AN ABSTRACT OF THE THESIS OF

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Title: Application of Ohmic Heating for Accelerating Fish Sauce Fermentation.

Abstract approved:

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Christina Ann Mireles DeWitt

Abstract approved:

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Jae Won Park

Fish sauce is a fermented condiment made primarily from anchovies and is popularly used in Asian countries as it provides unique salty and umami flavor. Its consumption is no longer limited to Southeast Asian countries, but extended to Europe, United States and Canada as a condiment in their food. Fish sauce fermentation is a time-consuming process at ambient temperatures and uses endogenous enzymes and microbes to completely degrade proteins to amino acids and/or di-peptides. Therefore, many trials

and research efforts have been made to accelerate fermentation by adding exogenous protease or inoculating microbes. However, further research was suggested as there were issues such as undesired off-flavor and aroma, loss of color, and excessive energy consumption when the methods were applied. Therefore, ohmic heating and Pacific whiting were applied in this research to accelerate the fish sauce fermentation and suppress the negative outcomes.

Ohmic heating is a heating system that can generate consistent and uniform heat in various heating rates through electrical resistance between two electrodes. Consistent and uniform heat was believed to accelerate the fermentation by increasing endogenous protease activity and microbial activity.

Pacific whiting (*Merluccius productus*) is highly sustainable and abundant in the Pacific Northwest as a marine stewardship council (MSC)-certified species. Although Pacific whiting is cost effective and highly sustainable species, Pacific whiting was not fully utilized until surimi production started in 1991-1992 due to extreme softening of the fillet caused by three major proteases (cathepsin L, cathepsin B, and cathepsin H). However, intense protease activity was believed to accelerate fish sauce fermentation by rapidly degrading proteins during initial stage of fermentation. In addition, Pacific whiting is a gadoid fish which has low histidine, a precursor of histamine, content. Therefore, substituting anchovies, scombroid fish species which contains abundant histidine, with Pacific whiting was believed to lower the histamine content in fish sauce.

In our research, Pacific whiting mince containing 25% salt was fermented in either water bath or ohmic heating at various temperatures sequentially for 8 weeks: 25°C for Week 1, 35°C for Week 2, and 55°C for Week 3 to 8. Different temperature conditions were applied for ohmic heating and water bath to provide optimum conditions for three major proteases in Pacific whiting (cathepsin L, B and H) and to mimic conventional heating method. Although identical temperature and incubation time were provided for both water bath sample and ohmic heating sample, a significant difference was found between the two samples. Brownness, taste value, and overall nitrogen content were significantly higher with ohmic heating than those with water bath ($p < 0.05$). The difference was hypothesized to be due to electroporation and uniform heat penetration

by ohmic heating. Regardless of heating methods, Pacific whiting fish sauce demonstrated significantly lower histamine content when compared with commercial fish sauce made with anchovies ($p < 0.05$).

This study discovered that ohmic heating can be utilized for fish sauce production as it significantly accelerates fermentation and add a value to the utilization of protease-laden Pacific whiting.

Application of Ohmic Heating for Accelerating Fish Sauce Fermentation

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APPROVED:

_____

Major Professor, representing Food Science and Technology

_____

Co-Major Professor, representing Food Science and Technology

_____

Head of the Department of Food Science and Technology

_____

Dean of the Graduate School

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Hyung Joo Kim, Author

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# CONTRIBUTION OF AUTHORS

Dr. Christina DeWitt and Dr. Jae Park provided important guidance to design the project, interpret the data, and made important edits and insight.

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**Chapter 1. Literature Review**

**1.1 Fish Sauce**

 **(1) Definition**

   Fermentation is a traditional and widely used food preservation method that not only improves food safety and shelf life, but also improves sensory and nutritional attributes (Zang, Xu, Xia, & Regenstein, 2019). Fish sauce is a traditional fermented seafood condiment which is made through decomposition of proteins in fish muscle or intestine into amino acids and peptides by enzymes. During fermentation, high salt concentration is applied in fish sauce not only to develop flavor but also suppress spoilage. In general, fish sauce demonstrates a clear brown color and has salty and umami flavor with distinctive odor (Lopetcharat, Choi, Park, & Daeschel, 2001; Nakano et al., 2017).


 **(2) Variety of fish sauce**

   Fish sauce is commonly produced using anchovies in Asia, but primarily in Southeast Asia. Its consumption is not limited to Southeast Asian countries, but extended to Europe, United States and Canada as a condiment in their food. Anchovy-based fish sauce is called differently by different countries: Budu (Malaysia), Patis (Philippines), Kecap-ikan (Indonesia), Nam-pla (Thailand), Shottsuru (Japan), Nuöc-mâm (Vietnam), Nngapi (Myanmar), Pissala (France), Garos (Greece), Colombo-cure (Pakistan and India), Yeesu (China), and Aekjeot (S Korea) (Table 1.1) (Lopetcharat, 1999; Zang, Xu, Xia, & Regenstein, 2019).


 **(3) Microorganisms involved in fish sauce fermentation**

   Physiological characteristics of microorganisms involved in fish sauce fermentation are halophilic as fermentation is carried out at high salt concentrations (nearly 25%) either aerobically or obligate anaerobically (Thongthai, McGenity, Suntinanalert, & Grant, 1992). These microorganisms and their microorganism-derived enzymes are known to enhance flavor and odor through protein degradation (Lee et al., 1987; Park, Cho, Koo, Oh, & Lee, 2000). Kim and Kim (1990) reported viable cells rapidly increased until the 30th day of fish sauce fermentation and then decreased rapidly (Fig. 1.1). At the primary stage of fermentation, *Micrococcus spp.*, *Halobacterium spp.*, and *Sarcina spp.* were the

dominant microbes whereas *Pediococcus spp.* and yeasts such as *Saccharomyces spp.* and *Torulopsis spp.* were the dominant microbes during the middle stage and the terminal stage, respectively.

### (4) Production

Fish sauce is produced by autolysis of proteins in fish meat and intestine by various protease enzymes (Beddows, Ardeshir, & Daud, 1979; Lopetcharat, Choi, Park, & Daeschel, 2001). Each protease enzyme involved in protein degradation during fish sauce fermentation has an optimum pH ranges. According to Lopetcharat, Choi, Park, and Daeschel (2001), alkaline proteinases such as trypsin and chymotrypsin have higher activity in neutral conditions whereas cathepsins are more active in acid conditions. Bersamin and Napugan (1961) discovered that the pH of fish sauce decreased from neutral pH ($\sim$7) to acidic pH ($\sim$5) during fermentation due to the large amount of organic acid generated. During the initial stage of fish sauce fermentation, trypsin and chymotrypsin are responsible for protein hydrolysis whereas cathepsins manage protein hydrolysis when pH drops to acidic conditions (Lopetcharat, Choi, Park, & Daeschel, 2001). However, the activity of proteases decreased during the fermentation as the substrate (high molecular weight proteins) was used up.

### (4.1) Traditional fermentation

In general, traditional fish sauce is made using anchovies after grinding whole fish and mixing it with salt approximately at 3:1 and then storing in underground concrete tanks or various fermentation containers for long term hydrolyzation. Traditional methods for fish sauce (Nam-pla in Thailand and Aekjeot in S Korea) preparation are demonstrated in Fig. 1.2. The primary process to be carried out is to grind raw fish and mix with salt. The fish species used as raw material for traditional fish sauces from different countries are various: *Stolephorus* spp., *Ristrelliger* spp., *Engraulis* spp., and *Decapterus* spp. for Nouc-mam; *Stolephrous* spp., *Ristrelliger* spp., and *Cirrhinus* spp. for Nam-pla; *Stolephorus* spp. for Budu; *Stolephorus* spp., *Clupea* spp., *Decapterus* spp., *Leionathus* spp. for Patis; *Stolephorus* spp., *Clupea* spp., *Leiagnathus* spp., *Osteochilus* spp., *Puntius* spp., and *Ctenops* spp. for Kecap-ikan; *Ristelliger* spp., *Cybium* spp., and *Clupea* spp. for

Colombo-cure; *Sardinella* spp., *Engraulis* pupapa, *Jelio* spp., *Carangidae* sp, and *Teuthis* spp. for Yeesui; *Scomber* colias for Garos; *Ahya pellucida, Gobius* spp., *Engraulis* spp., and *Atherina* spp. for Pissala; *Astroscopus japonicus* for Shottsuru; *Astroscopus japonicus*, and *Engraulis japonica* for Aekjeot (Table 1.1). Regardless of countries, the species used as raw materials are generally small scombroid fish species that are caught near the production site in massive amounts which allows manufacturers to easily obtain the raw material (Park, Cho, Koo, Oh, & Lee, 2000). Therefore, one of the critical conditions for manufacturing a high-quality fish sauce is to use fresh raw materials.

Other conditions utilized to produce a high-quality fish sauce is application of salt to reduce the water activity which would result in suppression of microorganisms that may cause spoilage. Microbial activity can be significantly different depending on the amount of salt used, fermentation temperature, and pH. In this aspect, the ratios between fish and salt for the production of different traditional fish sauce were examined (Table 1.1). The ratios between raw fish and salt used in traditional fish sauces from different countries are the following: 3:1～2 for Nouc-mam; 1～5:1 for Nam-pla; 3～5:1 for Budu; 3～4:1 for Patis; 6:1 for Kecap-ikan; 6:1 for Colombo-cure; 4:1 for Yeesui; 9:1 for Garos; 4:1 for Pissala; 5:1 for Shottsuru; 3～4:1 for Aekjeot (Lopetcharat, Choi, Park, & Daeschel, 2001; Zang, Xu, Xia, & Regenstein, 2019).

After mixing the raw fish and salt based on the ratios, fermentation is proceeded. Different fermentation periods are applied in the traditional fish sauces by different countries. Fermentation takes 3 to 12 months for Nouc-mam, Budu, Patis, and Yeesui, 5 to 12 months for Nam-pla, 6 months for Kecap-ikan, and 12 months for Colombo-cure, 8 days for Garos, 2 to 8 weeks for Pissala, 3 to 6 months for Shottsuru, and 12 to 18 months for Aekjeot (Park, Cho, Koo, Oh, & Lee, 2000).

Production of first grade fish sauce begins by adding salt to freshly ground fish and mixing it thoroughly. The salted fish gets fermented for a period of time and then filtered. Lastly, the filtrate is aged in sunlight for 2-4 weeks for color development. For secondary grade fish sauce, additional fermentation is held using residues from the prior process with the addition of salt water (concentration matches first fermentation concentration). After 1-4 months of fermentation, secondary grade fish sauce is filtered and collected. Just like the secondary grade fish sauce, third grade fish sauce is made through fermenting

the prior process with the addition of salt water. However, additional processes are applied for third grade fish sauce: boiling to extract proteins, adding caramel pigment to adjust the color to brown, and adding nitrogen-based additives to adjust the total nitrogen (Park, Cho, Koo, Oh, & Lee, 2000). To obtain the first grade S Korean fish sauce Aekjeot, boiling is often applied to sterilize and aggregate polymer peptides prior to filtration.

**(4.2) Rapid fermentation**

A negative aspect of the traditional fermentation method is that the fermentation period takes too long. Therefore, many researchers have tried to develop methods to accelerate the fermentation by either providing optimum conditions (temperature, pH, and salt concentration) for endogenous enzymes with strong activity (Gildberg, Espejo-Hermes, & Magno-Orejana, 1984; Gildberg, 1989; Gildberg, 2001; Lopetcharat, 1999; Yoshinaka, Sato, Tsuchiya, & Ikeda, 1983; Yu et al. 2014) or intentionally adding exogenous enzymes and providing optimum conditions (Akolkar, Durai, & Desai, 2010; Beddows and Ardeshir, 1979; Chaveesuk, Smith, & Simpson, 1993; Funatsu et al., 2000; Furutani & Satomi, 2013; Klomklao, Benjakul, Visessanguan, Kishimura, & Simpsonet, 2006; Rabie, Namir, Rabie, & Hassanien, 2018; Taoka et al., 2019; Uchida et al., 2005; Utagawa, 2012; Xu, Yu, Xue, Xue, & Ren, 2008; Yongsawatdigul, Rodtong, & Raksakulthai, 2007; Yu et al., 2014). To be specific, Gildberg, Espejo-Hermes, & Magno-Orejana (1984), Gildberg (1989), Xu, Yu, Xue, Xue, & Ren (2008), and Yu et al. (2014) applied acid, alkali, or adjusted the salt content whereas Gildberg (2001) provided optimum fermenting conditions for tryptic enzymes present in male Arctic capelin and Atlantic cod intestines in order to accelerate the fermentation were 26°C at pH 8.0. In addition, Lopetcharat (1999) discovered optimum fermenting conditions for cathepsin enzymes present in whole Pacific whiting and by-products were 50°C with 25% salt, and Yoshinaka, Sato, Tsuchiya, & Ikeda (1983) discovered optimum fermenting conditions for endogenous visceral enzymes present in sardine intestine to accelerate fermentation were 50°C at pH 8.0.

Additionally, there were also studies to accelerate fish sauce production by applying commercial proteolytic enzymes such as bromelain, papain, neutrase, alcalase, and protamex or inoculating microbes with strong protease activity such as *Aspergillus oryzae*

and *Aspergillus sojae*. To be specific, Akolkar, Durai, & Desai (2010) applied *Halobacterium* sp. SP1 as a starter culture, Beddows and Ardeshir (1979) applied plant proteases such as bromelain, papain and ficin, Chaveesuk, Smith, & Simpson (1993) applied trypsin and chymotrypsin, Funatsu et al. (2000), Furutani and Satomi (2013), and Utagawa (2012) added Koji mold, Klomklao, Benjakul, Visessanguan, Kishimura, & Simpsonet (2006) applied skipjack tuna (*Katsuwonus pelamis*) spleen, Rabie, Namir, Rabie, & Hassanien (2018) applied bromelaine, Taoka et al. (2019) applied *Lactobacillus plantarum* Strain N10, Uchida et al. (2005) applied soy sauce koji and lactic acid bacteria, and Yongsawatdigul, Rodtong, & Raksakulthai (2007) applied proteinases and bacterial starter culture in order to accelerate the fermentation. However, there were reports that the acceleration method brought up undesired off-flavor and aroma or loss of color of fish sauce. In addition, there has not been any research that aims to accelerate fish sauce production by taking advantage of the ability of ohmic heating to apply rapid and consistent heating system as well as accelerate the destruction of cell membrane through electroporation. The objective of this research, therefore, was to investigate the application of ohmic heating to fish sauce production using Pacific whiting (*Merluccious productus)*. This species was deemed appropriate for production of fish sauce because it is abundant and inexpensive. The low value for Pacific whiting results from high level of autolysis protease enzymes (cathepsin B, H, and L) that make its fillet soft and, therefore, difficult to market.

## (5) Factors affecting fish sauce quality
### (5.1) Fish species

During fish sauce fermentation, fish serves as substrates not only in enzymatic reactions but also in microbial reactions (Lopetcharat, 1999). The main fish species used in fish sauce manufacture are red-fleshed marine fish species such as anchovy (*Stolephorus spp.* or *Engraulis japonicus*), sardine (*Clupea pilchardus*), mackerel (*Rastrelliger spp.* or *Scomber colias*), jack mackerel (*Decapterus spp.*), gizzard shad (*Dorosoma spp.*), herring (*Clupea spp.*), and Japanese sardinella (*Sardinella spp.*) (Table 1.1). Besides red-fleshed marine fish species, carp (*Clarius spp.*), catfish (*Clarias spp.*), snakeheads (*Ophicephalus spp.*), squid (*Omnastrephis sloani* or *pacificus*), sandfish (*Astroscopus japonicus*) and

fresh water fish species such as *Cirrhinus spp.*, *Osteochilus spp.*, *Puntius spp.*, *Ctenops spp.* are known to be used in fish sauce manufacture (Lopetcharat, Choi, Park, & Daeschel, 2001).

In general, raw fish used in fish sauce fermentation differ from country to country as species that can be easily obtained and abundantly caught are used. Fish sauce is made by autolysis of proteins in fish meat and intestine by protease enzymes. Therefore, fish species used in fish sauce must have abundant autolytic enzymes with strong activity that can decompose fish protein (Park, Cho, Koo, Oh, & Lee, 2000). Depending on the fish species used as raw material, not only sensory properties such as odor and flavor but also nutritional properties can be impacted. Therefore, fish species used in fish sauce manufacture is a very important factor that can affect the quality of the product.

### (5.2) Freshness of fish

The freshness of fish is the primary factor that controls the quality of fish sauce (Park, Cho, Koo, Oh, & Lee, 2000). Raw fish used in fish sauce production are generally low-price species which are caught in large quantities in a single fishing trip without refrigerated storage. Therefore, freshness management is likely to be insufficient. However, suppressing microbial contamination and biogenic amine generation, the indicators of spoilage, from harvesting to offloading and to manufacturing, is demanded in order to produce high quality fish sauce.

### (5.3) Composition of salt

Salt is a chemical compound in which a sodium (Na) atom and a chlorine (Cl) atom are combined, and is a neutral salt with the molecular formula of NaCl. Salt is widely used in food processing as it not only provides salty taste but also a preservation effect through dehydration caused by osmotic pressure (Kim & Kang, 2021). During fish sauce fermentation, salt suppresses the growth of harmful microorganisms by lowering the water activity of raw fish. Therefore, salt is an essential ingredient in fish sauce fermentation.

In general, salt can be classified into either sea salt or rock salt based on the source. Due to the easy availability, sea salt is more often used in fish sauce fermentation. Sea

salt is obtained by evaporating and concentrating sea water using solar heat and wind.

According to Lopetcharat (1999), the major component of sea salt is sodium chloride which consists approximately 90% of sea salt. Other components that can affect the fish sauce fermentation are $CaSO_4$, $MgSO_4$, $MgCl_2$, and $CaCl_2$ which consists 0.24%, 0.17%, 0.3%, and 0.24% of salt, respectively (Suwanik, 1979). Wilaipan (1990) discovered that $CaSO_4$, $MgSO_4$, $MgCl_2$, and $CaCl_2$ in sea salt inhibits the diffusion of NaCl into fish, resulting in insufficient preservative action and rapid decomposition. Additionally, fish sauce fermentation can be accelerated due to the presence of the halophilic bacterias such as *Halobacterium* sp., *Halococcus* sp., and *Serratia salinaria* and proteases produced by the bacteria when sea salt is applied (Horie & Hinago, 1974).

### (5.4) Ratio between fish and salt

Another very important factor affecting the quality of fish sauce is the ratio between fish and salt. The ratio between fish and salt are 3:1～2 for Nouc-mam, 1～5:1 for Nam-pla, 3～5:1 for Budu, 3～4:1 for Patis, 6:1 for Kecap-ikan, 6:1 for Colombo-cure, 4:1 for Yeesui, 9:1 for Garos, 4:1 for Pissala, 5:1 for Shottsuru, and 3～4:1 for Aekjeot (Table 1.1) (Lopetcharat, Choi, Park, & Daeschel, 2001). The ratio between fish and salt varies depending on the country of manufacture as well as the size and freshness of the fish species used as raw material (Wilaipan, 1990). The difference in the ratio between the countries was believed to be due to the difference in climate in addition to the consumer's perception on the risk of consuming high salt concentrated condiment between countries.

### (6) Standards for fish sauce

The standards for fish sauce were established based on producing countries (China, Japan, S Korea, Thailand, Philippines, and Vietnam), importing countries (USA, Canada, and Europe), and Codex Alimentarius. Examination on the producing countries was conducted based on National Health Commission of the People's Republic of China (2022), Ministry of Health, Labour and Welfare of Japan (2022), Ministry of Food and Drug Safety of S Korea (MFDS) (2022a;b), Food and Drug Administration of Thailand (FDA Thailand) (2022),  Food and Drug Administration of Philippines (FDA Philippines) (2022), and Vietnam's Standards and Quality Institute (VSQI) (2022), respectively.

The survey on fish sauce standards of the importing countries was limited to the United States, Canada, and Europe. Survey on the importing countries was conducted based on United States Food and Drug Administration (FDA) (2022), Canadian Food Inspection Agency (CFIA) (2021), and European (EU) Food Safety Authority (2022). As there is no standard for fish sauce in the U.S., standards for seafood and processed seafood are presented as a substitute. The standards of Codex Alimentarius were compiled by examining the CODEX Alimentarius International Food Standards (2022).

The standards for fish sauce can be divided into sensory, physical, biological, and chemical standards. Fish sauce standards set in various countries and agency are demonstrated in Table 1.2. Among the chemical standards, factors that do not affect the fermentation process such as PCBs, heavy metals, and radioactivity were not included.

## (6.1) Standards on sensory properties

The sensory standards for fish sauce are divided into four properties: color, transparency, flavor, and odor. According to the standards used in Vietnam and S Korea, the product should have the appropriate unique brown color without any discoloration. In addition, transparency of product should be translucent and not turbid based on the CODEX Alimentarius International Food Standards (2022). Lastly, distinct objectionable off odor (rotten, putrid, rancid, gamey, pungent etc.) and off flavor (bitter, sour, metallic, taint, etc.) must not be detected based on the CODEX.

## (6.2) Standards on physical properties

The physical standards for fish sauce are divided into two properties: foreign matter and sediment. Foreign matter should not be detected based on the standards used in China, S Korea, and Vietnam. In case of sediment, only Thailand has regulation in which the sediment should be a natural substance that weighs below 0.1 g. Among the physical standards, sediment can be controlled by introducing a heat treatment prior to the filtration process and applying a metal detection process by passing the product in a bottle through a metal detector.

**(6.3) Standards on biological properties**

The biological standards for fish sauce are divided into nine properties: three hygiene indicators such as viable cell count, *E. coli*, and Coliform group, five food poisoning bacteria groups such as *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *Clostridium perfringens*, and mold. The viable cell standards of fish sauce were presented in two countries, Vietnam and China, which were $10^5$ CFU/g and number of sample (n)=5, maximum allowable number of sample units yielding results between m and M (c)=2, microbiological limit that separates good from marginally acceptable (m)=$10^4$ CFU/g, microbiological limit above which sampling results are unacceptable (M)=$10^5$ CFU/g, respectively. In the case of *E. coli*, the result should be negative in Vietnam and n=5, c=2, m=4 CFU/g, M=40 CFU/g in Canada. Standards for Coliform group are $10^2$ CFU/g in Vietnam, n=5, c=2, m=10 CFU/g, M=$10^2$ CFU/g in China, and n=5, c=1, m=0, M=0 in S Korea. Food poisoning bacteria *Staphylococcus aureus* should be negative in Vietnam, n=5, c=2, m=$10^2$ CFU/g, M=$10^4$ CFU/g in China, $10^4$ MPN/g in U.S., n=5, c=1, m=$10^3$ CFU/g, M=$10^4$ CFU/g in Canada. For *Salmonella* spp., standards in Canada, China, and U.S. indicate that *Salmonella* spp. should be negative. In case of, *Listeria monocytogenes* only U.S. has regulation which is negative detection. Similarly, *Vibrio parahaemolyticus* and *Clostridium perfringens* are also regulated in China and Vietnam, respectively, and the values are the standards are n=5, c=2, m=$10^2$ MPN/g, M=$10^3$ MPN/g and 10 CFU/g. Lastly, only Vietnam has standard on mold which is 10 CFU/g. In order to meet these biological standards for fish sauce, a heat treatment process prior to filtration could be introduced to aggregate polymer peptides and inhibit hygiene indicator bacteria, food poisoning bacteria and mold.

**(6.4) Standards on the chemical properties**

Detail values of chemical standards for fish sauce such as protein (%), PCBs, total nitrogen (%), amino acid nitrogen (g/100 g), ammonia nitrogen (g/100 g), NaCl (%), pH, histamine (mg/100 g), tar color pigment, heavy metal (mg/kg), and radioactivity

(Bq/kg) were investigated. However, factors that does not change during fermentation such as PCBs, heavy metal, and radio activity were not included.

The standards for protein content of commercial fish sauce by country of origin was 6% or above for a first grade and 4% or above for a regular grade in Thailand. In case of the CODEX, S Korea, Vietnam, and Thailand, total nitrogen content was used instead of protein content. Total nitrogen content must be 1.0% or above based on CODEX and standards from S Korea and Vietnam whereas the concentration must be 0.9% or above in Thailand.

Amino acid nitrogen standard and ammonia nitrogen standard are based on the percentage of amino acid nitrogen in total nitrogen. Amino acid nitrogen content indicates the amount of soluble amino groups in fish sauce whereas ammonia nitrogen content is an indicator of the breakdown of soluble protein and peptides into free amines (Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2006). Amino acid nitrogen must be 35% or above based on the standards used in Vietnam, 40% or above but less than 60% in Thailand, and 40% or above for the CODEX. For the ammonia nitrogen, the product must have 30% or lower concentration based on the standards in Vietnam.

Sodium chloride content must be above 24.5% for a first-grade product in Vietnam, 24% or above in the Philippines, and 20% or above based on CODEX and standards in Thailand. In the case of pH, pH of fish sauce product must range 5.0-6.5 based on CODEX, standards in Philippines, and Vietnam. Histamine content must be 400 mg/kg or lower according to the CODEX and standards used in Philippines and EU whereas Canada has much strict regulation by 200 mg/kg or less. Lastly, the tar color pigment, a pigment synthesized by extracted benzene or naphthalene from coal tar, must not be detected based on standards used in S Korea and Thailand.

As an appropriate standard for quality control of fish sauce, chemical standard was judged to be the most appropriate rather than sensory, physical, or biological standards. Therefore, it was believed that the quality of fish sauce can be controlled using total

nitrogen content, amino acid nitrogen concentration, sodium chloride concentration, pH, and histamine content as the standards.

### (7) Changes in composition during fermentation and compositional properties of the final product

Fish sauce is a seasoning product that contains a large amount of peptides and free amino acids derived from natural animal protein as it is processed by applying high concentration of salt to fish and aging it for a long time to liquefy the fish using autolytic enzymes and microorganisms (Lopetcharat, 1999).

### (7.1) Changes in components during fermentation

Cho and Choi (2000) studied the changes in total nitrogen content, amino acid nitrogen content, and percent of amino acid nitrogen in total nitrogen during anchovy fish sauce fermentation and sand lance fish sauce fermentation for 18 months (Fig. 1.3). Regardless of species, fish sauce demonstrated an increasing trend in total nitrogen content and amino acid nitrogen content as the fermentation proceeded. During fermentation from month 2-18, total nitrogen content, amino acid nitrogen content, and percentage of amino acid nitrogen in total nitrogen of anchovy fish sauce was 712-2,387 mg/100 mL, 300-1,442 mg/100 mL, and 42.1-60.4%, respectively. In the case of sand lance fish sauce, 1,400-1,825 mg/100 mL, 775-1,256 mg/100 mL, and 55.4-68.9% was obtained, respectively. The coefficient of correlation was above 0.99 and the results were believed to be due to degradation of proteins into peptides and free amino acids during fermentation.

Cho and Choi (2000) also studied on the changes in free amino acid concentration during anchovy fish sauce fermentation. Total free amino acids content demonstrated an increasing trend as the fermentation proceeded and the value increased from 7.9 to 9.1 g/100 mL during month 5.5 to 18 (Table 1.2). Among the free amino acids, glutamic acid demonstrated the largest increase in content and composition, followed by leucine,

alanine, lysine, isoleucine, and valine. However, after 18 months of fermentation, the free amino acid composition of the anchovy fish sauce was different from the free amino acid composition of the raw anchovy (Park, Cho, Koo, Oh, & Lee, 2000).

**(7.2) Comparison on components of commercial fish sauce**

Cho and Choi (2000) conducted research on the quality characteristics of commercial fish sauce made from four different countries (Thailand, Vietnam, Philippines and S Korea) (Table 1.3). The total nitrogen content of commercial fish sauce by country of origin was 441-1,900 mg/100 mL for Thailand, 865-2,199 mg/100 mL for Vietnam, 140-1,281 mg/100 mL for the Philippines, and 934-2,387 mg/100 mL for S Korea. When the total nitrogen standards (900 mg/100 mL for Thailand; 1,000 mg/100 mL for Vietnam, S Korea and CODEX) were applied, 2 out of 4 products in Thailand, 1 out of 7 products in Vietnam, and 1 out of 2 products in Philippines were below the standards. However, 19 out of 19 products met the regulations in S Korea.

The amino acid nitrogen content of commercial fish sauce by country of production was 338.4-1,570.6 mg/100 mL for Thailand, 629.3-1,643.0 mg/100 mL for Vietnam, 115.4-955.1 mg/100 mL for Philippines, and 570.5-1,442.2 mg/100 mL for S Korea. Based on the results from the total nitrogen content and the amino acid nitrogen content of commercial fish sauce produced in different countries, the percentage of amino acid nitrogen in total nitrogen can be calculated. Based on the CODEX (40% or higher) and the standard used in Vietnam (35% or higher), all products, regardless of the country of origin, satisfied the standard. However, when the standard used in Thailand (40% or higher, but less than 60%) was applied, all 4 products from Thailand, all 7 products from Vietnam, both products from Philippines, and 7 out of 19 products from S Korea were disqualified (Cho & Choi, 2000).

The sodium chloride concentration of commercial fish sauce by the country of origin was 24.1-29.9 g/100 mL for Thailand, 23.7-29.4 g/100 mL for Vietnam, 29.7-30.6 g/100 mL for Philippines, and 22.9-30.9 g/100 mL for S Korea (Cho & Choi, 2000). Based on the CODEX and the standard used in Thailand (20.0 g/100 ml or higher),

Philippines (24.0 g/100 mL), and Vietnam (24.5 g/100 mL or higher for a first grade), 3 out of 4 products from Thailand, 5 out of 7 products from Vietnam, both products from Philippines, and 17 out of 19 products from S Korea met every quality category.

According to Cho and Choi (2000), the pH of commercial fish sauce by the country of origin was 5.08-6.38 for Philippines, 4.66-5.93 for Thailand, 5.26-5.91 for Vietnam, and 5.44-6.79 for S Korea. Based on the CODEX and the standard used in Philippines (5.0-6.5) and Vietnam (5.0-6.5 in first grade), 2 out of 4 products from Thailand, all 7 products from Vietnam, both products from Philippines, and 17 out of 19 products from S Korea met every quality category.

The brownness of commercial fish sauce by the country of origin was 0.609-2.311 for Philippines, 1.213-3.419 for Thailand, 0.788-3.274 for Vietnam, and 0.547-1.447 for S Korea (Cho & Choi, 2000).

Cho and Choi (2000) and Lopetcharat, Choi, Park, & Daeschel (2001) reported the free amino acid contents of commercial fish sauce produced from different countries (Table 1.4). The free amino acid content of fish sauce by the country of origin was highest in fish sauce from S Korea (9.07 g/100 mL), followed by Vietnam (8.53 g/100 mL), Thailand (6.46 g/100 mL), Philippines (5.72 g/100 mL), and China (4.65 g/100 mL). The major free amino acids found were glutamic acid and lysine in fish sauce from China and Philippines; glutamic acid, alanine, and leucine in fish sauce from S Korea; glutamic acid and leucine in fish sauce from fish sauce from Vietnam; and glutamic acid, alanine and lysine in fish sauce from Thailand.

The flavor of fish sauce plays a major role in determining its quality (Kim and Kim, 1990). Among the flavor components of fish sauce, volatile acid content and volatile basic nitrogen content are generally high whereas volatile amine content and volatile alcohol content are very low. In general, low grade fish sauce products contain the low amount of volatile acids. The major flavor components in commercial fish sauce are volatile acids such as acetic acid, propionic acid. isobutyl acid, Isovaleric acid, valeric acid, and caproic acid, carbonyl compounds such as formaldehyde, acetaldehyde, acetone, isobutyl aldehyde, methyl ethyl ketone, and isovaleraldehyde, volatile amine

compounds such as mono-, di, tri-methylamine, ethylamine, isopropyl amine, propylamine, diethyl amine, and butylamine, and volatile alcohols such as methanol, ethanol, butanol, and amyl alcohol (Kim and Kim, 1990).

### (8) Usage

Fish sauce, due to its characteristic flavor and odor, is a popular condiment and often used as a dipping sauce or a salt replacement not only in Southeast Asia but also worldwide (Lopetcharat, Choi, Park, & Daeschel, 2001). Fish sauce can be stored at room temperature for a long period of time as its high sodium chloride content can prevent the spoilage of the product.

## 1.2 Ohmic heating

### (1) Mechanism

Ohmic heating is a heating method in which an alternation electric current passes through electrically conducing food product and the heat is generated internally due to electrical resistance of the food product giving a higher rate of temperature increase than that of conventional heating methods. Also, ohmic heating provides uniform heat distribution throughout the product. By using ohmic heating it is possible to overcome the problems of the microwave processing such as, crust formation, protein toughening, price, and portability. The first ohmic cooker for surimi gel testing developed in 1994-1995 at the OSU Seafood Lab (Astoria, OR) used a 2 cm diameter which is not usually used for the commercial industry. The 2nd generation cooker is a 3 cm i.d. tube versus the 2 cm i.d. of the 1st generation (Park and Reed, 2014). Then RAPSA (Rapid, Accurate, Portable Surimi Analyzer) were invented as a commercial version.

### (2) Effects on food

Similar to microwave heating, ohmic heating converts electrical energy into heat inside food. However, unlike microwave heating that can only heat food unevenly due to the limited heat penetration, ohmic heating can provide even heat to food between two electrodes as it does not have limitation on heat penetration. Additionally, ohmic heating

not only has ability to provide consistent heat but also has ability to heat liquid and solid at the same time, and no need to apply mechanical stirring as the heat is evenly distributed as long as runaway heat is blocked (Buffler, 1993; De Alwis & Fryer, 1990).

### (3) Applications

Ohmic heating, a new heating method, is being applied in various fields, such as heat sterilization of paste like high viscosity foods, heat sterilization of foods with a mixture of liquid and solid, thawing frozen foods, production and heat sterilization of surimi seafood, and heat sterilization of food containing particles (Cho, Kim, Kim, & Pyun, 1994; Park & Beliveau, 2014).

Ohmic heating has many advantages such as having no restriction on heat penetration, uniform heating, ability to heat liquid and solid at the same time, and no need to apply mechanical stirring as the heat is distributed identically. In this aspect, premium grade fish sauce can be produced with consistent heat conduction and ability to hold optimum temperatures for autolytic protease enzymes present in fish, when ohmic heating is applied. In addition, ohmic heating is highly energy efficient. Kurnia and Anjar (2021) stated that ohmic heating can convert 90% of the electrical energy into heat.

However, ohmic heating also has disadvantages as well. First of all, as the ohmic heating is relatively new heating system, studies to be referred are limited. One may have to develop their own protocols and heating conditions in order to apply the ohmic heating system into their research. In addition, ohmic heating may be difficult to be applied in heating samples with high fat content as the sample would be non-conductive due to lack of moisture and salt (Kurnia and Anjar, 2021).

### 1.3 Biogenic amine

Biogenic amines are biologically active low molecular weight nitrogen compounds formed from amino acids such as histidine, tyrosine, tryptophan, lysine, phenylalanine, arginine, ornithine, and so on (Park, Lee, & Mah, 2019; Wójcik, Łukasiewicz, & Puppel, 2020). Biogenic amines are generated by decarboxylation reaction of free amino acids in acidic conditions. Through the decarboxylation of lysine, ornithine, arginine, and histidine, biogenic amines such as cadaverine, purescine, agmatine, and histamine are

generated, respectively (Fig. 1.4) (Wójcik, Łukasiewicz, & Puppel, 2020). Depending on the chemical structure, biogenic amines can be classified as either aromatic amines (histamine, tyramine, serotonin, phenylethylamine, and tryptamine), aliphatic diamines (putrescine and cadaverine), aliphatic polyamines (agmatine, spermidine, and spermine), or volatile aliphatic amines (methylamine, ethylamine, isopentylamine, and ethanolamine) (Erdag, Merhan, & Yildiz, 2018). Biogenic amines are present and can be found in foods, especially when freshness deteriorates in seafood or processed seafood products (Ruiz-Capillas & Jimenez-Colmenero, 2010).

The intake of foods containing high concentrations of biogenic amines has been associated with toxic effects and can present a potential health hazard. The toxicity of biogenic amines is mainly related to histamine and tyramine (Świder, Roszko, Wójcicki, & Szymczyk, 2019). Histamine toxicity results from consuming more than a certain amount of stale red meat fish (tuna, mackerel, bonito, bluefish, anchovies and so on) which contains abundant histidine, a precursor of histamine, or stale processed red meat fish products. Due to the vasodilator effect of histamine, symptoms such as diarrhea, headache, abdominal pain, hypotension, flushing, and rash may appear. In general, these symptoms disappear within a few hours, but if they persist for a long time, antihistamine treatment must be taken (Ruiz-Capillas & Herrero, 2019). Tyramine is known to cause hypertension when ingested over a certain amount of stale fish or stale processed fish products that contains high dosage of tyrosine, a precursor of dopamine and noradrenaline. In addition, caution should be taken on other biogenic amines such as putrescine, cadaverine, spermidine, phenylethylamine, agmatine, and spermine as these biogenic amines have the

function of enhancing the toxicity of histamine (Wójcik, Łukasiewicz, & Puppel, 2020). The histamine safety standard for fish sauce is currently 200 mg/kg or less in Canada and 400 mg/kg or less in CODEX and EU. Recently, however, there are movements to strengthen the histamine standard.

In general, high histamine content is often found in fish sauce when stale red meat fish is used, when fish sauce is fermented with low salt concentration, or when there was a failure in controlling temperature. Therefore, histamine content in fish sauce should be controlled by either using fish species with low histidine such as Pacific whiting, cod, and

Alaska pollock, applying halophilic starter cultures (Zaman, Bakar, Jinap, & Bakar, 2011), or inoculating halophilic lactic acid bacteria (Wakinaka et al, 2019) to inhibit histamine accumulation.

## 1.4 Pacific whiting

### (1) Characteristics

Pacific whiting, also known as Pacific hake, is semi-pelagic schooling species of ground fish found off the West Coast of the United States and Canada (NOAA Fisheries, 2022). Morphological and ecological characteristics of Pacific whiting are demonstrated in Table 1.5. Pacific whiting is a round shape fish with silvery body color, black speckles, and black colored oral mucosa. Pacific whiting is known live around 15 years and spawns from January through March off south-central California, and feed upon shrimp, krill, and pelagic schooling fish species, such as eulachon and Pacific herring. This species is known to form a school in midwater between 164 and 1,640 feet off the west coast of the U.S. and Canada. The stocks of Pacific whiting can be divided into 3 groups: a migratory coastal stock, a central-south Puget Sound stock, and a Strait of Georgia stock

### (2) Annual catch

The coastal stock of Pacific whiting is managed through the bilateral Pacific Whiting Agreement between the United States and Canada. The coast-wide fishery Pacific whiting landings averaged 239,919 M/T from 1966 to 2020, with a low of 89,930 M/T in 1980 and a peak of 440,950 M/T in 2017 (Fig. 1.5) (Johnson, Edwards, Berger, & Grandin, 2021). Since 1987, The average catch (239,919 M/T) or higher was harvested after 1987 (excluding 10 years such as 1993, 2000-2004, 2009-2010, 2012 and 2015). Since 1990, the average Pacific whiting catch in U.S. coast has been 181,620 M/T (76.1% of total catch) whereas 58,299 M/T (23.9% of total catch) was caught from the coast of Canada. The average Pacific whiting coast-wide catch during 2011–2020 was 258,306 M/T in U.S and 66,799 M/T in Canada (Johnson, Edwards, Berger, & Grandin, 2021). Total catch in 2020 was 379,270 M/T, of which the catch in U.S. and Canada were 287,980 M/T and 91,362 M/T, respectively. Attainment in the U.S. was 67.8% of its quota and 87.4% in Canada.

**(3) Nutritional Composition**

Data on the nutritional components of Pacific whiting is very limited. Therefore, nutritional components and histidine, a precursor of histamine, of white-fleshed fish such as Pacific whiting, Pacific cod, and Alaska pollock and dark-fleshed fish which are widely used as raw materials for fish sauce, such as anchovy and sardine, were compared (Table 1.6). The proximate composition of Pacific whiting (*Merluccius productus*) was 80.5% moisture, 16.8% crude protein, 1.5% crude fat, and 1.0% ash, which was similar with other white-fleshed fish, Alaska pollock (*Theragra chalcogrammus*) and Pacific cod (*Gadus macrocephalus*). The proximate composition of Alaska pollock was 80.3% moisture, 17.5% crude protein, 0.7% crude fat, and 1.5% ash whereas that of Pacific cod was 79.6% moisture, 18.2% crude protein, 0.4% crude fat, and 1.4% ash. However, a significant difference was found between white-fleshed fish and dark-fleshed fish. The proximate composition of anchovy (*Engraulis japonicus*) and sardine (*Sardinops sagax*) were 73.4% and 69.2% moisture, 17.7% and 20.0% crude protein, 5.4% and 9.1% crude fat, and 1.5% and 1.2% ash, respectively (National Institute of Fisheries Science in Korea, 2018).

**(4) Usage**

In general, Pacific whiting is processed into headed and gutted product, fillet, or surimi. Processed Pacific whiting is then frozen prior to the distribution to prevent rapid protein degradation that may decrease the product quality. Therefore, methods to enrich and utilize the undervalued Pacific whiting is urgently needed. In this aspect, additional research needs to be conducted to develop methods to turn Pacific whiting into products that meet consumer trends such as HMR (home meal replacement) products or essential products used as an ingredient in making HMR such as sauces. Lopetcharat and Park (2002) conducted research to enrich the utilization of protease-laden Pacific whiting by developing a Pacific whiting fish sauce. However, it may be difficult to actually produce fish sauce from fresh Pacific whiting when costs to maintain the temperature are considered. In order to produce fish sauce in U.S, persistent heat is required unlike the Southeast Asian countries as the temperature is not high enough to ferment fish sauce. Therefore, further research is still required that can be actually encourage industries in

U.S to make fish sauce from Pacific whiting.

### (5) Autolytic enzymes

Fish autolytic enzyme refers to an enzyme that is present within the fish but degrades proteins in fish. Autolytic enzymes cleave polypeptides or proteins into small fragments by restricting the terminal peptide linkage and there are variety of autolytic proteinase present in fish muscle (Yongsawatdigul, Hemung, & Choi, 2014). Therefore, autolytic enzymes are regarded as negative factor in fish fillets or surimi as texture properties may rapidly degrade resulting in a decreased textural quality. However, autolytic enzymes can be regarded as positive and essential in fish sauce fermentation as amino acids generated by autolysis can contribute to microbial growth during primary stage of fermentation.

According to Fowler and Park (2015), major proteases found in Pacific whiting are cathepsins B, H, and L. Molecular weight, hydrolysis type, optimum conditions (pH and temperature), and target proteins of cathepsins present in Pacific whiting are described in Table 1.7.

### (5.1) Cathepsin B

Cathepsin B is well known lysosomal thiol proteinase which can be separated into two molecular components: cathepsin B1 and cathepsin B2. Cathepsin B1 is a thiol endopeptidase with molecular weights in the range of 24-28 kDa and demonstrates highest activity at pH 6 but unstable at pH 7 or above. Cathepsin B2 is an exopeptidase with molecular weights in the range of 47-52 kDa and hydrolyzes Bz-Gly-Arg (Hippuryl-Arginine) at pH 5.5-6.0, and demonstrates amidase activity at pH 5.6 (Yoshida et al., 2015).

Cathepsin B also plays an important role in intracellular proteolysis. Although optimum pH of cathepsin B may vary depending on types and substrates, optimum temperature is identical as 35℃. Cathepsin B manages degradation of myofibrillar proteins, such as myosin and actin, and sarcoplasmic proteins, such as hemoglobin and myoglobin.

### (5.2) Cathepsin H

Cathepsin H is a lysosomal cysteine peptidase exhibiting predominant aminopeptidase

activity but limited endopeptidase activity (Rawlings and Barrett, 1993). The enzyme is involved in the degradation of intracellular protein such as myosin and actin. The optimum pH and temperature of cathepsin H is pH 5 and 20°C in general, but may alter based on species.

**(5.3) Cathepsin L**

Research has demonstrated that most of cathepsin B and almost all of cathepsin H present in Pacific whiting mince can be removed with rinsing whereas cathepsin L, a protease attached to myofibrillar protein, is not removed. During the initial stage of fermentation, cathepsin B, H, and L are likely involved in protein degradation. However, from the middle stage of fermentation, water-soluble cathepsins (B and H) become solubilized in the liquid generated during fermentation and excreted from fish meat whereas cathepsin L is retained in the fish meat. Therefore, from the middle stage of fermentation, cathepsin B and H are believed to degrade proteins present in liquid portion whereas cathepsin L degrades proteins present in fish meat (An, Weerasinghe, Seymour, & Morrissey, 1994). Cathepsin L is known to have the highest activity at 55°C and can degrade myosin heavy chain, actin, α-actinin, troponin-T, and troponin–I (Coffey & de Duve, 1968).

Table 1.1. Country of origin, raw fish species, fish:salt ratio, and fermentation periods applied in various traditional fish sauce from different countries

| No | Fish sauce | | | | |
| | Name | Country of origin | Raw fish species | Fish:Salt ratio | Fermentation period |
| --- | --- | --- | --- | --- | --- |
| 1 | Nouc-mam | Cambodia | *Stolephorus* spp., *Ristrelliger* spp., *Engraulis* spp., *Decapterus* spp. | 3:1~2 | 3~12 months |
| 2 | Nam-pla | Thailand | *Stolephrous* spp., *Ristrelliger* spp., *Cirrhinus* spp. | 1~5:1 | 5~12 months |
| 3 | Budu | Malaysia | *Stolephorus* spp. | 3~5:1 | 3~12 months |
| 4 | Patis | Philippines | *Stolephorus* spp., *Clupea* spp., *Decapterus* spp., *Leionathus* spp. | 3~4:1 | 3~12 months |
| 5 | Kecap-ikan | Indonesia | *Stolephorus* spp., *Clupea* spp., *Leiagnathus* spp., *Osteochilus* spp., *Puntius* spp., *Ctenops* spp. | 6:1 | 6 months |
| 6 | Colombo -cure | India and Pakistan | *Ristelliger* spp., *Cybium* spp., *Clupea* spp. | 6:1 | 12 months |
| 7 | Yeesui | China | *Sardinella* spp. *Engraulis* pupapa, *Jelio* spp., *Carangidae* spp, *Teuthis* spp. | 4:1 | 3~12 months |
| 8 | Garos | Greece | *Scomber* colias | 9:1 | 8 days |
| 9 | Pissala | France | *Ahya pellucida, Gobius* spp. *Engraulis* spp. *Atherina* spp. | 4:1 | 2~8 weeks |
| 10 | Shottsuru | Japan | *Astroscopus japonicus* | 5:1 | 3~6 weeks |
| 11 | Aekjeot | S Korea | *Astroscopus japonicus, Engraulis japonica* | 3~4:1 | 12~18 months |

(Note) Lopetcharat K, Choi YJ, Park JW, & Daeschel M. 2001. Fish sauce products and manufacturing: A review. Food Rev Int 17, 65-88.

Table 1.2. Changes in the free amino acid content of anchovy fish sauce during fermentation
(mg/100 mL)

| Amino acid | Anchovy fillet[1] | | Fermentation period (month)[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5.5 | | 8.5 | | 13.5 | | 18.0 | |
| Taurine | | -[5] | 198.5 | (2.5)[3] | 198.5 | (2.3) | 207.2 | (2.4) | 213.4 | (2.4) |
| Aspartic acid | 3,527 | (15.1) | 679.7 | (8.6) | 58.1 | (0.7) | 28.0 | (0.3) | 16.2 | (0.2) |
| Threonine | 517 | (2.2) | 375.9 | (4.8) | 341.6 | (4.0) | 90.7 | (1.0) | 166.6 | (0.7) |
| Serine | 197 | (0.8) | 332.2 | (4.2) | 211.8 | (2.5) | ND[4] | | ND | |
| Glutamic acid | 2,151 | (9.2) | 1,122.5 | (14.2) | 1,693.0 | (19.7) | 1,803.0 | (20.4) | 1,857.8 | (20.5) |
| Proline | 596 | (2.6) | 302.1 | (3.8) | 315.4 | (3.7) | 321.7 | (3.6) | 323.2 | (3.6) |
| Glycine | 943 | (4.0) | 289.5 | (3.7) | 374.9 | (4.4) | 591.9 | (6.7) | 609.3 | (6.7) |
| Alanine | 1,647 | (7.1) | 998.9 | (12.7) | 1,123.1 | (13.1) | 1,234.0 | (13.9) | 1,268.8 | (14.0) |
| Cystine | 2,811 | (12.1) | 217.7 | (2.8) | 242.6 | (2.8) | 287.0 | (3.2) | 319.5 | (3.5) |
| Valine | 1,862 | (8.0) | 425.3 | (5.4) | 527.5 | (6.1) | 681.1 | (7.7) | 720.0 | (7.9) |
| Methionine | 599 | (2.6) | 105.4 | (1.3) | 120.4 | (1.4) | 133.7 | (1.5) | 145.8 | (1.6) |
| Isoleucine | 1,051 | (4.5) | 646.1 | (8.2) | 710.9 | (8.3) | 720.2 | (8.1) | 726.6 | (8.0) |
| Leucine | 1,570 | (6.7) | 591.5 | (7.5) | 1,038.3 | (12.1) | 1,217.7 | (13.7) | 1,285.5 | (14.2) |
| Tyrosine | 792 | (3.4) | 18.9 | (0.2) | 24.6 | (0.3) | 25.0 | (0.3) | 25.2 | (0.3) |
| Phenylalanine | 821 | (3.5) | 77.4 | (1.0) | 76.0 | (0.9) | 65.5 | (0.7) | 54.0 | (0.6) |
| Histidine | 1,016 | (4.4) | 399.0 | (5.1) | 399.0 | (4.6) | 341.3 | (3.9) | 294.5 | (3.2) |
| Lysine | 2,192 | (9.4) | 693.7 | (8.8) | 853.3 | (9.9) | 1,058.8 | (11.9) | 1,105.0 | (12.2) |
| Arginine | 1,038 | (4.4) | 407.1 | (5.2) | 279.7 | (3.2) | 57.8 | (0.7) | 38.8 | (0.4) |
| Total | 23,330 | (100.0) | 7,881.4 | (100.0) | 8,588.7 | (100.0) | 8,864.6 | (100.0) | 9,070.2 | (100.0) |

[1]Total amino acid contents (mg/100 g), [2]Free amino acid contents

[3]Percentages of each amino acid content based on the total content of amino acids

[4]ND: Not detected [5]-: Not determined

(Note) Cho YJ & Choi YJ. 2000. Special Report for Quality Estimation of Anchovy Sauce. Submitted to the Ministry of Marine and Fisheries, Seoul, Korea, pp. 260-297.

Table 1.3. Comparison on the chemical composition of commercial fish sauce manufactured from various countries

| Component | Country of origin | | | |
|---|---|---|---|---|
| | Philippines | Thailand | Vietnam | S Korea |
| Number of samples | 2 | 4 | 7 | 19 |
| Total nitrogen (TN) (mg/100 mL) | 140.0~1,281.0 | 441.0~1,900.0 | 865.0~2,199.0 | 934.0~2387.0 |
| Amino acid nitrogen (AN) (mg/100 mL) | 115.4~955.1 | 338.4~1,570.6 | 629.3~1,643.0 | 570.5~1,442.2 |
| AN/TN (%) | 74.6~82.4 | 76.7~83.5 | 72.8~76.7 | 52.9~65.8 |
| Color (at 453nm) | 0.609~2.311 | 1.213~3.419 | 0.788~3.274 | 0.547~1.447 |
| NaCl (g/100 mL) | 29.7~30.6 | 24.1~29.9 | 23.7~29.4 | 22.9~30.9 |
| pH | 5.08~6.38 | 4.66~5.93 | 5.26~5.91 | 5.44~6.79 |

(Note) Cho YJ & Choi YJ. 2000. Special Report for Quality Estimation of Anchovy Sauce. Submitted to the Ministry of Marine and Fisheries, Seoul, Korea, pp. 260-297.

Table 1.4. Free amino acid content and composition of fish sauce produced from various countries

(mg/100 mL)

| Amino acid | Manufactured country | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | China[a,1] | | S Korea[b,2] | | Phillipine[c,1] | | Thailand[d,1] | | Vietnam[e,1] | |
| Tau | 124.5 | (2.6) | 213.4 | (2.4) | 211.6 | (3.7) | 102.1 | (1.6) | 169.0 | (2.0) |
| Asp | 362.9 | (7.8) | 16.2 | (0.2) | 415.7 | (7.3) | 609.7 | (9.4) | 430.3 | (5.0) |
| Thr | 222.2 | (4.8) | 66.6 | (0.7) | 298.7 | (5.2) | 379.4 | (5.9) | 534.6 | (6.3) |
| Ser | 138.9 | (3.0) | N.D.[f] | | 274.3 | (4.8) | 260.4 | (4.0) | 393.3 | (4.6) |
| Glu | 823.1 | (17.7) | 1857.8 | (20.5) | 944.1 | (16.5) | 1205.1 | (18.7) | 3031.9 | (35.5) |
| Pro | 86.4 | (1.9) | 323.2 | (3.6) | 143.8 | (2.5) | 178.7 | (2.8) | 193.0 | (2.3) |
| Gly | 186.5 | (4.0) | 609.3 | (6.7) | 323.0 | (5.6) | 268.3 | (4.1) | 232.6 | (2.7) |
| Ala | 437.8 | (9.4) | 1268.8 | (14.0) | 506.9 | (8.9) | 670.8 | (10.4) | 328.9 | (3.9) |
| Cys | 115.2 | (2.5) | 319.5 | (3.5) | | ND | | ND | 38.1 | (0.5) |
| Val | 338.0 | (7.3) | 720.0 | (7.9) | 358.7 | (6.3) | 476.1 | (7.4) | 350.1 | (4.1) |
| Met | 159.5 | (3.4) | 145.8 | (1.6) | 217.3 | (3.8) | 167.0 | (2.6) | 294.6 | (3.4) |
| Ile | 282.5 | (6.1) | 726.6 | (8.0) | 355.7 | (6.2) | 298.4 | (4.6) | 511.4 | (6.0) |
| Leu | 375.4 | (8.1) | 1285.5 | (14.2) | 466.1 | (8.1) | 343.6 | (5.3) | 895.1 | (10.5) |
| Try | 38.4 | (0.8) | 25.2 | (0.3) | 58.4 | (1.0) | 37.2 | (0.6) | 44.9 | (0.5) |
| Phr | 176.2 | (3.8) | 54.0 | (0.6) | 201.5 | (3.5) | 226.7 | (3.5) | 129.5 | (1.5) |
| His | 99.8 | (2.1) | 294.5 | (3.2) | 222.8 | (3.9) | 269.7 | (4.2) | 307.3 | (3.6) |
| Lys | 667.7 | (14.3) | 1105.0 | (12.2) | 696.4 | (12.2) | 956.5 | (14.8) | 634.0 | (7.4) |
| Arg | 19.0 | (0.4) | 38.8 | (0.4) | 29.9 | (0.5) | 6.8 | (0.1) | 14.9 | (0.2) |
| Total | 4654.0 | (100.0) | 9070.2 | (100.0) | 5724.9 | (100.0) | 6456.5 | (100.0) | 8533.5 | (100.0) |

(Note) [1] Lopetcharat K. Choi YJ, & Park JW. 2001. Fish sauce products and manufacturing: A review. Food Rev Int 17, 65-88. [2] Cho YJ & Choi YJ. 2000. Special Report for Quality Estimation of Anchovy Sauce. Submitted to the Ministry of Marine and Fisheries, Seoul, Korea, pp. 260-297.

[a]Fish + salt, [b]Anchovy + salt, [c]Fish extract + salt, [d]Anchovy fish extract + salt, [e]Anchovy fish extract + salt, [f]ND: Not detected

Table 1.5. Morphological and ecological characteristics of Pacific whiting

| Item | | | Characteristics |
|---|---|---|---|
| · Scientific name | | | *Merluccius productus* |
| · Appearance | Shape | | Round form |
| | Color | Back | Silvery with black speckles on the back |
| | | Mouth | Black inside |
| · Biological | Lifespan | | Around 15 years |
| | Spawning | | Season: from January through March |
| | | | Area: off south-central California |
| | Feed | | Shrimp, krill, and pelagic schooling fish, such as eulachon and Pacific herring |
| · Habitat | | | School location: in midwater |
| | | | Depth: in water between 164 and 1,640 feet deep |
| · Location | | | Off the West Coast of the United States and Canada |
| · Population status | | | Three stocks<br> - A migratory coastal stock<br> - A central-south Puget Sound stock<br> - A Strait of Georgia stock |

(Source) NOAA Fisheries. 2022. Retrieved from https://www.fisheries.noaa.gov/region/west-coast on March 18, 2022.

Table 1.6. Proximate composition and histidine content of various fish species

| Fish | | Proximate composition (g/100 g) [2] | | | | | Histidine (mg/100 g) |
|---|---|---|---|---|---|---|---|
| | | Moisture | Crude protein | Crude lipid | Ash | Carbo-hydrate[1] | |
| White-fleshed fish | Pacific whiting[2] | 80.5 | 16.8 | 1.5 | 1.0 | 0.2 | - |
| | Alaska pollock[3] | 80.3 | 17.5 | 0.7 | 1.5 | 0.0 | 331 |
| | Cod[3] | 78.6 | 19.5 | 0.3 | 1.3 | 0.3 | 333 |
| Dark-fleshed fish | Anchovy[3] | 73.4 | 17.7 | 5.4 | 1.5 | 2.0 | 644 |
| | Sardine[3] | 69.2 | 20.0 | 9.1 | 1.2 | 0.5 | 1086 |

[1]Carbohydrate = 100 - (Moisture + Crude protein + Crude lipid + Ash)

(Note) [2] Kim JS & Park JW. 2004. Characterization of acid-soluble collagen from Pacific whiting surimi processing byproducts. J Food Sci 69, C637-C642.

[3] National Institute of Fisheries Science in Korea, 2018. Composition Table of Marine Products in Korea 2018. National Institute of Fisheries Science in Korea, Busan. Korea pp. 34-35, 44-45, 66-67, 134-135, 140-141, 152-153.

Chemical composition of fish can be varied significantly depending on the season (spring, summer, fall and winter). The references of Pacific whiting used in this table was collected in July. However, specific period of when the sample specimen was taken for Alaska pollock, cod, anchovy, and sardine were not mentioned.

Table 1.7. Properties of cathepsins B, H and L identified in Pacific whiting

| Cathepsin | Molecular weight | Hydrolysis type | Optimum | | Target protein |
|---|---|---|---|---|---|
| | | | pH | Temp. (°C) | |
| B1 | 24-28 kDa | Endoprotease, Exopeptidase | 5.0 | 35 | Myosin, actin, collagen |
| B2 | 47-52 kDa | Exopeptidase | 5.5-6.0 | 35 | Broad specificity |
| H | 28 kDa | Endoprotease, Exopeptidase | 5.0 | 20 | Actin, myosin |
| L | 24 kDa | Endoprotease | 3.0-6.5 | 55 | Actin, myosin, collagen, α-actinin, troponin-T, -I |

(Source) Park JW. 2005. Surimi and surimi seafood. CRC Press, 2nd ed, Boca Raton, Florida, USA. 233.
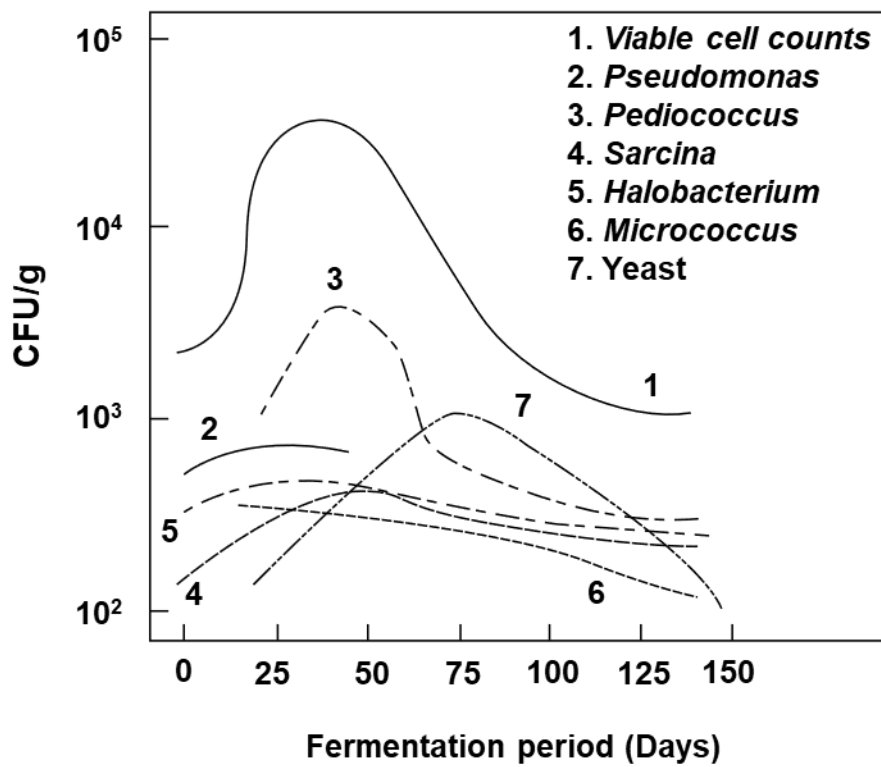
Fig. 1.1. Changes in microbes during the fermentation of S Korean anchovy sauce (Aekjeot).

(Note) Kim YM & Kim DS. 1990. Salt-fermented fish in Korea. Korea Food Research Institute, Wanju, Korea, p367-385.

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                    ┌─────────────┐
                    │  Raw Fish   │
                    └──────┬──────┘
                           │  -Salt addition
                           │  -Fermentation
                           │  -Heat treatment at 95°C for 20 min (only applied in Aekjeot)
                           │  -Filtration
            ┌──────────────┴──────────────┐
      ┌───────────┐              ┌───────────┐
      │  Extract  │              │  Residues │
      └─────┬─────┘              └─────┬─────┘
            │                          │  -Brine addition
            │                          │  -Fermentation
            │                          │  -Filtration
            │              ┌───────────┴───────────┐
     ┌───────────┐   ┌──────────────┐       ┌───────────┐
     │ First grade│   │ Second grade │       │  Residues │
     │ fish sauce │   │  fish sauce  │       └─────┬─────┘
     └───────────┘   └──────────────┘             │  -Fermentation
                                                   │  -Brine addition
                                                   │  -Boiling process
                                                   │  -Filtration
                                                   │  -Caramel or additives added
                                             ┌──────────────┐
                                             │  Third grade │
                                             │  fish sauce  │
                                             └──────────────┘
```
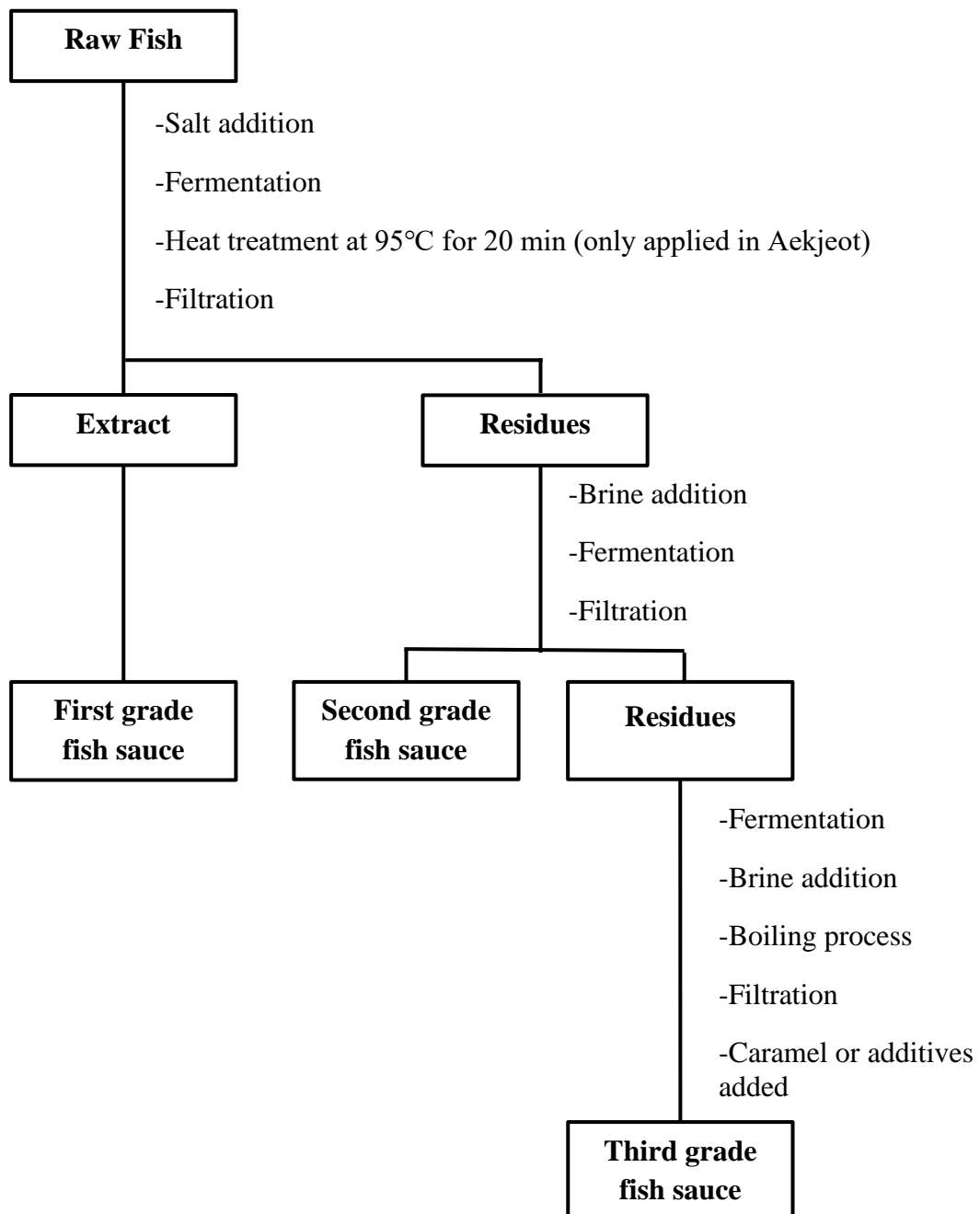
Fig. 1.2. Flow chart for preparation of traditional fish sauce (Nam-pla in Thailand and Aekjeot in S Korea).

(Note) Lopetcharat K. Choi YJ, & Park JW. 2001. Fish sauce products and manufacturing: A review. Food Rev Int 17, 65-88.
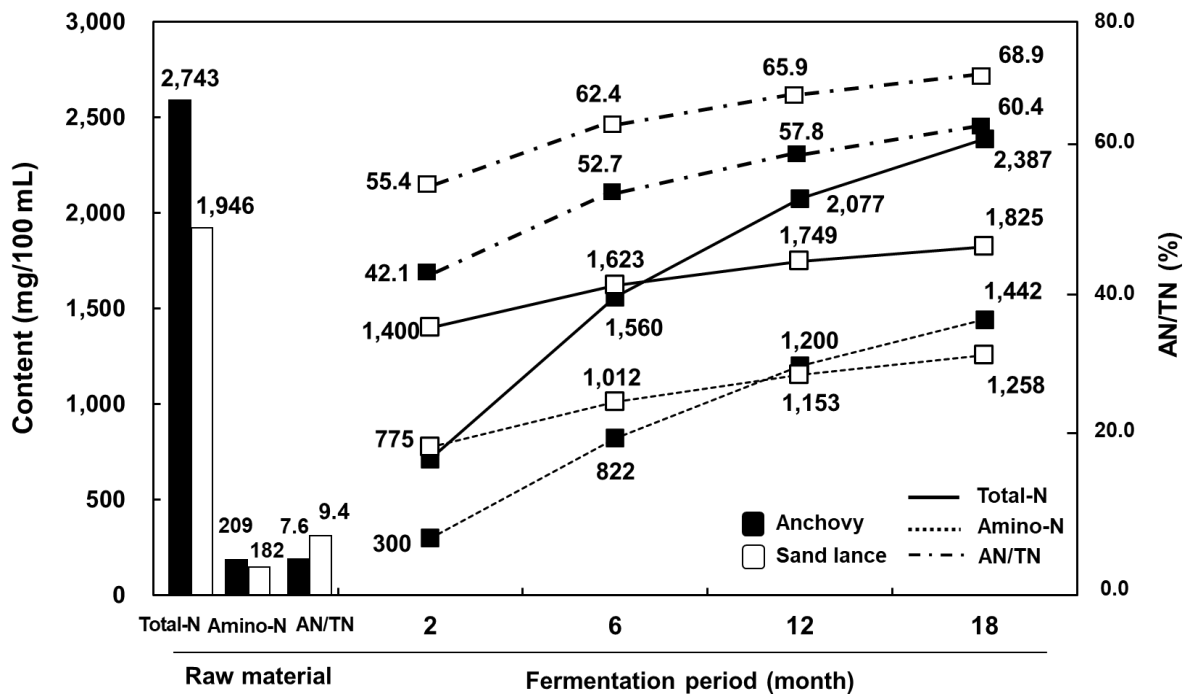
Fig. 1.3. Change in the total nitrogen (total-N) and amino acid nitrogen (amino-N) contents of fish sauce from anchovy and sand lance sauce during fermentation.

Anchovy; Total-N [Y= 1,217.8ln(x) + 716.43 (r=0.9995)], Amino-N [Y = 826.95ln(x) + 283.97 (r=0.9977)], AN/TN [Y= 13.375ln(x) + 42.627 (r=0.9915)]
Sand lance; Total-N [Y= 309.01ln(x) + 1,403.7 (r=0.9988)], Amino-N [Y = 347.49ln(x) + 773.41 (r=0.9998]], AN/TN [Y = 9.7186ln(x) + 55.42 (r=0.9993)]

(Note) Cho YJ & Choi YJ. 2000. Special Report for Quality Estimation of Anchovy Sauce. Submitted to the Ministry of Marine and Fisheries, Seoul, Korea, pp. 260-297.

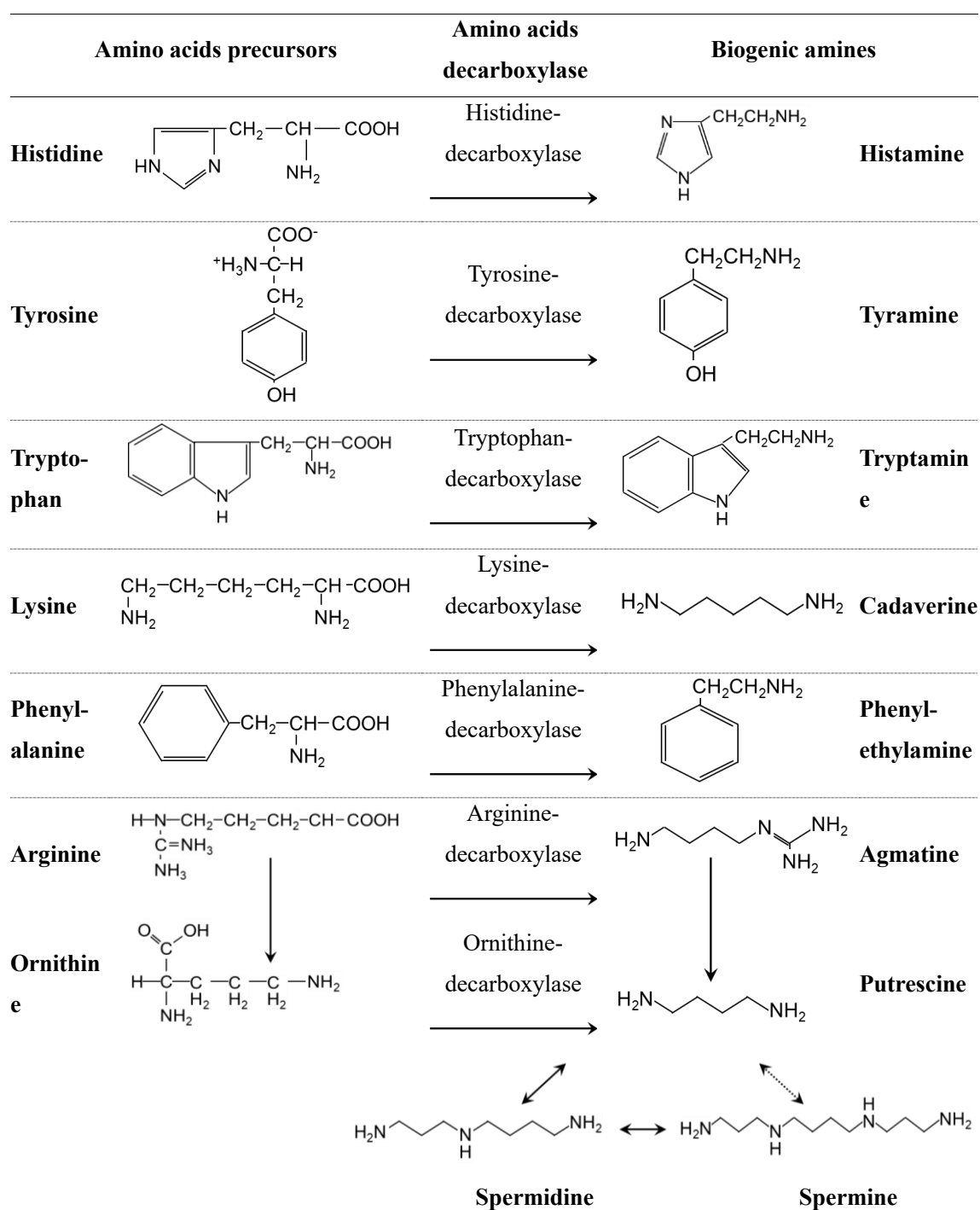| Amino acids precursors | Amino acids decarboxylase | Biogenic amines | |
|---|---|---|---|
| **Histidine** | Histidine-decarboxylase | | **Histamine** |
| **Tyrosine** | Tyrosine-decarboxylase | | **Tyramine** |
| **Trypto-phan** | Tryptophan-decarboxylase | | **Tryptamine** |
| **Lysine** | Lysine-decarboxylase | | **Cadaverine** |
| **Phenyl-alanine** | Phenylalanine-decarboxylase | | **Phenyl-ethylamine** |
| **Arginine** | Arginine-decarboxylase | | **Agmatine** |
| **Ornithine** | Ornithine-decarboxylase | | **Putrescine** |
| | | **Spermidine** | **Spermine** |

Fig. 1.4. Amino acid precursors and amino acid decarboxylase enzymes in the formation of biogenic amine in seafood.

(Note) Ruiz-Capillas C & Jimenez-Colmenero F. 2010. Biogenic Amines in Seafood Products. In Handbook of Seafood and Seafood Products Analysis. Nollet LML and Toldra F, ed. Taylor & Francis Group, New York, USA, pp. 833-834
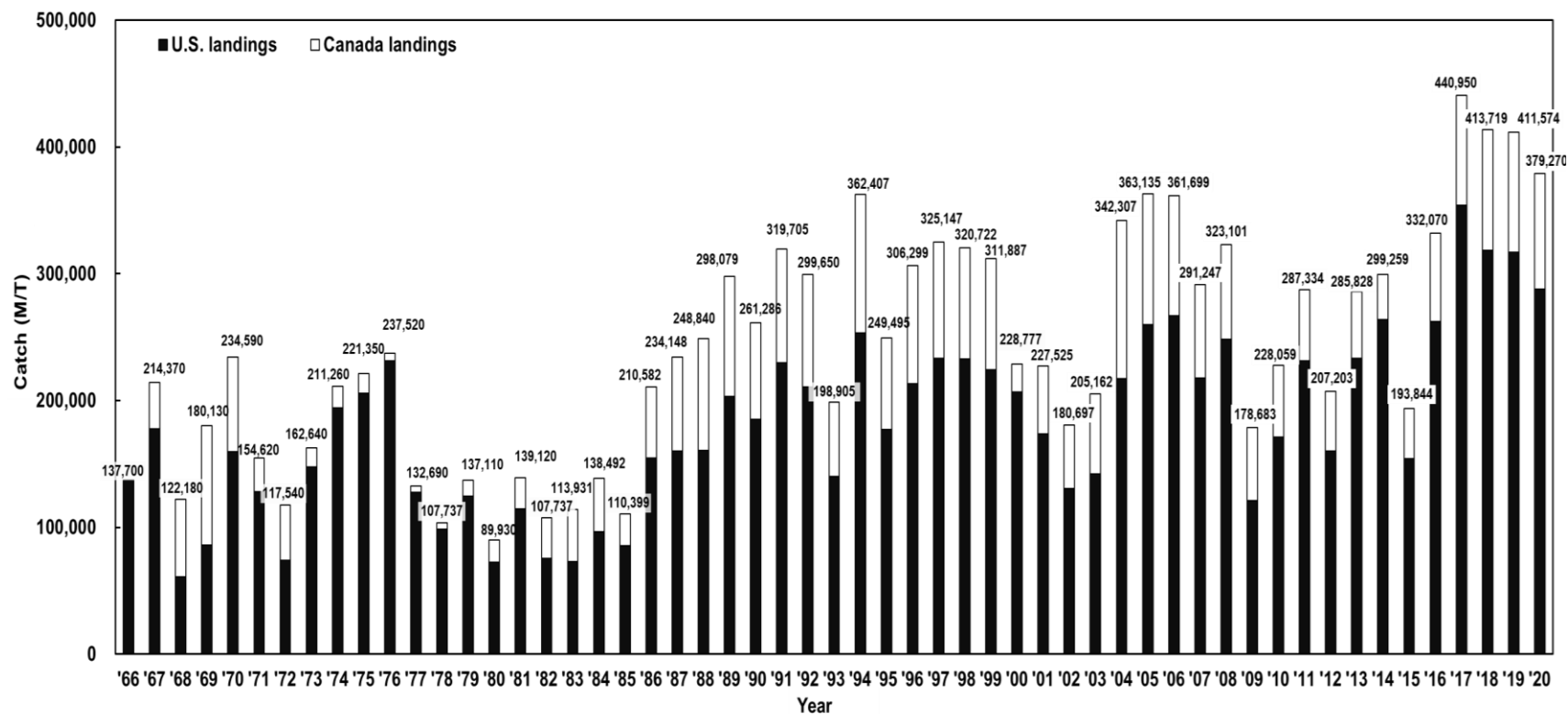
Fig. 1.5. Annual Pacific whiting catch in U.S. and Canada from 1966 to 2020.

(Source) Johnson KF, Edwards AM, Berger AM, & Grandin CJ. 2021. Status of the Pacific hake (whiting) stock in U.S. and Canadian waters in 2021. Prepared by the Joint Technical Committee of the U.S. and Canada Pacific Hake/Whiting Agreement, National Marine Fisheries Service and Fisheries and Oceans Canada. 269 p.)

Chapter 2

Application of ohmic heating for accelerating fish sauce fermentation

**Running title:** Application of ohmic heating in fish sauce fermentation

Hyung Joo Kim, Christina A. Mireles DeWitt*, Jae W. Park

OSU Seafood Research and Education Center, Oregon state University, United
States

* Corresponding Author

Email: christina.dewitt@oregonstate.edu

Department Food Science and Technology

OSU Seafood Research & Education Center, 2001 Marine Dr Ste 253,

Astoria, Oregon 97103, USA

Tel:+503-325-4531 Ext 3

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## 2.1 Abstract

Ohmic heating was applied to accelerate fermentation for fish sauce preparation using protease-laden Pacific whiting. Pacific whiting mince containing 25% salt was fermented in either water bath or ohmic heating at various temperatures sequentially for 8 weeks: 25°C for Week 1, 35°C for Week 2, and 55°C for Week 3 to 8. Different temperature conditions were applied to provide optimum conditions for three major proteases in Pacific whiting (cathepsin L, B and H). Although identical temperature and incubation time were provided for both water bath sample and ohmic heating sample, a significant difference was found between the two samples. Brownness, taste value, and overall nitrogen content were significantly higher with ohmic heating than those with water bath ($p < 0.05$). The difference was hypothesized to be due to electroporation and uniform & consistent heat penetration by ohmic heating. Regardless of heating methods, Pacific whiting fish sauce demonstrated significantly lower histamine content when compared with commercial fish sauce made with anchovies ($p < 0.05$). This study discovered that ohmic heating can be utilized for fish sauce production as it significantly accelerates fermentation and adds value to the utilization of protease-laden Pacific whiting.

**Key word:** Fish sauce, Ohmic heating, Pacific whiting, Cathepsin activity, Process acceleration

**2.2 Introduction**

Fish sauce is a fermented condiment made primarily from anchovies and is popularly used in Asian countries as it provides unique salty and umami flavor. Fish sauce is generally produced by fermenting anchovy mince with salt at approximately 3:1 at ambient temperature and the process generally takes 12 to 18 months (Lopetcharat, Choi, Park, & Daeschel, 2001). During fermentation, fish protein is degraded to di-peptides and amino acids by endogenous protease enzymes and salt-tolerant microorganisms.

Fish sauce fermentation is a time-consuming process at ambient temperatures and uses endogenous enzymes and microbes to completely degrade proteins to amino acids and/or di-peptides. Even though fish sauce is used in a small quantity as a condiment, salt concentration in fish sauce is enormously high at around 25%. Therefore, many trials and research efforts have been made to achieve two goals: accelerating fermentation and reducing salt content. To accelerate fermentation, various methods such as adjusting pH (Gildberg, Espejo-Hermes, & Magno-Orejana, 1984), adding exogenous protease (Chaveesuk, Smith, & Simpson, 1994), and inoculating microbes (Akolkar, Durai, & Desai, 2010) were used. To reduce salt content in fish sauce, methods such as electrodialysis (Jundee, Devahastin, & Chiewchan, 2012) and applying ethanol treatment (Liu et al., 2017) were applied. However, further research was suggested as there were issues such as undesired off-flavor and aroma, loss of color, and excessive energy consumption when those methods were applied.

Pacific whiting (*Merluccius productus*) is highly sustainable and abundant in the Pacific Northwest as a marine stewardship council (MSC)-certified species. However, Pacific whiting was not fully utilized until surimi production started in 1991-1992 due to extreme softening of the fillet during slow cooking. Texture softening is caused by three major proteases (cathepsin L, cathepsin B, and cathepsin H) in Pacific whiting flesh if not thermally processed rapidly (Yongsawatdigul, Hemung, & Choi, 2014). An, Weerasinghe, Seymour, & Morrissey (1994) reported different optimum conditions for each enzyme (55°C for cathepsin L, 20-37°C for cathepsin B, and 20°C for cathepsin H). Although surimi processing, which removes the majority of cathepsins except cathepsin L through washing, has utilized the problematic fish Pacific whiting successfully in a commercial scale, it still leaves a significant amount of solid byproducts and soluble proteins lost during washing. As the fish sauce processing starts with grinding whole fish (traditionally anchovies) and mixing with salt prior to incubation, it can utilize the fish maximally.

Ohmic heating generates consistent and uniform heat through electrical resistance between two electrodes. Various heating rates (extremely slow or fast) can be achieved depending on voltage gradients. Unlike conventional water bath cooking which demonstrates gradual temperature increase and uneven heat distribution, ohmic heating can distribute heat evenly and increase the temperature rapidly as massive heat is generated by current (Pongviratchai & Park, 2007). Yongsawatdigul, Park, Kolbe, Dagga, & Morrissey (1995) introduced ohmic heating to cook Pacific whiting surimi and overcame texture softening. Rapid and uniform heating in ohmic heating was the key for the success of Pacific whiting surimi. Additionally, Knirsch, Dos Santos, de

Oliveira Soares, & Penna (2010) reported electroporation of cell membranes may also occur in food during ohmic heating. Electroporation is defined as formation of pores in cell membranes due to an electric field and known as one of the dominant mechanisms of ohmic heating (An & King, 2007).

We hypothesized that combined effect of protease-laden Pacific whiting and uniform temperature distribution in combination with electroporation by ohmic heating could shorten the process of fish sauce production. We proposed to compare two fermentation heating methods: ohmic heating and water bath. We assumed that rapid and uniform heat generated by ohmic heating would significantly accelerate fish sauce fermentation without generating any undesired outcome. Biochemical properties such as brownness, pH, overall nitrogen content, and free amino acid content can be used as indexes of fish fermentation as they demonstrate either increasing or decreasing trend during the fermentation (Lopetcharat, Choi, Park, & Daeschel, 2001; Mueda, 2015; Tungkawachara, Park, & Choi, 2003). Therefore, biochemical properties of fish sauce from ohmic heating were evaluated and compared against fish sauce from water bath heating.

## 2.3 Materials and methods

### (1) Raw materials

Pacific whiting (*Merluccius productus*) was harvested off the Oregon coast in May – August 2021. Fresh whole fish (600–900 g) were randomly selected from Da Yang

Seafood (Astoria, OR, USA) and stored in ice before transporting to the Oregon State University Seafood Laboratory. Pacific whiting was then stored in cold room (4ºC) for 24 hr prior to sample preparation. Salt (Morton Salt, Chicago, IL, USA) was obtained from local grocery store and stored at room temperature.

### (2) Fish sauce preparation

Whole Pacific whiting without evisceration was rinsed using fresh water, cut into chunks (3-4 cm long) and chopped at 1,800 rpm using a silent cutter (UM 5 Universal, Stephan Machinery Corp, Columbus, OH, USA) for 1 min. Then salt was added constituting 25% of total weight and chopping continued at 3,600 rpm for 4 min. After chopping was completed, salted Pacific whiting mince was stuffed into either 200 mL PYREX jar with cap for water bath heating or a nylon cooking tube (3 cm diameter) for ohmic heating. Water bath heating was applied to mimic the conventional heating method used in Asian countries. Ohmic heating was applied to observe if enhanced heat penetration accelerated the fermentation process.

Ohmic heating was proceeded according to the method of Tadpitchayangkoon, Park, & Yongsawatdigul (2012) with some modifications. Ohmic heating apparatus was constituted by AC power pack Titan (model Mac-01, Compact Power Co., Yorba Linda, CA, USA), voltage transducer (VT8-007D, Ohio Semitronics Inc., Hilliard, OH, USA), current transducer (CT8-015DY101, Ohio Semitronics Inc), two titanium electrodes (3 cm diameter), thermocouple and temperature controller (Model CNi3254-C24, Omega Engineering Inc., Stamford, CT, USA). A thermocouple was inserted into the stuffed

nylon tube and pressure (276 kPa) was applied ensuring both electrodes have good contact with the salted fish mince inside the tube. The samples were heated to desired temperature at a frequency of 10 kHz at voltage levels of 100 V.

A thermocouple was inserted into the geometric center of the cooking tube to control the holding temperature during fermentation. A built-in temperature sensor was used for the water bath. To provide the optimum temperature conditions for the cathepsins, temperature was maintained at 25°C for Week 1, 35°C for Week 2, and 55°C for Week 3 to 8. Optimum incubation temperatures were selected according to An, Weerasinghe, Seymour, & Morrissey (1994). Time at temperature was selected based on preliminary trials (data not shown). When the fermentation process was over, samples were carefully removed from either nylon tube or culture bottle and liquid portion was collected by squeezing the sample using 8-fold cheesecloth. Laboratory hydraulic press (Fred S. Carver, Inc., New York, N.Y., U.S.A.) at 1,500 psi was also applied until every liquid portion was obtained. The liquid was then filtered using Whatman #1 filter paper (Whatman Int'l, Maidstone, U.K.). Additionally, a control sample (unfermented salted Pacific whiting mince) was used for pH and enzyme analysis. All fermented samples were stored at room temperature prior to analysis.

Two commercial fish sauce were purchased from a local grocery store to compare their characteristics with our samples. Both were manufactured from anchovies with one from Thailand and the other from Korea. Commercial samples were also stored at room temperature prior to analysis.

**(3) Sodium chloride content, brownness, and pH**

Sodium chloride content was determined according to the Volhard method (AOAC 937.13, 2000). The pH value was determined using a pH meter (AR15, Fisher Scientific, Atlanta, GA, USA). Brownness of the samples were measured according to the method of Lopetcharat & Park (2002). The degree of brown color, brownness, was determined using a spectrophotometer (UV-2401PC, Shimadzu, Japan) at 420 nm. For pH measurements, samples were diluted with deionized water (DI) to 1:10 before the analysis to weaken the high ionic strength in the undiluted fish sauce. The unit was calibrated with pH 4, 7, and 10 standard solutions prior to the measurement.

**(4) Total nitrogen, ammonia nitrogen, formol nitrogen, and amino acid nitrogen contents**

Total nitrogen and ammonia nitrogen in the fish sauce were determined according to the micro-Kjeldahl method (AOAC 940.25, 2000) with slight modifications. For total nitrogen, a Kjeldahl flask containing 1.5 g of sample, 15 mL of concentrated sulfuric acid, and 12 g of copper catalyst was digested at 410°C for 60 min. The copper catalyst was prepared using 3.5 parts potassium sulfate and 1 part copper sulfate pentahydrate. The digest was cooled and 40 mL of distilled water was added. The diluted digest was distilled following addition of 50 mL of 50% sodium hydroxide to the Kjeldahl flask. The generated steam was condensed into an Erlenmeyer flask containing 25 mL of trapping solution (4% boric acid, 0.1% methyl red, 0.1% bromocresol green, VWR Scientific, Radnor, PA). The solution was then titrated using 0.1 N hydrochloric acid.

For ammonia nitrogen, a Kjeldahl flask containing 1.5 g of sample was diluted with 40 mL of water. Distillation proceeded as described for total nitrogen.

Formol nitrogen content was determined by the method of Beddows, Ardeshir, & Daud (1976) with slight modifications. Fish sauce (1 mL) was diluted with 40 mL of deionized water (DI) and titrated to pH 7.0 with 0.1 N sodium hydroxide. Then, 10 mL of formalin solution (38%, v/v) was added. The solution was titrated with 0.1 N sodium hydroxide until it reached pH 8.5. Test results were calculated using the following equation:

Formol nitrogen (mg/100 mL) = 0.0014 * mL of NaOH * Titration factor * N of NaOH / sample weight (g)

Amino acid nitrogen was calculated by subtracting ammonia nitrogen content from formol nitrogen content.

**(5) Free amino acid content and taste value**

For free amino acids content, 50 mg 5'-sulfosalicylic acid was added to 1.0 mL fish sauce sample and held at room temperature for 30 min. Then the sample was centrifuged at 3,000 x g for 10 min. The supernatant was diluted with lithium citrate buffer (pH 2.2) to a final volume of 10 mL. The obtained solution was used as a sample for free amino acid composition analysis. The solution and standard amino acids were subjected to the auto amino acid analyzer (LKB-Biochrom. 30, Pharmacia-Biotech.,

Bucking hamshire, UK). Standard amino acids were used to identify the amino acid profile in fish sauce samples. Type and concentration of amino acids in fish sauce were obtained by comparing the retention time and peak area between the standards and the unknown compounds.

Taste value of amino acids were calculated using method of Cha and Cadwallader (1998). Taste threshold required for calculating amino acid taste value was obtained from Kato, Rhue, & Nishimura (1989). Taste value was calculated using the following equation:

Taste value = Free amino acid content * Taste threshold of free amino acid * 100

### (6) Cathepsin activity

Samples were dialyzed using method of Sinsuwan, Rodtong, & Yongsawatdigul (2008) with some modifications. Crude extract was collected by centrifugation at 8,000 x g at 4°C for 30 min. The extract was then dialyzed with 20 mM Tris–maleate (pH 7.0) at 4 °C overnight, using dialysis membrane sack with molecular weight cut-off 12 to 14kDa (Spectra/POR, regenerated cellulose, Thermo Fisher Scientific, Pittsburgh, Pa., U.S.A). Cathepsin L, B, and H activity of fish sauce was determined according to the method of Barrett & Kirschke (1981). Cathepsin activity was analyzed using 20 µM of Z-Phe-Arg-amino-7-methylcoumarin, Z-Arg-Arg-amino-7-methylcoumarin, and L-Arg-amino-7-methylcoumarin as substrates for cathepsin L, B, and H, respectively. Optimum temperature conditions used in the analysis were based on the results of An,

Weerasinghe, Seymour, & Morrissey (1994). Cathepsin L, B, and H analysis were conducted at 55°C, 35°C, and 20°C, respectively. The amount of methylcoumarin generated was observed using a spectrofluorometer (Perkin Elmer LS 50B, Waltham, MA) set at 370 nm excitation wavelength and 460 nm emission wavelength. Blank samples were prepared by adding the terminator (stopping reagent) prior to adding the substrate. One unit of the cathepsin activity was defined as one micromole of methylcoumarin released per min at the optimum pH and temperature conditions.

**(7) Histamine content**

Histamine content was determined according to the AOAC official histamine analysis method (AOAC, 2000).

**(8) Statistical analysis**

Statistical analysis was conducted using the analysis of variance (AOAC, 2000) on SPSS for Window version 19.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA test was used to analyze the statistical significance ($p < 0.05$) between sample means. The entire study was conducted using two biological replicates and three technical replicates to verify the results.

**2.4 Results and Discussion**

**(1) Sodium chloride content, brownness, and pH**

Sodium chloride is an essential ingredient in fish sauce as it not only provides salty flavor, but also contributes to the denaturation of fish protein (Gildberg, Espejo-Hermes, & Magno-Orejana, 1984). In addition, Lopetcharat, Choi, Park, & Daeschel (2001) stated that sodium chloride can also control pathogenic microbes during fermentation. Therefore, more than 20% sodium chloride is generally applied in fish sauce fermentation. The sodium chloride content of ohmic heating sample was 22.8 g/100 g whereas it was 23.2 g/100 g for water bath sample (Table 2.1). Samples did not have a significant difference ($p > 0.05$). This was believed to be due to the identical salt content applied in both samples. In addition, sodium chloride contents of commercial products were 24.7 g/100 g for product made in Korea and 22.6 g/100 g for product made in Thailand. The sodium chloride content of commercial products made did not have a significant difference with both of our Pacific whiting fish sauce samples ($p > 0.05$). According to Nakano et al. (2017), commercial fish sauce manufactured from different countries contained 11 to 25% of sodium chloride. The sodium chloride concentration of fish sauce should be 20% or above according to CODEX (CODEX Alimentarius International Food Standards, 2022) and regulations in Vietnam (Vietnam Standards and Quality Institute, 2022) and Thailand (Food and Drug Administration Thailand, 2022) on high grade fish sauce. Therefore, sodium chloride concentration of our samples satisfied the value suggested by regulations.

Brownness is one of the important quality factors in fish sauce industry as it not only determines the functionality of the color, but also can be used as an index of the maturity of fish sauce (Mueda, 2015). The appearance of Pacific whiting fish sauce fermented using different heating methods (water bath and ohmic heating) and commercial anchovy fish sauce with different country of origin (Thailand and Korea) are demonstrated in Fig. 2.1. The transparency of all four samples were very clear and there was not much difference between the samples. However, brownness of the ohmic heating treated sample was obviously darker than the water bath treated sample but much lighter than the commercial fish sauce samples. Brownness value of fish sauce made using ohmic heating was 1.65 whereas sample made using water bath was 1.18 (Table 2.1). Significant difference was found between the brownness value of the two samples ($p < 0.05$). In addition, brownness value of commercial fish sauce made in Korea and Thailand were 1.74 and 1.70, respectively. Regardless of the country of origin, brownness of commercial samples was significantly higher than that of our water bath sample ($p < 0.05$). However, no significance was found between the commercial samples and our ohmic heating sample ($p > 0.05$). During fish sauce fermentation, lipids in fish degrade into fatty acids which participate in Maillard reaction resulting in increased brownness (Mueda, 2015). Cho and Choi (2000) stated that brownness of commercial fish sauce made in Thailand and Korea were 1.231-3.419 and 0.635-2.349, individually. The brownness value of ohmic heating sample was within that range whereas the brownness of water bath sample was below the range.

In general, pH of fish sauce demonstrates a decreasing trend during fermentation due to free hydrogen ions, free amino acids, and organic acids generated (Lopetcharat, Choi,

Park, & Daeschel, 2001; Tungkawachara, Park, & Choi, 2003). The pH values of samples were 6.49 for water bath sample, 6.30 for ohmic heating sample, 7.18 for the raw material, 6.81 for the liquid extruded from the salted Pacific whiting mince prior to fermentation (control) (Fig. 2.2). Significant differences were found between the four groups ($p < 0.05$). In addition, pH values of commercial fish sauce were 5.44 and 5.69 for commercial fish sauce made in Korea and Thailand, respectively. Regardless of the heating methods, pH values of commercial fish sauce were significantly lower than that of our samples ($p < 0.05$). According to Amarra, Capanzana, Gironella, & De los Reyes (2021), pH is essential in determining the quality of fish sauce as it can not only repress the growth of pathogenic microorganisms, but also accelerates bacterial degradation. According to Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO) (2018), the final pH of fish sauce should be between 5.0-6.5. Our sample pH was within the FAO guideline. According to Nakano et al. (2017), pH value of commercial fish sauce manufactured from six different countries ranged from 4.48 to 5.97. Difference in pH between our samples and commercial fish sauce was likely due to different species (Pacific whiting vs. anchovies). Based on our results, pH of Pacific whiting was 7.18 whereas Kocatepe et al. (2019) stated pH of anchovy (*Engraulis encrasicolus*) was 6.42. The pH of fish decreases rapidly during post mortem as glycogen is rapidly degraded into lactic acid (Mol, Erkan, Uecok, & Tosun, 2007). As anchovy has dark reddish colored flesh that can hold abundant glycogen, initial pH of anchovy would be lower than Pacific whiting which has white colored flesh. In addition, a significantly different freshness of raw materials might have contributed to the difference in pH: it is not uncommon to store

anchovies in SE Asia at ambient temperature for 2-3 days while we used Pacific whiting held at refrigerated condition for 20-30 hr postharvest. Perhaps food grade additives often included at commercial fish sauce production could have affected the reduced pH. Food grade additives such as citric acid or sorbic acid is sometimes used in commercial fish sauce to lower pH and develop color (Lopetcharat & Park, 2002).

**(2) Total nitrogen, ammonia nitrogen, formol nitrogen, and amino acid nitrogen contents**

Nitrogen analysis is essential in fish sauce as nitrogen compounds, especially free amino acids, are the major group that contribute to taste and aroma of fish sauce (Xu, Yu, Xue, Xue, & Ren, 2008; Zhao, Jiang, Xu, & Xia, 2017). With the Kjeldahl method, it is possible to analyze the total nitrogen content as it breaks down the protein and peptides into free amino acids which are subsequently converted to ammonia. However, it is unable to determine the source of nitrogen (soluble protein/peptide vs. free amine) in the fish sauce. The ammonia nitrogen content is an indicator of the breakdown of soluble protein and peptides into free amines whereas the amino acid nitrogen content indicates the amount of soluble amino acid groups in fish sauce (Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2006). The nitrogen analyses were conducted to determine the source of nitrogen and to obtain amino acid nitrogen content, an indicator of taste and aroma of fish sauce.

During fish sauce fermentation, fish proteins are decomposed into peptides, free amino acids, and ammonia which can have a significant effect on taste and odor of fish

sauce. As nitrogen content in these compounds can be expressed using the total nitrogen content, total nitrogen content is regarded as one of the essential quality factors in fish sauce (Ciou, Hsieh, Lee, & Hsieh, 2020; Jiang, Zeng, Zhu, & Zhang, 2007; Zhao, Jiang, Xu, & Xia, 2017). Total nitrogen content of ohmic heating sample was 1,132.3 mg/100 g whereas that of water bath sample was 892.1 mg/100 mL (Table 2.2). A significant difference was found between the two samples ($p < 0.05$). Tungkawachara, Park, & Choi (2003) discovered that total nitrogen in fish sauce increased as the fermentation continued. A significant difference between the two samples was likely due to the accelerated fermentation through ohmic heating-driven uniform heat distribution in combination with electroporation. In addition, total nitrogen contents of commercial products were 821.1 mg/100 g for product made in Korea and 1,485.9 mg/100 g for product made in Thailand. The total nitrogen content of ohmic heating sample was significantly higher ($p < 0.05$) than the commercial fish sauce made in Korea whereas the content was significantly lower ($p < 0.05$) than the fish sauce produced in Thailand. According to the CODEX regulation (CODEX Alimentarius International Food Standards, 2022) and regulations in Vietnam (Vietnam Standards and Quality Institute, 2022) and Korea (Ministry of Food and Drug Safety, 2022) on fish sauce, total nitrogen content should be 1,000 mg/100 mL or above. The regulations were only satisfied by ohmic heating sample. In addition, Cho and Choi (2000) reported that the total nitrogen content of fish sauce products made in Thailand and Korea was 441 – 1,900 mg/ 100 mL and 934 – 2,195 mg / 100 mL, respectively.

The major component of the taste of fish sauce is free amino acids obtained by hydrolysis rather than proteins or polymer peptides (Cho and Choi, 2000). The free

amino acid content can be obtained by subtracting ammonia nitrogen content, an indicator of protein and peptide degradation into free amines, from formol nitrogen content (Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson, 2006). Formol nitrogen content of ohmic heating sample and water bath sample was 481.9 mg/100 g and 305.8 mg/100 g, respectively. In addition, ammonia nitrogen content of ohmic heating sample and water bath sample was 142.3 mg/100 g and 128.4 mg/100 g, respectively. Significant difference was found between the two samples in formol nitrogen content ($p < 0.05$) whereas significant difference was not found in ammonia nitrogen content ($p > 0.05$). Klomklao, Benjakul, Visessanguan, Kishimura, & Simpson (2006) stated that formol nitrogen content and ammonia nitrogen content in fish sauce increased as the fermentation continued due to the protease enzymes that break down proteins to ammonia. Based on the formol nitrogen content and ammonia nitrogen content, ohmic heating sample was believed to have much more intense flavor and aroma than water bath sample.

Amino acid nitrogen is not only used as an indicator of level of protein cleavage but also as an indicator of taste and aroma in fish sauce (Chaveesuk, Smith, & Simpson, 1994). Amino acid nitrogen content of ohmic heating sample was 339.6 mg/100 g and that of water bath sample was 177.4 mg/100 g (Table 2.2), and they were significantly different ($p < 0.05$). According to Tungkawachara, Park, & Choi (2003), amino acid nitrogen content increased as fermentation continued due to the polypeptide degradation. Like what was discussed above, a significant difference in amino acid nitrogen content between the two of our samples was likely due to the accelerated fermentation caused by electroporation and more uniform heat generated through

ohmic heating. According to Cho and Choi (2000), amino acid nitrogen content of commercial fish sauce produced in Thailand and Korea was 338.4-1,570.6 mg/100 mL and 570.5-1,191.3 mg/100 mL, respectively. The obvious difference between results from our research and results from the past research were believed to be due to differences in the fermentation period. As we have only conducted 8 weeks of fermentation to see the different trends between water bath heating sample and ohmic heating sample, our fish sauce samples may not have been fully fermented as the commercial products.

### (3) Free amino acid content and taste value

Fish sauce is a fermented condiment with a unique flavor and aroma which are mainly from soluble peptides and free amino acids. Therefore, free amino acid is one of the major factors that determine the quality of fish sauce and demonstrates an increasing trend as fermentation proceeds (Tungkawachara, Park, & Choi, 2003). According to Benjakul and Morrissey (1997), the major amino acids in Pacific whiting are glutamic acid, aspartic acid, lysine, leucine, arginine, and alanine. The free amino acid contents of our samples were 2,412.8 mg/100 g for ohmic heating sample whereas it was 1,192.0 mg/100 g for water bath sample (Table 2.3). A significant difference was found between the two samples ($p < 0.05$). The free amino acid contents of commercial fish sauce samples were 2,974.4 mg/100 g for the product made in Korea whereas it was 5,018.7 mg/100 g for the product made in Thailand. Regardless of the heating conditions, the two commercial samples had significantly higher free amino acid

contents then our samples ($p < 0.05$). The significant difference between our samples and commercial samples was believed to be due to different raw materials (Pacific whiting vs. anchovies) and different fermentation period. Perhaps food grade additives such mono sodium glutamate which are often included at commercial fish sauce production to enhance flavor and aroma could have affected the free amino acid content. Tungkawachara, Park, & Choi (2003) reported that free amino acid content of Pacific whiting fish sauce that has been fermented for 9 months was 3,837.4 mg/100 mL. The major free amino acids in ohmic heating sample were glutamic acid, alanine, leucine, lysine, and arginine whereas it was taurine, alanine, leucine, lysine, and arginine for water bath sample. Tungkawachara, Park, & Choi (2003) also reported that major free amino acids in Pacific whiting fish sauce fermented for 0 months were glutamic acid, alanine, leucine, lysine, and arginine. Major free amino acids in commercial fish sauce samples were glutamic acid, γ-amino butyric acid (GABA), α-aminobutyric acid, and lysine for commercial product made in Korea whereas aspartic acid, glutamic acid, alanine, and lysine were the major amino acids in commercial product made in Thailand. Difference in major amino acid components were believed to be due to difference in the raw material, fermentation conditions, and application of food grade additives.

Free amino acids are one of the major substances that determine the flavor of seafood. Therefore, taste value was calculated based on free amino acid concentration and taste threshold in order to examine the major free amino acid groups that contribute to the flavor and aroma of fish sauce. Kato, Rhue, & Nishimura (1989) reported that aspartic acid (3 mg/100 mL) has the lowest threshold, followed by glutamic acid (5 mg/100

mL), histidine (20 mg/100 mL), methionine (30 mg/100 mL), valine (40 mg/100 mL), arginine and lysine (50 mg/100 mL). The total taste value of our fish sauce samples that has been fermented for 8 weeks was 92.32 for ohmic heating sample whereas that of water bath sample was 35.77 (Table 2.4). A significant difference was found between the samples ($p < 0.05$). The total taste values of commercial fish sauce samples were 175.58 for product made in Korea and 300.23 for product made in Thailand. Regardless of heating conditions used in fermentation and country of origin, total taste values of commercial samples were significantly higher than our samples ($p < 0.05$). Again, likely indicating longer fermentation time may be needed to achieve equivalent taste values. Based on the results, commercial fish sauce samples were believed to have much intense flavor compared to our samples.

### (4) Cathepsin activity

Cathepsins are proteases located in the lysosomes and can be distinguished according to the active sites (Chéret, Delbarre-Ladrat, De Lamballerie-Anton, & Verrez-Bagnis, 2007). Cathepsin L, B, and H are cysteine-proteases and cause degradation of intracellular fish protein during the fermentation (Tungkawachara, Park, & Choi, 2003). Although protein denaturation is regarded as negative in fillets or surimi, protease activity is regarded as positive in fish sauce as more amino acids that implies unique taste and flavor are generated (An, Weerasinghe, Seymour & Morrissey, 1994).

Activities of cathepsin L, B, and H were measured (Fig. 2.3). Prior to fermentation, cathepsin H was the dominant group whereas cathepsin L was the least active.

According to Porter, Koury, & Stone (1995), cathepsin B is the dominant cathepsin group in Pacific whiting. Difference in the dominant cathepsin group between the current research and research from the past was likely due to different sample type (whole Pacific whiting vs. Pacific whiting fillet) and presence of salt (salted Pacific whiting vs. non-salted Pacific whiting). Activity of all 3 cathepsin groups demonstrated decreasing trend regardless of heating methods after the fermentation. Regardless of the heating conditions, cathepsin H had lowest activity after fermentation. The decreasing trend of cathepsin activity during fish sauce fermentation was believed to be caused by inhibition from end products (Bu et al., 2021). A significant decrease in cathepsin H activity indicates that cathepsin H is the major protease involved in whole Pacific whiting fish sauce fermentation. In addition, significant difference was found between our two samples in cathepsin L activity ($p < 0.05$). This was believed to be due to the difference caused by heating stability (uniform heat vs. non-uniform heat). According to An, Weerasinghe, Seymour, & Morrissey (1994), cathepsin L has highest activity at 55$^\circ$C. During sample preparation, our samples were held at 55$^\circ$C using either ohmic heating or water bath during week 3 to 8. However, water bath would not have maintained the temperature uniformly due to its limitation whereas ohmic heating would have maintained 55$^\circ$C, an optimum temperature for cathepsin L, resulting in more inhibitors generated.

**(5) Histamine content**

Histamine is a biogenic amine which could cause allergic reactions when consumed (Kohn, 2014). According to the Food and Agriculture Organization of the United Nations (FAO), & World Health Organization (WHO) (2018), histamine in fish sauce must be below 40 mg of histamine in 100 g of fish sauce. A significant difference ($p >$ 0.05) was found between the two incubation treatments for the histamine content: 0.41 mg of histamine/100 g of fish sauce for ohmic heating and 0.23 mg of histamine/100 g of fish sauce for water bath (Fig. 2.4). The significant difference was believed to be due to different heating methods: ohmic heating vs. water bath. As identical Pacific whiting mince was used for both heating methods, content of histidine, a precursor of histamine, would be identical prior to fermentation. According to Visciano, Schirone, & Paparella (2020), histamine is generated through microbial enzymatic activities. Therefore, rapid and consistent heat generated from ohmic heating was believed to have accelerated the microbial enzymatic activities resulting in a significant difference. In addition, histamine contents of commercial products were 30.61 mg of histamine/100 g of fish sauce for Korea and 14.38 mg of histamine/100 g of fish sauce for Thailand. A significant difference was found between the commercial samples and our lab scale samples ($p < 0.05$). This difference was believed to be due to different raw materials: Pacific whiting vs. anchovies. Commercial fish sauce samples were manufactured using anchovies, which are scombroid fish with abundant histidine that can transform to histamine during fermentation (Kim, Wei, Clemens, & An, 2005). However, Pacific whiting is gadoid fish (Hubalkova, Kralik, Tremlova, & Rencova, 2007) with lower amounts of histidine and therefore should not produce a significant level of histamine.

## 2.5 Conclusions

Fermentation index allows one to determine the degree of fermentation. In fish sauce fermentation, brownness, overall nitrogen content, and taste value can be regarded as measures contributing to the fermentation index. Higher fermentation indexes were obtained when ohmic heating was applied in fish sauce fermentation. Additionally, using Pacific whiting as raw material significantly decreased the histamine content in fish sauce. Therefore, applying ohmic heating in Pacific whiting fish sauce fermentation can not only accelerate the process, but also add a value to the utilization of protease-laden Pacific whiting. However, further research is still needed to determine whether high proteolytic activity in Pacific whiting actually contributes to the acceleration of fish sauce fermentation and to discover the optimum fermentation conditions with possibly extended incubation time to maximize the yields using ohmic heating for Pacific whiting fish sauce.

## 2.6 Acknowledgements

Table 2.1. Comparison on the sodium chloride content and brownness of commercial anchovy fish sauce and Pacific whiting fish sauce fermented for 8 weeks using either ohmic heating system or water bath

| Sample type | | Sodium chloride | Brownness |
|---|---|---|---|
| Raw material | Country of origin / Heating method | (g/100 mL) | (A420 nm) |
| Anchovy | Korea | $24.7\pm0.2^a$ | $1.74\pm0.00^a$ |
| | Thailand | $22.6\pm0.4^a$ | $1.70\pm0.00^a$ |
| Pacific whiting | Ohmic heating | $22.8\pm1.3^a$ | $1.65\pm0.08^a$ |
| | Water bath | $23.2\pm1.3^a$ | $1.18\pm0.04^b$ |

All data are reported as the mean±SD (n = 2). Mean values in each column with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).

Table 2.2. Comparison on the total nitrogen, formol nitrogen, ammonia nitrogen, and amino acid nitrogen contents of commercial anchovy fish sauce and Pacific whiting fish sauce fermented for 8 weeks using either ohmic heating system or water bath

| Nitrogen (N) (mg/100 g) | Commercial anchovy fish sauce (Produced country) | | Pacific whiting fish sauce (Fermentation apparatus) | |
|---|---|---|---|---|
| | Korea | Thailand | Ohmic heating | Water bath |
| Total-N | $821.1\pm3.4^a$ | $1,485.9\pm10.9^c$ | $1,132.3\pm1.17^b$ | $892.1\pm85.0^a$ |
| Formol-N | $656.0\pm1.1^c$ | $1,111.3\pm0.9^d$ | $481.9\pm1.1^b$ | $305.8\pm2.2^a$ |
| Ammonia-N | $204.8\pm2.1^b$ | $381.7\pm2.5^c$ | $142.3\pm8.3^a$ | $128.4\pm5.3^a$ |
| Amino acid-N | $451.2\pm1.1^c$ | $729.6\pm0.9^d$ | $339.6\pm8.3^b$ | $177.4\pm5.3^a$ |

All data are reported as the mean±SD (n = 2). Mean values in each row with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).

Table 2.3. Comparison on the free amino acid content of commercial anchovy fish sauce and Pacific whiting fish sauce fermented for 8 weeks using either ohmic heating system or water bath

| Amino acid (mg/100 g) | Commercial anchovy fish sauce (Produced country) | | Pacific whiting fish sauce (Fermentation apparatus) | |
|---|---|---|---|---|
| | Korea | Thailand | OHS | WB |
| Phosphoserine | 6.0±0.9[a] | 13.0±0.7[b] | 23.0±2.2[c] | 21.7±10.6[c] |
| Taurine | 45.0±7.7[a] | 60.3±1.9[b] | 95.9±0.3[d] | 81.9±2.4[c] |
| Aspartic acid | 149.2±5.3[c] | 400.9±1.2[d] | 97.8±0.2[b] | 37.8±0.7[a] |
| Hydroxyproline | 3.4±2.6[a] | 2.4±2.0[a] | 0.4±0.1[a] | 0.8±0.7[a] |
| Threonine | 61.2±0.7[b] | 275.1±0.5[d] | 111.3±0.2[c] | 46.2±0.4[a] |
| Serine | 45.4±1.2[a] | 157.1±0.5[c] | 90.0±0.1[b] | 41.1±0.5[a] |
| Glutamic acid | 533.3±7.6[c] | 585.4±1.3[d] | 168.9±0.6[b] | 51.2±0.7[a] |
| α-Aminoadipic acid | - | 21.9±0.5[b] | 7.1±0.5[a] | - |
| Proline | 51.0±11.0[c] | 88.5±0.3[d] | 36.3±0.6[b] | 16.6±1.0[a] |
| Glycine | 101.3±0.4[c] | 203.6±0.6[d] | 43.4±0.2[b] | 24.9±1.2[a] |
| Alanine | 194.4±1.0[b] | 408.4±1.0[d] | 170.6±0.2[b] | 85.3±0.4[a] |
| Citrulline | 71.9±11.1[c] | 257.2±0.7[d] | 5.9±0.1[b] | 3.2±0.2[a] |
| α-Aminobutyric acid | 240.5±13.7[a] | 329.5±47.1[b] | - | - |
| Valine | 143.7±1.2[c] | 354.5±0.7[d] | 139.4±2.4[b] | 58.5±0.6[a] |
| Cystine | - | 10.9±0.1[b] | 10.9±0.6[b] | 6.2±0.4[a] |
| Methionine | 69.3±0.9[b] | 155.7±0.3[d] | 88.1±1.5[c] | 33.4±3.7[a] |
| Isoleucine | 126.6±2.6[c] | 238.3±0.5[d] | 103.4±1.8[b] | 39.3±3.5[a] |

| | | | | |
|---|---|---|---|---|
| Leucine | 195.9±4.3[b] | 311.9±0.8[d] | 277.7±1.8[c] | 127.2±4.1[a] |
| Tyrosine | 26.3±6.6[a] | 77.7±0.1[b] | 107.6±1.9[c] | 36.0±9.4[a] |
| Phenylalanine | 83.3±0.7[b] | 205.5±0.3[d] | 103.4±3.0[c] | 44.5±2.2[a] |
| β-Alanine | 2.0±0.2[a] | 2.2±0.0[a] | 18.1±6.0[b] | 2.1±0.1[a] |
| β-Aminoisobutyric acid | - | 2.8±0.1[a] | 46.3±6.4[c] | 22.5±4.7[b] |
| γ-Aminobutyric acid | 523.6±2.2[b] | 1.2±0.0[a] | - | - |
| Ethanolamine | 1.9±0.1[a] | 2.5±0.1[b] | 7.7±0.2[c] | 9.4±0.9[d] |
| Hydroxylysine | 1.9±0.6[a] | 11.8±1.0[c] | 9.7±0.3[b] | 7.8±2.6[b] |
| Ornithine | 62.9±0.9[d] | 46.3±0.2[c] | 15.7±0.5[b] | 6.4±0.1[a] |
| Lysine | 188.2±4.0[b] | 546.4±1.5[d] | 341.1±0.9[c] | 179.3±0.9[a] |
| Histidine | 28.1±1.7[b] | 141.0±0.5[c] | 5.6±0.3[a] | - |
| 3-Methylhistidine | - | 3.2±0.5 | - | - |
| Anserine | 15.7±10.2[a] | 81.2±7.4[d] | 71.5±0.6[c] | 55.8±4.3[b] |
| Carnosine | - | 16.2±4.6 | - | - |
| Arginine | 2.4±1.6[a] | 6.1±4.1[a] | 216.0±21.0[c] | 152.9±27.2[b] |
| Total FAA | 2,974.4±41.8[c] | 5,018.7±53.2[d] | 2,412.8±28.1[b] | 1,191.7±7.7[a] |

All data are reported as the mean±SD (n = 2). Mean values in each row with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).

Table 2.4. Comparison on the taste value of commercial anchovy fish sauce and Pacific whiting fish sauce fermented for 8 weeks using either ohmic heating system or water bath

| Amino acid | Taste threshold value | Commercial anchovy (Produced country) | | Pacific whiting sauce (Fermentation apparatus) | |
|---|---|---|---|---|---|
| | | Korea | Thailand | OHS | WB |
| Arginine | 50 | 0.05±0.03[a] | 0.12±0.08[a] | 4.32±0.42[c] | 3.06±0.54[b] |
| Aspartic acid | 3 | 49.72±1.76[c] | 133.63±0.39[d] | 32.61±0.08[b] | 12.60±0.25[a] |
| Threonine | 260 | 0.24±0.00[b] | 1.06±0.00[d] | 0.43±0.00[c] | 0.18±0.00[a] |
| Serine | 150 | 0.30±0.01[a] | 1.05±0.00[d] | 0.60±0.00[b] | 0.27±0.00[c] |
| Glutamic acid | 5 | 106.65±1.52[c] | 117.09±0.26[d] | 33.78±0.13[b] | 10.23±0.14[a] |
| Proline | 300 | 0.17±0.04[c] | 0.30±0.00[d] | 0.12±0.00[b] | 0.06±0.00[a] |
| Glycine | 130 | 0.78±0.00[c] | 1.57±0.00[d] | 0.33±0.00[b] | 0.19±0.01[a] |
| Alanine | 60 | 3.24±0.02[c] | 6.81±0.02[d] | 2.84±0.00[b] | 1.42±0.01[a] |
| Valine | 40 | 3.59±0.03[c] | 8.86±0.02[d] | 3.49±0.06[b] | 1.46±0.02[a] |
| Methionine | 30 | 2.31±0.03[b] | 5.19±0.01[d] | 2.94±0.05[c] | 1.11±0.12[a] |
| Isoleucine | 90 | 1.41±0.03[c] | 2.65±0.01[d] | 1.15±0.02[b] | 0.44±0.04[a] |
| Leucine | 190 | 1.03±0.02[b] | 1.64±0.00[d] | 1.46±0.01[c] | 0.67±0.02[a] |
| Phenylalanine | 90 | 0.93±0.01[b] | 2.28±0.00[d] | 1.15±0.03[c] | 0.49±0.02[a] |
| Lysine | 50 | 3.76±0.08[b] | 10.93±0.03[d] | 6.82±0.02[c] | 3.59±0.02[a] |
| Histidine | 20 | 1.40±0.09[b] | 7.05±0.03[c] | 0.28±0.01[a] | - |
| Total | - | 175.58±3.50[c] | 300.23±0.82[d] | 92.32±0.50[b] | 35.77±0.28[a] |

The taste threshold value was quoted from Kato et al. (1989). Taste value = Free amino acid content (mg/100 g) / taste threshold (mg/l00 g).

|                          Pacific whiting fish sauce                          |  |                    Commercial anchovy fish sauce                    |  |
|---|---|---|---|
|                              (Heating apparatus)                              |  |                           (Country of origin)                           |  |
| Water bath | Ohmic heating | Thailand | S Korea |

Figure 2.1. Photo of Pacific whiting fish sauce fermented for 8 weeks using either water bath or ohmic heating, and commercial anchovy fish sauce.
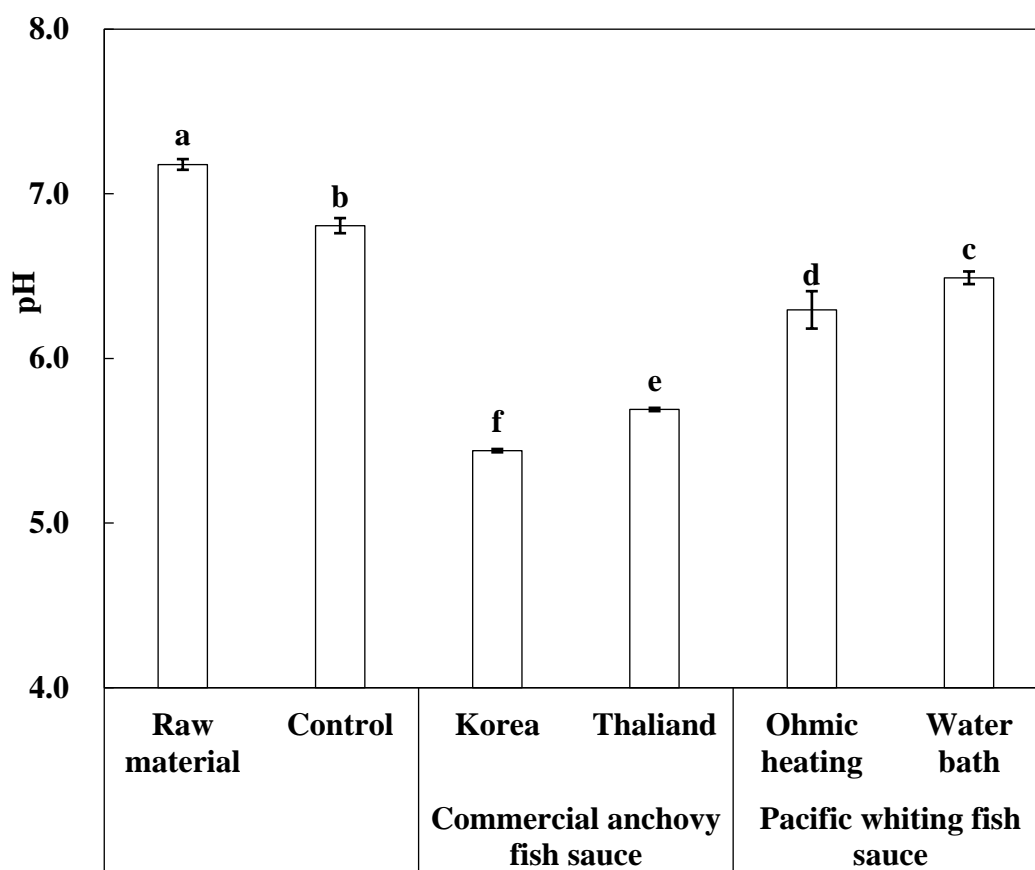
Figure 2.2. The pH of raw Pacific whiting, control (salted Pacific whiting mince prior to fermentation), commercial anchovy fish sauce, and Pacific whiting fish sauce samples fermented for 8 weeks with different heating conditions (n = 2). Results labeled with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).
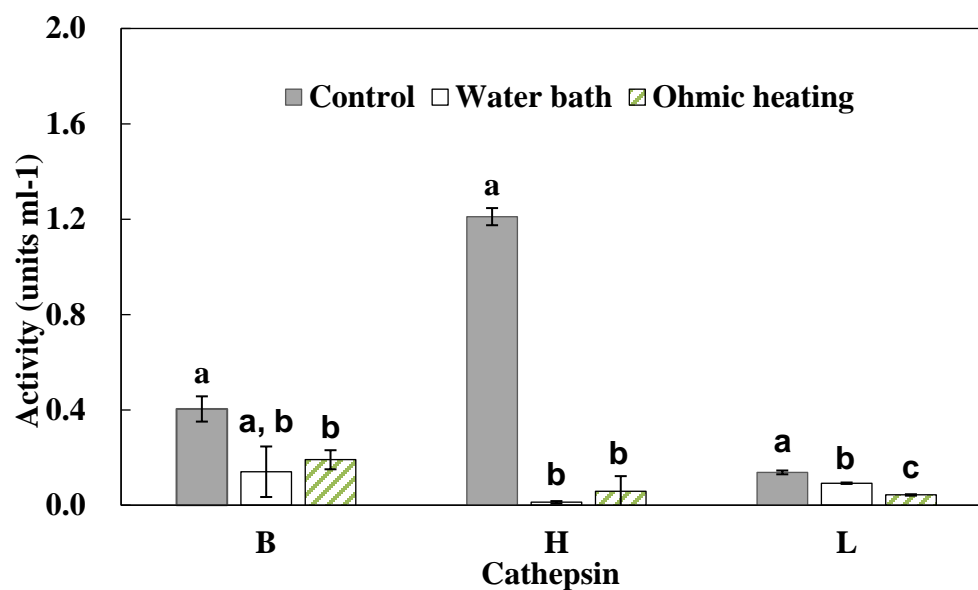
Figure 2.3. Cathepsin activity of fish sauce measured at before and end of fermentation with different heating conditions (n = 2). Statistical analysis was conducted individually between each cathepsin groups. Results labeled with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).
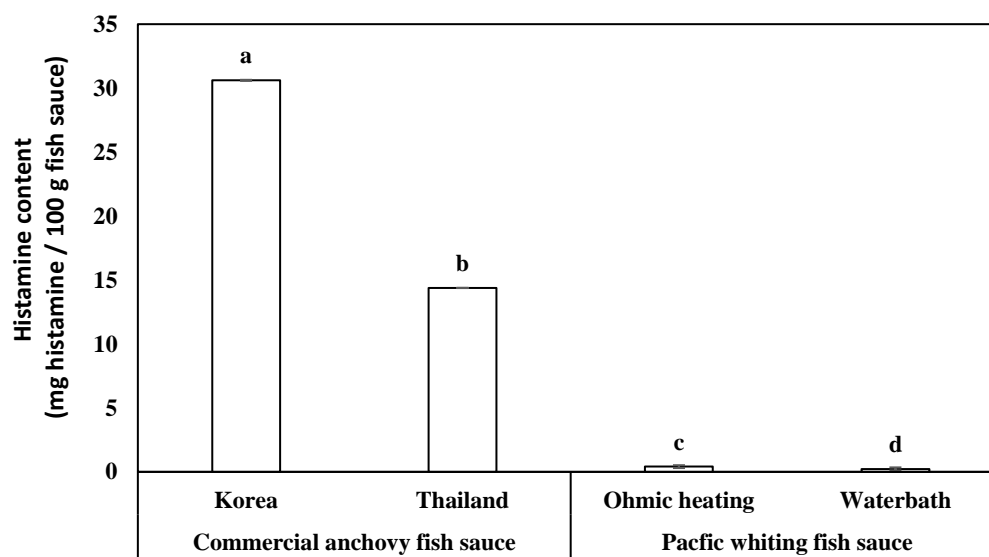
Figure 2.4. Histamine content of commercial anchovy fish sauce and Pacific whiting fish sauce fermented for 8 weeks with different heating conditions (n = 2). Results labeled with same letter indicates that values were not significantly different among the fermentation conditions (p > 0.05).

Chapter 3. General Conclusions

Nowadays, demands on fish sauce is no longer limited to Asian countries. Therefore, it is important to encourage seafood industries to start up fish sauce business. Starting up fish sauce business is not only important as the global fish sauce market is rapidly growing but also as it adds values to cost effective fish species such as Pacific whiting. Although Pacific whiting is one of the most important and abundant commercially fished groundfish in the American Pacific Northwest, it is difficult to sell Pacific whiting as fillet due to the extreme texture softening caused by proteases. Therefore, Pacific whiting, an abundant and nutritious resource, can be utilized differently in a way to take advantage of its texture softening proteases.

In this research, application of ohmic heating successfully accelerated Pacific whiting fish sauce fermentation. Unlike previous research for shortening the fermentation time that brought up undesired negative outcomes, applying ohmic heating did not demonstrate any negative outcomes based on the chemical analysis. The result of this study proved that the application of ohmic heating into fish sauce manufacture can add a value to the utilization of protease-laden Pacific whiting. In addition, ohmic heating is practical as its energy is retained efficiently within the fermentation container. In order to maintain the fermentation temperature for countries where the temperature is not as hot as in Southeast Asia, the use of ohmic heating would be very efficient for fish sauce production. Therefore, this research is believed to contribute an exciting means highly to the seafood industry for making fish sauce out of the undervalued fish species.

However, further studies are still needed as there were few limitations on this study. First of all, a research that could verify how ohmic heating contributes to accelerating fish sauce fermentation needs to be conducted. Based on the previous studies, we hypothesized ohmic heating accelerates fish sauce fermentation through electroporation and uniform heat penetration. However, as fish sauce is a fermented condiment and involve microbes, additional microbial analysis such as total cell count

during initial stage of fermentation must be conducted to verify potential effects of ohmic heating upon the microbes.

Secondly, a research that could verify the fact that cathepsin H is the dominant protease during Pacific whiting fish sauce fermentation should be conducted. We hypothesized cathepsin H is the dominant protease as it demonstrated highest activity prior to fermentation but lowest activity after fermentation. However, cathepsins may not have contributed greatly once heating profile exceeded its optima. Therefore, further research needs to be conducted using each optimum temperature ($25^oC$, $35^oC$ or $55^oC$) during the whole fermentation process to verify the dominant cathepsin.

Thirdly, further research to verify the fact that using Pacific whiting as raw material for fish sauce can lower the histamine content needs to be conducted. As the methods used in the commercial anchovy fish sauce and our Pacific whiting fish sauce samples were completely different, histamine content would need to be tested again using anchovy fish sauce made using ohmic heating and water bath.

Lastly, the current research needs to be repeated to make sure that the results are accurate. Due to the limitation on number of available ohmic heating system, only two biological replicates were prepared and used in this research. Therefore, additional replicates need to be prepared and tested to make sure that our results were accurate.

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