"Characterization of Salt-fermented Shrimp Paste from the Philippines"

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Ghent, 21 August 2013

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The author

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Anna Rose Pilapil
Preface

Fish is a food. Indeed! But looking for a thesis topic appropriate for a person with a fisheries background for a degree in the major field of food technology was not an easy job. You need to fit yourself to apples, dough, chocolates, or creams when, in the first place, you’ve been working on fishes. Not to mention the hard work needed to cope up with the relatively new courses you need to complete for two years.

But thanks to Prof. Katleen Raes and Prof. John Van Camp for offering us a free topic - any subject related to indigenous fermented products known to the student. I got to see the light at the end of the tunnel for my search. But I should not forget the person who led me to that tunnel. Special thanks to my friend Jihan.

This thesis is about the salt-fermented shrimp paste from the Philippines. Filipinos called it bagoong alamang, a mouth-watering condiment usually eaten with unripe mangoes or added to cuisines. This work is an extensive characterization of the product added with a fermentation simulation done in lab scale. To some point, this is a benchmark study for this particular fermented product but is also an additional literature for its scarce written resources.

This Master’s degree, along with this thesis, sprung out from a humble ambition of upholding development in the fish technology department of the College of Fisheries of Mindanao State University (MSU-Main Campus) – my Alma Mater and Employer. And most importantly, the completion of this thesis is a visible proof of what prayers and grace can do.
Acknowledgment

"For I know the plans I have for you," declares the LORD, "plans to prosper you and not to harm you, plans to give you hope and a future." - Jeremiah 29:11

"You are my hiding place; you will protect me from trouble and surround me with songs of deliverance." - Psalm 32:7

First and foremost, big thanks to the government of Belgium and VLIR-UOS for providing financial assistance for my graduate studies in the whole duration of my stay here in the country.

My sincere gratitude to my promoter, Professor Katleen Raes for granting and allowing me to work on this topic. Thank you for diligently correcting my papers, and for the constructive suggestions.

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To Ellen V., Ferrielle, Bram and Maaike. You were my comrades in the lab. Thank you for every conversation and laughter we've shared. Thanks for opening parts of your life story to me. Thanks also to Nguyen for all the helpful suggestions and for allowing me to use some of your paraphernalia in the lab.

To Catalina and Zhou Zhou – thank you for the close bond of friendship that was formed throughout my thesis period. Thank you for staying late with me and sometimes helping me wash the glassware. I will surely miss those days.

My heartfelt appreciation to our IUPFOOD Coordinators, Prof. Marc Hendrickx, Prof. Koen Dewettinck, Dr. Chantal Smout and ir. Katleen Anthierens. And to the most supportive staff Miss Ruth van den Driessche and Katrien Verbist. Thank you for the all-out support.

To my IUPFOOD classmates and friends, especially to Jihan, Joel, Chirag, Emelia, Getnet, Victor, Clarisse and Christine. Thank you for believing in me when I'm posting announcements, for all the encouraging messages, for checking on me and for scolding me sometimes. Thank you for assuring me that I have you at my back.

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I owe my greatest gratitude to my spiritual family both here in Belgium and back in the Philippines. Thanks to International Community Church in Gent (ICCG) and International Church of Evangelicals in Leuven (ICEL) for providing me spiritual nourishment and
support while I'm here. To my Free Methodist Church (FMC-Butuan) and Christian Group Ministries, Inc. (CGMI) family, who never fail to check on me and uplifts my spirit when I’m down. Thank you for all the faithful prayers and encouraging words. Special thanks to my Nanays Annie Mero, Jane Flores, Charry Dadang and Mailyn Perez. You never failed to stand as my spiritual mother and prayer partners.

To my Alma Mater & Employer – Mindanao State University, Main Campus especially to the College of Fisheries. Thank you for all the help during the processing of my application papers and support during the whole duration of my graduate studies. Thank you for being a family and not just a colleague. Thank you that all my triumphs are also your joy.

To my beloved family, Mama, Papa and Ading, and to my friends & relatives. You are always with me through bad and good times, in ups and downs. Thanks for being a joy and source of inspiration. Thank you also for all the love and support. To my late sister, Ennenn, I always remember you in all my achievements. I always dreamed of celebrating them with you.

And everything will not be possible without the grace and orchestration of my Lord and Savior Jesus Christ. Thank You for the unconditional and amazing love that You’ve given to me through the Cross. You have never left nor forsaken me despite my unfaithfulness. You did not just paved the way for my academic advancement but You’ve also catered my medical needs. Thank you for teaching me how to live in full dependence on You away from my comfort zone. You’ve been so gentle in dealing with my rough edges. All the glory, highest praise and adoration You alone deserve.

"You are worthy, our Lord and God, to receive glory and honor and power, for You created all things, and by Your will they were created and have their being." – Revelation 4:11
Abstract

Shrimp paste or bagoong alamang is an important fermented commodity in the Philippines and in this research, it was comprehensively characterized. Pastes samples were procured in the Philippines and were brought to Belgium for biochemical analyses. Samples were obtained both from traditional manufacturers and from a local commercial supermarket. The traditionally prepared shrimp pastes were made around the months of February and March, approximately ripened for 6 months. Production date was not known for the commercial shrimp paste. All samples were analyzed for chemical composition, tests for pH, water activity, salt content, non-protein nitrogen (free and total NPN), fatty acid composition, mineral content, TBARS for lipid oxidation and biogenic amines. Bagoong alamang (traditional & commercial) showed a content of protein (14.40±0.93g/100g), fat (1.10±0.43g/100g), SAFA (16.17±1.61%), MUFA (34.66±2.77%), and PUFA (37.89±4.65%). The mean pH value of the products is 7.47±0.01. The samples were shown to be stable with A_w and NaCl content of 0.71±0.01 and 13.93±0.05g/100g respectively. Although samples were processed in the same country, different results for other parameters like TBARS (3.76±0.86µgMDA/g), total NPN (3.81±0.60gN/100g), free NPN (1.75±0.46gN/100g), biogenic amines and mineral content were observed.

Using the shrimp species (Crangon crangon) available in Belgium, the fermentation process done in the Philippines was simulated. The set-up made use of cooked, frozen, and fresh raw shrimps. Starter cultures involving strains of lactic acid bacteria were employed to determine its possible activity in the fermentation process. Series of experiments revealed that enzymes naturally present in the raw material and from the microorganisms adhering to the fresh raw shrimps are key players in the fermentation process. Based on the obtained pH values (7.47±0.40) of the shrimp pastes, there could be concluded that no acid fermentation had occurred.
Table of Contents

Copyright .................................................................................................................. ii
Preface .................................................................................................................. iii
Acknowledgment ................................................................................................... iv
Abstract ................................................................................................................ vi
List of Abbreviations .............................................................................................. ix
List of Figures .......................................................................................................... x
List of Tables ........................................................................................................... xi
Chapter 1. Introduction ......................................................................................... 1
Chapter 2. Related Literature ............................................................................. 3
   A. What is fermentation? .................................................................................... 3
   B. Fermentation of fish .................................................................................... 5
   C. Fermented fish products in Indonesia ....................................................... 6
   D. Fermented fish products in Myanmar ....................................................... 9
   E. Fermented fish products in Thailand ....................................................... 9
   F. Fermented products in Malaysia ............................................................. 11
   G. Fish fermentation in Africa ....................................................................... 13
   H. Fermented fish products in the Philippines ........................................... 15
   I. Fermented shrimp paste in Philippines ................................................... 17
   J. Traditional food fermentations and its development ............................ 18
Chapter 3. Materials and Method .................................................................... 20
   A. Collected samples from the Philippines ................................................... 20
   B. Gross composition .................................................................................... 21
   C. pH (Bendall, 1978) .................................................................................. 24
   D. Water activity ($A_w$) ............................................................................ 24
   E. Salt content (Volhard, 976.18, AOAC-1995) ......................................... 24
   F. Total Non-Protein Nitrogen (Oddy, 1974) .............................................. 25
   G. Free Non-Protein Nitrogen (Oddy, 1974) .............................................. 27
   H. Biogenic amines (Malle et al., 1996) ....................................................... 27
   I. Mineral content (Binsan et al., 2007) ....................................................... 28
   J. Fatty acid composition ............................................................................. 29
   K. TBARS (Mercier et al., 2004) ................................................................ 31
   L. Fermentation experiments ........................................................................ 32
   M. Statistical analysis ...................................................................................... 34
Chapter 4. Results and Discussion ................................................................... 35
   A. Raw materials ............................................................................................. 35
B. Characterization of shrimp paste ................................................................. 35
C. Fermentation of shrimp paste on lab-scale .................................................. 47

Chapter 5. Conclusions and Recommendations ............................................. 59
Chapter 6. List of References ........................................................................ 61
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAB</td>
<td>acetic acid bacteria</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>(A_w)</td>
<td>water activity</td>
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<tr>
<td>BA</td>
<td>biogenic amines</td>
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<tr>
<td>BHT</td>
<td>butylhydroxytoluene</td>
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<tr>
<td>Ca</td>
<td>calcium</td>
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<tr>
<td>cfu</td>
<td>colony forming units</td>
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<tr>
<td>Cu</td>
<td>copper</td>
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<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
</tr>
<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
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<td>FAME</td>
<td>fatty acid methyl esters</td>
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<tr>
<td>Fe</td>
<td>iron</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>ILVO</td>
<td>Instituut voor Landbouw- en Visserijonderzoek</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>K</td>
<td>potassium</td>
</tr>
<tr>
<td>LAB</td>
<td>lactic acid bacteria</td>
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<tr>
<td>Mg</td>
<td>magnesium</td>
</tr>
<tr>
<td>Mn</td>
<td>manganese</td>
</tr>
<tr>
<td>MRS</td>
<td>the Man, Rogosa and Sharpes’ medium</td>
</tr>
<tr>
<td>MUFA</td>
<td>monounsaturated fatty acids</td>
</tr>
<tr>
<td>Na</td>
<td>sodium</td>
</tr>
<tr>
<td>NaCl</td>
<td>sodium chloride salt</td>
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<tr>
<td>NPN</td>
<td>non-protein nitrogen</td>
</tr>
<tr>
<td>PCA</td>
<td>plate count agar</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RBC</td>
<td>Rose Bengal Chloramphenicol Agar</td>
</tr>
<tr>
<td>SAFA</td>
<td>saturated fatty acids</td>
</tr>
<tr>
<td>TBARS</td>
<td>thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TSA</td>
<td>tryptic soy agar</td>
</tr>
<tr>
<td>TVB-N</td>
<td>total volatile basic nitrogen</td>
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<tr>
<td>Zn</td>
<td>zinc</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1  Planktonic shrimps utilized in shrimp pastes

Figure 2  Raw material for salt-fermented shrimp paste (a); shrimp paste from local producers (b, c, d); and commercially produced (e)

Figure 3  Fermented cooked shrimps in clay pots without starter culture

Figure 4  Fermented cooked shrimps in clay pots - a) without starter culture; b) with *Lactobacillus brevis*; c) with *Lactobacillus plantarum*; d) with *Lactobacillus sakei*

Figure 5  Fermentation of cooked shrimps using *Lactobacillus plantarum* as starter in Schott bottles – a) without water; b) with water

Figure 6  Fermentation of cooked shrimps with supernatant from previously produced paste

Figure 7  Fermentation of raw yet frozen shrimps with the use of *Lactobacillus plantarum* as starter in flasks – a) without water; b) with water

Figure 8  Fermentation of raw, fresh shrimps with the use of *Lactobacillus plantarum* as starter in Schott bottles – a) without culture (control); b) with culture (*Lactobacillus plantarum*), without water; b) with culture (*Lactobacillus plantarum*), with water

Figure 9  Fermentation using raw, fresh shrimps without starter culture - a) drained; b) not drained without water; c) not drained with water
List of Tables

Table 1  Chemical composition of some fermented fish products in Indonesia
Table 2  Microbial count (cfu/g) of some fermented fish products in Indonesia
Table 3  Chemical composition (g/100g fresh sample) of some fermented fish products in Myanmar (Tyn, 1993)
Table 4  Chemical composition of some fermented shrimp products in Thailand (Faithong et al., 2010)
Table 5  Chemical composition of known fermented products in Malaysia (Karim, 1993)
Table 6  Chemical composition of some fermented products in Africa (adapted from Anihouvi et al., 2012)
Table 7  Chemical composition (g/100g wet weight) of some fermented products in the Philippines (adapted from Montaño et al., 2001)
Table 8  Characterization of the samples and manufacturers
Table 9  Chemical composition of shrimp paste traditionally produced in some parts of the Philippines
Table 10 Biogenic amines (expressed as mg/kg) present in the fermented shrimp pastes from Philippines
Table 11 Mineral composition of shrimp paste traditionally produced in some parts of the Philippines
Table 12 Fatty acid profile (g/100g FAME) of shrimp paste traditionally produced in some parts of the Philippines
Table 13 Comparison of fatty acid profiles (g/100g FAME) from different studies done on shrimp paste in the Philippines
Table 14 Fermentation of cooked shrimps without starter culture
Table 15 Fermentation of cooked shrimps with starter culture
Table 16 Fermentation of cooked shrimps with the use of Lactobacillus plantarum as starter
Table 17 pH of the cooked shrimps with supernatant from previous fermentation
Table 18 Microbial count of cooked shrimps with supernatant from previous fermentation
Table 19 Microbial count of raw yet frozen shrimp with Lactobacillus plantarum as starter
Table 20 Microbial count of raw, fresh shrimps - with and without starter culture
Table 21 pH of fresh, raw shrimp without starter culture
<table>
<thead>
<tr>
<th>Table 22</th>
<th>Microbial count of the fermentation set-up using fresh, raw shrimps without starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 23</td>
<td>Chemical composition of fermented shrimp pastes produced in the lab</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

Fermentation is a technique of food preservation which dates back in the early part of history. It employs locally available raw materials and less complicated procedures. Fermentation, as reviewed by Steinkraus (2002), performs the following functions in food processing:

a) develop various flavor, aroma and texture in foods;
b) preserve food through increasing and lowering of pH and/or high salt concentrations;
c) improve nutritional quality by enhancing digestibility and biological enrichment with vitamins, amino acids, and fatty acids;
d) detoxify food substrates during processing; and
e) decrease cooking period.

Fermented products have been classified in various ways. Different authors categorized them depending on the commodity used and by their functional basis. Steinkraus in his researches, classified fermentations in 1) protein meat substitutes from vegetables like legumes or cereal; 2) high salt/meat-flavored sauce and paste fermentations; 3) lactic acid fermentations; 4) alcoholic fermentations; 5) acetic acid fermentations; 6) alkaline fermentations; and 7) leavened breads (Steinkraus, 1997, 2002).

Fish fermentation, a high salt fermentation, is not a commonly followed preservation technique. It is only utilized in countries where these food resources are abundantly available. Philippines is one of those countries. The technology is simple and the operating cost is low (Peralta, 2008). Fermented fish products in the Philippines are essential elements of the people’s diet. They used it as a condiment or as a vital ingredient of their cuisines (Montaño et al., 2001; Peralta et al., 2005, 2008). Fish fermented products in the country are categorized according to their salt content. Those with higher salt concentration (15-20%) are used as side dish or condiment. On the other hand, those with lower salt concentration are accompanied with carbohydrate source like rice. They are eaten as such. This aspect of fermentation in the country is considered to be rural, seasonal as it only depends on the availability of the raw material and is traditional (Olympia, 1992).

Salt-fermented shrimp paste or bagoong alamang is a well-known salt-fermented product in the Philippines. It is the equivalent to the belacan of Malaysia, terasi udang of Indonesia and kapi of Thailand. Though it is popular, less attention to the production process and to quality parameters had been given to it. Despite the abundant use of these products, its quality is not well standardized, especially on the traditionally manufactured products. The technology
that has been used by these producers are mostly not research-based and described in literature. Studies focusing on the product and the process are rather scarce. Transfer of knowledge about the production depends from families to families, and is passed on from one generation to another. They just mastered them by experience. So the quality of the same fermented products is not the same for one seller to another (Olympia, 1992; Putro, 1993). Effort on studies regarding the quality and safety aspect of salt-fermented shrimp paste is still essential.

The objectives of this study were 1) an in-depth characterization of the salt-fermented shrimp paste that are traditionally and commercially produced in the Philippines, 2) simulation of the fermentation process carried out in the Philippines under controlled conditions with the intention of standardizing the process flow and to gather information on the type of microorganisms involved in the process.
Chapter 2. Related Literature

A. What is fermentation?

Fermentation is a metabolic process in which complex organic substances are degraded into simpler compounds due to the action of microorganisms and enzymes (Chojnacka, 2010). Traditionally, fermentation is a commonly utilized method for food preservation. It has been widely accepted because of its simplicity, efficiency and lower cost compared to freezing or other modern methods, making it highly practised in developing and far-off locations (Battcock & Azam-Ali, 1998). Steinkraus (1997) explicitly defined fermented foods as food matrices associated with an outgrowth of microorganisms. These microorganisms produce enzymes such as amylases, proteases and lipases which are responsible for the hydrolysis of respectively polysaccharides, proteins and lipids. As a result, food products with distinct sensorial and nutritional qualities desirable for consumers are obtained. What man is enjoying in his table - from condiments like meat-flavoured sauce, paste, and vinegar; cheese, leavened bread, yoghurt, alcoholic beverages, sausages, pickled vegetables - are all products obtained by fermentation (Steinkraus, 1997).

Commercial scale fermentations are categorized as 1) solid-state or 2) submerged. In the solid-state type, fermenting microflora can proliferate even at less-water condition like bread-making, soy paste production, tempeh, gari, and other commercially important traditional foods. The second type, the submerged, involves more water like in slurry, brine or sugar solutions. Examples of submerged fermentation are pickles, yoghurt, beers and wines. These two main types can further be partitioned in aerobic or anaerobic fermentations. Koji preparation in soy sauce production is an example of an aerobic fermentation while yoghurt and sausage are examples of anaerobic fermentation (Chisti, 2010).

Bourdichon et al. (2012) comprehensively enumerated the major roles that fermentation is contributing to food processing. It includes preservation and promote food safety through the production of compounds such as lactic acid, acetic acid, bacteriocins, together with lowering the water activity of food, resulting in an inhibition of spoilage and pathogenic bacteria (Ross et al., 2002; Gaggia et al., 2011; Adams & Mitchell, 2002; Adams & Nicolaides, 2008); and enhancing nutritional (van Boekel et al., 2010; Poutanen et al., 2009) and sensorial quality of foods (Marilley & Casey, 2004; Smit et al., 2005; Lacroix et al., 2010; Sicard & Legras, 2011). Steinkraus (2002) also explained that fermentations directly or indirectly improve the nutritional quality of foods by biological enrichment. The process can elevate the protein content in food items like rice (tape ketan in Indonesia), increase availability and promote
balance of amino acids (methionine in Indian \textit{idli}) and vitamins (thiamine in \textit{tape ketan}) essential to human’s diet.

There is a wide range of fermented foods in the world. Each country has their special type and own way of producing it (Battcock & Azam-Ali, 1998). With this, different authors classify fermented foods in different ways. Steinkraus (2002) categorized them as followed:

a. Textured protein meat substitutes like the \textit{tempe} and \textit{ontjom} from Indonesia.

b. High salt/savoury sauces and pastes like soy sauce, Japanese miso, Philippine \textit{taosi}, \textit{patis} and \textit{bagoong}, Korean’s \textit{kanjang} and \textit{joetkal}, and Malaysian’s \textit{belacan}.

c. Lactic acid fermentations like in pickled vegetables, yoghurt, cheese and sourdough.

d. Alcoholic beverages including wines and beers.

e. Acetic acid or vinegar fermentations. Wine vinegars are commonly known in this category.

f. Alkaline fermentation like Nigerian \textit{dawadawa} and Japanese \textit{natto}.

g. Leavened bread like the yeast and sourdough bread from the West and Middle east.

h. Unleavened bread

It was stressed out that the distinction between these categories is not that clear and may have overlap.

Food fermentation is mostly carried out by bacteria, yeast and molds. However, in their paper, Bourdichon et al. (2012) enumerated microorganisms that have a significant contribution to the fermentation process as followed:

a. \textit{Actinobacteriaceae} - This group includes \textit{Brachybacterium alimentarium} and \textit{Brachybacterium tyrofermentans} (Schubert et al., 1996), \textit{Microbacterium gubbeenense} (Bockelmann et al., 2005), \textit{Brevibacterium aurantiacum}, \textit{Brevibacterium linens}, \textit{Brevibacterium casei} (Leclercq-Perlat et al., 2007), \textit{Corynebacterium casei}, \textit{Corynebacterium variabile} (Bockelmann et al., 2005) and \textit{Micrococcus luteus} which are involved in cheese ripening, and \textit{Micrococcus lylae} for meat fermentation (Bonnarme et al., 2001; Garcia Fontan et al., 2007).

b. \textit{Firmicutes} - Genus \textit{Carnobacterium} (Hammes et al., 1992) are utilized for dairy and meat; \textit{Tetragenococcus} and \textit{Weissella} for meat, fish, cabbage, cassava, and cacao (Collinset al., 1993); \textit{Lactobacillus} and \textit{Leuconostoc} for coffee and vegetables; \textit{Staphylococcus} for meat (Nychas & Arkoudelos, 1990), \textit{Lactococcus} for cheese (Ouadghiri et al., 2005); \textit{Streptococcus} as ripening cultures for cheese (Georgalaki et al., 2000); and \textit{Bacillus} species on cacao (Schwan & Wheals, 2010) and soy beans (Kubo et al., 2011).
c. **Proteobacteriaceae** - *Acetobacter* and *Glucanacetobacter* not just for vinegar but also in cacao and coffee (Sengun & Karabiyikli, 2011), and *Zymomonas* for the fermentation of alcoholic beverages (Rogers et al., 1984; Escalante et al., 2008).

d. **Fungi** – Yeast species like *Cyberlindnera, Dekkera, Hanseniaspora, Lachancea, Metschnikowia, Schizosaccharomyces, Schwanniomyces, Starmerella, Trigonopsis*, and *Wickerhamomyces* are employed for wine and beverages; and *Aspergillus, Guehomyces, Mucor, Neurospora, Rhizopus*, and *Zygosaccharomyces* are among the fungi species for soy and vegetable fermentation.

**B. Fermentation of fish**

Fish and fishery products are not considered as most common raw material for fermentation. Although fermented fish products are characterized by their putrid smell, they are still required by some consumers because of its taste. Fermented fish is highly popular in Asian countries because of excessive presence of the resources and the prevalence of an often tropical climate with high humidity and temperatures, providing a good environment for the fermentation process. Each country has its unique way of preparing fermented fish products. The traditional method of fish fermentation is considered as natural or spontaneous. Some Asian countries identify fermented fish products as an important food item and condiment. Since the diet of the majority of the Asian population is not mainly comprised of meat and dairy products, they use fermented fish products as an important source of protein and salt intake. Fermented fish products are added to dishes, especially vegetable ones, during the cooking process to enhance flavor, as they give an umami and salty taste to the dishes (Anihouvi et al., 2012).

Small sardines, shrimps, shellfish, squid and fish eggs are among the top species utilized as resource for fermented seafood (Steinkraus et al., 1993). Balachandran (2001) described fish fermentation as a process by which the proteins in the fish material are disintegrated into simpler forms because of the action of endogenous enzymes and microorganisms in the fish which leads to stable food products, even at room temperature (quoted in Kakati & Goswami, 2013). Steinkraus (2002) stated that the proteases, inherent to fish and shrimp, produce short chain peptides and free amino acids giving its desirable taste and flavour (cited in Faithong et al., 2010). Most of the fish fermentations are not based on scientific experiences, but are carried out in small scale at household level by trial and error. The technique is just passed on from generation to generation.
In literature, only a few articles are written about fish fermentation in which generally traditional chemical analyses on the final product or biochemical changes within the fermentation period are described. The most important aim of those studies was to get insight in chemical composition and microbial flora of fish fermentations (Steinkraus et al., 1993).

C. Fermented fish products in Indonesia

Fermented fish products in Indonesia have gained popularity in the market along with dried salted fish products. Shrimp paste (trasi), fish paste (pedah), fermented dry salted marine catfish (jambal roti), freshwater fish (bekasam) and fish sauce (kecap ikan) are among on the list. Pedah is a product of salting, drying and fermenting fishes like mackerel. Raw materials must be wet and are placed in layers in baskets or similar containers. The holes in the basket would allow slow draining, leading to maturation of the product and the development of its characteristic flavor and texture. The whole fermentation process of pedah takes about 2-3 months at a temperature of 29°C, though the time frame is still dependent on the individual processors. Pedah has distinct qualities compared to other salted, fermented fish products (table 1). It is a fatty product, with reddish brown color and a cheesy flavor. Pedah, according to Desnair et al. (2009), has a pH of 6.0 after 6 days of fermentation and Aw value of 0.75 at a salt concentration of 50% (w/w). The study mentioned that pedah is an intermediate moisture food and its fermentation process is considered as spontaneous leading to final products with differing quality (quoted in Petrus, 2013). Idawati (1996) stated that species of Lactobacillus, Leuconostoc and Streptococcus were isolated from the product (cited in Irianto, & Irianto, 1998).

Shrimp paste, or termed as trasi or trasi udang, is a well-known ingredient and condiment in Indonesia. It is prepared from the planktonic shrimp Schizopodes or Mytis species (figure 1) which are abundantly caught in this area. Fermented shrimp paste, is more preferred by the local people, above the fermented fish paste (trasi ikan). It is commonly eaten added with chili and garlic (Putro, 1993). The production process of fermented shrimp paste, as described by Putro (1993), consists of several steps. To prevent the raw material from spoiling, the shrimps are immediately mixed with salt (10%) even on board. After reaching the shore, more salt (around 5%) is added. The shrimps are then drained by spreading them in mats and sundried for 1-3 days until the moisture content is decreased to 50%. Further processing techniques like kneading and mixing are then applied. Food colorants such as Carthamine D and Rhodamine B can be added to obtain the desired color (purple-red to dark
brown). The batter is then allowed to mature until the expected aroma is achieved. In other Indonesian areas, like for instance Java Island, shrimps are subjected to a pre-cooking treatment before the real fermentation process starts. Salt (15%) is added to the raw or pre-cooked shrimps and then it is sun-dried for one day. The salted shrimps then underwent mincing, mixing and thorough kneading after which the obtained paste is sun-dried and further kneaded. More salt is added according to manufacturer’s taste, and then the paste is pressed into cylindrical containers and allowed to ferment until the needed aroma is reached. Differences in the quality of the fermented shrimp paste are observed from one place to another, even if the raw materials used were the same (Putro, 1993). This is due to the natural fermentation procedure allowing the natural microbial flora and endogenous enzymes to carry out their functions. Trasi has a chemical composition shown in table 1 and is characterized by a very high salt content (23%). Putro (1993) cited more authors giving more description about the product. It was mentioned that the pH and total volatile basic nitrogen (TVB-N) values did not significantly changed during a 7 day fermentation process, from 6.35-6.51 and 209–241mgN/100g respectively (Budhyatni et al., 1982). More than 130 different volatile compounds were also detected in shrimp paste, contributing to the specific sensory characteristics i.e. hydrocarbons, carbonyls, fatty acids, esters, sulphur compounds, pyrazines and amines (causing a cacao-like odor), volatile fatty acids and ammonia (resulting in acidic and ammoniacal aroma) (Moelhojardjo, 1972).

![Figure 1. Planktonic shrimps utilized in shrimp pastes](http://www.imas.utas.edu.au/zooplankton/image-key/malacostraca/pelacanida/myidacea.html)
Table 1. Chemical composition of some fermented fish products in Indonesia

<table>
<thead>
<tr>
<th></th>
<th>Shrimp paste (trasi)*</th>
<th>Fish paste (pedah)**</th>
<th>Fish sauce (kecap ikan)***</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisture (g/100g sample)</strong></td>
<td>30-50</td>
<td>52.71-53.94</td>
<td>64.12-70.88</td>
</tr>
<tr>
<td><strong>Ash (g/100g sample)</strong></td>
<td>10-40</td>
<td>15.96-16.90</td>
<td>16.30-21.82</td>
</tr>
<tr>
<td><strong>Crude Protein (g/100g sample)</strong></td>
<td>20-40</td>
<td>20.15-21.54</td>
<td>6.11-6.40</td>
</tr>
<tr>
<td><strong>Carbohydrates (g/100g sample)</strong></td>
<td>3.5-5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Crude Fat (g/100g sample)</strong></td>
<td>2-5</td>
<td>1.25-1.37</td>
<td>1.68-1.99</td>
</tr>
<tr>
<td><strong>NaCl (g/100g sample)</strong></td>
<td>23</td>
<td>10-13</td>
<td>nd</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>nd</td>
<td>6.0</td>
<td>5.01-5.28</td>
</tr>
</tbody>
</table>

Sources: * Moeljohardjo, 1972 as cited in Putro, 1993; ** Desnair et al., 2009 as cited in Huda, 2012; ***Desnair et al., 2007 as cited in in Huda, 2012; nd – not determined

Fish sauce, or locally termed as *kecap ikan*, is manufactured from fishes belonging to the *Sardinella* family. Fish is added with 25-30% dry salt and is stored without stirring until the fermentation process is completed. After a maturation period of 10 months to one year, the fermented product is filtered. Before bottling, brown sugar and some spices are added (Putro, 1993). The microbial flora present at the end of the fermentation of the shrimp paste, fish paste and fish sauce are shown in table 2.

Table 2. Microbial count (cfu/g) of some fermented fish products in Indonesia

<table>
<thead>
<tr>
<th></th>
<th>Shrimp paste (trasi)</th>
<th>Fish paste (pedah)</th>
<th>Fish sauce (kecap ikan)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Plate Count (x 10^3)</strong></td>
<td>248</td>
<td>1.64</td>
<td>0.80-0.85</td>
</tr>
<tr>
<td><strong>Staphylococcus (x 10^5)</strong></td>
<td>21.8</td>
<td>67.0</td>
<td>30</td>
</tr>
<tr>
<td><strong>Mold (x 10^1)</strong></td>
<td>81.0</td>
<td>5.0</td>
<td>0.8-36</td>
</tr>
<tr>
<td><strong>Yeast (x 10^2)</strong></td>
<td>7.1</td>
<td>23</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: Poernomo et al., 1984 as cited in Putro, 1993

Fermented products not having uniform quality from different areas are the major problem observed in the country. More studies are still necessary to standardize quality and safety (Putro, 1993).
D. Fermented fish products in Myanmar

Fermentation of fish is a preservation technique that has a long traditional history in Myanmar. A common product is called nga-pi. It is either prepared from fish or shrimp, grounded, salt is added, and allowed to dry for 3-4 days. Maturation of the product takes 3-6 months, and is performed in earthen jars or vats. The chemical characteristics of the fish paste are given in table 3. The fish paste is recognized as a good source of essential amino acids (lysine, leucine, valine, phenylalanine, methionine, isoleucine and threonine), minerals and vitamins (Duchateau et al., 1953 as cited in Tyn, 1993). Also the liquid by-product of nga-pi is used. The localities are collecting it as fish sauce called ngan-pya-ye. The liquid is kept in storage tanks and allowed to age from several months to one year to develop the required flavor. After the maturation period, it is boiled in steel pans for 4-6 hours. The boiling process aims to decrease the microbial contamination, as well as to reduce the final moisture content of the product to 55-60%.

Table 3. Chemical composition (g/100g fresh sample) of some fermented fish products in Myanmar (Tyn, 1993)

<table>
<thead>
<tr>
<th></th>
<th>Shrimp paste</th>
<th>Fish paste</th>
<th>Fish sauce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>40</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

E. Fermented fish products in Thailand

Thailand is a country with longer periods of dry season than rainy periods, so food fermentation is a technique to preserve the food and to cater problems of food supply. Fermented products from the country are generally based on family traditions and differ from each location (Paludan-Muller et al., 2002). Fish sauce (nam-pla), shrimp paste (kapi) and salt-fermented fish (plaa-raa) are the common fermented products of the Thai people. The Thai fermented products can be divided into three categories: a) fish/shrimp with a large quantity of salt, b) fish with salt and carbohydrate source and c) fish with salt and fruit. Popular products that contain high amount of salt include nam-pla (fish sauce), kapi (shrimp paste) and budu (fish sauce). Plaa-raa and plaa-som are included in the second category while plaa-mum is an example from the third category.
Nam-pla can be made from different fish species, but local people have a high preference for anchovies. Some producers also use molluscs like mussel to produce a similar product. The ratio of the salt and fish is respectively 1:3 for nam-pla and 1:5 for kapi. Differences on the salt and fish ratio depend on customer’s preferences. The endogenous microbial flora of nam-pla are Staphylococcus and Bacillus. In a study of Crisan et al. (1975), only Bacillus species were isolated from the nam-pla, with a sequence of different species during the fermentation process (as quoted in Phithakpol, 1993). During the first month, only Bacillus licheniformis was identified to thrive the product. After 7 months fermentation, Bacillus cereus along with Bacillus licheniformis flourished, while at the end of the ripening period, Bacillus megaterium and Bacillus subtilis were found. Plaa-raa on the other hand is a fermented fish product with added rice. Fish and shrimps commonly utilized are freshwater species. The rice, which can be glutinous or just the normal type, is first roasted and then added to the fish after several days of salt-fermentation. The rice is added as carbohydrate source, supporting the growth of microorganisms, e.g. lactic acid bacteria. The carbohydrates in the rice reacts with the salt, creating a specific taste. Roasting the rice contributes to the brown colour of the final product. Staphylococcus epidermidis, Micrococcus, Pediococcus halophilus and Bacillus are bacteria identified as contributors in the fermentation process of plaa-raa. Other products in the same category as plaa-raa contain cooked instead of roasted rice. The cooked rice is usually minced first before added to the fish. Examples of these products are plaa-som, koong-som and som-fak (Phithakpol, 1993). Fish added with salt and fruit belongs to the last classification of fermented products in Thailand. This group is comprised only of khem-bak-nad and plaa-num which uses pineapple and papaya respectively. Khem-bak-nad utilizes long, thin pieces of fish, mixed with rock salt (1:5 product:salt ratio), placed in containers and set aside overnight. Later, the fish is then removed and added with sliced pineapple. The resulting mixture is then contained in bottles in which it is fermented for 3 months. Plaa-mum, on the other hand, uses minced fish, salt (3:1 ratio) and roasted rice; and is mixed and packed in containers for 1.5-2 months. After which, the fish is also removed, mixed with papaya and packed again. The product is eaten either raw or cooked (Phithakpol, 1993).

In a recent study done by Faithong et al. (2010), kapi, koong-som and jaloo, which are also fermented shrimp products, were characterized. These products are using nearly the same species as used in other Asian shrimp products. Kapi is similar to nam-pla except that it is made specifically from the planktonic shrimp species Acetes vulgaris. It is mixed with a ratio salt to shrimp of 1:3-1:5, after which it is sun-dried to obtain lower moisture content and allowed to ripen until 2 months. Endogenous microbial flora associated with its production are Pediococcus halophilus, Staphylococcus aureus and Staphylococcus epidemis. Jaloo is prepared the same way as kapi with the exception of the drying process. In koong-som
production, palm-sap sugar concentrate is added. The chemical composition of these Thai shrimp products is given in table 4. The protein and salt content of koong-som is lower compared to jaloo. The moisture content of kapi is significantly lower compared to koong-som and jaloo. Koong-som has the lowest salt and protein content, 2.74–3.81% and 6.4-7.3% respectively. On the other side, its moisture content (73-81%) is similar with jaloo. Koong-som is also more acidic (pH 3.7-3.9) compared to kapi (7.4-7.7) and jaloo (7.24-7.38).

Table 4. Chemical composition of some fermented shrimp products in Thailand (Faithong et al., 2010)

<table>
<thead>
<tr>
<th></th>
<th>Kapi</th>
<th>Koong-Som</th>
<th>Jaloo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g/100g sample)</td>
<td>36.8-50.0</td>
<td>73-81</td>
<td>70.5-78.0</td>
</tr>
<tr>
<td>Crude Protein (g/100g sample)</td>
<td>20-25</td>
<td>6.4-7.2</td>
<td>9.8-14.2</td>
</tr>
<tr>
<td>Crude Fat (g/100g sample)</td>
<td>1.5-2.2</td>
<td>&gt;0.55</td>
<td>nd</td>
</tr>
<tr>
<td>Carbohydrates (g/100g sample)</td>
<td>2.3-13</td>
<td>6.90-15.75</td>
<td>3.2-4.3</td>
</tr>
<tr>
<td>NaCl (g/100g sample)</td>
<td>20-25</td>
<td>2.74-3.81</td>
<td>4.5-5.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.4-7.7</td>
<td>3.7-3.9</td>
<td>7.24-7.38</td>
</tr>
</tbody>
</table>

nd- not determined

F. Fermented products in Malaysia

The country of Malaysia is bestowed with fishery products enough for its population. Postharvest losses have led to the realization that fish preservation techniques such as fermentation are important. However, the fermentation technique needs to be improved as it is still artisanal and thus carry out on small scale.

Known fermented products of Malaysia include fish sauce (budu), fermented fish incorporated with roasted rice, tamarind and salt (pekasam), and shrimp paste (belacan). The chemical composition of the mentioned fermented products is given in table 5. Budu is the dark brown supernatant extracted from salt fermented fish which is similar to any other fish sauce produced in other Asian countries. It is the equivalent of patis in the Philippines, nam-pla in Thailand and ngapi of Myanmar. It is usually made from anchovies (Stolephorus commersoni and Stolephorus indicus) which is washed first with seawater and mixed with salt in a salt-fish ratio of 1:2-1:3. The fermentation process, which is anaerobic, is performed in earthen pots or concrete containers with occasional stirring, and stored at room temperature (±27°C). The maturation period of the product takes about 3-12 months during
which the typical aroma is formed. The dark brown colored liquid, which is formed during the fermentation, is then mixed with the remaining fish residues and boiled together with palm sugar to eliminate the unpleasant odor. Tamarind is also added to *budu* to improve the flavor and to decrease the pH, so the outgrowth of putrefactive bacteria is inhibited. Finally, the whole mixture is filtered before it is placed in their respective containers (Karim, 1993). Consumption of *pekasam*, another known fermented product of Malaysia, is limited to only some parts of the country. It is prepared with cleaned fish (from freshwater source), to which 30-50% salt is added, and set aside overnight. After draining the product, roasted rice (50% w/w) and tamarind are added. The mixture is placed tightly in earthen containers and fermentation is performed for 4 weeks. The addition of rice provides additional carbohydrates, creating a suitable environment for lactic acid bacteria to steer the fermentation. As a result, acid is produced, lowering the pH to 4.5-6.0, and contributes to the distinct flavor, desirable color and longer preservation. Aside from being a carbohydrate source, the roasted rice also minimizes the fishy odor of the product and provides the distinct color. Shrimp paste (*belacan*) is the known salt-fermented shrimp paste in the country. It is grayish-pink to grayish-purple in color; and has buttery, stingy taste (Hajeb & Jinap, 2012). It is made in a similar way as the *trasi* in Indonesia and *bagoong alamang* in Philippines. Shrimps (*Acetes* and *Mysid* species) are mixed with 10-15% salt. Then the mixture is sun-dried in mats for 5-8 hours to reduce the moisture content to 50% and placed in containers to ferment for 7 days. The difference of the product from the rest around Asia comes from the extra step in the production. At the end of the 7 days fermentation, the product is comminuted, sun-dried for 8 hours, minced, and packed into balls or blocks. Khairunnisak et al. (2009) and Jinap et al. (2010) mentioned that *belacan* possess substantial concentration of glutamate and 5’-nucleotides glutamate which contributed to its strong umami taste. This made it as one of the umami flavor source in Malaysian dishes (as quoted in Hajeb & Jinap, 2012). Merican (1971) & Merican et al. (1980) mentioned that *Flavobacterium, Corynebacteria, Brevibacterium, Lactobacillus* and *Bacillus* species are among the microorganisms that thrived in this product (as cited in Karim, 1993). Karim (1993), however, pointed out that lactic acid bacteria, micrococci, bacilli and halophilic bacteria are the ones dominating the product.
Table 5. Chemical composition of known fermented products in Malaysia (Karim, 1993)

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish sauce (budu)</strong></td>
</tr>
<tr>
<td><strong>Fish with rice</strong></td>
</tr>
<tr>
<td><strong>Shrimp paste</strong></td>
</tr>
<tr>
<td><strong>Moisture (g/100g sample)</strong></td>
</tr>
<tr>
<td><strong>Crude Protein (g/100g sample)</strong></td>
</tr>
<tr>
<td><strong>Crude Fat (g/100g sample)</strong></td>
</tr>
<tr>
<td><strong>NaCl (g/100g sample)</strong></td>
</tr>
<tr>
<td><strong>pH</strong></td>
</tr>
</tbody>
</table>

**G. Fish fermentation in Africa**

Fermentation of fish products in parts of Africa has been an old and popular activity. It is one of the interventions used to provide a solution regarding food security issues in the continent. The used fermentation methods are strongly related to the African culture. Just like in Asia, fermentation technology in the area is not based on scientific experiences and is only used on household scale. Thus, the techniques used in African fish fermentation differ from one region to another. They depend on factors like availability of salt since other places are landlocked, and to the food habits of the local people. The people closely incorporate fermentation with salting and drying, and the process period ranges from hours to days. They make use of wooden vats, concrete tanks, oil drums, plastic barrels, earthen pots and others. They also bury them in grounds. Tuna, tilapia, catfish, triggerfish, rays, sea bream, mackerel and perch are among the fish species utilized for fermentation. In African countries like Sudan, less than 30% of their catch from rivers and lakes is allotted to fermentation. Coastal areas like Cote d’Ivorie, Ghana and Senegal ferment fresh fish like tuna, and thawed, low-grade fishes which were previously frozen in cold stores. The products are usually highly salted (Essuman, 1992).

Fermented products in the continent are known with different names from each country of origin. Some examples are lanhouin in Benin and in Togo; Ghana produces momone, koobi, kako and ewule; other places like Gambi call it guedj; Senegal has tambadiang and yet. Lanhouin is a fermented fish which stays whole and firm even after the 2-9 days of fermentation. Croaker, threadfin, and mackerel are the common species used for this product. First, the fish is cleaned and eviscerated. It is then left overnight (10-15 hours) in containers without water. The processors termed this particular step as the ripening period as it affects the texture and aroma of the produced fish. Then the fish is washed with seawater
and salted from the belly part to the gills and to the rest of the body. The products are then contained in vats, earthen jars, baskets and cans, which will later be covered with cloth or buried in the soil at 2 meters depth to ferment. The fermentation takes usually 3-8 days. Processors then remove the excessive salt by washing and the product is then sun-dried for 4 days. Halophilic species of *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus* and *Corynebacterium* comprised the microbial flora of *lanhouin*. *Bacillus* species are mainly *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus mycoides* and *Bacillus cereus*; and *Staphylococcus* species like *Staphylococcus lentus* and *Staphylococcus xylosus* are known to dominate the population. *Momone* is also a fermented fish product which is prepared of fishes like catfish and barracuda, similar as used for *lanhouin*. Also, the process of *momone* is similar to that of *lanhouin*. The difference is that for *momone*, the fish is cut into pieces or sliced on the dorsal side. The microbial populations taking part in the fermentation are *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Pediococcus*, *Staphylococcus*, *Klebsiella*, *Debaryomyces*, *Hansenula* and *Aspergillus* (Anihouvi et al., 2012). The chemical composition of both products is also very similar, as shown in table 6. There are many other fermented products that are popular in Africa, but still little attention was given to them. Examples of those are *djege* and *djadan* from Mali, *gyagawere* and *adjonfa* from Cote d'Ivorie, *fessiekh*, *terkeen* and *mindeshi* from Sudan and Chadian *salanga* (Anihouvi et al., 2012).

Table 6. Chemical composition of some fermented products in Africa (adapted from Anihouvi et. al., 2012)

<table>
<thead>
<tr>
<th></th>
<th>Lanhouin</th>
<th>Momone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisture (g/100g sample)</strong></td>
<td>51.1-56.6</td>
<td>54.8-57.6</td>
</tr>
<tr>
<td><strong>Crude Protein</strong></td>
<td>24.6-26.5</td>
<td>25.2-26.2</td>
</tr>
<tr>
<td>(g/100g sample)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aw</strong></td>
<td>0.71-0.77</td>
<td>0.65-0.73</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.3-7.6</td>
<td>7.5-7.8</td>
</tr>
<tr>
<td><strong>NaCl</strong></td>
<td>5.2-7.3</td>
<td>5.7-5.8</td>
</tr>
<tr>
<td>(g/100g sample)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fermentation products also post a hazard on public health. There are prevailing problems on sanitation and hygienic practices. Although, this is also the case for other countries producing fermented fish products, however, Africa has higher concern on its potable water. The water that they use is from natural sources and is usually contaminated (Essuman, 1992).
H. Fermented fish products in the Philippines

The Philippines is an archipelagic country and is known to be a haven of fish and other fish products. Given those resources, the Filipino population used several techniques for its preservation, of which one of them is fermentation.

Fermented fish products in the country are divided into 2 groups. A first group contains those products with a high salt content (15-20%) like fermented fish/shrimp paste and sauce. They are commonly used as condiment. The second group of products has a low salt content and includes fermented rice fish/shrimp mixture, that when fermented, becomes acidic and contains a cheese-like aroma (Olympia, 1992). The rice fish/shrimp paste is only known in some parts of the country and has shorter shelf-life compared to fermented fish products used as condiment due to its low salt content (Mabesa & Babaan, 1993).

Marine fishes are utilized to prepare fish sauce and fish paste. Sanchez (1977) enumerated that the common species used are anchovies (*Stolephorus comersonii*), round scad (*Decapterus macrosoma*) and sardines (*Sardinella fimbriata*) (as cited in Mabesa & Babaan, 1993). Fish paste, locally known as *bagoong*, is a partial or complete fermentation of fish. The used fish is washed and drained thoroughly. After adding salt in a salt to fish ratio of 1:3 or 2:7, the fish is allowed to ripen for days to months. It can be eaten raw or cooked, depending on consumers’ desire (Olympia, 1992). Fish paste contains high amount of protein, approximately 8-25%, and therefore it is considered as a source of protein of Filipinos, especially the financially challenged people. Main problems faced in its production are the long fermentation period which lasts from 3 weeks to a year; and the high microbial load of the product (Mojica et al., 2005). Olympia (1992) described the total viable count of bacteria of the product to decrease with respect to time. Initially, aerobic microorganisms dominate at the start of the process followed by microaerophilic and anaerobic microorganisms. Unfortunately, during the process both desired and undesired microorganisms proliferate. In the study of Mojica et al. (2005), pH of fish paste was found out to be 6-6.8. Other chemical characteristics of the product are shown in table 7. *Patis* or fish sauce is the liquid decanted from the fermented fish paste. It is a clear liquid with a yellowish to brown color. Hydrolysis of the fish flesh is mainly done by endogenous enzymes, leading to the increase in peptides and amino acids in the liquid part. From the beginning of the process until the 17th day of the fermentation, amino-nitrogen and total volatile bases (TVB) increase. The total bacterial count was also seen to have decreased rapidly up to sixth months of ripening, and declined upon reaching termination. Isolated microorganisms were identified to be facultative anaerobes (Olympia, 1992). Sanchez (1983) as cited in Itoh (1993) identified the following microorganisms from an aged fish sauce: *Micrococcus calpogenes*,
*Micrococcus roseus, Micrococcus varians, Staphylococcus epidermis* and *Staphylococcus saprophyticus*. These microorganisms were found at the end of the fermentation process, although at the start of the fermentation, mainly *Bacillus* species such as *Bacillus coagulans, Bacillus megaterium* and *Bacillus subtilis* were present.

Added fermented product, which is uniquely utilized only in some parts of the Philippines, is lactic acid fermented rice and shrimp. It is termed by the locality as *balao-balao*. Shrimps like *Penaeus indicus* and *Macrobranchium species* are involved in its fermentation. To the shrimps, 20% of salt is generally added, and allowed to stand overnight. It is then drained well, and mixed with cooled cooked rice. The mixture, throughout the 7-10 days of fermentation period, becomes acidic and the shrimp’s shell reddens and softens (Olympia, 2002). Solidum (1997) stated that during the early stage of fermentation, within the 5th day, the product is predominated with gram-positive cocci while acid-producing rods are mainly observed on the last stage of the fermentation period (as cited in Mabesa & Babaan, 1993).

Fish fermented with rice is also well-known, named as *burong isda*. It is made of fish, rice and salt. Adding angkak or red rice, which is fermented by the mold *Monascus purpureus*, is optional. Producers commonly use fishes like catfish and tilapia. During fermentation, the fish flesh and bones become very soft. Preparation as well as consumption is similar to the methods used for fermented rice and shrimp (Olympia, 1992). The fermentation process takes days or weeks to complete. The microorganisms that were isolated from the natural fermentation process on the product were *Pediococcus acidilactici* and *Leuconostoc paramesenteroides* (Mabesa & Babaan, 1993). Olympia et al. (1992) also found starch hydrolyzing lactic acid bacteria on the product. A succession of lactic acid bacteria during the fermentation process could be observed: in a first step *Streptococcus* was observed, followed by *Pediococcus*, and then by *Lactobacillus* and *Leuconostoc*. However, growth of *Pediococcus* and *Leuconostoc* may vary or sometimes they appear at the same time. It has been alleged that these lactic acid bacteria come from the fish itself. Chemical analysis of their research showed that pH of *burong isda* decreases as the fermentation proceeds. After 10 days of fermentation, a final pH of 4.2 was reached, due to the accumulation of lactic acid produced from the carbohydrates. At the end of this period, the product is ready to be consumed (Olympia et al., 1992).
I. Fermented shrimp paste in Philippines

Locally known as *bagoong alamang*, fermented shrimp is a common food product in the country. It is regularly consumed by the people living in coastal regions and as a condiment by those living in urban areas. *Bagoong alamang* is mostly made from *Acetes species*. The production is similar to the production of fish paste. Whole or grounded shrimps are mixed with salt in a salt to fish ratio of 1:3. Sometimes, coloring agents are added (Mabesa & Babaan, 1993). The supply of the raw material for fermented shrimp paste is not the whole year round. It is seasonal and not even fixed every year. The supply is also changing from area to area depending on its exposure to prevailing winds like monsoon. A high amount of catch is possible usually during the earlier part of the rainy season. They are not hard to catch because they usually form aggregates during their spawning period. The shrimps group themselves together based on maturity, light availability and temperature of the water (Ruddle, 1993). In the study of Montaño et al. (2001), fatty acid composition and some chemical quality parameters of shrimp paste were analyzed. Chemical composition of the product is shown in table 7.

The level of total amino acids in the shrimp paste had been observed to increase from the initial day of fermentation to the 10th day. It was best seen on the quantity of asparagine, taurine, leucine, alanine and lysine. These amino acids are credited to give the distinct flavor of the product (Montaño et al., 2001; Kim et al., 2003). The research identified aspartic acid, glutamic acid, alanine, leucine and lysine as the main amino acids present in a 3-month fermented shrimp paste. For microbiological characterization, a study was done by Dalmacio et al. (2011) on fermented products in the country, either plant or animal based. PCR-denaturing gradient electrophoresis was used for this particular assessment. Lactic acid bacteria (LAB) and acetic acid bacterial (AAB) species, which were dominating in the samples, were identified. Specifically, the LAB enumerated were *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus panis*, *Lactobacillus pontis* and *Weissella cibaria*. Among the AAB, *Acetobacter pomorum*, *Acetobacter ghanensis*, *Acetobacter orientalis*, and

### Table 7. Chemical composition (g/100g wet weight) of some fermented products in the Philippines (adapted from Montaño et. al., 2001)

<table>
<thead>
<tr>
<th></th>
<th>Shrimp Paste</th>
<th>Fish Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>33.2</td>
<td>34-39.6</td>
</tr>
<tr>
<td>Ash</td>
<td>43.9</td>
<td>37.1-46</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>0.91</td>
<td>0.60-1.57</td>
</tr>
<tr>
<td>NaCl</td>
<td>24.4</td>
<td>19-23.5</td>
</tr>
</tbody>
</table>
Acetobacter pasteurianus were present. Particularly for fermented shrimp paste, Lactobacillus panis, which is a heterofermentative LAB, and Lactobacillus fermentum were identified. Bacillus spp. and Acetobacter orientalis were also recognized. Nieto (1982) verified that quality of the bagoong is affected by the salt to shrimp ratio and the time elapsed between salting and standing time. The studies revealed that a ratio salt to shrimp of 1:6 showed the best sensorial quality. The optimum combination for the maturing time was between 5-7 days at 31°C.

J. Traditional food fermentations and its development

For products like bread and cheese, and alcoholic beverages like wine and beer, production process has been well-developed and already set on commercial scale. But in areas like Asia, Africa and Latin America, the fermentation technology is underdeveloped and considered as indigenous. Local fermented foods are produced under varying process conditions from one locality to another. Aside from being indigenous, it is also not well-documented (Battcock & Azam-Ali, 1998).

Most of the traditional fermented products in these developing countries are accounted as natural fermentations and are frequently produced in a non-controlled condition. The risk is that it is difficult to control the process and spoilage microorganisms can be incorporated in the process leading to unsafe products (Battcock & Azam-Ali, 1998). Nowadays, research done on fermented products is more into using starter cultures.

Caplice & Fitzgerald (1999) and Hugas & De Vuyst (2000) explained that consuming starter cultures is a good method to optimize the fermentation process (as quoted in Hwanhlem et al., 2011). It improves the sensorial quality and microbial stability of fermented food products. A starter culture is defined by Saithong et al. (2010) to be a microbial preparation with high count of cells, either made from one pure strain or mixed population, which is supplemented to raw food substrate to assist in the fermentation process. It had been noted to be of use in important food groups like dairy products and fermented fruits, vegetables, meat and fish. Basic criteria of a good starter for fermented products according to Holzapfel (1997) are accelerated acidification, ability to improve the production process, enhance the sensorial characteristics of the final product and food safety (as cited in Hwanhlem et al., 2011). Starters are produced naturally from the original food product by keeping a sample from the previous fermentation process or in a ready-to-use, highly concentrated commercial form (Hansen, 2002). Saithong et al. (2010) specified that LAB are considered as the common
and dominating species in most of the fermented products. Kobayashi et al. (2004) as well as Savadogo et al. (2004) concluded that the organic acids, hydrogen peroxides and bacteriocins, produced by these bacteria, kept the pH of the food low which inhibited the growth of spoilage microorganisms resulting in a good preservation of the food and an extension of shelf-life (as quoted in Hwanhlem et al., 2011). Though the technology is not yet fully grasped by traditional producers of fermented products, Hwanhlem et al. (2011) mentioned that the use of starter cultures has been used as a requirement to optimize small-scale fermentation processes in Thailand.
Chapter 3. Materials and Method

A. Collected samples from the Philippines

Salt-fermented shrimp pastes weighing 250 grams each were purchased from 3 local producers in the province of Agusan del Norte, Philippines, as well as one commercially produced fermented shrimp paste.

Shrimps that were used for the preparation of the different salt-fermented shrimp pastes belonged to the *Acetes* species (figure 2a). Concerning the traditional prepared pastes, shrimps were collected from the catch of the local fishermen. After removing visible debris, stones and other small fishes, the shrimps were placed on fine mesh nets and sun-dried for 1-2 hours. In a plastic pail or tub, shrimps and salt were mixed together in a ratio by weight of 3 to 1. If shrimps and salt are mixed in pails, this is done by hand covered with gloves, while wooden paddles are used if the mixing is done in tubs. The mixture is then transferred to empty rice sacks and allowed to drain for about 2 days. In case there is not enough liquid left, the mixture is transferred back into clean pails to which a small amount of color additive is added, only to provide the product of a pink color. Products which are in the pails are first covered with plastic bags before placing the final cover of the pail. The mixture is then stored in *nipa* houses (native houses in the country made from bamboo sticks and covered with *nipa* leaves) for a ripening period of weeks to months. Products are already sold to consumers after one week of ripening, but it is advised to carry out a ripening period of at least 4 months. The traditional pastes (figure 2b, c & d) collected were approximately 5 months old. Also, bottles of the commercially prepared (figure 2e) shrimp paste *Julu* were procured from the same province (no manufacturing date and batch number were specified). All the samples were purchased on July 29th-30th, 2012. All collected samples were preserved under low temperature conditions (±4°C) and transported by plane to Belgium where they were stored at -20°C until analyses.
B. Gross composition

a. Dry matter content (ISO 1442-1973)

Instruments:
Drying oven at 105°C
Analytical balance
Desiccator

Reagents:
95% ethanol

Procedure:
To aluminum foil recipients, 15 grams of sea sand was added and recipients were placed in a preheated oven at 105°C for one hour, after which they were cooled down in a desiccator for at least 30 minutes and weighed (M₀). Then, 5 grams of shrimp paste was added to the aluminum recipients and weighed again (M₁). Samples were then mixed with 5ml of ethanol and placed in the oven for 3.5 hours after which they were cooled down in a desiccator for about 45 minutes to one hour and weighed (M₂). Dry matter content was calculated as:

\[
\% \text{DM} = \frac{(M₂-M₀)}{(M₁-M₀)} \times 100
\]
Where:
\[
\% \text{DM} = \text{gram dry matter per 100 gram sample} \\
M_0 = \text{mass of the preheated sea sand in the aluminum recipient (g)} \\
M_1 = \text{mass of sand and sample in the aluminum recipient before drying (g)} \\
M_2 = \text{mass of sand and sample in the aluminum recipient after drying (g)} 
\]

Samples were measured in duplicate and individually kept in extraction thimbles for the determination of crude fat content by the Soxhlet method.

\subsection*{b. Crude protein content by Kjeldahl method (ISO 937-1978)}

**Instruments:**
- Analytical balance
- Nitrogen-free paper
- Destruction tubes
- Digestion system
- Distillation unit
- Titration unit

**Reagents:**
- Kjeldahl tablets (which consist of 235g Na\text{2}SO\text{4}, 4g CuSO\text{4}.5H\text{2}O and 5g Selenium powder)
- Chemically prepared sulphuric acid (H\text{2}SO\text{4})
- Tashiro solution (1g methyl red and 0.5g methylene blue dissolved in 500ml ethanol)
- Phenolphthalein as color indicator
- 32\% Sodium hydroxide (NaOH)
- 0.16M Boric acid (10g H\text{3}BO\text{3} added with 0.4L distilled water and 0.2L ethanol for 1 liter solution)
- 0.1M Hydrochloric acid (HCl)

**Procedure:**

On nitrogen-free paper, 1 gram of sample was weighed and placed in a destruction tube. Then, 20ml of H\text{2}SO\text{4} and a Kjeldahl tablet were also added to the destruction tube. The mixture was heated in the destruction chamber for approximately 2 hours until it turned into a clear solution. After cooling down the tubes, 50ml of distilled water and 3-4 drops of phenolphthalein indicator were added. The destruction tube was then installed in the distillation unit which was previously cleaned with distilled water. To each tube, 32\% of NaOH was supplied until the solution turned brown. In the outlet of the distillation unit, a flask with 50ml boric acid was placed to collect the distillate. Each sample was distilled for 4 minutes. The purple boric acid solution turned from purple to gray to green. The flask with the distillate was then titrated with 0.1N HCl until the color turned back to purple. The amount of HCl titrated was recorded. All samples were measured in duplicate and per analysis a blank was included. The protein content was calculated as:
Where:

\[ E = \left( \frac{(V - Vb) \times 14 \times N \times 6.25}{Vs \times 1000} \right) \times 100 \]

- \( E \) = crude protein content (g/100g fresh matter)
- \( V \) = volume of HCl titrated for the sample (ml)
- \( V_b \) = volume of HCl titrated for the blank (ml)
- \( Vs \) = weight of the sample (g fresh sample)
- \( N \) = normality of HCl
- \( 14 \) = molecular weight of nitrogen (g/mol)
- \( 6.25 \) = protein conversion factor

**c. Crude fat content by Soxhlet method (ISO 1444-1973)**

**Instruments:**
- Extraction thimbles which contain dried sample
- Cotton wool
- Extraction flasks
- Soxhlet apparatus
- Analytical balance
- Drying oven
- Desiccator

**Reagents:**
- Petroleum ether

**Procedure:**

First, extraction flasks were dried at 105°C for 1 hour after which they were cooled down in a desiccator and weighed (=K1). Dried samples, obtained after the dry matter analysis (ISO 1442-1973), together with the aluminum foil recipients were placed in extraction thimbles and covered with cotton wool. The thimble was then placed in the Soxhlet apparatus and petroleum ether was added in such a way that the extraction flask contained two times the volume of the thimble holder. The apparatus was heated and the dried sample was extracted with petroleum ether for 6 hours. After 6 hours, practically all the fat was extracted and found at the bottom of the extraction flask. The procedure continued until all the extracted fat remained in a minimum amount of petroleum ether. Finally, the extraction flasks were dried at 105°C during 1 hour after which they were cooled down in a desiccator and weighed (=K2). Fat content was calculated using the formula:

\[ V = \frac{K2 - K1}{M1 - M0} \]

Where:

- \( V \) = % fat in the fresh matter
- \( K1 \) = weight of the empty flask
- \( K2 \) = weight of the flask plus fat
- \( M0 \) = mass of the preheated sea sand (g) from the dry matter analysis
- \( M1 \) = mass of sand and sample before drying (g) from the dry matter analysis
C. **pH** (Bendall, 1978)

**Instruments:**
- Ultra Turrax
- pH meter (Consort C830)

**Reagents:**
- Bendall solution (9.3mg iodoacetic acid \([C_2H_3IO_2]\), 2ml of 0.1 M NaOH and 11.175g potassium chloride (KCl) were added, diluted to 1L and adjusted to pH 7)

**Procedure:**
The pH measurements were done in a similar way as described for solid products like meat. In a plastic sample cup, 5ml of chilled (0°C) Bendall solution was added to 1g of sample and homogenized for 1 minute using an Ultra Turrax (13500rpm). The pH of the homogenate was measured using a pH meter (temperature of measurement was set to 0°C). The measurement was done in duplicate.

D. **Water activity (A\(_w\))**

**Instrument:**
- AquaLab, Series 4TE Decagon Devices SN 540001787

**Procedure:**
The \(A_w\) value was measured at 25°C using the chilled mirror dewpoint technique. The samples were first thawed and placed in the oven at 22°C for approximately 30 minutes to decrease the temperature differences between the sample and the instrument. Approximately 5 grams of sample were weighed in a plastic sample cup and after which the cup was placed in the sealed chamber of the AquaLab equipment containing a mirror. After cooling down the mirror, a photoelectric cell detects the change in reflectance from the time condensation occurs on the mirror. After equilibrating the sample with the head-space of the sealed chamber, the relative humidity of the air in the chamber is the same as the water activity of the sample. At equilibrium, the temperature is recorded and finally the \(a_w\) value is calculated by the AquaLab equipment using the vapor pressure chart. All samples were measured in duplicate.

E. **Salt content** (Volhard, 976.18, AOAC-1995)

**Instruments:**
- Conical flasks
- Boiling water bath
Filter paper  
Titration unit  

Reagents:  
Carrez A (8.25g Potassium ferricyanide $[K_3Fe(CN)_6]$ is dissolved in distilled water up to 100ml mark)  
Carrez B (22g zinc acetate $[C_4H_{10}O_6Zn]$ and 3ml anhydrous acetic acid were added with distilled water to prepare 100ml solution)  
Nitric acid indicator (124g ferrous ammonium sulfate hexahydrate $H_8FeN_2O_8S_2.6H_2O$ dissolved with 280ml concentrated nitric acid $[HNO_3]$ and 720ml distilled water)  
0.1M Silver nitrate (16.99g silver nitrate ($AgNO_3$) was dissolved in distilled water for 1L solution)  
0.1M Ammonium thiocyanate (7.615g $NH_4SCN$ in distilled water for 1L solution)  

Procedure:  
In a 250ml conical flask, 10 grams of sample was weighed. After adding 100ml distilled water with a temperature of approximately 70°C, the flasks were placed in a boiling water bath during 20 minutes. After cooling the flasks to room temperature, 2ml of both Carrez A and Carrez B were added, then the flasks were shaken vigorously. The sample solution was then allowed to set for 30 minutes before it was filtered. The filtrate was collected in a 250ml volumetric flask. After rinsing the conical flask with distilled water, the filter was also washed with distilled water until the volumetric flask was filled to the mark. In duplicate, 25ml of the extract was mixed with 5ml nitric acid indicator and 25ml 0.1M silver nitrate. After shaking vigorously, the solution was titrated with 0.1M ammonium thiocyanate until a stable orange-brown color was seen. A blank, containing 25ml distilled water instead of extract, was also prepared. The analysis was done in double for each sample and blank. Salt content was calculated as:  

\[
\%NaCl = \frac{(V_b - V) \times N \times 58.43}{V_s}
\]

Where:  
$\%NaCl$ = salt content  
$V$ = volume of $NH_4SCN$ titrated for the sample (ml)  
$V_b$ = volume of $NH_4SCN$ titrated for the blank (ml)  
$N$ = normality of $NH_4SCN$  
58.43 = molecular weight of NaCl (g/mol)  
$V_s$ = weight of the fresh sample (g)  

F. Total Non-Protein Nitrogen (Oddy, 1974)  

Instruments:  
Ultra Turrax  
Closed boiling water bath  
Drying Oven at 105°C  
Spectrophotometer
Reagents:
0.6M Perchloric acid (HClO₄)
8.4M HCl solution
15 M NaOH solution
Extraction solution with pH 5.8 (mixture of 250ml propionic acid [C₃H₆O₂], 250ml 2-Methoxyethanol [C₃H₈O₂], 350ml distilled water and 100ml NaOH (15M) and diluted to 1L flask)
Ninhydrin solution (5g ninhydrin [C₉H₆O₄] in 1L extraction solution)
Reducant solution (50mg ascorbic acid [C₆H₈O₆] dissolved in 50ml distilled water)
Leucine stock solution (dissolving 74.8mg L-leucine [C₆H₁₃NO₂] in 100ml distilled water)
Standard solution (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml stock solution diluted to 100ml distilled water)
60% ethanol solution

Procedure:
First, 5 grams of samples were homogenized with 0.6M perchloric acid (PCA) for 1 minute using Ultra Turrax (8000rpm). It was done to precipitate the proteins and extract the highest amount of non-protein nitrogen (NPN) as possible. For each sample, one PCA extraction was made. Two ml of each extraction was added with 5ml HCl and stored in the oven for 24 hours for hydrolysis. Samples were neutralized with a NaOH solution to obtain a pH between 4 and 10, transferred to 50ml flask and then diluted with distilled water to the mark. From each sample, 1ml of diluted (100 times dilution) PCA extract was transferred in duplicate in test tubes. Standard series and the blank (distilled water) in duplicates were also placed in test tubes. Then, 100µl of reductant solution and 1ml of ninhydrin solution were added to the tubes and mixed using a vortex. All the test tubes were then covered with aluminum foil and placed in a boiling water bath for 20 minutes. After cooling down to room temperature, 5ml of ethanol solution was added and the solution was mixed using a vortex. After 15 minutes, all tubes were mixed again and absorbance of the samples was measured at 570nm. All samples were analyzed in duplicate. Total NPN was calculated using the following formula:

\[
N_{total} = \left( \frac{X \times d + 50 \times 100 \times 100}{V_s \times DM \times 1000} \right) / 1000
\]

Where:
\( N_{total} \) = total \( \alpha \)-NH₂-N content in dry matter (gN/100g)
\( X \) = the amount of total \( \alpha \)-NH₂-N obtained from the calibration curve
\( d \) = dilution factor
\( DM \) = dry matter (g DM per g fresh sample)
\( V_s \) = weight of the fresh sample used in the perchloric extract (g)
G. Free Non-Protein Nitrogen (Oddy, 1974)

Procedure:
Free NPN was measured using the same procedure with total NPN, except for the hydrolysis part. One ml of diluted (50 times dilution) PCA extract from each sample was transferred in duplicate in test tubes. Standard series and the blank (distilled water) in test tubes were also prepared. To every test tubes 100µl of reducing solution and 1ml of ninhydrin solution was added and mixed using a vortex. All were then covered with aluminum foil and placed in a boiling water bath for 20 minutes. After cooling down, 5ml of ethanol solution was added and the solution was mixed using a vortex. After 15 minutes, all tubes were mixed again and the samples’ absorbance was measured with spectrophotometer at 570nm. Analysis was done in duplicate. Free NPN was calculated using the following formula:

\[
N_{\text{free}} = \frac{X \cdot d \cdot 100 \cdot 100}{Vs \cdot DM \cdot 1000} / 1000
\]

Where:
- \(N_{\text{free}}\) = free α-NH₂-N content in dry matter (g/100g)
- \(X\) = the amount of free α-NH₂-N obtained from the calibration curve
- \(d\) = dilution factor
- \(DM\) = dry matter (gDM per g fresh sample)
- \(Vs\) = weight of the fresh sample used in the perchloric extract (g)

H. Biogenic amines (Malle et al., 1996)

Instruments:
- High-Performance Liquid Chromatography HPLC (Shimadzu)
- Ultra turrax
- Centrifuge machine
- Vortex

Reagents:
- 0.2M Perchloric acid (17ml 70% HClO₄ added with 983ml HPLC water)
- Internal standard (IS) – (prepared from 320mg of 1,7 diaminohexane dihydrochloride dissolved in 50ml HPLC water)
- Dansylchloride (187.5mg of C₁₂H₁₂ClNO₂S in 25ml acetone)
- Saturated sodium carbonate (44g Na₂CO₃ in 200ml HPLC water)
- L-Proline (10%w/v)
- Toluene
- Acetonitrile (pure C₂H₃N Biosolve brand)
- Stock solution (10000ppm of biogenic amines- putrescine, cadaverine, histamine, tyramine, spermidine, spermine, tryptamine, 2-phenylethylamine. Stored at -18°C)
- Standard series (biogenic-amine-free fish matrix from whiting [Merlanguis merlangus] with a concentration of 0 to 400ppm)
Procedure:

a. **Sample preparation**

The sample preparation and analysis were conducted in ILVO (Instituut voor Landbouw- en Visserijonderzoek) of Flanders. Five grams of the shrimp paste sample was weighed in sample cups and added with 10ml perchloric acid and 100µl of IS. The sample was then mixed using the Ultra turrax and centrifuged set (4°C, 14000rpm) for 5 minutes. The supernatant was collected for further analysis.

b. **Dansylation**

Dansylation is the derivatization of amino acids using dansylchloride (Tapuhi et al., 1981). To 100µl sample supernatant, 300µl of (Na$_2$CO$_3$) and 400µl of dansylchloride were added. The sample was mixed using a vortex and stored at a dark place with temperature of 60°C for 5 minutes after which the sample was cooled with cold water and supplemented with 100µl of L-proline. The addition of L-proline neutralized the excess dansylchloride. The sample was mixed again using vortex and placed again in dark place at room temperature for 15 minutes. Sample was taken out from the dark area and added with 500µl toluene. The upper layer was later collected and evaporated under N$_2$ gas. Acetonitrile amounting to 200µl was also added to the sample and was filtered.

c. **HPLC analysis**

All the samples were analyzed utilizing HPLC with Kromasil C18 column. Elution of the sample was performed using a water/acetonitrile gradient at a rate of 1ml/min. To the machine, 20µl of sample was injected and was measured at 254nm.

I. **Mineral content** (Binsan et al., 2007)

Instruments:
- ICP – OES (Inductively Coupled Plasma Optical Emission Spectroscopy) – VARIAN VISTA-MPX ICP-OES
- Muffle oven
- Crucibles
- Filter paper
- Volumetric flasks

Reagents:
- Concentrated nitric acid (HNO$_3$)
- Double distilled water (DDW)
- Blank (1% Nitric acid – 1ml HNO$_3$ in 100ml solution)
- ICP Multi-element standard solution
- Working standards (10, 20, 50, 100ppm standards prepared by dissolving 1ml ICP standards in 10, 20, 50 and 100ml double distilled water)
Procedure:

Concentration of macroelements like calcium, magnesium, sodium, and potassium; and microelements copper, iron, manganese and zinc was determined. In labeled and dried crucibles, 5 grams of samples were weighed. They were then heated using a Bunsen burner to eliminate the organic matter. All the crucibles were then placed overnight in the muffle oven set at 550°C. Crucibles were then taken out and allowed to cool down at room temperature. To the samples, 5ml nitric acid was added, mixed and filtered in 10ml volumetric flasks. Crucibles were washed with DDW and filtered. More DDW was added until the mark. Samples were further diluted up to 1000 times for the analysis of elements Ca, Mg and K; and 10000 times for Na. Blank, working standards and all the samples (in doubles) were then analyzed using ICP-OES containing a plasma and auxiliary flow is 15L/min and 1.5L/min respectively. Pump rate was 15rpm and the nebulizer pressure was set at 1kPa. For each element, absorbance was measured at 5 different wavelengths. Mineral content was calculated using the formula:

\[
\text{Conc} = \frac{(c \times d)}{1000} \frac{1}{V_s}
\]

Where:
- Conc = concentration of each element (mg/g sample)
- c = calculated concentration by ICP instrument (mg/L)
- d = dilution factor (ml)
- Vs = weight of the fresh sample (g)

J. Fatty acid composition

a) Extraction of fatty acids from shrimp paste (Folch et al., 1957)

Instruments:
- Glass extraction tubes with screw caps
- 100ml Volumetric flasks
- Separatory funnels
- Filters
- Analytical balance
- Centrifuge
- Jet filter pump
- Rotating evaporator
- Test tubes with screw caps

Reagents:
- Chloroform/Methanol solution (C/M) - 2:1 v/v
- 0.1% Butylhydroxytoluene (BHT) in chloroform (1g BHT in 1L chloroform)
Procedure:

After weighing 5 grams of frozen sample in an extraction tube, 25ml C/M solution and 3ml 0.1% BHT solution were added. Samples were homogenized for 1 minute using an Ultra Turrax (13500rpm). The dispersing unit of the Ultra Turrax was then washed with 10ml C/M solution which was collected in the extraction tube. The homogenate was left overnight in a dark area at room temperature. The content of the extraction tube was then filtered and collected in a 100ml volumetric flask. The extraction tube was washed 2 times with 10ml C/M and poured on the same filter. After draining the filter, the filter was rinsed with 5ml C/M. After 10 minutes, the filtrate was divided into two test tubes to which 15ml distilled water had been added. The volumetric flask was washed with 5ml C/M and the content was added to one of the 2 test tubes. The tubes were then centrifuged for 10 minutes at 3000rpm. Using a water jet filter pump, the upper layer of the solution was removed. Content of both tubes was transferred to one separatory funnel using a glass funnel. Each tube was washed with 5ml C/M and the remaining liquid was added to the funnel. After 20 minutes, 2 separated layers could be noticed. The bottom layer was collected in a rotavapor flask. The glass funnel, as well as the inside of the separatory funnel, were washed with 5ml C/M. After 20 minutes, a separation into 2 layers could be seen and the bottom layer was collected again in the rotavapor flask. The extracts were evaporated with the aid of a rotating evaporator (water bath: 40°C) and the fat was re-dissolved in 10ml chloroform. The remaining extracts were stored in test tubes in the freezer. All extractions were made in duplicate.

b. Fatty acid methylation (Raes et al., 2001)

Instruments:
15ml tubes with teflon stopper
Tip tubes
Pasteur pipette
GC vials and cover
Oven set at 50°C
Centrifuge
Vortex
Evaporator

Reagents:
0.5N NaOH in Methyl alcohol (20g NaOH is dissolved in 1L MeOH)
HCl in MeOH (0.5L HCl is mixed in 0.5L MeOH)
Internal standard (IS) solution (2mg C19:0/ml hexane)

Procedure:

The extracts which were previously stored in the freezer, were allowed to warm up to room temperature. To 1ml extract, 1ml IS was added. The solution was then evaporated under
nitrogen, after which 3ml of 0.5N NaOH/MeOH was added. The solution was stirred with a vortex and kept at 50°C for 30 minutes. Then, 2ml HCl/MeOH solution was added and the tube was stirred again and kept at 50°C for 10 minutes. Tubes were then shaken and cooled to room temperature. The Fatty Acid Methyl Esters (FAME) were extracted by adding 2ml hexane and 2ml distilled water. The solution was stirred using a vortex and centrifuged for 5 minutes at 2000 rpm. The upper layer was then removed using a Pasteur pipette and transferred to a tip tube. The FAME extraction was done twice. Hexane was then evaporated under nitrogen. Finally, FAME were re-dissolved in 1ml hexane, stirred with a vortex and transferred in vials for storage in the freezer.

The FAME was analyzed using Gas Chromatography using a HP88 Agilent column (60mx0.25mmx0.2µm). The injector and detector temperature, flow rate over column and injection volume were 250°C, 280°C, 2ml/min and 1µl respectively. Initially, the column temperature was 120°C and was held for 1 minute and increased to 175°C at a rate of 6°C/min. Later, at a rate of 2°C/min, the temperature was elevated to 210°C with a holding time of 6.5 minutes. Then, temperature further increased to 230°C at a rate of 5°C/min and hold at 230°C for 5 minutes. Concentration of each fatty acid was determined as followed:

\[
\text{Conc} = \left(\frac{X}{Y}\right) \times 100
\]

Where:
- Conc = concentration of each fatty acid (g/100g FAME)
- X = peak area of the specific fatty acid as reflected in the GC report
- Y = total amount of area of known and unknown fatty acids (excluding BHT and IS)

K. **TBARS** (Mercier et al., 2004)

**Instruments:**
- Sample cups
- Analytical balance
- Ultra turrax
- Boiling water bath
- Vortex
- Spectrophotometer

**Reagents:**
- 50mM NaOH (2g NaOH per L of distilled water)
- TBA reagent (1g 2-thiobarbituric acid in 100ml 50mM NaOH)
- TCA reagent (2.8g trichloroacetic acid [C₂H₇Cl₃O₂] is dissolved in 100ml distilled water)
- 2% BHT solution (dissolving 2g BHT in 100 ethanol)
- 100mM phosphate buffer (50g trisodium phosphate (Na₃PO₄·12H₂O) dissolved in 1L distilled water and pH adjusted to 7 using phosphoric acid)
- Standard stock solution (60µl of 1,1,3,3-tetramethoxypropane in 100ml distilled water)
Standard work solution (stock solution is diluted 40 times and prepared 0-5-10-15-
20-30% work solution)
n-butanol

Procedure:
In duplicate, 5 grams of each shrimp paste sample was weighed in a plastic sample cup to
which 50ml of 0.1M phosphate buffer was added. The sample was homogenized using an
Ultra turrax at 8000rpm for 1 minute. The Ultra turrax dispersing unit was then rinsed with
10ml of the same buffer solution which was collected together with the homogenate. In test
tubes, 0.5ml homogenate or standard was incubated with 10µl BHT solution, 0.25ml TBA and
0.25ml TCA in a boiling water bath during 10 minutes. After incubation, the samples were
allowed to cool down to room temperature. Then, 2ml n-butanol was added to the tubes and
they were stirred with a vortex. All the samples were then centrifuged at 6000rpm for 5
minutes. The upper layer of the solution was then collected in cuvettes and the absorbance
was measured with a spectrophotometer at 535nm. Also a blank (pure n-butanol) was
included. Malondialdehyde concentration was calculated as followed:

\[ TBA = \left( \frac{c}{1000} \right) \times 72 \times \frac{v}{m} \]

Where:
\( TBA \) = thiobarbituric acid number (µg malondialdehyde/g fresh sample)
\( c \) = calculated concentration of malondialdehyde obtained from the calibration curve
(µmol/l)
\( 72 \) = molecular weight of malondialdehyde (µg/µmol)
\( v \) = volume of phosphate buffer used (ml)
\( m \) = weight of the fresh sample (g)

L. Fermentation experiments

The fermentation process of the shrimp paste was executed on lab-scale. Since raw,
uncooked shrimps were not available in Belgian fish shops or on fish markets, cooked
shrimps were initially used during the first fermentation experiments. In a first set-up, 100
grams of cooked shrimps were drained, pounded and salted with a 1:3 (w/w) salt-to-shrimp
ratio. Fermentation conditions were framed from what is traditionally done in the Philippines.
The salted shrimps were covered in clay pots for 10 days at 30°C (Peralta et al., 2005). After
10 days, the fermented product was sampled for microbial analysis. Paste amounting to 10
grams was placed in a stomacher bag and added with 90ml sterile saline water (0.85%). The
samples were then homogenized in a stomacher for 1 minute. One ml of the homogenate
was then transferred to test tubes with 9ml sterile saline water and was mixed using a vortex
(-2 dilution). Up to -10 dilutions were prepared. From each dilution (-2, -4, -6, -8 and -10),
0.1ml sample was plated (spread plate) in Man, Rogosa and Sharpe (MRS) Agar and incubated at 30°C for 2 days. MRS medium was used to check on the growth of LAB.

Another experiment was set-up where starter cultures were added to the product, i.e. *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus sakei*. From a pure culture, 0.25ml inoculum was cultured in the 10ml MRS broth and allowed to grow for 24 hours at 30°C. Those strains were added to assist the fermentation process. One ml of the prepared inoculum was added to the samples (100g cooked shrimps mixed with salt in a ratio 1:3), put in clay pots, before placing them in the fermentation room (to obtain 10⁶ cfu/ml at time 0h). Resulting samples were again diluted in the same manner (up to -6 dilutions) and were plated in MRS (incubated for 2 days at 30°C) and also in Tryptone Soya Agar (TSA) for halophilic bacteria (incubated for 10 days at 35°C).

When utilizing clay pots, it was observed that the produced shrimp paste was very dry, which possibly had an effect on the growth of the employed LAB starter cultures. The next fermentation set-up was performed in sterilized Schott bottles to increase the surface area for salt penetration on the shrimp meat, and to avoid drying of the paste. Fresh inoculum of *Lactobacillus plantarum* was added as starter culture to the cooked shrimps to obtain 10⁶ cfu/ml at the start of the experiment. To half of the flasks sterile water (100ml) was added, to the other half of the flask no additional water was added. The sterile condition ensured that the microorganisms that carried out the fermentation would be the starter culture and the ones naturally present on the shrimp and salt, and not the microorganisms from the flask or tap water. Samples taken after 10 days incubation were plated out on MRS and Plate Count Agar (PCA) media. MRS plates were incubated for 2 days at 30°C and PCA plates for 5 days at 30°C.

The last experiment using cooked shrimps was done under the same conditions but with 1ml of the supernatant obtained from the fermentation using fresh and raw shrimps as starter culture (see experimental set-up below). The paste produced was plated out on a wider range of media, i.e. MRS, PCA at 22°C and 30°C, TSA, Rose Bengal Chloramphenicol (RBC) Agar at 37°C and incubated for 2, 5, 10 and 7 days respectively. For each set-up, pH was measured.

The same trial set-up was conducted once with raw, yet frozen shrimps that were bought in the local supermarket. The shell of the shrimps was removed and only the flesh was utilized. It was acclimatized and placed in the incubation room at 30°C for two hours. Samples were
added with *Lactobacillus plantarum* as starter (10^6 cfu/ml) and were tested with and without the addition of sterilized water. MRS and TSA media were used for the microbial count.

In May, ILVO of Flanders was able to provide raw shrimps for the experiments. From the institution, it was brought to the lab and was fermented the next day. The shrimps were pounded using mortar and pestle, and were added with the same ratio of salt (1:3 w/w). The shrimp samples were not drained. Similar to the former set-ups, to the raw shrimps, no starter culture was added (control), or *Lactobacillus plantarum* culture (10^6 cfu/ml) was added, both with and without water. Experiments were all done under the same fermentation conditions. Bacterial count was checked using MRS and PCA. The supernatant obtained from the shrimp paste produced in this experiment was used as starter for the last set-up of the cooked shrimps.

Finally, a trial was done with the raw shrimps but without utilizing starter cultures. Three types of samples were performed: drained (20 minutes), not drained without water and not drained with water. The products obtained after the 10-day fermentation were analyzed on MRS, TSA, PCA and RBC at the same incubation conditions. Chemical composition was determined on the shrimp paste acquired from the last experiment of which results were compared on the shrimp paste brought from Philippines.

**M. Statistical analysis**

Shrimp paste samples were collected from 4 sources, i.e. 3 traditionally and 1 commercially produced. From each source, 3 replicates (n=12) were collected. SPOTFIRE S+82 software was used to analyze the data statistically. Normality of data distribution and equality of variance were considered using Modified Levene and Kolmogorov-Smirnov tests. For hypothesis testing, One-way ANOVA (parametric) and Kruskal Wallis (non-parametric) tests were utilized. Significant differences between the results from the different sources of shrimp paste were determined. Tukey's test was used in conjunction with ANOVA to find which means are significantly different from each other. All the used statistical tools were set at 5% level of significance.
Chapter 4. Results and Discussion

A. Raw materials

Salt-fermented shrimp paste samples were traditionally and industrially produced. Three of it were purchased from local producers and one from a local supermarket, all from the southern part of the Philippines during July-August 2012. Descriptions of the samples are presented in table 8. Names of the producers and their years of experience in making bagoong alamang are also included.

<table>
<thead>
<tr>
<th>Name of Manufacturer/Brand</th>
<th>Date Manufactured</th>
<th>Region of Origin</th>
<th>Other Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Acedo (3-4 years experience)</td>
<td>March 2012</td>
<td>Misamis Oriental, Phil</td>
<td>1:3 salt:shrimp ratio with 1:40 sugar:product ratio with food coloring</td>
</tr>
<tr>
<td>B. Amay (22 years experience)</td>
<td>February 2012</td>
<td>Agusan del Norte, Phil</td>
<td>2:5 salt:shrimp ratio liquid drained then added back food coloring added no sugar added</td>
</tr>
<tr>
<td>C. Candole (27 years experience)</td>
<td>February 2012</td>
<td>Agusan del Norte, Phil</td>
<td>2:5 salt:shrimp ratio food coloring added no sugar added</td>
</tr>
<tr>
<td>D. Julu brand (commercial)</td>
<td>Unspecified</td>
<td>Unspecified source</td>
<td>with food coloring amount of salt and water added not specified in the label no sugar added</td>
</tr>
</tbody>
</table>

B. Characterization of shrimp paste

Chemical composition of the different shrimp pastes are given in tables 9, 10 and 12. Gross composition of the product including dry matter, protein and fat content was first measured (table 9). Dry matter content of traditionally prepared pastes varied between 38 and 53g/100g. The one produced commercially showed an average value of 48.1g/100g. All products were significantly different in their dry matter content (p<0.001). Dry matter of a food is linked to the content of other components like fat, carbohydrates, protein and others. Shrimp paste from sample A has been added with sugar which contributed to its high dry matter content. The lowest dry matter content was measured in product B, maybe due to the fact that the drained liquid was added back to the batter before fermentation took place. The
dry matter content of the samples is lower than the ones reported by Montaño et al. (2001), i.e. 66.4–67.2g/100g on shrimp paste that was also available in a local market in the northern part of the Philippines. Also, shrimp paste produced in other countries showed higher dry matter content, like the shrimp paste from Indonesia which is 50-70g/100g (Putro, 1993), from Malaysia with 60-70g/100g (Karim, 1993), from Myanmar with 60g/100g (Tyn, 1993) and 50-60g/100g for kapi of Thailand. However, the dry matter content of the studied shrimp paste is higher than Thailand’s jaloo and koong-som, both having dry matter values between 20-30g/100g (Faithong et al., 2010).

The protein content of the product samples varied between 12.9–15.1g/100g. The level is close to the range of protein in fresh shrimp which is 17-21g/100g, depending on the species (Tag El-Din et al., 2009). The protein content derived from the samples is similar to values reported for shrimp paste of Myanmar which incurred 18g/100g protein content (Tyn, 1993) and Thailand, specifically jaloo (9.8-14.2g/100g) (Faithong et al., 2010). Other products like trasi udang in Indonesia (Putro, 1993) and belacan in Malaysia (Karim, 1993) have a much higher protein content (20-40g/100g) compared with the bagoong alamang being analyzed here. The same is true for kapi of Thailand with a protein content of 20-25g/100g. Koong-som, also from Thailand, has much lower protein levels, around 6-7g/100g (Faithong et al., 2010). The protein content between the samples is significantly different (p<0.001) with the highest protein content for product C and the lowest content for product B. Anihouvi et al (2012) mentioned that protein content in fermented products may vary and is influenced by the proteolytic activity of the microorganisms and enzymes during the process. Proteins are degraded to amino acids and peptides which are carried along with the water that is extracted out from the substrate.

Analysis on the fat content of the samples provided values from 0.80-1.56g/100g which are in agreement to the reported fat content of marine shrimp species of 1.75g/100g (Tag El-Din et al., 2009). These values coincide with what Montaño et al. (2001) reported from analyzing the same kind of product, which garnered a fat level of 0.91±0.08g/100g. The figures are also close to what was obtained in similar products as ngapi of Myanmar (Tyn, 1993), kapi of Thailand (Faithong et al., 2010) and belacan of Malaysia (Karim, 1993), containing 1.5, 1.5-2.2, 1.4-2.6g fat/100g respectively. It can be noticed that sample A has the lowest value among the traditionally prepared pastes, although not significantly different with the other studied shrimp pastes (p=0.05). However, this value matches with koong-som from Thailand (Faithong et al., 2010) with fat level less than 0.55g/100g. To both of these products (koong-som and sample A), sugar has been added.
Table 9. Chemical composition of shrimp paste traditionally produced in some parts of the Philippines

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Dry Matter (g/100 g sample)</td>
<td>52.92±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude Protein (g/100g sample)</td>
<td>14.79±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude Fat (g/100 g sample)</td>
<td>0.80±0.18</td>
</tr>
<tr>
<td>pH</td>
<td>7.75±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;ω&lt;/sub&gt;</td>
<td>0.70 ±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaCl (g/100g)</td>
<td>14.01±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total NPN (gN/100g DM)</td>
<td>3.36±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free NPN (gN/100g DM)</td>
<td>1.53±0.23&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS (µgMDA/g)</td>
<td>2.48±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation; n=3. Values within a row with different superscripts (a-d) denotes significant differences from each other, p ≤ 0.05

Other parameters analyzed were pH, A<sub>ω</sub> and NaCl content (table 9). The majority of the shrimp pastes manufactured in Asian countries (belacan, kapi and jaloo) have neutral pH varying between from 7.2-7.8 (Karim, 1993; Faithong et al., 2010). Mizutani et al. (1992) also noted that pH of shrimp paste is neutral around 7.5±0.3, which is higher than the pH of soybean paste, which is around 5.0. Further analysis showed that shrimp paste had total amino acid (12.5±3.8%), glutamic acid (1.7±0.8%) and total organic acid (1.47±0.90%) content, twice the amount available in soy sauce. But in shrimp paste, these organic acids are present in the form of salts, and thus not give the sour taste as in soy sauce (Mizutani et al. 1992). Faithong et al. (2010) acknowledged that volatile based compounds, like ammonia and other protein degradation products formed during fermentation, are also responsible for the high pH of these products. However, a risk is present since a high pH will allow the growth of bacteria and the occurrence of anaerobic proteolysis, resulting in the production of amines (Sarojnalini & Suchitra, 2009). In the study conducted, the traditionally made bagoong alamang gave a pH value of 7.6-7.7, significantly higher than the industrial produced one (p<0.001) (table 9). These values are close to what was previously published. On the other hand, the pH of the commercially produced one had a significantly lower pH value of 6.8. The value from the commercial shrimp paste is comparable with the one reported by Budhyatni (1982), i.e. a pH of 6.35-6.51 for trasi udang in Indonesia (as cited in
The A_w value, an important parameter for the estimation of shelf-life of a product, was also determined in all samples. All of the samples had an A_w of 0.70-0.73, as exemplified in table 9 (p<0.001). These values are lower than the ones reported by Nieto (1982) on the same product (0.75 to 0.89). The values got from the analysis were expected since shrimp paste has a number of water-binding solutes other than the high salt content present. Proteins and peptides from proteolysis, carbohydrates, including other inorganic substances are responsible for lowering the water activity (Montaño et al., 2001). This is clear, for shrimp paste A, to which sugar was added. This low A_w value restricts the growth of spoilage microorganisms, thus prolonging the shelf-life of the product (Montaño et al., 2001).

Water activity in correlation with salt content has an essential role in the quality and stability of the food product, especially of fishery products which are highly perishable. Salt extends preservation by lowering the A_w value, which in turn provides an unsuitable environment for spoilage microorganisms (Majumdar & Basu, 2010). Bagoong alamang from samples B, C and D showed similar salt contents, while sample A contained significantly more salt (p<0.05). The salt content is similar to the amount reported by Olympia (1992) for condiments like shrimp paste in the Philippines (15g/100g), by Mizutani et al. (1992) which showed average values of 17.5g/100g, and by Karim (1993) reporting salt content of belacan of Malaysia between 13-25g/100g. However, the results retrieved are lower than what Montaño et al. (2001) obtained for bagoong alamang (24.4g/100g). Products of the same kind from Indonesia, Myanmar and kapi of Thailand also have a double amount of salt - 22, 25 and 20-25g/100g respectively (Putro, 1993; Tyn, 1993; Faithong, 1993). Jaloo and koong-som, both based on shrimps, have significantly lower salt concentration, ranging from 2.7–5.4g/100g (Faithong et al., 2010). These differences can be attributed to the different salt-shrimp ratio that each manufacturer is using and its accuracy during the production process.

Non-protein Nitrogen (NPN) are short chain peptides, free amino acids, amines, amine oxides, nucleotides, urea and quaternary ammonium salts which are released during protein hydrolysis. These compounds are accountable for flavor and taste formation of fermented products (Kim et al., 2003; Faithong et al., 2010; Ikeda, 1979 [as quoted in Hui et al., 2012]). The disintegration of proteins leads to the release of biologically active peptides and amino acids. In crustaceans like fresh shrimp' muscle, NPN generally accounts for 20-50% of its total nitrogen content (Hui et al., 2012). But in the fermented product analyzed, it exceeds, probably due to the hydrolyzing activities during fermentation. Total NPN value may include all the products from the 24-hour acid hydrolysis performed in the sample while free NPN content pertains to the α-amino nitrogen readily present (not peptide-bound) in the product. The values for total NPN (table 9) ranged between 3.4-4.6gN/100g dry matter (1.6-1.8gN/100g sample) while the values for the free NPN arrayed from 1.53 to 2.13gN/100g dry
matter (0.6-0.9gN/100g sample), with significant differences between the different samples (p<0.05). In the analysis done by Kim et al. (2003) on fermented sauces from shrimp by-products, total NPN level was only 0.3-1.05gN/100g sample and 0.15-0.60gN/100g sample for the free amino nitrogen. It can be observed that the values obtained from the analysis on fermented shrimp paste in this study were higher than those reported by Kim et al. (2003). This has been explained by Kim et al. (2003) mentioning that the hydrolyzing capacity of the proteases and peptidases are more evident in products with a lower salt content compared to those with higher salt concentration. The shrimp paste analyzed in this study only contains 13.93±0.05g/100g NaCl while the shrimp sauce utilized Kim et al. (2003) contained 30g/100g NaCl. In the research conducted by Majumdar et al. (2006) on fermented Indian shad, it was observed that a change in NPN (total and free) content can be affected by maturation period and sample source. The NPN value of the product increased during the ripening stage. Majumdar et al. (2006) also cited the research of Rahman et. al (1999) on Ilish fish and Hernandaz-Herrero et al. (2002) on anchovy, observing the same trend. This supports the obtained total and free NPN values. It can be seen that shrimp paste from sample B and C have higher values in both total and free NPN since they have been produced a month earlier than sample A. A conclusion is difficult to give for the commercial ones since manufacturing date was not specified for the product. Tungkawachara et al. (2006) linked the increasing trend of NPN at maturation to the degradation of polypeptides. Voskresensky (1965) reasoned out that during ripening, there is diffusion of NPN compounds from the fish into the brine (as cited in Majumdar et al., 2006). Faithong et al. (2010) mentioned that NPN content may differ in fermented products because of different raw materials, procedure applied and the time spent to complete the fermentation process.

Biogenic amines (BA) have been an issue in seafoods because of scombroid food poisoning (Yongjin et al., 2007). These amines are volatile compounds which are products of decarboxylation of free amino acids during bacterial spoilage. Biogenic amines are expected to be present in high levels in fish products like fish paste and sauce according to Brink et al. (1990) and Yangsawatidigul et al. (2004) (as quoted in Zhong-Yi, et. al., 2010) due to the microorganisms involved in the fermentation process and also due to its high protein content (Suzzi & Gardini, 2003). In the analysed salt-fermented shrimp pastes, phenylethylamine, putrescine, cadaverine, histamine, tyramine and spermidine were detected (table 10). These BA enumerated are anticipated to be present in fermented (Suzzi & Gardini, 2003) and sea foods (Lehane & Olley 2000 as cited in Prester, 2011) like shrimp paste. The amount of phenylethylamine had a wide range of values between 4.4-31.6mg/kg, although it was only detected in samples B and D. Tyramine, cadaverine and putrescine are among the BA that are linked to microbial growth and product spoilage (Suzzi and Gardini, 2003; Lehane & Olley
2000, as reported in Prester, 2011). In the research of Suzzi & Gardini (2003), French sausages, characterized with high microbial count, contained 400mg/kg of putrescine and 270mg/kg of tyramine. Also, Bover-Cid et al. (2000) reported values on temperature-abused sausages for tyramine, putrescine and cadaverine of 250, 80, and 340mg/kg respectively. In a commercial Thai fermented minced fish, a wide range of values was also reported – 17-275mg/kg for putrescine, 20.2-328mg/kg for cadaverine and 76-225mg/kg for tyramine (Riebroy et al., 2004). In a study done by Tsai et al. (2006) on fermented shrimp paste from Taiwan, values for putrescine was 5.0-118ppm, 80±45ppm for cadaverine and 3.7±5.1ppm for tyramine. Recently, Saaid et al. (2009) got high values for putrescine and tyramine in shrimp sauces, 331 and 449mg/kg respectively. Putrescine, cadaverine, and tyramine were present in samples B, C and D with concentrations 36-417, 57-534 and 114-188mg/kg respectively. Results obtained from samples B and D (table 10) were noticeably higher for the three mentioned amines compared to the previously mentioned studies. However, values from sample C were close to what Tsai et al. (2006) got, except for the tyramine. Considering the high concentration of these amines, it can be said that the shrimp pastes analysed could be in the initial state of deterioration but are still acceptable. Based on the review of Prester (2011), there are still no suggested limitations for putrescine and cadaverine set for humans. Nonetheless, Shalaby (1996) mentioned that values between 100-800mg/kg of tyramine are still acceptable based on Good Manufacturing Practice (as cited in Saaid et al., 2009). Histamine, a biogenic amine mostly studied in fish and other fishery products due to its toxicity issues (Prester, 2011), was detected in low amounts in the samples (1.07-25mg/kg). It is at the lower range from what was retrieved by Tsai et al. (2006) which was 20-1180 (382±402) mg/kg. Hernandez-Herrero et al. (1999) pointed out that incorrect handle and storage of raw material leads to high histamine count (as quoted in Tsai et al., 2006). This supports the low content of this amine in the analyzed samples. Shrimps that were used by the manufacturers are immediately mixed with salt and processed as soon as it reaches the shore. Also, the histamine level of the shrimp paste analyzed is still acceptable based on the limit set by Codex Alimentarius for fishery products which is 200mg/kg (Vallejos et al., 2012). Spermidine was only seen in paste from sample D. However, no biogenic amines were identified from sample A. These results lower than the detection limit; can be credited to the characteristic of the shrimp paste. Sample A has the highest salt content and lowest $A_w$ value (table 9) which could have affected the decarboxylation activity of the microorganisms present (Suzzi & Gardini, 2003 & Zarei et al., 2011 as cited in Visciano et al., 2012). It is also exhibited in table 10 that the analysis was not able to detect tryptamine and spermine in all the samples. Visibly, there is a significant variance in the results of the analysis between samples ($p<0.05$). This outcome had been confirmed by Suzzi & Gardini (2003). In their study, they have mentioned that even in similar products, differences on the BAs present can
still differ. This was attributed to some factors like quality of raw materials, endogenous microflora, hygienic quality during production of the personnel and environment, and the precursors (free amino acids) present. These factors were also mentioned by Singh et al (2012) in their review on fermented meat products.

Table 10. Biogenic amines (expressed as mg/kg) present in the fermented shrimp pastes from Philippines

<table>
<thead>
<tr>
<th>Amines</th>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptamine</td>
<td></td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td></td>
<td>nd</td>
<td>31.6±6.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>4.4±1.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>Putrescine</td>
<td></td>
<td>nd</td>
<td>417±25.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.5±1.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111±4.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cadaverine</td>
<td></td>
<td>nd</td>
<td>534±34.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.9±3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>149±4.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td>nd</td>
<td>25.0±3.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.77±1.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.07±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyramine</td>
<td></td>
<td>nd</td>
<td>188±37.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127±3.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114±1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spermidine</td>
<td></td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>9.9±0.55</td>
<td></td>
</tr>
<tr>
<td>Spermine</td>
<td></td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

<sup>nd</sup> =not detected

Results are expressed as mean ± standard deviation; n=3. Values within the row with the different superscripts (a-d) denotes significant difference from each other, p ≤ 0.05.

Shrimp is also known to be rich in minerals like calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), chromium (Cr), potassium (K), phosphorus (P), and sodium (Na) (Oksuz et al., 2009). Shrimps utilize these minerals for their various physiological needs. In the study conducted by Oksuz et. al (2009) on fresh shrimps from Turkey, amounts for the macronutrients like Ca, Na, K and magnesium (Mg), were obtained of 0.32-0.50, 0.57-0.88, 0.64-1.0 and 0.38-0.58g/kg respectively. For microelements such as Mn, Cu, Zn and Fe, the group retrieved values 0.145-0.729, 2.2-2.83, 5.87-6.1 and 2.0-18mg/kg respectively. Values for these micro and macronutrients in fermented shrimp paste from the Philippines are shown in table 11. For almost all elements, the levels obtained are higher than what was shown by Oksuz et al. (2009) on fresh shrimps. Only the value of zinc was close to what was obtained from the fermented samples. However, in the work of Binsan et al. (2008) on shrimp extract paste from white shrimp (<i>Litopenaeus vannamei</i>), the mineral contents were close to
the ones obtained in this study, except for Zn and Na. In case of sodium, a high level is expected because of the salt that was added for the fermentation (Binsan et al., 2008). In fermented products like fish sauce, regardless of the fermentation period, Kim et al. (2003) gave values for calcium of 0.20-1.24g/kg sample, magnesium 0.027-0.043g/kg, and iron 3-28mg/kg. Kim et al. (2003) also pointed out that the calcium contained in the shells was partially liquefied and included in the solution contributing to its high amount in the fermented shrimp paste. The mineral content between the different samples was also significantly different, except for zinc and sodium (table 11). Varying element content in the product could reflect the contamination level to which area the raw material was harvested (Oksuz et al., 2009; Tag El-Din et al., 2009). Sodium level of the product can be connected to its salt content. When calculated, Na values ranged between 2.61-2.79mol/kg sample and NaCl values between 2.38-2.39mol/kg sample. Generally, outcome from the analysis exemplified that shrimp paste, as it is highly consumed by the locality, can be good source of dietary minerals like calcium and iron (Ravichandra et al., 2009). Mineral content is also associated with safety, quality of raw material and for labeling requirements of the product (Oksuz et al., 2009).

Table 11. Mineral composition of shrimp paste traditionally produced in some parts of the Philippines

<table>
<thead>
<tr>
<th>Macroelements (g/kg sample)</th>
<th>Samples</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td></td>
<td>1.27±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.25±0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>8.08±0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.99±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.98±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.49±0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td>63.63±4.87</td>
<td>59.98±2.44</td>
<td>61.64±5.18</td>
<td>64.17±4.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td>2.48±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.19±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.53±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microelements (mg/kg sample)</th>
<th>Samples</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td></td>
<td>&lt;0.5</td>
<td>1.36±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58±0.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td>5.88±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.07±0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.81±3.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.48±2.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td>6.75±1.06</td>
<td>6.92±0.55</td>
<td>6.23±0.78</td>
<td>5.94±0.43</td>
<td>0.36</td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td>24.76±6.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.78±4.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.55±5.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.99±2.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation; n=3. Values within the row with the letter superscripts (a-d) denotes significant difference from each other, p ≤ 0.05.
In table 1, only those individual fatty acids counting for at least 0.1% of total FAME are presented. The SAFA mainly present are lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0). Myristoleic acid (C14:1), palmitoleic acid (C16:1), elaidic acid (C18:1t), oleic acid (C18:1c9), cis-vaccenic acid (C18:1c11), gadolienic acid (C20:1) and nervonic acid (C24:1) are among the MUFA which are the most important ones. The PUFA which are noticed to be in abundance in shrimp pastes are α-linolenic acid (C18:3ω3, ALA), eicosapentaenoic acid (EPA; C20:5ω3), docosapentaenoic acid (C22:5ω3) and docosahexaenoic acid (DHA; C22:6ω3), linoleic acid (C18:2ω6), γ-linolenic acid (C18:3ω6), eicosadienoic acid (C20:2ω6), dihomo-γ-linolenic acid (C20:3ω6) and arachidonic acid (C20:4ω6). The fatty acid profile of the different shrimp pastes was significantly different (p < 0.001, except for C14:1; p=0.051) as shown in table 1.

Total SAFA, MUFA and PUFA (table 1) ranged between 32.7-38.8, 15.13-18.5, and 31-40.7 g/100g FAME respectively. The results obtained from this study are in agreement with the results of Montaño et al. (2001) which garnered values of 47.3±4.0%, 18.0±1.4%, and 34.7±5.4% for each group of fatty acids for the same product. These values are also very similar to the ones obtained for fresh, untreated shrimps as reported by Montaño et al. (2001), Tag El-Din et al. (2009) and Fatima et al. (2012). This suggests that during fermentation and during the whole production process, no real changes in fatty acid composition occur. The fatty acid profile of the raw material was not significantly affected by the salting procedure (Montaño et al., 2001). PUFA can be further divided in omega3 (ω3) and omega6 (ω6) fatty acids. Tag El-Din et al. (2009) reported values of 38.4% of ω3 and 2.1% of ω6 fatty acids in fresh marine shrimps. Montaño et al. (2001) on the other hand, got lower values of 22.6±4.8% for ω3 but higher values of 9.2±0.1% for ω6 fatty acids in the same kind of fresh product. The values obtained in this study (table 1) are quiet similar to those observed by Montaño et al. (2001), i.e. 25.2±4.8% (ω3) and 9.5±0.6% (ω6). Sample D showed a significantly lower amount of ω3 fatty acids and ω6 fatty acids compared to the other samples. This is clearly observed in the significant lower values of individual PUFA, i.e. C20:5ω3; C22:5ω3; C22:6ω3 and C20:4ω6; while the precursor fatty acids C18:3ω3 and C18:2ω6 were significantly higher in sample D. Sample D was also significantly different in SAFA and MUFA compared to the other samples. The major differences were a significantly higher proportion of C12:0; C16:0 and C16:1 in sample D compared to the other samples. There could have been some degradation of the unsaturated fatty acids in sample D because of the heat treatment it received during bottling (Sathivel et al., 2003 as quoted in Hui et al., 2012). The difference in its amount of SAFA could be attributed to the region where the raw material came from, which may have affected the shrimp’s diet (Oksuz et al., 2011). But no
strong conclusion can be drawn from it since no information was retrieved from the label of the commercial product (sample D). Results on fatty acid analysis of the shrimp paste from other local studies are shown in table 13. Taking into consideration each fatty acid, except those not identified, results are within the range of values reported earlier. Montaño et al. (2001) and Tag El-Din et al. (2009) along with Oksuz et al. (2009) confirmed that shrimp meat contains a high amount of EPA and DHA.

Table 12. Fatty acid profile (g/100g FAME) of shrimp paste traditionally produced in some parts of the Philippines

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.08±0.01a</td>
<td>0.13±0.003a</td>
<td>0.10±0.02a</td>
<td>1.04±0.04b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.21±0.01a</td>
<td>4.35±0.02c</td>
<td>3.58±0.011c</td>
<td>3.62±0.01d</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C16:0</td>
<td>19.54±0.07a</td>
<td>20.83±0.08d</td>
<td>20.64±0.01b</td>
<td>23.53±0.19c</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C18:0</td>
<td>7.75±0.06a</td>
<td>6.41±0.29b</td>
<td>7.19±0.09c</td>
<td>7.95±0.10a</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.57±0.04a</td>
<td>0.55 ±0.003a</td>
<td>0.54±0.02a</td>
<td>1.53±0.01b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C22:0</td>
<td>1.18±0.02a</td>
<td>1.08±0.05b</td>
<td>1.12±0.05ab</td>
<td>0.84±0.002c</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.40±0.01a</td>
<td>0.30±0.01b,c</td>
<td>0.33±0.02b</td>
<td>0.28±0.03c</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation; n=3. Values within the row with the different superscripts (a-d) denotes significant difference from each other, p ≤ 0.05.
Table 13. Comparison of fatty acid profiles (g/100g FAME) from different studies done on shrimp paste in the Philippines

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Untreated*</td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>0.8±0.2</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C14:0</td>
<td>4.10±0.6</td>
<td>4.80±0.3</td>
<td>3.0-3.1</td>
</tr>
<tr>
<td>C16:0</td>
<td>25.00±2.9</td>
<td>27.70±0.4</td>
<td>19.8-20</td>
</tr>
<tr>
<td>C18:0</td>
<td>11.00±0.8</td>
<td>10.10±0.1</td>
<td>7.7-7.8</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.47±0.05</td>
<td>1.01±0.02</td>
<td>0.70</td>
</tr>
<tr>
<td>C22:0</td>
<td>2.80±0.4</td>
<td>1.94±0.03</td>
<td>ND</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.70±0.2</td>
<td>0.52±0.01</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:1</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C16:1</td>
<td>6.60±0.7</td>
<td>11.70±0.2</td>
<td>6.5-6.6</td>
</tr>
<tr>
<td>C18:1t</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C18:1c9</td>
<td>10.80±0.7</td>
<td>10.10±0.1</td>
<td>7.70</td>
</tr>
<tr>
<td>C18:1c11</td>
<td>nd</td>
<td>nd</td>
<td>2.1</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.40±0.1</td>
<td>0.34±0.02</td>
<td>0.90</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.28±0.04</td>
<td>nd</td>
<td>0.5-0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5-0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3ω3</td>
<td>2.15±0.05</td>
<td>1.23±0.02</td>
<td>1.8-1.9</td>
</tr>
<tr>
<td>C20:5ω3</td>
<td>11.00±1.6</td>
<td>10.90±0.1</td>
<td>11.0-11.1</td>
</tr>
<tr>
<td>C22:5ω3</td>
<td>0.3±0.3</td>
<td>0.47±0.02</td>
<td>0.6-1.6</td>
</tr>
<tr>
<td>C22:6ω3</td>
<td>11.50±3.3</td>
<td>10.10±0.5</td>
<td>17.1-17.3</td>
</tr>
<tr>
<td>C18:2ω6</td>
<td>2.90±0.2</td>
<td>2.52±0.03</td>
<td>1.40</td>
</tr>
<tr>
<td>C18:3ω6</td>
<td>0.16±0.06</td>
<td>0.25±0.06</td>
<td>nd</td>
</tr>
<tr>
<td>C20:2ω6</td>
<td>0.44±0.03</td>
<td>0.41±0.06</td>
<td>nd</td>
</tr>
<tr>
<td>C20:3ω6</td>
<td>0.10±0.1</td>
<td>0.19±0.04</td>
<td>nd</td>
</tr>
<tr>
<td>C20:4ω6</td>
<td>5.80±0.6</td>
<td>5.88±0.07</td>
<td>4.7-4.8</td>
</tr>
</tbody>
</table>

*nd – not determined
*Untreated means unfermented shrimp
**Values are in range in initial-final 10-day experiment.
***Values are in range in a 360-day fermentation experiment.
TBARS in the different shrimp pastes analyzed ranged from 2.48 to 4.43µgMDA/g sample (table 9). A similar shrimp paste product of Thailand, *kapi*, has a lower TBA value. Faithong & Benjakul (2012) reported values between 0.12 and 0.21µgMDA/g sample during the period of 12 months. On the other hand, these values attained from the experiment are comparatively lower than what Majumdar et al. (2006) got from their study on fermented fish. Fresh *Illich* got a TBA value of 6.38µgMDA/g which eventually increased to 17.30µgMDA/g after adding salt. It did fluctuate during the process but ended up to a level of 18.23µgMDA/g at the end of fermentation process for 150 days. The authors also cited other studies like Chang et al. (1994) reporting a value of 45.9µgMDA/g for 4-week old salt-fermented sandfish; and Hernandaz-Herrero et al. (2002) on salted anchovy which has values increasing from 8.64-12.57µgMDA/g after 7 weeks of study. However, it is difficult to compare exact values for TBARS measurements between different laboratories. It can only be concluded that the TBARS values of this study are within the range of values reported for similar products in literature. From table 9, it is visible that there is a significant difference in the TBA value from each source of shrimp paste (p<0.001). Sample A contained a considerably low TBA value. Fat content of the sample cannot be utilized as a factor since fat level of the 4 samples is not significantly different (p>0.05). Therefore, it can be credited to other parameters like the fermentation period, amount of PUFA and metal ions such as Fe and Cu. PUFA are very prone to oxidation (Binsan et al., 2008; McIntyre & Hazen, 2010) while iron and copper act as prooxidants (Mizushima, 1977; Saiga et. al., 2003 [as cited in Carrasco-Castilla et. al., 2012]). In a study of Ruiz-Capillas et al. (2012) on dry fermented sausages and Shirahigue et al. (2010) on chicken meat, TBA value was noted to be affected by the length of processing and storage time. Sample A compared to samples B and C had been produced later (table 8). Although the fatty acid profile is similar between samples A, B and C, the fat content of sample A is lower, meaning less amount of PUFA present (expressed as mg/100g shrimp paste). Taken this into account, with the lower content of iron, the lower TBARS value can be explained for sample A. Although the proportion of PUFA is significantly lower in sample D, as well as the fat content, the high TBARS value of sample D could be caused mainly by the significantly higher mineral content, especially of iron and copper (table 11).

Based on the results for chemical composition, there can be concluded that even with the same fermented product, and from the same country, quality may still vary. Each manufacturer had their own processing technique yielding their own kind of product. Shrimp pastes with different quality - both chemical and nutritional quality - are produced.
C. Fermentation of shrimp paste on lab-scale

Trial fermentation experiments were done in the laboratory. The major aim of these fermentation experiments were to 1) imitate the Philippine production process in order to have a production process which can be performed in a more standardized way; and 2) get insight in the microbial sequence during the production process, as until now no literature on the steering microbial flora is available for fermented shrimp paste.

Raw, uncooked shrimps are not available in Belgian markets, so only cooked shrimps were available to use. Fermentation conditions were based from what is usually done by shrimp paste manufacturers in the Philippines (Peralta et al., 2005). The cooked shrimps (100 grams) were drained, pounded, added with sea salt in a ratio of 1:3 (salt to shrimp) and were drained again. The shrimps were then stored in clay pots at 30°C and covered with plastic freezer bags to promote anaerobic conditions. In table 14, it is shown that the pH of the product is alkaline (8.64-8.69) before the fermentation process. It did not change after the procedure (8.60-8.68). At the end of the fermentation period (figure 3), no growth was observed on MRS-plates. This implied that there was no acid fermentation going on, thus no lactic acid fermentation. Taking these results into account, the use of a starter culture was considered.

Table 14. Fermentation of cooked shrimps without starter culture

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Microbial Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
</tr>
<tr>
<td>A</td>
<td>8.64</td>
<td>8.68</td>
</tr>
<tr>
<td>B</td>
<td>8.69</td>
<td>8.60</td>
</tr>
</tbody>
</table>

Figure 3. Fermented cooked shrimps in clay pots without starter culture
Sarojnalini & Suchitra (2009) named a number of authors (Apilado & Mabisa, 1991; Joshi et al., 1991; Aryanta et al., 1991; Joshi & Rudra Setty, 1994; Asiedu & Sanni, 2002; Paludan et al., 2002) utilizing starter cultures with the aim of assisting and speeding up the fermentation process of a particular food product. This has been the goal of introducing LAB strains in the sample. The second set-up made use of *Lactobacillus plantarum*, *Lactobacillus sakei* and *Lactobacillus brevis*. *Lactobacillus plantarum* is widely used in several fermented products (de Vries et al., 2006) and known to be halotolerant, while the *Lactobacillus sakei* and *Lactobacillus brevis* have been used in meat (Gory et al., 2001; Nguyen et al., 2009), also a protein-rich raw material. Therefore, those 3 bacterial strains (6log cfu/ml) were added to cooked shrimps with similar production flow as in the first experiment. When the pH was measured, no significant change was noticed even when a starter was added to the cooked shrimps compared to the control treatment, as well as during the time of incubation (table 15). Similar pH-values (8.6-8.7) as in the first experiment were observed. There was still no considerable growth observed on MRS after 10 days fermentation. However, a slight growth was observed on the plates with TSA. After the fermentation period, it was observed that the products were dried-out (figure 4). This can be due to the absorption of water by the clay pots. The no-growth observed in the MRS medium and the basic pH can be due to high salt condition of the substrate. Adams (2009) mentioned that even certain species of LAB are salt tolerant, their growth and metabolism are hampered decreasing acid generation during the fermentation.

Table 15. Fermentation of cooked shrimps with starter culture

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>pH Day 0</th>
<th>pH Day 10</th>
<th>Microbial Count (cfu/g) MRS</th>
<th>Microbial Count (cfu/g) TSA**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.85</td>
<td>8.23</td>
<td>&lt; 10³</td>
<td>6 x 10³</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td>8.85</td>
<td>8.11</td>
<td>&lt; 10³</td>
<td>5 x 10³</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>8.43</td>
<td>8.35</td>
<td>&lt; 10³</td>
<td>3 x 10³</td>
</tr>
<tr>
<td><em>L. sakei</em></td>
<td>8.43</td>
<td>8.20</td>
<td>&lt; 10³</td>
<td>7 x 10³</td>
</tr>
</tbody>
</table>
Another fermentation experiment was set-up, using glass bottles instead of clay pots. Also, no draining of water from the raw material was applied and additional water (100ml) was added to avoid drying of the shrimps during incubation. Thus, in this experiment, sterilized glass bottles were utilized and sterile water was added (figure 5). *Lactobacillus plantarum* (6log cfu/ml) was added as starter culture. The sterile condition ensured that the microorganisms that carry out the fermentation will be the starter culture and the ones naturally present on the shrimp and salt, and not the microorganisms from the flask or tap water. It can be seen from table 16 that the pH was not alkaline but neutral, varying between 7.28-7.51, for both samples in the flasks with and without water. This change in pH (going to neutral) could be due to the diluting effect of the liquid that was diffused out from the shrimps after salt was added. On MRS plates, similar results were obtained. There was again a decreased number of LAB after 10 days of incubation. On the other hand, PCA reflected a

Figure 4. Fermented cooked shrimps in clay pots - a) without starter culture; b) with *Lactobacillus brevis*; c) with *Lactobacillus plantarum*; d) with *Lactobacillus sakei*
different outcome. There was an one-log increase in the microbial count of the product supplemented with water. The available water could have provided a suitable environment for the growth of the microorganisms that were present. However, it is not known which microorganisms were growing out.

Table 16. Fermentation of cooked shrimps with the use of *Lactobacillus plantarum* as starter

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>Microbial Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
</tr>
<tr>
<td>Without water</td>
<td>nd</td>
<td>7.51</td>
</tr>
<tr>
<td>With water</td>
<td>7.49</td>
<td>7.28</td>
</tr>
</tbody>
</table>

*nd* - not determined

Figure 5. Fermentation of cooked shrimps using *Lactobacillus plantarum* as starter in Schott bottles – a) without water; b) with water

A final trial was performed with the use of cooked shrimps. But instead of employing starter culture, backslopping was done. One ml of the supernatant from the shrimp paste of fermented fresh and raw shrimps was added to the substrate (results of this experiment are described below). No water was added to the samples, and the process still occurred under the same conditions as the earlier set-ups. The pH of the product, as seen in table 17, is still neutral starting from 7.51-7.56 then lowering to 7.41-7.45 at the end of the 10-day incubation
period. Due to time constraints, the microbial counts were measured at day 1 instead of day 0. The repetitions of this experiment vary extensively in microbial counts after 10 days of incubations, which limit to take strong conclusions. Flask A showed a two-log increase in the microbial growth for PCA at 22°C and 30°C, and for the TSA, while no growth or a decrease in bacterial colonies was observed after 10 days for flask B (table 18). The reason for this difference in results is not clear. Both flasks (figure 6) were incubated in a similar way, at the same time.

Table 17. pH of the cooked shrimps with supernatant from previous fermentation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.51</td>
<td>7.41</td>
</tr>
<tr>
<td>B</td>
<td>7.56</td>
<td>7.45</td>
</tr>
</tbody>
</table>

Table 18. Microbial count of cooked shrimps with supernatant from previous fermentation

<table>
<thead>
<tr>
<th>Sample</th>
<th>MRS Day 1</th>
<th>MRS Day 10</th>
<th>PCA*** (22°C) Day 1</th>
<th>PCA*** (22°C) Day 10</th>
<th>PCA (30°C) Day 1</th>
<th>PCA (30°C) Day 10</th>
<th>TSA Day 1</th>
<th>TSA Day 10</th>
<th>RBC^ Day 1</th>
<th>RBC^ Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 10³</td>
<td>&lt; 10³</td>
<td>5.35 x 10⁵</td>
<td>1.18 x 10⁷</td>
<td>3.64 x 10⁵</td>
<td>1.05 x 10⁷</td>
<td>5.0 x 10⁵</td>
<td>1.1 x 10⁷</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 10³</td>
<td>&lt; 10³</td>
<td>2.18 x 10⁵</td>
<td>2.0 x 10³</td>
<td>1.65 x 10⁵</td>
<td>1.0 x 10³</td>
<td>1.81 x 10⁵</td>
<td>2.0 x 10³</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

For RBC: “-“no growth; “+” with growth
According to Kiesvaara (1975) and Park (1995), salt-fermentation of fish is mainly steered by the action of enzymes, inherent to the fish or produced by the microorganisms, which are responsible for hydrolyzing the proteins (as cited in Kim et al., 2002). Catharina et al. (1999) (as mentioned in Jiang et al., 2007) also supported this statement. They stated that hydrolyzing activity of proteolytic enzymes in the fish is an important stage in the process. Aside from the enzymes from the fish, halotolerant microorganisms according to Faithong et al. (2010) are also able to produce enzymes which aid the degradation process and which are still active at high salt concentrations. At this period, formation of flavor and aroma happened; and the desired consistency is achieved. Amino acids, peptides, and nucleotides are some of the products formed during fermentation. So the use of cooked shrimps was not a good option in testing the fermentation activity of the shrimps. Cooking may deactivate these enzymes and eliminate the natural microflora assisting the process. However the availability of non-cooked shrimps is very limited in Belgium.

The same trial set-up was conducted once with raw yet frozen, shrimps (figure 7) which can be bought in supermarkets. The product was tested with and without the addition of water. The pH of the end-products (table 19) of the fermentation process resulted in a much lower pH compared to the cooked shrimps (table 18). Similar trends as with the cooked shrimps were observed for the microbial counts. Though the state of the raw material is different (cooked compared to raw but frozen), it still portrayed similar trends in terms of bacterial growth. Due to the freezing process, endogenous enzymes and naturally present microflora will be inactivated, thus negatively influencing the hydrolysis process.

Figure 6. Fermentation of cooked shrimps with supernatant from previously produced paste
Table 19. Microbial count of raw yet frozen shrimp with *L. plantarum* as starter

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>Microbial Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
</tr>
<tr>
<td>Without water</td>
<td>ND</td>
<td>5.91</td>
</tr>
<tr>
<td>With water</td>
<td>ND</td>
<td>6.0</td>
</tr>
</tbody>
</table>

From ILVO (Oostende, Belgium), raw, uncooked shrimps were obtained in May. From the institution, the shrimps were brought to the lab in sea water and the fermentation was started the next day. The shrimps were pounded using mortar and pestle, and the same ratio of salt as before was added. The experimental design (figure 8) was as followed: 1) flask without the addition of starter culture (control), 2) with culture (*Lactobacillus plantarum*; 6log cfu/ml) and water added and 3) with culture (*Lactobacillus plantarum*; 6log cfu/ml) without water added. In table 20, it was shown that the control had a pH of 7.30 which slightly decreased to 7.21 after the fermentation period. The same pH profile was measured in the product with culture but without water. On the other hand, there was an increase of pH from 7.48 to 7.73
for the product that was inoculated with *Lactobacillus plantarum* and with water added. No outgrow of microorganisms was observed on both MRS and PCA agar, independent of the treatment.

![Figure 8. Fermentation of raw, fresh shrimps with the use of *Lactobacillus plantarum* as starter in Schott bottles – a) without culture (control); b) with culture (*Lactobacillus plantarum*), without water; b) with culture (*Lactobacillus plantarum*), with water](image)

Table 20. Microbial count of raw, fresh shrimps - with and without starter culture

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>Microbial Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>7.30</td>
<td>7.21</td>
</tr>
<tr>
<td>Without culture, no water</td>
<td>7.23</td>
<td>7.21</td>
</tr>
<tr>
<td>With culture, without water</td>
<td>7.48</td>
<td>7.73</td>
</tr>
<tr>
<td>With culture, with water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A new experiment with raw shrimps was set-up, without the use of starter cultures. All fermentation conditions were still the same except that the product was allowed to ferment naturally. The effect of the adding additional water was tested again. The first set of flasks (figure 9a) contained product that was drained for 20 minutes; the second set (figure 9b) was not drained and not supplemented with water; and the last set of flasks (figure9c) was also
not drained but added with water. Table 21 shows that the flasks with less water (drained and not drained, without water) had a pH around 6.5-6.7, a little lower than the pH of the product in the flasks with water added (7.1-7.3). The pH after 10-day incubation was for all treatments the same and around 7.2-7.3. There was again a shift of pH to neutral just like what was observed in the set-up done with cooked shrimps. The water, both from the shrimp flesh and the ones which were added, tend to dilute and neutralize the pH of the substrate. The microbial count of this particular experiment is shown in table 22. There was a decrease in the number of microorganisms that was inoculated in MRS, PCA, and TSA concerning the products which contained less water while flasks with water showed an increase in amount of microorganisms. Results incurred from the RBC did not show any trend.

The pH of the products (cooked, fresh yet frozen, and raw, uncooked) which is high, approaching neutral, even after the 10-day of fermentation process signifies the type of microorganisms that thrive in to this kind of product. As the pH is not low (pH < 7.0), no acid fermentation took place so the role of LAB in the product is minimal (de Vries et al., 2006). However, the high pH can insinuate that Bacillus species are involved in the fermentation. In a study of Sarojnalini, & Suchitra (2009) on ngari, a fermented fish, a pH of 6.49±0.01 was measured. It’s microbial profile revealed that Bacillus were present, specifically Bacillus coagulans, Bacillus pumilis, Bacillus subtilis and Bacillus pantothenticus suggesting that it has an essential role in the fermentation process. Sand & Crisan (1974) pointed out that these bacteria have high proteolytic and lipolytic activity attributing to the distinct flavour and aroma of fermented fish products (as cited by Sarojnalini & Suchitra, 2009). Anihouvi et al (2012) also identified same Bacillus species in lanhouin and momone, both fermented fishes, which got pH values of 7.3 to 7.8. Same results were recently found by Kakati & Goswami (2013) in some fermented fish in India. The study isolated Bacillus bacteria in products like

Figure 9. Fermentation using raw, fresh shrimps without starter culture - a) drained; b) not drained without water; c) not drained with water
shidol and hentak with pH value ranging from 6.1 to 6.4. Thapa et al. (2004) and Adams (2009) mentioned that the presence of Bacillus in salt-fermented products like the ones mentioned is credited to its capacity to form spores and thus survive in high salt condition. Yeasts like Candida and Saccharomyces species were also identified in some fermented fish products (Thapa et al., 2004) but its involvement in the fermentation process of the shrimp paste can’t readily be discussed because of the scarcity of literature.

Table 21. pH of fresh, raw shrimp without starter culture

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Drained</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.56</td>
</tr>
<tr>
<td>B</td>
<td>6.60</td>
</tr>
<tr>
<td>Not drained without water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.73</td>
</tr>
<tr>
<td>B</td>
<td>6.58</td>
</tr>
<tr>
<td>Not drained with water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.29</td>
</tr>
<tr>
<td>B</td>
<td>7.15</td>
</tr>
</tbody>
</table>

Table 22. Microbial count of the fermentation set-up using fresh, raw shrimps without starter

<table>
<thead>
<tr>
<th>Condition</th>
<th>Microbial Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRS</td>
</tr>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Drained</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&lt; 10³</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 10³</td>
</tr>
<tr>
<td>Not drained without water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&lt; 10³</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 10³</td>
</tr>
<tr>
<td>Not drained with water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&lt; 10³</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 10³</td>
</tr>
</tbody>
</table>

For RBC: “-”no growth; “+” with growth
Berna et al. (2005) tried to explain the decreasing trend of microbial count during the fermentation procedure (as cited in Jiang et al., 2007). At the earlier stage of the process, the microorganisms still have the opportunity to proliferate and provide the initial degradation since the salt added has not yet been totally dissolved and infiltrated into the substrate. After some time, most of the bacterial population, including the spoilage bacteria, is eliminated and only the halophilic ones remained.

The paste of the last fermentation set-up utilizing fresh, raw material was subjected to proximal and chemical analyses, similar to the ones performed on the shrimp paste brought from the Philippines. In table 23, all the outcomes are shown. It can be noticed that the values are within the range of the results depicted in table 9. Pastes which were drained (sample 1), and not drained, without water (sample 2) have dry matter, protein, salt content, total and free NPN levels close to the values of the analyzed shrimp paste from the Philippines. However, the sample which was added with water before the fermentation has low dry matter (27±0.08g/100g), protein (8.57±0.57g/100g) and NaCl content (12.05±0.36g/100g). In agreement with the determined shrimp paste from the Philippines, the pastes resulting from the last fermentation set-up also gave a neutral pH ranging from 7.17-7.26. With the data gathered, it was seen that samples with added water gave a different value compared to those without. It did not just affect the microbial quality of the product (table 21) but also changed its chemical characteristics. However, non-parametric statistical tests have shown that each sample do not significantly differ from each other. This can be caused by the small sample size.

Table 23. Chemical composition of fermented shrimp pastes produced in the lab

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Dry Matter</strong> (g/100 g sample)</td>
<td>42.72±0.41</td>
<td>41.65±0.81</td>
</tr>
<tr>
<td><strong>Crude Protein</strong> (g/100g sample)</td>
<td>13.82±1.41</td>
<td>12.07±0.88</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.26±0.02</td>
<td>7.19±0.02</td>
</tr>
<tr>
<td><strong>NaCl (g/100g)</strong></td>
<td>13.12±0.15</td>
<td>13.07±0.07</td>
</tr>
<tr>
<td><strong>Total NPN</strong> (gN/100g DM)</td>
<td>2.94±0.95</td>
<td>5.25±0.76</td>
</tr>
<tr>
<td><strong>Free NPN</strong> (gN/100g DM)</td>
<td>0.85±0.17</td>
<td>1.04±0.13</td>
</tr>
<tr>
<td><strong>TBARS</strong> (µgMDA/g)</td>
<td>1.44±0.33</td>
<td>1.82±0.42</td>
</tr>
</tbody>
</table>

*Samples: 1 – drained; 2 – not drained, without water; 3 – drained, added with water*
Although the products of the last fermentation set-up (samples 1 and 2) resemble the biochemical properties of the shrimp paste brought from the Philippines, they appear differently, in terms of color and structure (figure 2 and figure 9). A number of reasons can be pointed to these differences. First, the type of shrimps that were utilized in the lab (Crangon crangon) was different from those fermented back in the Philippines (Acetes sp.). Secondly, their size also differs. As the shrimps used in the lab set-up were larger than the ones from the Philippines, they contained more melanin causing a dark colored fermented product (Kim, et al., 2000 as quoted in Manheem, 2012). A third reason could be the fact that the proteolytic activity of the endogenous enzymes in the shrimps had been affected during the time between harvest and fermentation. The raw material available for the lab experiments was fermented only after 24 hours or more after harvest, unlike in the Philippines where the shrimps were already added with salt and fermented just few hours after harvesting. The degree of freshness of the raw material may have affected the activity of the enzymes. Also, the endogenous enzyme activity can be different between the two types of shrimp species. Lastly, the pastes fermented in the lab were produced without adding food coloring.

To take final conclusions, fermentation conditions should be more controlled and more replications of the experiment should be carried out. Microbial count using different media and pH can be monitored at a regular interval to determine changes in function of time. Isolation and identification of the specific microorganisms that are involved in the fermentation process could be interesting.
Chapter 5. Conclusions and Recommendations

Chemical composition showed that shrimp paste manufactured in the Philippines, either traditionally or commercially produced, is a stable product. Its $A_w$ and salt content are respectively low and high enough to limit the growth of spoilage microorganisms. The product is also rich in PUFA, minerals (Na, Ca, and Fe) and protein content which are all essential in man's diet.

Although fermented fish products cover a high demand in the country, results obtained in this study have shown that there is still a deficiency in quality standardization. All sources were different from each other in all measured parameters. The difference can strongly be pointed to the fact that each manufacturer has its own secret recipe in producing the same product. Though respondents were asked with the procedure they employed in the product, it is still expected that they will not divulge their family secret during the interview. Main reasons for quality differences are integrity of the shrimp used – all Acetes species or mixed with other fish species; accuracy in measuring the ingredients added and equipment used, difference in drying rate and used raw materials.

Results from the fermentation set-up showed that enzymes, from both the shrimps and present bacteria, and the microflora inherent to the product probably have very important roles in the fermentation process. The neutral pH of the paste produced suggested that no acid fermentation took place. Activity of LAB is restricted. Based on the obtained pH values and literature, it can be concluded that Bacillus species is involved in the fermentation process. Likewise, it was found out that following given fermentation conditions, paste that was produced in the lab can still have similar biochemical quality with the original fermented product in the Philippines.

In future research, same biochemical analyses can be carried out on the fermented product during the whole fermentation period (day 0 to day 10) to observe any relevant changes. Analysis on the amino acid composition of the product can be done to check if it is a good source of essential amino acids and to see how concentrations vary between sources. These amino acids can also be linked to the concentration of biogenic amines present in each sample. Moreover, a study on the microbial succession in the product can be conducted to have clear idea on the bacterial species that are initiating and dominating the whole fermentation process. This is one limitation of the study since the procurement of the product happened when the raw material was out of season. A wider research covering different areas in the country producing fermented shrimp paste could be interesting. Finally,
since the paste that was produced from the available shrimps here in Belgium didn’t have high sensorial quality; shrimp sauce production can be considered. Though it entails a long fermentation period, it can be a venue for a further study on the use of starter cultures. Shrimp sauce can be a good alternative to food condiments available in Belgium or around Europe. The product can be a good source of proteins, amino acids and essential fatty acids. Careful consideration on its salt content should be done to make it legally accepted. This action also can help to decrease the fish discard in Belgium. Europe has been strengthening its effort on dropping the fish discard rate in the next years. Fish fermentation is another approach that can be applied to catch with less commercial value which opens opportunities for researches on fish fermentation in Europe.
Chapter 6. List of References


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