

A review on fish sauce processing, free amino acids and peptides with sensory properties

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Abstract

Fish sauce is a well-known condiment for its delicious flavour developed through a fermentation process. It is generally used as an additive for flavour enhancement in cooking. The fish sauce comes in several appearances, ranging from a clear liquid with light colour and texture to a dark and cloudy liquid. This product is produced by a long-duration of fermentation process by the combination of fish and salt. This high salt fermentation began with the action of enzymes from fish muscle and its digestive tracts, then continued with halophilic bacteria activity to further break down fish proteins in producing a liquid product with a mixture of soluble proteins, peptides and amino acids. This review is comprised of two sections, the first section covers various processing methods of fish sauces, mainly in the Asia region, namely *Nam-pla*, *Nuoc-mam*, *Yu-lu*, *Ishiru*, *Shottsuru*, *Bakasang* and *Budu*. These fish sauces roughly involve applying salt for fermentation, with different fish species, salt concentration, fermentation duration and processing techniques. The later section covers taste-contributing components of fish sauces in the scope of amino acids and peptides. Five of the major amino acids found to taste in the fish sauce, glutamic acid, threonine, alanine, methionine and histidine, are also reviewed in this paper. Sensory peptides in fish sauces shown in this paper are peptides with salt taste enhancement properties and peptides exhibiting sweet, sour, bitter, umami and kokumi tastes.

1. Introduction

Fish sauce is a brown to grey liquid with a unique aroma and flavour produced as the end product of fish fermentation. It is commonly used as a flavouring additive in cooking and often consumed as a condiment in daily meals (Saisithi, 1994; Rosma *et al.*, 2009; Montero *et al.*, 2017). There has been a record of fish sauce consumption during the Roman and Athenian eras during the fifth century, namely *Garum* (Skara *et al.*, 2015; Aquerreta, Astiasarn, and Bello, 2002) as well as in ancient Greece, *Aimeteon* (Ishige, 1993). *Garum* was produced by the activity of halotolerant enzymes from the viscera of a fresh mackerel in breaking down its blood and its salted innards for roughly two and up to nine months. Meanwhile, *Aimeteon* was produced by the viscera and blood of tunny fish (Beddows *et al.*, 1979; Beddows, 1998; Aquerreta *et al.*, 2002; Nielsen, 2004).

Nowadays, various fish sauce products are widely used worldwide, predominantly in Southeast Asia and some other parts of the globe (Adewumi, 2018).

Fish sauces are known by different names throughout different countries, such as *Nam-pla* (Thailand), *Bagoong* (Philippines), *Bakasang* (Indonesia), *Shottsuru* (Japan), *Aekjot* (Korea), *Yu-lu* (China), *Colombo cure* (India), *Ngapi* (Burma) and *Budu* (Malaysia) (Kuda and Miyawaki, 2010; Sim *et al.*, 2015). These varieties of fish sauce products have different fish species used as the primary raw material, salt concentrations, storage temperatures, storage containers and processing techniques, resulting in a final product with unique characteristics of different smells, tastes and colours (Lopetcharat *et al.*, 2007). Previous research has found that fish sauce contains a high

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concentration of nitrogen-based ingredients, with essential amino acids such as lysine, leucine, valine, isoleucine, threonine, valine, and phenylalanine accounting for 80% of the total (Saisithi, 1994; Je *et al.*, 2005; Ahmad *et al.*, 2019). Even though fish sauce is not considered a conventional functional food due to its high salt content, it can be used as a source of biologically active substances (Ichimura *et al.*, 2003; Fukami *et al.*, 2004; Najafian and Babji, 2018).

Since fresh fish are highly susceptible to microbial contamination and spoilage (Gram and Huss 1996; Adams, 2010), processing techniques especially fermentation, become a popular choice in developing countries for their low cost, ease of preparation and high acceptability among consumers. Today, fermented foods, specifically fish sauces, are in great demand to enhance food flavour or mask tainted food taste. Looking at the global capacity, the fish sauce had increased to more than 1100 kilometric tonnes (KMT) as recorded in 2015 compared to previous years of 1000 KMT. Meanwhile, in 2018, the global fish sauce market had a total value of 2300 Million USD and is expected to reach 3050 million USD by the end of 2025. As for fish sauce-producing countries, Thailand is the largest supplier and consumer with a production market and consumption market share of 44.8% and 26.9% respectively in 2015. Followed by Vietnam with a production market share of 37.7% and 22.4% sales market share. Another country involved is

China, sharing 6.7% of the sales market (More, 2021).

Differences in fish species, ingredients, and processing will produce each country's unique product, which possesses its different taste, appearance and chemical profile, including free amino acids and peptides (Yoshida, 1998; Lopetcharat *et al.*, 2007). This article will focus on the processing of fish sauce, with five well-known fish sauces reviewed, including fish sauces from Thailand (*Nam-pla*), Vietnam (*Nuoc-mam*), Japan (*Ishiru* and *Shottsuru*), China (*Yu-lu*), Indonesia (*Bakasang*), and Malaysia (*Budu*). The free amino acids and peptides with sensory properties in fish sauces will be discussed in the second section of this article. This paper incorporated all prior publications containing the terms "fish sauce," "peptides," "free amino acid," and "sensory."

2. Fish sauce processing

Variety production of fish sauce generally has the same concept, salting, natural enzyme hydrolysis, and bacterial fermentation. Briefly, after draining liquid from freshly harvested fish, it is mixed with salt at a determined ratio, as shown in Table 1. Then the mixture is dumped into the tank with a layer of salt at the bottom, then layered with salt at the top of the tank to prevent any parts of the fish exposed to air that will eventually result in spoilage. The tank is closed and left to ferment based on the manufacturer's standard operating

Table 1. Summary of fish sauce processing

Country Origin	Fish Sauce	Fish Species	Fermentation Duration	Fish; salt (w/w)	References
Thailand	<i>Nam-pla</i>	<i>Stolephrous</i> spp. <i>Ristrelliger</i> spp. <i>Cirrhinus</i> spp.	5-12 months	1-5:1	Ray and Montet (2015); Garnjanagoonchorn
Vietnam	<i>Nuoc-mam</i>	<i>Stolephorus</i> spp.	9-12 months	3:1-3:2	Nghia <i>et al.</i> (2017)
China	<i>Yu-lu</i>	<i>Engraulis japonicus</i> , <i>Channa asiatica</i>	6-12 months	3:1	Jiang <i>et al.</i> (2007); Wang <i>et al.</i> (2018)
Japan	<i>Ishiru/ Shottsuru</i>	<i>Astroscopus japonicus</i> , <i>Clupea pilchardus</i> <i>Omnastraphis sloani</i> , <i>Omnastraphis pacificus</i>	12 months	5:1	Ohshima and Giri (2014); Ray and Montet (2015)
Indonesia	<i>Bakasang</i>	<i>Stolephorus</i> spp. <i>Clupea</i> spp. <i>Leiagnathus Osteochilus</i> spp. <i>Sardinella</i> spp. <i>Engraulis</i>	2 months	5:1.5-3.5	Ijong and Ohta (1996); Ray and Montet (2015)
Malaysia	<i>Budu</i>	<i>Stolephorus</i> spp. <i>Sardinella</i> spp. <i>Decapterus macrosoma</i>	3-12 months	3-5:1	Beddows (1985); Klamkiao <i>et al.</i> (2006) Huda (2015)

procedure (Lopetcharat *et al.*, 2007). Anchovy, sardine, menhaden, and mackerel are widely used as primary raw materials in Asian fish sauces (Wang *et al.*, 2018). Table 1 shows a summary of various fish sauce processing techniques and their parameters. During fermentation to produce a fish sauce, fish muscle tissues were progressively broken down into smaller units by the action of proteolytic fish gut enzymes (Montero *et al.*, 2017). These small and finite units will then become a substrate for lactic acid bacteria to start fermenting (Hall, 2002; Devanthi *et al.*, 2019). A high concentration of salt is added to provide means of reducing water activity to prevent fish spoilage. Simultaneously, the high salt condition creates a favourable environment for halotolerant microorganisms which were found responsible to continue protein breakdown by fermentation. It is a continuous process after enzymatic proteolysis occurred at the early stages of fermentation (Martin, 1994; Steinkraus, 1997; Ohshima and Giri, 2014).

2.1 Nam-pla

Since Thailand is the largest fish sauce producing country, the processing applied is more towards large-scale commercialization involving proper by-product handling to produce several grades of fish sauce. Figure 1 shows the processing flow of fish sauce in Thailand. *Nam-pla* is made from major species of *Stolephorus* spp. and small fish like *Ristrelliger* spp. which are salted at a 5:1 to 1:1 (fish to salt) ratio depending on the size of the fish (Garnjanagoonchorn, 2016). The mixture was then placed into the fermentation tank with a woven bamboo mat and heavy rocks were placed on top as a preventive measure against fish mixture floating during fermentation. During the months of fermentation, the brown supernatant is extracted from the mixture. Some manufacturers remove the liquid using an outlet at the tank's bottom, which helps separate liquid and sediment. The resulting liquid is then allowed to ripen in the sun for two weeks to three months to cultivate a pleasing fragrance and flavour, resulting in a high-quality *Nam-pla* (Panda *et al.*, 2011).

After filtration, the remaining sediment is mixed with brine solution and fermented for up to 4 months, yielding a second-grade *Nam-pla* after liquid extraction. After producing second-grade *Nam-pla*, the remaining sediments are combined with saturated brine to produce a lower-grade fish sauce. The overall nitrogen content of these second and lower-grade products is enhanced by incorporating *Meiki* water, which is a rich source of glutamic acid (by-product in the making of monosodium glutamate, MSG). This ingredient is commonly used to meet the nitrogen content requirements for Thai fish

sauce (Lopetcharat *et al.*, 2007). It is then boiled and filtered, since it is commercialized, the transparent liquid produced is often improvised with additives such as sugar, caramel, and monosodium glutamate to change the colour taste.

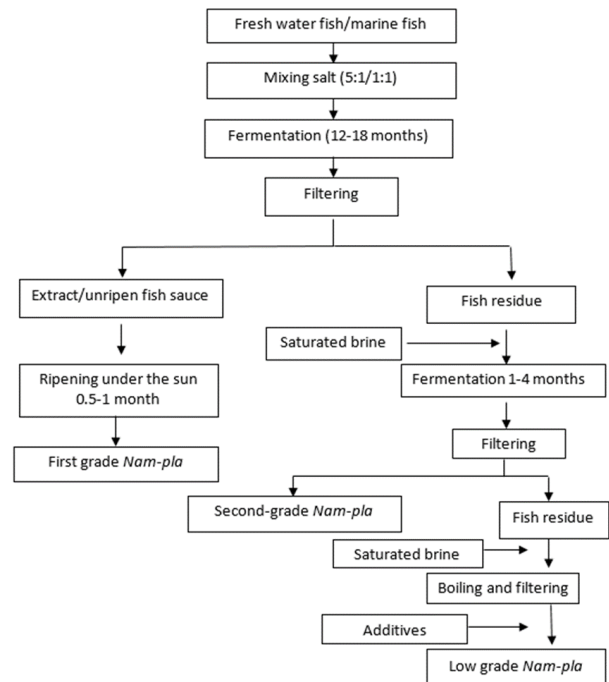


Figure 1. Processing of *Nam-pla* as adapted from (Wilaipan, 1990).

2.2 Nuoc-mam

Nuoc-mam is a clear and brownish Vietnamese fish sauce with a sharp scent. It is primarily developed in North and South Vietnam's coastal regions. According to traditional processing, fresh fish is kneaded and pressed using a hand, mixed with layers of salt with fish to the salt ratio of 3:1, and the mixture is dumped into a jar and buried in the ground with a tightly closed lid. It is left to ferment for 16 to 18 months, supernatant acquired after the fermentation is the *Nuoc-mam* (Beddows, 1985; Lopetcharat *et al.*, 2007; Anh, 2015). According to Nghia *et al.* (2017), processing a good quality *Nuoc-mam* begins with selecting fish species (only *Stolephorus* spp. used) and salting on board right after the catch. Next, the fermentation process is implemented in wooden vats with liquid recirculation through the fish for 9 to 12 months. After extracting the amino acid-containing liquid from the vats, the resulting fish residue is recombined with brine water and allowed to circulate for secondary fermentation. Then, depending on the nitrogen concentration, the collected liquid from the first fermentation is mixed with the liquid formed during secondary fermentation to create the final product of *Nuoc-mam*. Figure 2 illustrates modern *Nuoc-mam* processing.

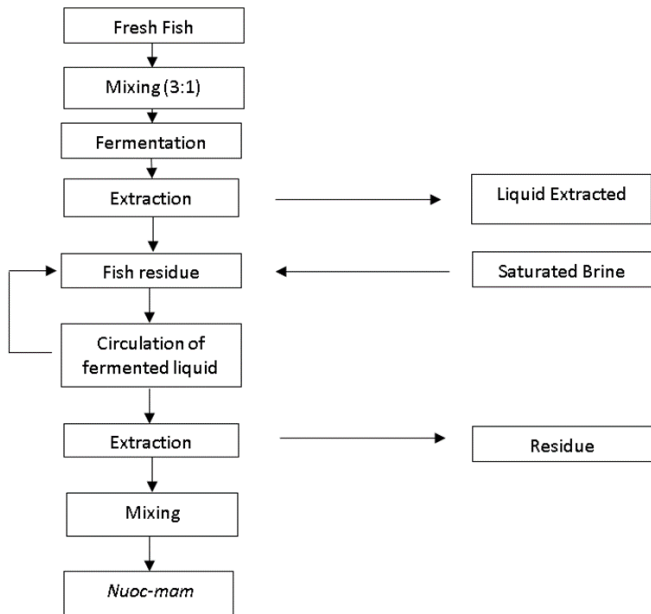


Figure 2. Processing of *Nuoc-mam* as adapted from (Nghia et al., 2017).

2.3 *Yu-lu*

Yu-lu is used extensively in the provinces of Guangdong and Fujian as a condiment in cooking (Jiang et al., 2007). Like other fish sauces, it is produced by fermentation of anchovies or other small marine fish with an approximately 3:1 fish-to-salt ratio. According to Wang et al. (2018), these mixtures of anchovy and salt could also be openly fermented at 25°C rather than fermenting in an enclosed tank. Fermentation time varies according to the producer's processing operation but is usually between 6 and 12 months. However, in the circumstances of higher salt concentration added to the mixture, the fermentation time could be extended due to decreased enzymatic operation (Saisithi, 1994). After the fermentation duration, the upper part liquid is extracted by filtration and ripened under the sun for two weeks. The liquid is then boiled, at this stage, the fish sauce liquid is added with flavouring ingredients such as sugar to improve the taste and produce a darker appearance (Lopetcharat, 2007). Finally, the resulting liquid is filtered before bottling to obtain a clear liquid as a final product. Figure 3 shows the traditional processing of Chinese fish sauce *Yu-lu*, adapted from Wilaipan (1990) and Wang et al. (2018).

2.4 *Ishiru*

Ishiru is a popular Japanese fish sauce mainly produced in Ishikawa prefecture. There are two versions of *Ishiru*, one with the base ingredient from a whole sardine and the other prepared by squid guts (Lopetcharat et al., 2007). Both versions undergo the fermentation process under high-salt conditions, which is shown in Figure 4. Sardine *Ishiru* is prepared by mixing 20% salt (w/w), and the mixture is fermented for

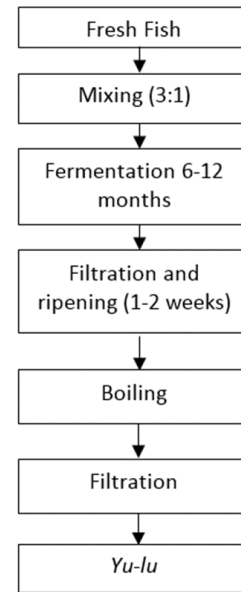


Figure 3. *Yu-lu* processing, adapted from Wilaipan (1990) and Wang et al. (2018).

approximately 6 to 12 months. Meanwhile, squid *Ishiru* is prepared with 18% salt (w/w) and left to ferment for two years (Ohshima and Giri, 2014). After fermentation, the accumulated liquid at the lower layer is taken using their drainage system. This liquid is then deproteinized by boiling and filtered. Finally, the resulting liquid is taken as a finished product (Kuda, 2015).

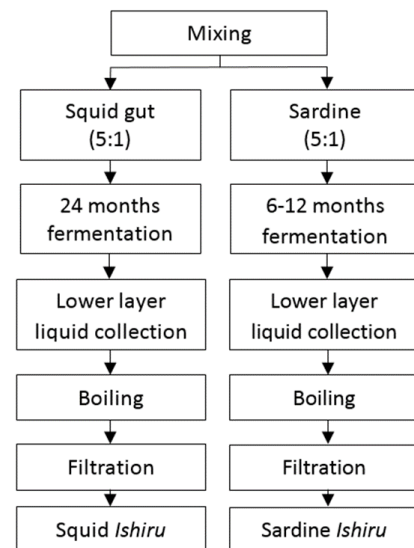


Figure 4. *Ishiru* processing adapted from (Lopetcharat et al., 2007) and (Ohshima and Giri, 2014).

2.5 *Shottsuru*

Shottsuru is another type of Japanese fish sauce commonly used in Akita prefecture (Mouritsen et al., 2017). It is made from sandfish, sardine, anchovy, horse mackerel, pacific mackerel and small shrimp. According to Ohshima and Giri (2014), small shrimp (mysid) is used to tint the sauce, with 10% of mysid mixed with fish meat before salting. The fish-to-salt ratio could be

within 3:1 or 7:2, and the mixture is left to ferment for 12 to 18 months in cement tanks. The liquid is then extracted, boiled and filtered through sea sand, and subsequent liquid is left to settle or age for a few months. Then the supernatant is gradually taken out. At this stage, *Shottsuru* is usually improved by flavouring addition such as soy sauce, caramel and seasoning. Figure 5 shows the processing flow of *Shottsuru*.

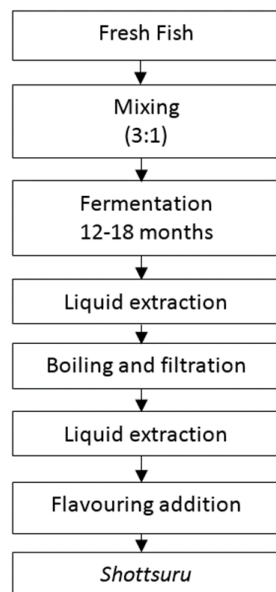


Figure 5. *Shottsuru* processing adapted from (Ohshima and Giri, 2014) and (Mouritsen et al., 2017).

2.6 Bakasang

Bakasang is a traditional Indonesian fish sauce made by the fermentation of whole small sardines. According to Ijong and Ohta (1995), *Bakasang* has widely accepted as an eating habit of Eastern Indonesian people, especially on North Celebes Island. Traditional *Bakasang* processing usually was prepared at home, which begins with salting, in which the fish-to-salt ratio ranges from 5:1.5 up to 5:3.5 (Ray and Montet, 2015). The mixture is then packed into bottles and stored in a warm location, usually near a fire source at a temperature of 300°C to 500°C. In about 3 to 6 weeks, the fermentation process is complete, and *Bakasang* is obtained (Ijong and Ohta, 1996). Figure 6 shows the traditional processing of *Bakasang*.

2.7 Budu

Budu is a dark brown liquid of fish sauce mainly produced on the East Coast of Peninsular Malaysia, particularly in Terengganu and Kelantan. It has been used as a dipping sauce for daily meals in Malaysia and a flavouring agent in various dishes and gravies (Huda, 2015). The processing of the *Budu* is quite similar to fish sauce production from other countries, the difference is that the product is adjusted to have a darker appearance by the addition of sugar, with a thick liquid consistency.

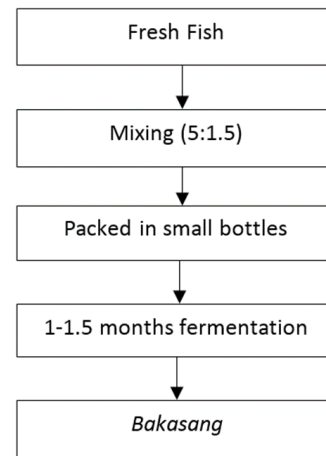


Figure 6. *Bakasang* Processing adapted (Ijong and Ohta, 1995) and (Ray and Montet, 2015).

Referring to Figure 7, the process begins with the salting process of cleaned anchovy at fish to a salt ratio of 3:1 (w/w) and is kept in an enclosed polyethylene tank to ferment under the sun for six and up to 12 months (Jedah and Ali, 1999; Klomklao et al., 2006; Nadiah et al., 2014) at approximately 30°C. Next, the mixture of fish and salt is added with flavouring and additives before fermentation; meanwhile, according to Rosma et al. (2009), some manufacturers improved the *Budu* with additives after 12 months of fermentation. These additives include tamarind, palm sugar, monosodium glutamate and several flavouring compounds. After the filtration process, the dark and brown liquid resulting from fermentation is known as ‘unprocessed *Budu*’, it is then boiled, left cooled and bottled.

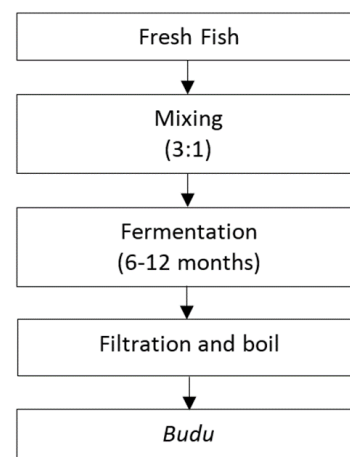


Figure 7. *Budu* Processing as adapted from (Rosma, 2009) and (Nadiah et al., 2014).

3. Sensory components in fish sauce

Sensory components are responsible for interaction with the human sense of taste and odour. Taste can be described as the chemical sense perceived by the taste buds in the mouth, meanwhile, the sense of odour is the perceived chemical sense in olfactory buds in the nose (Nishimura and Kato, 2009). According to Chan and Cheung (2010), there are five primary tastes: sweetness,

sourness, saltiness, bitterness, and umami. Three of these basic tastes, namely sweet, salty and umami, are associated with carbohydrates, minerals and amino acids. Meanwhile, sour taste perception is often linked to spoiled food and bitter perception of toxic substances. Thus, the integration of signals between a sense of smell and a sense of taste can be perceived as flavour (Laing and Jinks, 1996). In this review, the scope of taste will be focusing on the properties of amino acids and peptides in fish sauce in contributing to taste perception.

There have been multiple studies relating active taste components in various foods, including the role of histidine in giving both umami and sourness to the taste of dried skipjack, valine in the bitterness of sea urchin roe, glycine in the sweetness of the lobster, and the role of proline and methionine in the overall taste of Vietnamese fish sauce (Konosu, 1974; Fuke and Konosu, 1991; Park, Endoh, Watanabe *et al.*, 2002; Nishimura and Kato, 2009). In another study, an octapeptide, Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala extracted from beef has been documented to contribute a delicious and savoury taste, elimination of two amino acid residues in the peptide Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala resulted in the change of taste into sour (Yamasaki and Maekawa, 1978; Yamasaki and Maekawa, 1980). Meanwhile,

dipeptides in cheese were found to impart an umami taste, including Arg-Pro, Arg-Asp, Asp-Asp and Asp-Glu (Zhao *et al.*, 2016).

Catabolism of amino acids and lipid autooxidation during fermentation result in the extended release of aromatic compounds in fermented foods, particularly fish sauce (Linden and Lorient, 1999; Marilley and Casey, 2004; Mouritsen *et al.*, 2017), contributing to dominant notes of 'ammoniacal,' 'cheesy,' and 'meaty' in fish sauces (Dougan and Howard, 1975). According to Giri *et al.* (2010), Trimethylamine (TMA) has been identified as responsible for 'ammoniacal' notes in *Nam-pla* and *Nuoc-mam*. Oxidation of lipid during fermentation in releasing aldehydes of 3-methyl-butanal and hexanal were reported for 'meaty' notes in fish sauce (Fukami *et al.*, 2002; Dehaut *et al.*, 2014). According to Russo (2020), volatile fatty acids were found among the most abundant, including butanoic acid, 2 and 3-Methylbutanoic acid, providing 'cheesy' and 'fatty' notes in Italian fish sauce.

3.1 Free amino acids

As amino acids were observed to elevate significantly after fermentation (Itou *et al.*, 2006), along

Table 2. Free amino acid with major taste properties in fish sauces.

Fish sauce	Amino Acid	Amount (mg/100 g)	Predominant Taste Properties	Reference
<i>Nam-pla</i>	Glutamic Acid	950	Sour	Chan and Cheung (2010); Park, Watanabe, Endoh <i>et al.</i> (2002); Yoshida (1998)
	Threonine	460	Sweet	
	Alanine	700	Sweet	
	Histidine	320	Bitter	
	Methionine	230	Bitter	
<i>Nuoc-mam</i>	Glutamic Acid	1370	Sour	Yoshida (1998); Chan and Cheung (2010)
	Threonine	700	Sweet	
	Alanine	1010	Sweet	
	Histidine	1360	Bitter	
	Methionine	270	Bitter	
<i>Yu-lu</i>	Glutamic Acid	828.4	Sour	Yoshida (1998); Jiang <i>et al.</i> (2007); Chan and Cheung (2010);
	Threonine	304	Sweet	
	Alanine	506	Sweet	
	Histidine	194	Bitter	
<i>Ishiru</i> (sardine)	Glutamic Acid	1383	Sour	Yoshida (1998); Chan and Cheung (2010)
	Threonine	608	Sweet	
	Alanine	506	Sweet	
	Histidine	194	Bitter	
	Methionine	255	Bitter	
<i>Bakasang</i>	Glutamic Acid	727	Sour	Ijong and Ohta (1996); Yoshida (1998); Lopetcharat <i>et al.</i> (2007)
	Threonine	60	Sweet	
	Alanine	484	Sweet	
	Histidine	44	Bitter	
	Methionine	360	Bitter	
<i>Budu</i>	Glutamic Acid	620	Sour/Umami	Kimizuka <i>et al.</i> (1987); Yoshida (1998); Khairunnisak <i>et al.</i> (2009); Chan and Cheung
	Threonine	229	Sweet	
	Alanine	406	Sweet	
	Histidine	190	Bitter	

with a high percentage of amino acids in total nitrogen (%) observed in various fish sauces from Thailand, Vietnam, China and the Philippines (Nakano *et al.*, 2017), it has been recorded those amino acids give a direct contribution to taste on their own or by working in synergy with other components (Chan and Cheung, 2010). Amino acids, in general, possess primary taste properties, which aspartic acid and glutamic acid in singular form are attributed to sour taste. These amino acids in the derived form of salt are characterized as umami, savoury and meaty with a chicken broth-like taste (Linden and Lorient, 1999). Meanwhile, L-amino acids with hydrophobic side chains, particularly leucine, isoleucine, tyrosine, and valine, are attributed to bitter taste (Temussi, 2012). On the other hand, the D-form of amino acids, such as proline, alanine, lysine, glycine, serine, and threonine, is usually attributed to a sweet taste.

Free amino acids are described as amino acids that are not embedded or bound to proteins (Oshimura and Sakamoto, 2017). These free amino acids are commonly low in most foods and not considered to affect yielding tastes directly. However, the level may be heightened significantly during fermentation or even storage to a point in giving out the taste of food (Kato *et al.*, 1989; Itou *et al.*, 2006). According to Park, Endoh, Watanabe *et al.* (2002), the strong and complex taste of the fish sauce results from many compounds, mainly free amino acids. The study found glutamic acid, threonine, alanine, valine, histidine, proline, tyrosine, cysteine, methionine, and pyroglutamic acid to have significant taste characteristics in fish sauce. Table 2 shows the free amino acid content that has the most significant impact on fish sauce flavour.

According to Table 2, glutamic acid in its dissociated state elicits sour stimuli (Chan and Cheung, 2010), this taste perception can be reasoned with the dissociation of its acidic properties in the taste cell membrane. However, the synergistic effect of glutamic acid with high salt content in fish sauces exhibits an umami taste. This was found to comply with Park, Endoh, Watanabe *et al.* (2002). Complete removal of glutamic acid in Vietnamese fish sauce synthetic extract resulted in total loss of umami and sweetness, making an overall flatter taste compared to extract without glutamic acid removal. The role of glutamic acid in its synergistic effect on umami taste has been shown in other foods, including bean paste in Japan, soy sauce in Korea and oyster sauce in Malaysia (Kawai *et al.*, 2009; Khairunnisak *et al.*, 2009; Kang *et al.*, 2011).

In terms of sweet-tasting amino acids, both threonine and alanine have a sweet predominant taste. Almost all D

-configured amino acids possess a strong sweet taste rather than a bitter taste (Yoshida, 1998; Chan and Cheung, 2010). According to Park, Endoh, Watanabe *et al.* (2002), in the omission test of alanine known as taste active component in almost all seafood, removing the synthetic extract resulted in a slight decrease in umami and sweetness, increasing bitter and astringent taste. As for threonine removal, the test observed a reduction of the overall 'fish sauce like' taste. Shirai *et al.* (1997) have documented threonine as one of the ingredients with taste contributing factor in squid (*Gonatopsis Borealis*) at 13 mg/100 g. Compared to the threonine content of all fish sauces ranging from 60 to 700 mg/100 g, as in Table 2, threonine in fish sauce could be identified as one of the significant components of taste contributing properties.

As for histidine and methionine, these amino acids on their own have a predominant taste of bitter (Chan and Cheung, 2010). However, in fish sauce, histidine contributes to bitterness, umami and 'fish sauce like' taste, where removal of this particular amino acid increased the sweetness and astringent taste of *Nuoc-mam* (Park, Endoh, Watanabe *et al.*, 2002). Meanwhile, methionine somehow contributes to the sweetness and overall taste of the fish sauce. Methionine also has been shown to portray taste properties in seafood, including sea urchins and lobster (Fuke and Konosu, 1991; Shirai *et al.*, 1997). The difference in perception of free amino acids in the fish sauce when compared to its predominant taste might be related to the synergistic interactions that affect taste and flavour, where some free amino acids enhance umami and savoury when combined in a mixture with the sodium salt of glutamate and aspartate (Fuke, 1994; Lioe *et al.*, 2005).

3.2 Peptides

Other than amino acids, peptides also have a role in the food taste profile. Previous studies in cheese products have shown the formation of sweet and broth taste from peptides, while medium-sized peptides were found in giving out bitter tastes in Swiss cheeses (Hammond and Biede, 1979). According to Arai *et al.* (1973), α -Glutamyl di- and tripeptides, especially those containing asparagine, threonine, and serine, enhance the umami flavour of the cheese. Other than that, peptide 7-L-glutamyl-S-(prop-l-enyl)-L-cysteine isolated from chives, and 7-L-glutamyl-S-(prop-l-enyl)-L-cysteine sulfoxide isolated from onions were found to elicit a biting taste sensation (Solms, 1997). Taste description of peptides identified in multiple studies in fish sauces including *Nuoc-mam*, *Nam-pla*, *Pla-ra*, *Yu-lu*, *Shottsuru*, *Ishiru* and *Garum* is shown in Table 3.

Peptides in the fish sauce also were described to

Table 3. Sensory peptides in fish sauces

Fish Sauce	Peptide sequence	Taste properties	Reference
<i>Nuoc-mam</i>	Tyr-Pro-Orn	Bitter	Park, Ishida, Watanabe <i>et al.</i> (2002)
	Val-Pro-Orn	Bitter	
	Gly-Pro-Orn-Gly	Flat	
	Ala-Pro	Bitter / Flat	
	Asp-Glu	Sour / Umami	
	Asp-Phe	Flat	
	Glu-Pro	Flat	
	Glu-Phe	Flat	
	Gly-Phe	Bitter	
	Gly-Tyr	Bitter	
	Val-Pro	Sweet	
	Tyr-Pro	Sour	
	Phe-Pro	Bitter	
	Val-Pro-Glu	Sour	
	Glu-Met-Pro	Flat	
	Asp-Met-Pro	Umami	
<i>Nuoc-mam</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012); Miyamura <i>et al.</i> (2015)
<i>Nam-pla</i>	Arg-Pro	Salt taste enhancing	Schindler <i>et al.</i> (2011)
	Arg-Ala	Salt taste enhancing	
	Ala-Arg	Salt taste enhancing	
	Arg-Gly	Salt taste enhancing	
	Arg-Ser	Salt taste enhancing	
	Arg-Val	Salt taste enhancing	
	Val-Arg	Salt taste enhancing	
	Arg-Met	Salt taste enhancing	
<i>Nam-pla</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012); Miyamura <i>et al.</i> (2015)
<i>Pla-ra</i>	γ -Glu-Val-Gly	Kokumi	Phewpan <i>et al.</i> (2019)
<i>Yu-lu</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012); Miyamura <i>et al.</i> (2015)
<i>Shottsuru</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012)
<i>Ishiru</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012)
<i>Garum</i>	γ -Glu-Val-Gly	Kokumi	Kuroda <i>et al.</i> (2012)
			Miyamura <i>et al.</i> (2015)

portray specific tastes. According to Park, Ishida, Watanabe *et al.* (2002), in the study of Vietnamese fish sauce *Nuoc-mam*, the taste characteristics of peptides were classified into sour/umami, umami, flat, sweet and bitter, as shown in Table 3. The study has shown that peptides characterizing sour tastes are Asp-Glu, Tyr-Pro and Val-Pro-Glu. The finding of the study seemed to comply with the list of sour peptides in Kirimura *et al.* (1969), where dipeptides containing Glu and/or Asp tend to produce a sour taste in water. This sour taste perception can be perceived due to proton binding from acidic amino acid dissociation of the peptide interacting with the taste membrane (Nishimura and Kato, 2009).

Referring to Table 3, peptides with flat or almost tasteless are Gly-Pro-Orn-Gly, Asp-Phe, Glu-Pro, Glu-Phe and Glu-Met-Pro. According to Arai *et al.* (1973), one of the bland taste groups of dipeptides containing glutamyl residue at the N-terminus is Glu-Pro, which complied with the taste characteristic of Glu-Pro in the study (Park, Ishida, Watanabe *et al.*, 2002). Meanwhile,

bitter peptides found in *Nuoc-mam* are Tyr-Pro-Orn, Val-Pro-Orn, Ala-Pro, Gly-Phe, Gly-Tyr and Phe-Pro. Almost all peptides containing hydrophobic amino acids of L-Phe, L-Tyr, L-Trp, L-Leu, L-Val, and L-Ile produce a bitter taste. Furthermore, the sequence of amino acids has its role in the intensity of bitterness, where Phe-Pro, as found bitter in *Nuoc-mam*, is more bitter when compared Pro-Phe (Shiraishi *et al.*, 1973; Nishimura and Kato, 2009).

The umami peptide identified in the Vietnam fish sauce is only Asp-Met-Pro, meanwhile, the sweet-tasting peptide is Val-Pro. According to Nishimura and Kato, Asp or Glu containing peptides produce a sour taste in water, however, Glu-Asp and Glu-Glu may produce umami perception in an aqueous solution of NaCl (2009). The sweet-tasting peptide Val-Pro found in the *Nuoc-mam* may be the outcome of the combination of L-configured Pro with a predominant taste of sweet and bitter. It could also result from D-configured valine, which generates a strong sweet taste (Chan and Cheung,

2010; Nishimura and Kato, 2009). In another study, a series of arginyl dipeptides isolated from *Nam-pla*, namely Arg-Pro, Arg-Ala, Ala-Arg, Arg-Gly, Arg-Ser, Arg-Val, Val-Arg and Arg-Met, which were identified as salt taste-enhancing molecules (Schindler *et al.*, 2011). These might be related to the function of L-configured arginine in enhancing salty taste, which has been documented in the study regarding Gouda cheese maturation (Toelstede *et al.*, 2009).

Recent interest in the scope of taste-contributing peptides has brought a new idea in characterizing a new kind of sensory perception. Since foods sometimes have continuity characteristics, complexity and 'mouthfulness' perception are limited to be explained by the five basic sensory tastes (Wang *et al.*, 2020). According to a study, it has been discovered that an extract of garlic contains a specific substance that improves and strengthens thickness, 'mouthfulness' and continuity perception when added to an umami solution. Analysis of the extract revealed the components responsible for attributes observed, which it is indicated by sulfur-containing compounds such as S-allyl-cysteine sulfoxide (alliin), S-methyl-cysteine sulfoxide, γ -glutamyl-allyl-cysteine, and glutathione (γ -glutamyl-cysteinyl-glycine) (Tan *et al.*, 2015; Nakamoto *et al.*, 2018). These substances with specific properties were then referred to as kokumi substances that is producing kokumi taste.

According to Yang *et al.* (2019), γ -glutamyl peptide, as one of the kokumi substances, does not give out kokumi taste on its own, only eliciting astringency in water. However, when kokumi-active substances are mixed into a solution comprised of several basic tastes (sweet, salty, sour, bitter and umami), the kokumi taste can be perceived. At the same time, the kokumi taste could not be detected from a solution containing basic tastes without kokumi substances. There is a study to understand the mechanism of kokumi sensation, in which γ -glutamyl peptides stimulate the calcium-sensing receptor (CaSR) to produce kokumi taste perception. The study also recognized a positive association between γ -glutamyl peptide CaSR activity with perceived kokumi intensity (Ohsu *et al.*, 2010; Miyamura *et al.*, 2015).

The γ -glutamyl peptide has been studied in a variety of foods, including common beans, black gram, soybean, garlic, cheese, soy sauce, yeast extract and fish sauces (Liao *et al.*, 2013; Bing *et al.*, 2014; Tan *et al.*, 2015; Miyamura *et al.*, 2015; Hillmann *et al.*, 2016; Nakamoto *et al.*, 2018; Shibata *et al.*, 2018). Meanwhile, in commercial fish sauce products, the most vital kokumi substance, γ -Glu-Val-Gly, have been determined on *Nam-pla*, *Yu-lu*, *Pla-ra*, *Shottsuru*, and *Garum* (Kuroda

et al., 2012; Miyamura *et al.*, 2015; Phewpan *et al.*, 2019). Table 3 shows fish sauces with sensory peptide sequences identified, including kokumi peptide.

As shown in Table 3, kokumi peptides γ -Glu-Val-Gly are present in *Nuoc-mam*, *Nam-pla*, *Pla-ra*, *Yu-lu*, *Shottsuru*, *Ishiru*, and *Garum*. Since the early fermentation process involves rapid proteolysis by fish enzymatic guts then followed by microbial activity, the kokumi peptide was likely produced the same way. Furthermore, according to Kuroda *et al.* (2012), the sequence of Val-Gly was found to be present in several fish muscular proteins using a database search, the proteins including myosin, actin, collagen and creatine kinase (Herrero *et al.*, 2000; Saito *et al.*, 2001; Wilson, 2003). Thus, it is assumed that the sequence of Val-Gly is released during protein breakdown via enzyme activity at the early fermentation stage.

Suzuki *et al.* (2007) described the role of dipeptide Val-Gly as a substrate for γ -glutamyltransferase (GCT) in the subsequent fermentation process by the action of microbes, where it can be expected that γ -Glu-Val-Gly was biosynthesized by GCT produced by multiple bacteria, including *Bacillus* spp., *Pseudomonas* spp., *Halobacterium* spp. and *Staphylococcus* spp. (Lopetcharat *et al.*, 2007; Yuen *et al.*, 2009). Also, the presence of this specific peptide has been statistically correlated with the microbial community in a fermented fish product, where *Tetragenococcus* spp. and *Lentibacillus* spp. were identified in generation γ -Glu-Val-Gly (Phewpan *et al.*, 2019).

4. Conclusion

Different versions of fish sauces may have different formulations with processing flow, however the fundamental for the fermentation process remains the same which involves the application of salts onto the fish. Previous studies have shown the presence of free amino acids like glutamic acid, and peptides such as kokumi peptides contributing to its sensorial activity in multiple varieties of fish sauces. However, this knowledge of Malaysian fish sauce *Budu* is relatively absent. Further study is also required on the mechanism of γ -Glu-Val-Gly generation during fermentation and how this peptide can be applied to give benefit the food industry.

Conflict of interest

There is no conflict of interest.

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