish company or supplier they originate, let alone from which are or ship: so therefore, fish contaminated with microorganisms from different areas are mixed in with other fish with no way of telling where any contaminated finfish originated. Furthermore, given the number of people and places involved before the fish reach the consumers, it is very difficult to know from which places contaminated fish might have originated or who may have caused the contamination of the fish. This problem causes difficulties for control of fish contamination; additionally, the consumers do not know where the fish originated to help them decide on their purchase.

The findings of this research have identified that the relationships between traders are mainly based on personal ties or that they may be associated with the provision of credit between one trader and the traders at the next level without food safety assurance or mechanisms available to coordinate between traders; so therefore, no one can control the quality or food safety of the whole distribution chain. Furthermore, due to the small scale nature of the DFDCs[5,6,7] only the first level traders are registered as companies under the control of the food safety regulatory establishment of the Ministry of Agriculture and Rural Development (MARD). From the second level traders and subsequent levels down to the bottom level middlemen traders, effective food safety control is beyond the reach of the administrative system of

MARD. In the local retail markets, there is open access for everyone to sell their products. Retailers do not need licenses to sell the fish, they just pay a fee to use the market's facilities. Therefore, provincial governments just regulate the first level trader who is registered as a business company, but they have no records or effective control over sub-level traders or retailers who contribute significantly to food safety effects in the distribution chain. This problem may be a major challenge for the food safety management of the seafood industry in Vietnam.

3.3 Time and temperature measurement

The results of observations revealed that temperature abuse occurred at all of the stages along the distribution chain (Table 1). Vietnamese regulations require that raw fish materials must be kept refrigerated under 4^0C. However, in this study, the internal temperature of almost all fish samples, except those in Trading Establishment, were higher than 4^0C. It showed that temperature control of raw fish in fishing ports, establishments and fish markets was non-compliant. The most serrious non-compliance occurred in Retail markets. Fish were exposed for long hours (average more than 9 hours) at high temperature (average temperature $>10^0$C) to display for selling. Limitation to the amount of time that the seafood can be exposed to temperatures higher than 4^0C may be necessary to avoid a problem related to time abuse of seafood.

Time and temperature abuse at one step alone may not lead to an unsafe product. However, time and temperature abuse that occurs at successive processing steps is critical for safety. The cumulative time and temperature abuse may lead to sufficient amounts of pathogenic bacterial and their toxins at unsafe levels of pathogenic bacteria or their toxins in food because of multiplications and producing toxins of bacterial. Moreover, time and temperature abuse are the most important predisposing factors leading to form histamine in the Scombridae and Scomberesocidae fish families. The non-compliance of controlling the amount of time and temperatures at fish markets in this study would permit pathogenic bacteria growth or toxin production.

Table 1: Time and temperature kept of fish batches evaluated in fishing ports, establishments, fish markets, and transportation

Places	n^a	Mean± SD^c	Percentage (%) of samples kept				Time (hours)	
			$\leq 4^0$C	$4^0<$ and $\leq 7^0$C	$7^0<$ and $\leq 10^0$C	$>10^0$ C	n^b	Mean ± SD
Fishing ports	600	8.50 ± 2.58	3.7	27	50.8	18.5	60	1.30 ± 0.85
Trading Establishments	300	5.76 ± 2.23	21.0	46.7	32.3	0	30	6.99 ± 3.57
Wholesale markets	300	7.41 ± 2.15	6.3	39.3	41.3	13.0	30	0.90 ± 0.33
Retail markets	1200	10.56 ± 3.06	1.3	10.1	37.3	51.2	60	9.29 ± 1.99
Transportations	1200	8.52 ± 2.46	2.8	24.8	46.6	25.9	60	1.27 ± 0.98

Note: n^a numbers of samples; n^b numbers of fish batches from fishing ports; Mean ± SD^c : mean of temperature at each place

3.4 Potential contaminant sources in the DFDCs

Four main vectors are identified in the diagram of contamination mechanisms (see Figure 2). The first vectors are insects, rodents, and animals; the second vectors are represented by the physical environment, including water, waste water, waste, dust, contaminated containers, tools, and working surfaces. The third vectors are constituted of people who are acting in DFDCs. The last vectors include endogenous microorganisms in the fish and initial contamination of fish during the processing of the catches and its storage on fish boats. These vectors are created from potential contaminant sources in the whole distribution chain. The potential contaminant sources originate from factors that include physical environmental factors, consumer factors, food safety management factors, and legal aspects of food

safety. In order to ensure fish safety in DFDCs, it is essential to understand the contaminant sources and identify the contextual factors related to DFDCs.

Physical environment factor

The current physical environment of the DFDCs has shown that inadequate infrastructure conditions directly affect safe handling practices of fish distributors[8]. The results of several studies[3,4,5,7,9] revealed that inadequate infrastructure is constantly subject to the element of risk, and impacts directly on workers and fish by increasing contamination risks from unhygienic practices.

Food safety management factors and legal aspects of food safety factors

Food safety management systems were important factors identified in this study as influential for fish safety in the DFDCs. The findings of this study show that most fish businesses or fish enterprises did not have effective food safety management systems in place. The ineffective food safety management systems were evidenced through the weakness of management commitment to food safety and role modelling of food safety practices. The inadequate physical environment and infrastructures and serious non-compliance of documents related to the food safety of DFDCs[5,7,8,9], were clear indicators of the weakness of managements' commitment to food safety. Furthermore, one of the important requirements for fish safety procedures is recall and traceability; there were no labels found in fish batches received from fish boats. If the fish are contaminated, there is no way of tracing them once they have been traded.

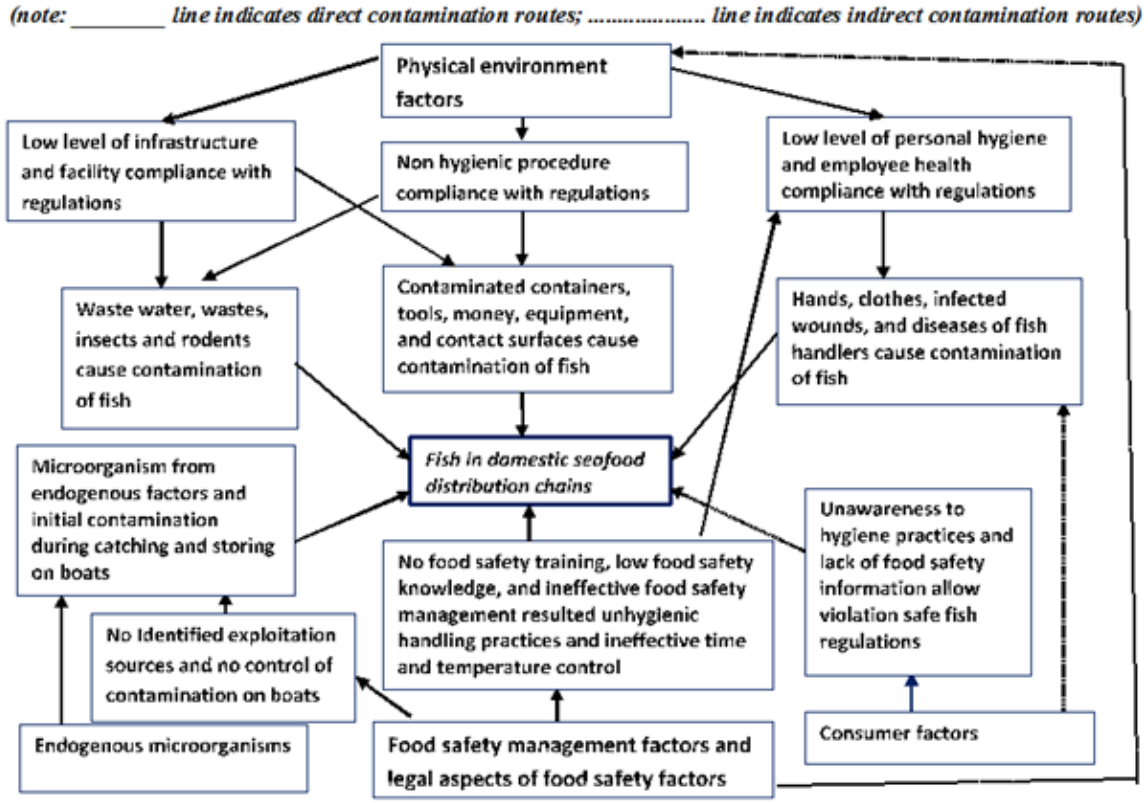
Consumer factors

Other factors that influence motivation for improving fish safety come from the consumer. In the current situation of high contamination of fish in the DFDCs, these results may show that consumer awareness of fish hygiene and safety is limited. Therefore, it may be a barrier to improving food safety for the DFDCs. Education in this regard will be one of the strong driving forces for improving food safety in the DFDCs. The role of consumer associations should be increased in order to develop their more independent and constructive voices in the future.

5. CONCLUSION

In the study, the distribution chain has been mapped from selected fishing ports to local retail markets. Operations related to raw fish and fish distribution have been identified. After landing, the fish raw material has to go through several stages, including fishing ports, traders' establishments, and wholesale markets, before entering retail markets. This study identified a number of issues in the implementation of safe fish handling within the domestic fish distribution chains, and these issues have provided the mechanism of microbiological contamination factors and their routes. It is of prime importance to analyse the food safety problems along the whole distribution chain and to suggest changes in order to improve fish safety for Vietnamese people.

Figure 2: Diagram of mechanism of microbiological contamination factors and their routes



REFERENCE

1. Le, S. X., & Nguyen, Q. T. K. (2012). *Household consumption of food fish in the Mekong Delta, Vietnam*. Paper presented at the Fisheries conference 5th Cantho University.
2. Lem, A., Tietze, U., Ruckes, E., & Anrooy, R. v. (2004). Fish markets and credit in Vietnam. In F. a. A. O. o. t. U. Nations (Ed.). Rome.
5. MARD. (2008). Identification of fish post-harvest research and development priorities in Vietnam. In P.-h. a. M. C.-F. S. P. Support (Ed.). Hanoi.
6.Vo, L. T. T. (2003). Quality management in shrimp supply chain in the Mekong Delta, Vietnam: Problems and measures. In A. C. (Ed.). Hanoi, Vietnam: Centre for International Management and Development Antwerp and Centre for ASEAN studies.
7. Pham, V. T. H. (2008). Overview of the seafood supply chain in Vietnam. In Institute for Fisheries Economics and Planning (Ed.), *Reform of the trade policy and fisheries, supply chain restructuring, improving consumer demand for sustainable management of fisheries: a case study in the Vietnam*. Hanoi: Institute for Fisheries Economics and Planning.
8. Ruddle, K. (2011). "Informal" Credit Systems in Fishing Communities: Issues and Examples from Vietnam. *Human Organization, 70*(3), 224-233.
9. Tuong, L. P., Pham, T. N., Nguyen, T. T. D., Duong, T. L., & Pham, V. H. T. (2009). Fisheries subsidies, supply chain and certification in Vietnam. Hanoi: United Nations Environment Programme.
10. Luu, P. H. (2015). *Compliance with seafood safety standards within the Vietnamese seafood distribution chain.* (PhD Traditional thesis), Queensland University of Technology, Queensland University of Technology.
11. Luu, H. P., Dunne, P. H., Pearse, W., Davies, B. (2016). Seafood safety compliance with hygiene regulations within Vietnamese domestic distribution chains. *British Food Journal* 118(4), doi:10.1108/BFJ-07-2015-0234.

FACTORS AFFECTING FOOD SAFETY PRACTICES OF SEAFOOD DISTRIBUTORS WITHIN VIETNAMESE DOMESTIC DISTRIBUTION CHAINS.

Phuc Hong Luu, Phan Thi Thanh Hien
Department of Food Technology, Nha Trang University, Vietnam.
Email:*phuclh@ntu.edu.vn*

1. Introduction

The government of Vietnam has recognised that ultimate responsibility for seafood safety lies with the distributors. In 2009 national technical regulations (QCVN) were developed and issued by the Ministry of Agricultural and Rural Development [1]. The regulations have a strong focus on the physical environment, personal hygiene and food safety training in an effort to protect food safety for consumers and to enhance the quality of Vietnamese seafood. However, emphasis is placed on food handler behaviour. Food safety standards are more likely to be improved when all factors affecting seafood safety, including food handling practices, are controlled [2].

There are few studies on the Vietnamese DSDCs. No systematic research has been conducted regarding the factors that affect food safety practices among domestic seafood distributors. Therefore, the aims of this research were to evaluate food safety practices of seafood distributors and investigate the associations between six interrelated factors including seafood distributor characteristics; training in food safety; knowledge of food safety; attitudes to food safety; concern of managers, co-workers, and consumers about food safety; and the working environment; on the food safety practices of seafood distributors in the DSDCs in Vietnam.

2. Materials and methods

Qualitative in-depth interviews were conducted with 11 participants including seafood handlers, retailers, and owners or traders in the DSDCs and food safety experts. Four focus group discussion were held, involving a total of 30 participants including seafood handlers, retailers, and owners or traders. In-depth interviews and focus group discussions were held to identify factors that would be most relevant for inclusion in the questionnaire and to explore how to use terminology/words in the survey questionnaire that are appropriate for fish distributors in Vietnam.

The questionnaires were developed using information from literature and the interview and focus group findings. The questionnaires were administered orally in-person, to 180 participants including 30 middlemen traders, 60 seafood handlers, and 70 retailers between May to June 2013. The participants were working in 6 fishing ports, 9 fish markets and 32 trading establishments in Khanh Hoa province, Ba Ria Vung Tau province, and Ben Tre in Vietnam. The participant were randomly selected on each day of data collection and participation was voluntary.

The questionnaires consisted of seven parts. Part one included questions about demographics (e.g. age, gender, income from their current job, education level, type of settlement, and number of years in the seafood business) and whether the participant had experienced a fish-borne illness. Part two explored prior training and awareness of procedure manuals for food safety and hygiene in the DSDCs.

Part three assessed knowledge of microbiologic hazard development, identifying fish contamination and fish-borne illness, knowledge of safe temperatures, and personal hygiene. Part four of the questionnaire focused on the participants' attitude toward seafood safety and hygiene. Part five of the questionnaire related to the concern was shown by managers, co-workers and consumers about food

safety and hygiene. Part six focused on satisfaction with working conditions and jobs including appropriate physical environment; cleanliness and sanitisation of structural environments; time pressure; and satisfaction with their job in terms of job character and organisational matters.

The final part of the questionnaires assessed participants' habits.

Data analysis

Statistical analysis was performed using SPSS (version 21) for Windows for all variables. Identification of bivariate associations between food safety practices and factors utilised correlation analysis, with Pearson's *r* for continuous factors (such as food safety attitude, satisfaction of working environment and job), Point-bi-serial correlation coefficients were used for dichotomous factors (such as gender), and a Spearman correlation was used for categorical variables with more than two levels (such as food safety knowledge and "work as"), respectively.

3. Result

Characteristics of the sample: Of the 180 participants, 66.7% were female and 33.3% were male. The majority of participants (65.5%) were 18 – 40 years of age and 68.9% of them had attained a secondary school level of education. On average participants had worked in the seafood industry for 6.5 years (range 4 -7 years). A large proportion of participants (95.6 %) classified themselves as permanent workers.

Food safety practices: Overall 60.5 % of participants 'rarely' implemented personal hygiene practices expected of food handlers. More than half of the respondents (58.6 %) said they 'rarely' conducted activities that would prevent contamination of fish. Approximately half of the participants said they 'sometimes' controlled time and temperature during fish handling.

Food safety training: Nearly all participants (97.2%) received some job-related training when they commenced. However, no participants (0%) had received training regarding food safety and hygiene.

Knowledge of food safety: Overall, the food safety and hygiene knowledge of seafood distributors was poor. The majority of participants selected the incorrect answer or identified that they did not know the answer for most of the food safety topics.

Attitudes to food safety risks and controls: It was found that only 18.3% of participants believed food safety was an important responsibility in their job, with the majority disagreeing with this statement (51.7%) or expressing uncertainty (30.0%). Similarly, only 21.1% of participants believed that it was important to learn more about food safety, with the majority disagreeing (50.5%) or expressing uncertainty (28.3%).

Concern of Managers, co-workers, and consumer about food safety: Most of respondents were "never" and "rarely" told or encouraged to implement safe food handling practices by managers/authorities, co-workers, or consumers. Indeed, no respondents said they were "often" or "always" reminded to employ food safety practices.

Participants' satisfaction of with their working conditions, work pace and job: The participants reported satisfaction with their working conditions, work pace and the job in general with a mean from 4.93 to 5.32. This may indicate that fish distributors are uncertain about satisfaction with their job.

Bivariate associations: Results from the statistical analysis regarding factors that may be related to food safety practices among seafood distributors have been examined widely. Most variables examined had no statistical significant relationship with total scores for food safety and hygiene practices with the exceptions being consumers who returned products due to food safety, which resulted in significant differences ($P < .05$); gender of seafood distributors ($P < .05$); duration of work (employment) ($P < .05$), and job position ($P < .01$).

Discussion

Food safety practices: The study also revealed that most of the seafood handlers disregarded illnesses, such as sore throats with fever, diarrhoea, or skin infections, and continued to work. Infected food handlers are well known sources for the transfer of enterotoxigenic pathogens (e.g. enterotoxigenic *E. coli* (ETEC)), and harmful viruses (e.g. Hepatitis A) to food [3]. By disregarding illnesses, seafood distributors increase the risk of food-borne illnesses to their consumers from their products.

More than half of the seafood distributors reported that they 'rarely' washed fish containers, working surfaces, tools and equipment with detergent/disinfectant/sanitiser, even though these tasks must be implemented according to the regulations [1]. Extensive research has shown that poor cleaning practices result in accumulated bacteria which may pose risks if transferred to the food during storage or handling [4,5].

Time and temperature control are critical for limiting the growth of bacteria and therefore in ensuring raw seafood safety. The results of this study indicate many seafood distributors are not controlling time and temperature.

Food safety knowledge and attitudes: The findings of this study suggest that the food safety knowledge of seafood distributors is also very poor. In particular, most of the seafood distributors had little knowledge about personal hygiene and 95.6% of distributors could not correctly identify (i.e. answered incorrectly or did not know) how pathogenic microorganism contamination of fish can occur. The lack of participants' knowledge on critical topics such as personal health and hygiene may be why the food safety practices discussed in the previous section are so inadequate.

These results are not particularly surprising given no seafood distributors had participated in any food safety training courses. The majority of participants had completed other training relevant to their job, which may indicate business owners and managers place greater importance on other aspects of the business (e.g. worker safety, efficiency) than they do food safety.

The score for attitude toward fish safety responsibility and fish-borne illness causing disease seems to indicate negative attitudes in this area. This might explain the inadequate food safety practice of seafood distributors; that they take their health for granted and they view themselves as not at risk or threatened by potential fish-borne disease. The discrepancy between food safety and hygiene attitudes and food safety and hygiene practices found in this study, was also in agreement with the findings of other studies about food hygiene knowledge, attitudes, and practices in hospitals in Iran, Italy, and Turkey [6,7]. The results of this study may explain that the lack of food safety training may lead to the seafood distributors adopting the knowledge, attitudes, and practices or behaviours empirically based on their skills and experiences in daily working. Therefore they implement food safety practice as their own knowledge and experiences.

Attitude of managers regarding food safety: The findings of this study revealed that no information was given to workers by managers or authorities, which demonstrated concern for food safety or hygienic handling by seafood distributors. This situation explains the findings' that almost all seafood distributors did not know anything about regulations relating to food hygiene and safety or employees' health and hygiene and that 100% of seafood distributors did not attend any food safety training. Therefore, although food safety regulations have been developed by the Vietnamese government to reduce the risks of fish-borne illness [9], it may be more difficult for these regulations to be applied to seafood distributors due to the low food safety commitment of managers and authorities in the DSDCs.

Factor affecting the food safety practices: In the factor related to consumers, the more frequently consumers returned fish products to seafood distributors because of food safety matters, the higher the

scores for food safety and hygiene practice that were gained by seafood distributors. The association between food safety and hygiene practice and consumers returning products could suggest that consumers were the main factors that could affect the practices of seafood distributors. In the context of seafood distribution chains, effective trading, or profits, is the only concern for seafood distributors. Therefore, consumers are the main targets for their business. Implementation of the food safety and hygiene practices of seafood distributors may affect the willingness of consumers to buy, and seafood distributors, therefore, try to achieve better food safety practices in this context. This revealed that the increased awareness of consumers about food safety may be an important point to promote food safety and hygiene practices of seafood distributors, one that may lead to the motivation of seafood distributors to learn about food safety practices and enhance their food safety attitude.

Another significant difference between food safety and hygiene practice and duration work (employment) was also found in this study.

The last significant factor associated with food safety practice was job position. Retailers seemed to achieve a higher practice score compared to seafood handlers. This may be explained by the fact that retailers were always directly in contact with consumers at retail markets, and thus, they needed to be aware of better food safety practices. On the other hand, the seafood handlers always worked at handling the seafood at fishing ports and indirectly traded with consumers; therefore, their awareness of food safety and hygiene practice may be less attention than that of retailers.

4. Conclusion

The findings of this study clearly indicate that general knowledge and attitudes to food safety and hygiene were at low level in most of the seafood distributors. The poor food safety and hygiene practices of seafood distributors have been affected directly by their low levels of knowledge of food safety and hygiene and by the lack of commitment to food safety from managers and authorities within the DSDCs. Developing and implementing training in food safety for seafood distributors and having effective management commitments, are the first steps in building effective food safety management systems for the DSDCs. In addition to training knowledge and practice, education for food safety responsibility and regulation obligations of food distributors is necessary. One significant factor affecting food safety and hygiene practice, the consumer, should be emphasised in the strategies for improving food safety among seafood distributors in the DSDCs.

References

1. MARD. (2009b). *QCVN 02-01: 2009/BNNPTNT - "Fisheries Food Business Operators - General condition for food safety". Provision on National Technical Regulation No 02-01:2009/BNNPTNT dated of September, 2009.*: Retrieved 14 March, 2013 from http://www.government.vn/portal/page/portal/chinhphu/hethongvanban?class_id=1&_page=163&mode=detail&document.
2. Green, L. R., & Selman, C. (2005). Factors impacting food workers' and managers' safe food preparation practices: A qualitative study. *Food Protection Trends, 25*(12), 981-990.
3. World Health Organization, W. (1999). Strategies for implementing HACCP in small and/or less developed bussiness (Report of WHO consultation). Retrieved 28, March, 2014, from Food Safety Program, WHO, http://www.who.int/foodsafety/publications/strategies-haccp/en/

4. Harrison, W. A., Griffith, C. J., Ayers, T., & Michaels, B. S. (2003). Bacterial transfer and cross-contamination potential associated with paper-towel dispensing. *American journal of Infection Control, 31*(7), 387-392. doi: 10.1067/mic.2003.81
5. Kassa, H., Harrington, B., Bisesi, M., & Khuder, S. (2001). Comparisons of microbiological evaluations of selected kitchen areas with visual inspections for preventing potential risk of foodborne outbreaks in food service operations. *Journal of Food Protection, 64*(4), 509-513.
6. Ehiri, J. E., Morris, J. G., & Mcwen, J. (1997). Evaluation of food hygiene training course in Scotland. *Food Control, 8*(3), 137-147. doi: 10.1016/S0956-7135(97)00005-4
7. Rennie, M. D. (1995). Health education models and food hygiene education. *Journal of Royal Society of Health, 115*(2), 75-79. doi: 10.1177/146642409511500203
8. Buccheri, C., Casuccio, A., Giammanco, S., Giammanco, M., La Guradia, M., & Mamnina, C. (2007). Food safety in hospital: Knowledge, attitudes and practices of nursing staff of two hospitals in Sicily, Italy *BMC Health Services Research, 7*, 45-56. doi: 10.1186/1472-6963-7-45
9. Tokuc, B., Ekuklu, G., Berberoglu, U., Bilge, E., & Dedeler, H. (2009). Knowledge, attitude and self-reported practices of food service staff regarding food hygiene in Edirne, Turkey. *Food Control, 20*, 565-568. doi: 10.1016/j.foodcont.2008.08.013.

SEAFOOD SAFETY COMPLIANCE WITH HYGIENE REGULATIONS WITHIN VIETNAMESE DOMESTIC DISTRIBUTION CHAINS.

Authors*:* Phuc Hong Luu, Vuong Van Tran. Corresponding author: Email*:phuchongluu@gmail.com*
- Department of Food Technology, Nha Trang University, Vietnam.
Keywords: food safety compliance, seafood safety, microbiological quality, raw fish.

1. Introduction

Seafood is very susceptible to contamination throughout the distribution chain. In Vietnam, seafood was the second highest cause of recorded food poisoning events during 2007 – 2013 (1). The government of Vietnam has recognised and attempted to address issues about seafood safety. Since 1998, regulations have required national registration of seafood traders and compulsory implementation of food safety regulations (2). Unfortunately, there are only a few studies that examine the extent of compliance with food safety regulations in Vietnam, especially in the seafood industry.

Whilst there is limited literature regarding compliance in the Vietnamese DSDCs, relevant studies have been conducted in other countries. The aims of this study were to determine the levels of compliance with government regulations in terms of physical environments and documents related to the hygiene procedures of the DSDCs, and to evaluate the food safety practices of fish distributors in the DSDCs. Additionally, the microbiological quality of raw finfish at points along the distribution chains has been assessed and compared with the national standards.

2. Methodology

The study assessed three components of the DSDC, infrastructure conditions and documents related to hygiene procedures, handling practices of seafood handlers, and microbiological quality of finfish.

Checklist method for assessing infrastructure conditions and documents related to hygiene procedures: The infrastructure assessment included three stages: development of the checklist, pilot study, and main survey. The compliance level of buildings, structures, and facilities was determined in the first part by visually inspecting them compared to the requirements in government regulations.

Notational analysis method for assessing handling practices of seafood handlers: The workers' food handling and hygiene practices were observed directly. The method used for observation was Notational Analysis, as these workers were handling and preparing raw fish for sale. The notational analysis technique was used to record the behaviour of food employees by Clayton and Griffith (2004) (3), who adapted the technique from the field of sports science. This method focuses on actions related to contamination. Fish handlers chosen for observation were randomly selected on each day of data collection. In total, 135 fish handlers were observed across 6 fishing ports, 10 trading establishments, and 8 fish markets; 60 handlers were working in fishing ports and establishments, and 75 were working in retail fish markets.

Assessment of microbiological quality of finfish: One hundred and thirty-five samples (n = 135) were purchased from six fishing ports and nine fish markets in in Khanh Hoa province, Ba Ria Vung Tau province, and Ben Tre province in Vietnam. Of these, 60 fish samples were taken from fishing ports and 75 from fish markets. The samples collected at the markets were 'tracked' from the fishing ports at the loading area and accompanied to the fish markets. Methods described in the US Food and Drug Administration (FDA) – Bacteriological Analytical Manual Online (4) were used in the bacteriological tests.

3. **Results and discussion**

Assessment of infrastructure conditions and documents related to hygiene procedures: It can be seen from Table 1 that non-compliances were observed at all fishing ports, trading establishments, and fish markets. The percentage of compliant sub-items in the building modules was the highest at 20.3%. Serious non-compliances at fish markets and trading establishments was only 6.4% and 10.1% respectively. In the facility module, the percentage of non-compliant and seriously non-compliant sub-items was quite high at fishing ports, trading establishments, and fish markets. There was notably no or a very low percentage of compliant sub-items in the surveyed places.

Similar to building module and facility module, the percentages of compliant sub-items in the documents related to hygiene procedures was low, i.e. 2.5% and 0.3% for fishing ports and fish markets respectively; whereas, the percentages of seriously non-compliant sub-items was quite high for fishing ports, fish trading establishments, and fish markets.

In general, the assessment of infrastructure, documents and records related to hygiene procedures in fishing ports, trading establishments, and markets has revealed a large number of indicators of non-compliance and serious non-compliance with government regulations. The findings of this study are similar to that of Lem *et al.*, (2004) (5) who point out that the poor infrastructure of distribution chains has affected the quality and prices of fish. The reports from MARD and several other reports also claimed that the hygiene conditions of fishing ports and fish markets were problems that needed to be addressed and improved (6,2,8) in unhygienic environments with poor personal hygiene or health management is likely to lead to the presence and growth of microorganisms and to an increase in the risks of contamination of raw fish in the distribution chains.

Table 1: The percentage of compliant sub-items evaluated using a checklist in fishing ports, trading establishments, and fish markets

Places (n = number of places)	**Building** (number of sub-items)			**Facilities** (number of sub-items)			**Documents related hygiene procedures** (number of sub-items)		
	CP (%)	NCP (%)	SNCP (%)	CP (%)	NCP (%)	SNCP (%)	CP (%)	NCP (%)	SNCP (%)
Fishing ports (n =6)	20.3	51.0 (16 sub-items)	28.7	-	31.3 (18 sub- items)	68.8	2.5	23.5 (27 sub-items)	74.1
Fish trading establishments (n = 32)	18.4	71.5 (9 sub-items)	10.1	2.3	71.5 (12 sub-items)	10.1	-	3.4 (30 sub-items)	96.6
Whole sale and retail fish markets (n = 12)	18.0	75.6 (13 sub-items)	6.4	1.8	39.9 (15 sub-items)	58.3	0.3	12.3 (27 sub-items)	87.3

CP – Compliance; NCP – Non-compliance; SNCP – Serious non-compliance

Assessment of estimates of seafood handling practices: The majority of times fish handlers contaminated their hands involved “touching floor”, “touching contaminated tools and utensils”, and “touching other contaminated surfaces”. Other acts likely to cause contaminated hands were “after coughing, sneezing, using tobacco, eating”, “after touching bin/waste”, “after touching bare body parts”, and “after using toilet room”.

Results were treated as average hand contamination frequency per fish handler. The average number of times hand contamination occurred per fish handler with gloved hands was the highest number as the fishing ports (51.08 times). However, the average number of times fish handlers attempted to wash their hands per fish handler was the lowest at 0.06 times.

Important findings of this research include the high number of times that hands or tools were contaminated and the very low number of times fish handlers' washed their hands or cleaned tools and work surfaces. The high number of occurrences of hand and tool contamination may be explained by two factors: firstly, most fish handlers at fish markets and fish handlers undertaking sorting at fishing ports were observed to be sitting on low chairs, therefore, their hands and/or tools were frequently in contact with the floors; moreover, due to unhygienic conditions surrounding fish handlers such as accumulated solid waste and wastewater on the floor, the hands of fish handlers or tools frequently made contact with contaminated surfaces. Secondly, the fish handlers may not be aware of microbiological hazards associated with fish, therefore, the risks of touching contaminated objects or putting tools on contaminated surfaces are not understood and are normal practices.

The low frequency of washing hands or tools after they become contaminated may result from two main factors. Firstly, lack of awareness hazards for fish from contaminated hands or tools may result in fish handlers' failure to wash their hands or tools. Secondly, even if the handlers do understand the hazards associated with contamination, they could not implement hand washing frequently because of the unhygienic conditions surrounding them, and inadequate facilities and soap for personal hygiene and cleaning. Many researchers have shown that there are a number of factors that are barriers to good hand hygiene practices including that hygiene facilities may be inaccessible or insufficient (8). As previously noted, the hygiene facilities and water infrastructure in fishing ports, trading establishments, and fish markets are insufficient and inconvenient for workers.

Assessment of microbiological quality of fin fish: The mean APC for samples obtained from the ports were 5.31 log cfu/g, with a range of 4.16 to 6.91 log cfu/g, and for markets 5.21 log cfu/g, with a range from 3.09 to 6.88 log cfu/g (Table 2). According to microbiological standards for raw fish accepted by the Ministry of Health in Vietnam (2007) (10) the APC level should not exceed 6 log cfu/g. Of the 60 samples from the fishing ports and 75 samples from the fish markets, 10% and 12% respectively had APC levels that exceeded the standard.

The coliform and faecal coliform levels in the fish samples obtained from the fishing ports and fish markets are summarised in Table 3. The mean coliform counts for fish in the ports and markets were 167.17 and 124.86 MPN/g, respectively. The mean faecal coliform was 84.05 MPN/g in ports and 99.27 MPN/g in markets. All fish samples were positive for coliform and faecal coliform contamination. In the 60 samples from fishing ports, 26.7% of the samples did not comply with microbiological standards used. Similarly, in the 75 samples from fish markets, 14.7% did not comply with the standards for coliform levels.

Table 2: Prevalence of APC, *Cl. Perfringen*, and *Salmonella* spp. in raw finfish from fishing ports and local retail markets

No. of samples in place	Microbiological test	Percentage (%) of samples in the indicated intervals (log cfu/g)								Range (log cfu/g)	Mean (log cfu/g)	No of positive samples (%)
		ND	0-1	1-2	2-3	3-4	4-5	5-6	>6			
60 in fishing ports	APC						40.0	50.0	10.0	4.16-6.91	5.31	
	Cl. perfringen	20.0	5.0	61.7	13.3					ND-2.72	1.67	
	Salmonella	95.0										5.0
75 in markets	APC					8.0	34.7	45.3	12.0	3.09-6.88	5.21	
	Cl. perfringen	29.3	5.3	49.3	16.0					ND-2.81	1.72	
	Salmonella spp.	88.0										12.0

Note: ND: not detected.

Table 3: Prevalence of Coliform, Fecal coliform, and *S. aureus* in raw finfish from fishing ports and local retail markets

No. of samples in place	Microbiological test	Percentage (%) of samples (MPN/g)						Range (MPN/g)	Mean (MPN/g)	No of negative samples (%)
		< 3	3-10	10-10^2	10^2-10^3	10^3-1100	> 1100			
60 in fishing ports	Coliform		1.7	56.7	35.0	5.0	1.7	5.47 - >1100	167.17	
	Fecal coliform		13.3	63.3	21.7	1.7		5.43 - >1100	84.05	
	S. aureus	6.7	6.7	78.3	8.3			<3 – 264.3	45.14	6.7
75 in markets	Coliform		5.3	74.7	13.3	5.3	1.3	6.40 - >1100	124.86	
	Fecal Coliform		10.7	74.7	9.3	4.0	1.3	6.53 - >1100	99.27	
	S. aureus	8.0	5.3	70.7	16.0			<3 - 404	65.88	8.0

C. perfringens was found in 80 and 70.7 percent of samples and with a mean count of 1.67 and 1.72 log cfu/g from the fishing ports and fish markets, respectively (Table 2). According to the microbiological standard of Vietnam (Ministry of Health, 2007), among the samples from fishing ports and fish markets analysed, 13.3% and 16.0% of the samples respectively exceeded 2 log cfu/g and therefore are considered of unacceptable microbiological quality.

Staphylococcus aureus was isolated in 93.3% and 92% of fish samples with a range of less than 3 to 264.3 MPN/g and less than 3 to 404 MPN/g from fishing ports and fish markets, respectively (Table 5). The percentage of the samples from the fishing ports and fish markets that exceeded the microbiological standards of Vietnam (10) was 8.3% and 16% respectively.

Contamination with *Salmonella* was detected in 5% of samples from the fishing ports and 12% of samples from fish markets (Table 4). According to the microbiological standard of Vietnam (Ministry of Health, 2007), the presence of *Salmonella* spp. in 25 g samples of raw fish is regarded as unacceptable.

Overall, approximately 42 percent and 39 percent of the samples from the fishing ports and local retail markets respectively were classified as unacceptable based on the microbiological standards of Vietnam. The unacceptable results were due mainly to high levels of coliforms. Some of the unacceptable samples were due to the high levels of *C. perfringens* and *S. aureus* in them, along with the presence of *Salmonella*. A previous study by Ha and Pham (2006) found that 20 percent of fish samples used at canteens in hospitals, factories and schools were unacceptable. The study by Ha and Pham (2006) found lower percentages of unacceptable samples compared to this study. Seasonal differences, different methods of fish preservation and other methodological differences may account for the different results. However, the conclusions that there are high levels of pathogenic bacteria contained in fish from local markets is consistent.

5. Conclusion

This study found most aspects of physical infrastructure and documentation related to hygiene procedures in fishing ports, fish trading establishments, and fish markets in the Vietnamese DSDCs are noncompliant. The practices of fish handlers were determined as creating a high risk of contamination of raw fish due to hand contamination and work surface and tool contamination. The study revealed unacceptable microbiological quality in most fish samples from ports distributing to markets through different distribution chains. Working in unhygienic environments and the poor hygiene practice of distributors may promote contamination. The authorities and domestic fish businesses should pay more attention to practical actions to solve these problems. To assist in this regard, strategies for improving food safety in the DSDCs in Vietnam and other settings have been recommended. Improving food safety in the DSDCs is a necessary and urgent matter for Vietnamese government agencies to consider.

References

(1) Vietnam Food Administration. (2015). *Report of Food safety and hygiene in Vietnam 2007 -2013*. Hanoi: Vietnam Food Administration.
(2) Ministry of Agricultural and Rural Development (MARD). (2008). *Identification of fish post-harvest research and development priorities in Vietnam* available at: www.fishenet.gov.vn (accessed 15 March, 2012).
(3) Clayton, D. A., & Griffith, C. J. (2004), "Observation of food safety practices in catering using notational analysis" *Bristish Food Journal*, Vol *106*, *No* 3, p 211-227. doi: 10.1108/00070700410528790
(4) Food and Drug Administration (FDA). (2011). "Bacteriological analytical manual online", available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm (accessed 15 March, 2012).
(5)Lem, A., Tietze, U., Ruckes, E., & Anrooy, R. v. (2004). Fish markets and credit in Vietnam. In F. A. O. Nations (Ed.). Rome.
(6) Doan, A. V. (2010), "Solutions and invesments for fishing ports". *Vietnamese Journal of Resoures and Enviroments*, Vol *5*, p 28.
(7) Nguyen, Q. (2014), "De Gi fishing ports: no environment management" *Dan Viet (vietnamese Journal)*.
(8)Green, L. R., Radke, V., Mason, R., Burshnell, L., Reimann, D. W., Mack, J. C., . . . Selman, C. A. (2007). Factors Related to food worker hand hygiene practices. *Journal of Food Protection, 70*(3), 661-666. Retrieved from www.cdc.gov
(9) Ha, T. A. D., & Pham, T. Y. (2006), "Study of *Salmonella*, *Campylobacter*, and *Escherichia coli* contamination in raw food available in factories, schools, and hospital canteens in Hanoi, Vietnam", *Annals New York Academy of Science*, Vol 1081, 262-265. doi: 10.1196/annals.1373.033.
(10)Ministry of Health, (2007). *Standard for microbilogy and chemical contamination in food - No 46/2007/QD-BYT* Hanoi: Ministry of Health in Vietnam, available at: http://vfa.gov.vn/van-ban-phap-luat/ban-hanh-quy-chuan-ky-thuat-quoc-gia-doi-voi-o-nhiem-vi-sinh-vat-trong-thuc-pham-164.vfa. (accessed 13 April, 2012)

SHELF-LIFE EVALUATION OF FRESH CATFISH (*PANGASIUS HYPOPHTHAMULS*) FILLETS AT DIFFERENT STORAGE TEMPERATURES

Tong Thi Anh Ngoc[a*] *and Ly Nguyen Binh*[a]

[a]*Department of Food Technology, College of Agriculture and Applied Biology, Can Tho University, Vietnam*

[*]*Corresponding author e-mail address: ttangoc@ctu.edu.vn*

Abstract

The freshness of fish is a big concern to fishery stakeholders. To improve the quality of fresh fish, it is important to evaluate microbiological, physicochemical and sensory changes for further determination of shelf life of fish during storage. Therefore, the fillets of catfish (*Pangasius hypophthamuls)* stored at 0, 4, 8, 12^{o}C were assessed at the regular time intervals. The changes of quality were strongly dependent on the storage temperatures. The shelf-life of catfish fillets stored at 0, 4, 8, 12^{o}C was therefore prolonged up to 21, 11, 7 and 3 days, respectively. In addition, total volatile basic nitrogen correlated well (r = 0.953) with the total microbial counts while the total microbial counts also correlated well (r = 0.905) with lactic acid bacteria. However, the weak correlation was shown between the total volatile basic nitrogen and the lactic acid bacteria (r = 0.087). These results obtained can be used as a reference tool to improve fishery quality management and to minimize the economic losses as well.

Key words: *Pangasius hypophthalmus*, fresh catfish fillet, quality changes, shelf life, temperature

1. Introduction

Pangasius hypophthalmus (Tra fish or catfish) is a good source of various nutrients namely protein quality, fatty acid, minerals, etc. that required for human health[1-4]. This has become appreciated by consumers from different markets all over the world. Up to date, *Pangasius* products, particularly frozen forms, are exported to 145 countries[5]. Besides frozen *Pangasius*, thawed *Pangasius* fillets considered as (re)fresh fillets have become increasingly popular in exported countries.

Product quality and shelf life of fishery products, rapid deterioration of fish is mainly caused by microbiological spoilage leading to reduced shelf life and economic loss. Microbiological spoilage can be manifested in visible growth, textural changes or off-odors and off-flavors[6]. The production of these off-odors depends not only on the intrinsic characteristics related to chemical composition of fish, but also on the extrinsic parameters of storage temperature[7]. In general, fish is kept in chilled storage and preferable on ice. The storage temperature influences the growth of spoilage microbiology. More specifically, the growth of *Pseudomonas* spp., *Shewanella putrefaciens*, and *Psychrobacter immobilis* were promoted under chilled and iced condition[8]. In contrast, abusive temperature can create an optimal environment for strong microbiological spoilage such as *Photobacterium phosphoreum* or other microorganisms which are able to produce biogenic amines[7].

In addition to temperature, storage conditions (i.e. aerobic storage, vacuum, modified atmosphere packaging) also impact on the shelf life of fish products. Modified atmosphere packaging (MAP), which employs elevated CO_2 and /or reduced O_2 concentration, is commonly applied storage method to extend the shelf life of fishery products. So far, only one study determined the shelf life of thawed *Pangasius* fillets stored under different MAP conditions at 4^oC[9]. On the other hand, the commercial aquaculture of *Pangasius* is still a young industry in Vietnam, which partly explains the little information concerning the shelf life and the quality of *Pangasius* during storage, particularly with the fresh *Pangasius*. The objective of the present study was to investigate the effect of temperature conditions on microbiological, physicochemical and sensory quality of fresh *Pangasius* fillets during storage.

2. Materials and method

2.1 Experimental set-up

The fillets of *Pangasius hypophthalmus* were bought from a company located in Hau Giang province processed *Pangasius* for export. After purchasing, the *Pangasius* fillets were stored in ice and transported in insulated boxes to the Laboratory of Microbiology and Biotechnology of Food Technology Department, Can Tho University for further storage experiments within 1 hour. Each fillet ranged from 150 to 200 g was then packed aseptically in PA (polyamide) package combined with vacuum level of 60% by means of vacuum packaging machine (Tecnovac 5100H, Italy). The size of 19 ×16 cm and thickness of 85 µm PA package was applied to the samples packed. The PA package was supplied by Cam Dao Company (District 3, Ho Chi Minh, Vietnam). The control samples were fillets without packaging, stored under air. The packaged fillets were stored in a refrigerator at 0, 4, 8, 12 ± 1.0°C for a period of 25 days. On a regular basis (day 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 of storage), *Pangasius* samples were randomly selected for the assessment of pH, the total volatile compound content, microbiological quality and sensory quality.

2.2 Analytical methodology

On a regular basis of storage periods, different parameters of pH, total volatile basic nitrogen content, microbiological analysis and sensory quality were determined in triplicate. The analytical methodology was based on TCVN 8338:2010[10]. pH was measured with a pH-electrode (Mettler Toledo, Schwerzenbach, Switzerland). Total volatile basic nitrogen (TVB-N) was based on steam distillation of an alkalized samples and absorbed by boric acid solution (1%) and further titration using sulfuric acid solution (0.1N) described by Antonacopoulos and Vyncke [11]. For microbiological analysis, a 25g composite sample from different parts of each fillet sample was transferred aseptically to a stomacher bag by means of sterile scalpels and tweezers. 225ml of sterile Maximum Recovery Diluent (MRD, Merck, Darmstadt, Germany) was added and the mixture was homogenized for 1 minute. Further decimal dilutions were prepared in MRD. The total mesophilic counts and lactic acid bacteria counts were determined by pour plating the decimal dilutions on Plate Count Agar (PCA, Merck, Darmstadt, Germany) and on de Man Rogosa Sharp agar (MRS, Merck, Darmstadt, Germany) (with an additional over layer), respectively. The pour plates were incubated for 3 days at 37°C to determine the total mesophilic and lactic acid bacteria counts. With regards to sensory evaluation, there were nine panelists participated in the sensory tests. The sensory evaluation was assessed using a scoring scale from 1 to 5 including odor, color and texture[12].

2.3 Statistical analysis

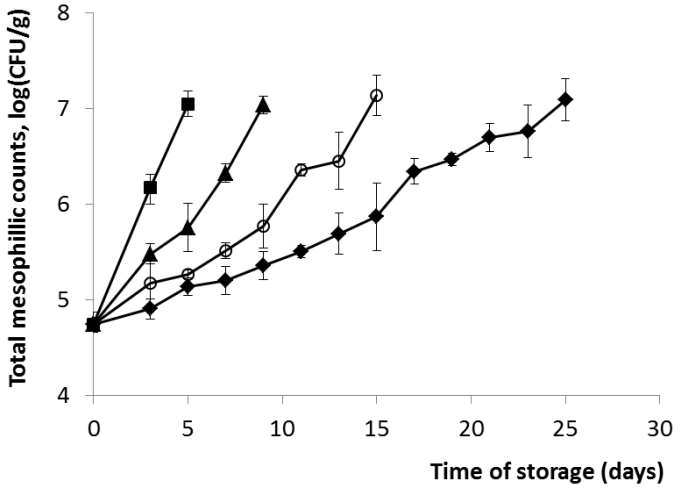
All experiments were performed in triplicate. The results of microbiological analysis of the fillets were expressed as log CFU/g. Results are reported as mean value ± standard deviation of these triplicate analyses. Cross-correlation were tested by means of the non-parametric Spearman rank order correlation coefficients (r) two tailed test ($\alpha = 0.05$) in SPSS version 20 (IBM Inc., Chicago, Ill., USA).

3. Results and discussion

The protein, lipid and water content of *Pangasius* fillets samples using in the present study were 16.23%, 1.71% and 80.56%, respectively (data not shown). It is a good source of nutrient for microbial growth and subsequently induced physicochemical and sensory changes.

3.1 Effect of storage at different temperatures on microbiological quality

A



B

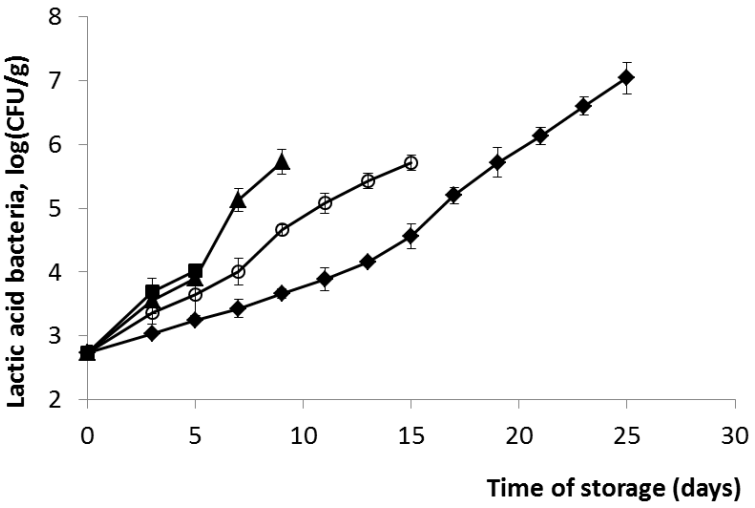
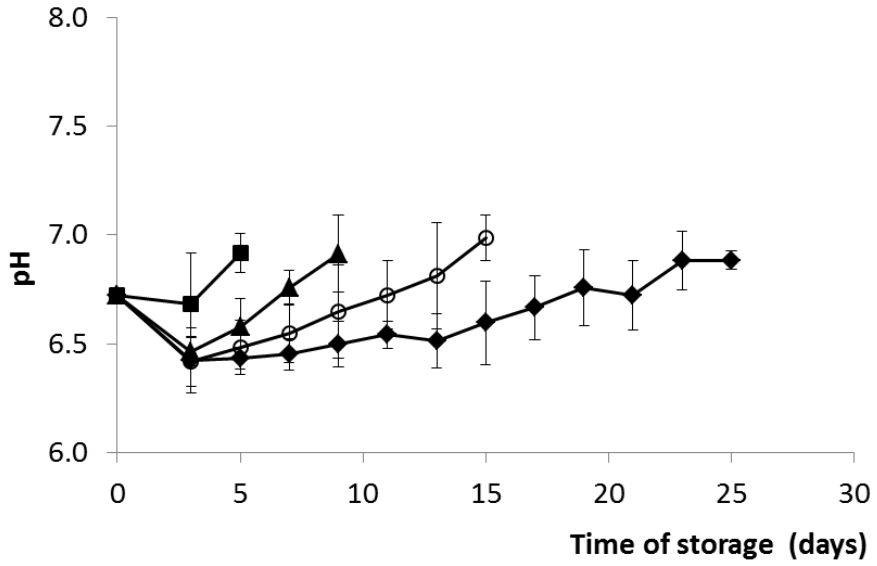


Figure 1. Evolution of total mesophillic counts (A) and lactic acid bacteria (B) of fresh *Pangasius* stored at 0°C (◆); 4°C (○); 8°C (▲) and 12°C (■). Bars represent the standard deviation of three independent samples

The evolution of total mesophillic counts and lactic acid bacteria is showed in Fig 1A and 1B, respectively. The growth rate of those microbial counts depended on the temperatures stored. During storage, the lower temperature would have resulted in the lower microbial evolution. The initial total mesophillic count was 4.7 ± 0.1 log CFU/g at day 0 and on day 5 increased to 5.1 ± 0.1, 5.3 ± 0.0, 5.8 ± 0.3 and 7.0 ± 0.1 log CFU/g on samples stored under 0, 4, 8 and 12°C, respectively. The initial total mesophillic count was consistent with the previous study of thawed *Pangasius*[9, 13].The total mesophillic count upper limit of acceptability, which is 7.0 log CFU/g for freshwater fish as *Pangasius*[14], was exceeded after 25, 15, 9 and 5 days of storage at 0, 4, 8 and 12°C, respectively. The use of low temperature extended significantly the rate of microbiological growth, mainly attributed to differences in the lag phases between 0-4°C and 8-12°C. On the contrary, the number of lactic acid bacteria appeared somewhat no lag phase during storage as the initial lactic acid bacteria was 2.7 ± 0.1 log CFU/g followed by a rapid increase to 3.2 ± 0.0; 3.6 ± 0.3; 3.9 ± 0.1; 4.0 ± 0.1 log CFU/g on samples after 5days storage under 0, 4, 8 and 12°C, respectively. The lactic acid bacteria may result in cross contamination and environmental contamination during processing[15]. The present study, *Pangasius* fillets were packed polyamide (PA) under vacuum of 60%. As a result, the lactic acid bacteria became dominant towards the end of shelf life. A strong correlation was observed between the total mesophillic counts and lactic acid bacteria (r = 0.905). The lactic acid bacteria which are tolerant to CO_2 considered to dominating spoilage organisms on MAP freshwater fish as *Pangasius*[9, 16].

3.2 Effect of storage at different temperatures on physicochemical quality

A



B

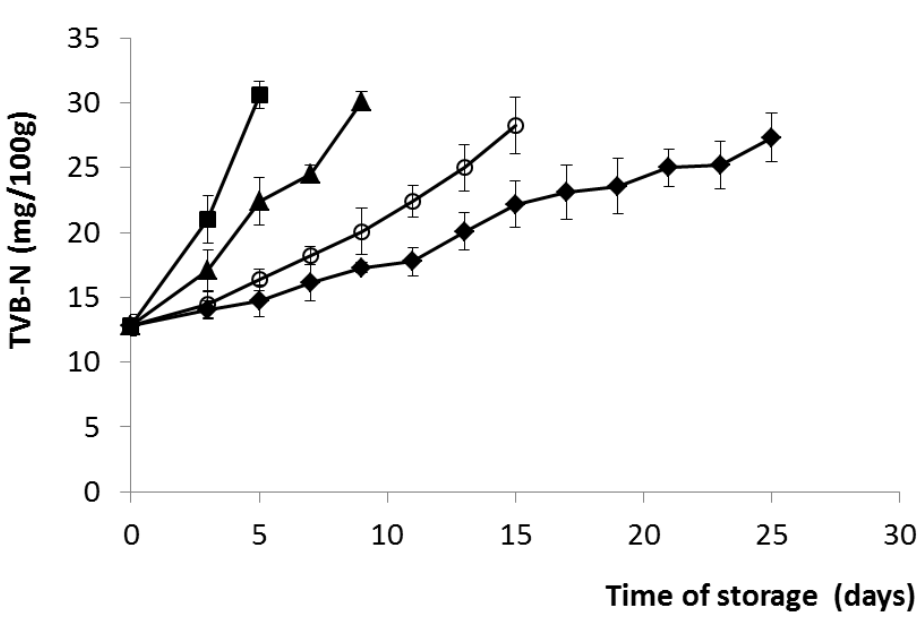


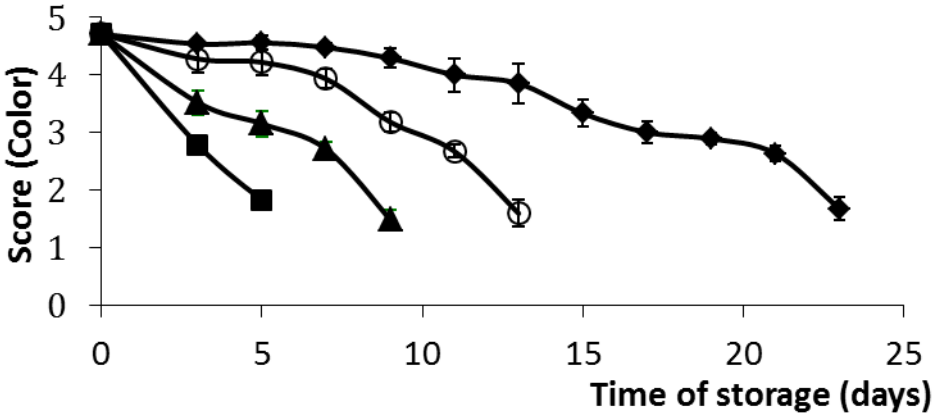
Figure 2. Evolution of pH (A) and total volatile basic nitrogen (TVB-N) (B) of fresh *Pangasius* stored at 0°C (◆); 4°C (○); 8°C (▲) and 12°C (■). Bars represent the standard deviation of three independent samples

The evolution of pH and TVB-N (total volatile basic nitrogen) content in *Pangasius* fillets samples stored at 0, 4, 8, 12°C is depicted in Fig 2 A and Fig 2 B, respectively. As can be seen in Fig 2 A, the initial pH was 6.72 and decreased slightly between 6.46 and 6.68. Thereafter, the pH increased throughout the storage period. The rates of pH change increased with the temperature storage, i.e. pH of 6.43 (at 0°C), 6.48 (4°C), 6.58 (8°C) and 6.92 (12°C) when *Pangasius* fillets stored at day 5. The total volatile basic nitrogen (TVBN) is a quality parameter indicating spoilage of fish during storage[17]. The TVB-N

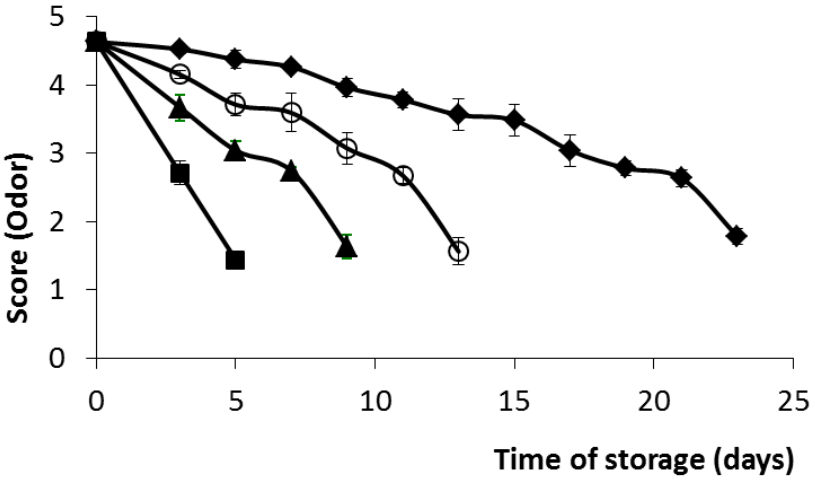
content of *Pangasius* samples kept under different temperatures is depicted in Fig 2 B. Similar results were observed in pH evolution, the TVB-N change was higher at high temperature than at low temperature. The value of TVB-N was 12.84 ± 0.81 mg/100g at day 0. At day 5, the TVB content increased to 14.71 ± 1.21, 16.34 ± 0.81, 22.41 ± 1.85 and 30.58 ± 1.07 mg/100g of *Pangasius* fish during storage at 0, 4, 8 and 12^{o}C, respectively. Afterward, they continued to increase to 27-31 mg/100g at the end of shelf life. The results obtained can be explained that microbiological growth with a shorter lag phase at higher temperature condition inducing the faster evolution of TVB-N. As the TVB-N content associated with fishery product spoilage, an increase in the TVB-N content in those samples indicated the stage of substantial spoilage of the muscle[18]. A noticed finding in the present study is the TVB-N content correlated well with the total mesophillic counts (r = 0.953). In contrast, a weak correlation was observed between the TVB-N content and lactic acid bacteria (r = 0.087). It is suggested that the TVB-N can be used as an index to predict the total microbial counts on *Pangasius* fish during storage.

3.3 Effect of storage at different temperatures on sensory quality

A



B



C

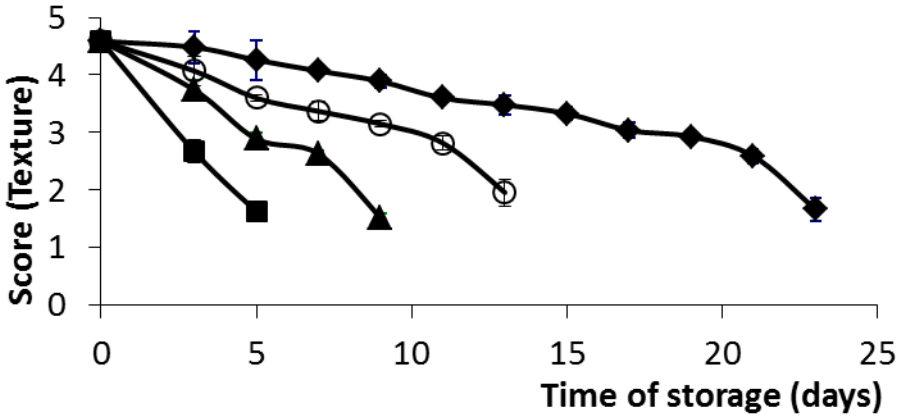


Figure 3. Sensory evaluation of color (A), odor (B) and texture (C) of fresh *Pangasius* during storage at 0°C (◆); 4°C (○); 8°C (▲) and 12°C (■). Bars represent the standard deviation of three independent samples

The results of sensory attributes (color, odor and texture) of *Pangasius* fillets are shown in Fig 3A, 3B, and 3C. Fresh *Pangasius* had white color, no off-odor and hard texture were therefore considered to possess very high acceptability (score of 4.6-4.7 at day 0). The sensory score for color, odor and texture of fresh fillets stored under different temperatures decreased with time of storage. Among them, the samples stored at 0°C were preferable with the highest score compared to those other samples stored at higher temperature (i.e. 4, 8 and 12°C) during storage time. The sensory quality, is normally applied in estimating the quality of fish, had a correlation with chemical and microbiological quality[19]. Therefore, in addition to the sensory quality, the limits of acceptability were based on chemical quality (i.e. TVB-N of 35 mg N/100g) and microbiological quality (i.e. total aerobic counts of 7 log CFU/g) as a tool to estimate the shelf life of fresh *Pangasius* during storage. The shelf-life of *Pangasius* fillets stored at 0, 4, 8, 12°C was up to 21, 11, 7 and 3 days, respectively.

4. Conclusion

Temperature had a great impact on the shelf life of *Pangsius* fillets stored in PA packaging with vacuum level of 60%. The use of low temperature might retard the microbiological and physicochemical changes, leading to decrease spoilage and prolong the shelf life of fresh *Pangasius* fillets. The *Pangasius* stored at 0°C was observed the optimal temperature for shelf life extension. More importantly, the present study shows that TVB-N value is valid quality index for prediction the microbiological quality of *Pangasius* fillets.

5. Acknowledgments

This research was funded by NAFOSTED, Vietnam (FWO.2011.32). We would like to thank Ms. Nguyen Thi Nghi for conducting experiment and Le Duy Nghia for practical assistance.

6. References

1. Karl H, Lehmann I, Rehbein H, Schubring R. International Journal of Food Science & Technology. 2010;45:56-66
2. Szlinder-Richert J, Usydus Z, Malesa-Ciećwierz M, Polak-Juszczak L, Ruczyńska W. Chemosphere. 2011;85:1725-1733
3. Usydus Z, Szlinder-Richert J, Adamczyk M, Szatkowska U. Food Chemistry. 2011;126:78-84
4. Ruiz-de-Cenzano M, Beser U, Cervera M, De la Guardia M. Ecotoxicology and Environmental Safety. 2013;95:195-201
5. VASEP. http://www.pangasius-vietnam.com/378/Daily-News-p/About-Pangasius.htm. Accessed on 15th August 2014. 2014
6. Gram L, Dalgaard P. Current opinion in Biotechnology. 2002;13:262-266
7. Olafsdottir G, Lauzon HL, Martinsdóttir E, Oehlenschläuger J, Kristbergsson K. Journal of Food Science. 2006;71
8. Gennari M, Tomaselli S, Cotrona V. Food Microbiology. 1999;16:15-28
9. Noseda B, Islam MT, Eriksson M, Heyndrickx M, De Reu K, Van Langenhove H, Devlieghere F. Food Microbiology. 2012;30:408-419
10. TCVN. 2010; Reference number: TCVN 8338: 2010
11. Antonacopoulos N, Vyncke W. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 1989;189:309-316
12. Meenakshi V, Narayanan K, Venkataraman R. Journal of Biomedical Sciences and Research. 2010;2:254-257
13. Tong Thi AN, Samapundo S, Devlieghere F, Heyndrickx M. International Journal of Food Contamination. 2016;3:17
14. ICMSF. Kluwer Academic/Plenum Publishers, Londen, Great Britain. 2002
15. Tong Thi AN, Noseda B, Samapundo S, Nguyen BL, Broekaert K, Rasschaert G, Heyndrickx M, Devlieghere F. International Journal of Food Microbiology. 2013;167:144–152
16. Arkoudelos J, Stamatis N, Samaras F. Food Microbiology. 2007;24:728-735
17. Connell J. Control of fish quality. 1990;2
18. Masniyom P, Benjama O, Maneesri J. International Food Research Journal. 2013;20:1401-1408
19. Karungi C, Byaruhanga Y, Muyonga J. Food Chemistry. 2004;85:13-17