

## **Fermented Fish Products: A Review on the Manufacturing Process, Technological Aspect, Sensory, Nutritional Qualities and Metabolite Profiles.**

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KEYWORDS	ABSTRACT
Fermented fish products Umami taste Technological aspects Sensory Metabolites profiles	Fermented fish products are traditional foods which have a long history of use as condiment and side dishes in many countries especially Southeast Asia and Asian countries. Today, fermented fish products are gaining importance and have a great potential in the food industry as general populace start to realize their unique characteristics. The indigenous fermented fish products are renowned for their umami taste and special aroma characters besides possess high nutritional values. This review article focuses on the manufacturing process of various fermented fish products, their technological aspect, sensory and nutritional qualities. In addition, the research works carried out on metabolites profiles of fermented fish products is also reviewed. This review paper provides an extensive knowledge and broad information on fermented fish products which can be used as reference to improve the products quality.

### **1.0 INTRODUCTION**

In many parts of the world, fermented fish products are very popular especially in Southeast and East Asian countries. These countries are including Malaysia, Thailand, Vietnam, Cambodia, Indonesia, Korea and China. Due to their characteristic flavor, fermented fish products are widely used as traditional seasoning and food condiments (Tsai et al. 2006). Fermented fish products are typically composed of the mixture of fishes and salt which have been undergo fermentation process for duration of 6 to 12 months or longer (Lopetcharat et al. 2001). Normally, the fermentation process requires a long time to make sure the solubilisation and development of flavor and colour of the product (Kim et al. 1997). During this period, the meat will be auto-digested by the enzymes in the fish itself and results in different categories of products such as

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sauce, paste and solid (Jiang et al. 2007; Tadashi et al. 1991). These traditional products own different names according to countries.

In Malaysia, fermented fish sauce is named as budu. Meanwhile, the other names of fermented fish sauce are bakasang (Indonesia), patis (Philippine), yu-lu (China), and nampla (Thailand) (Sim et al. 2009). Fermented shrimp paste is also one of the indigenous fermented fish products and extensively used in those countries. It is as well comes with different names such as belacan (Malaysia), kapi (Thailand), ngapi (Burma) trasi (Indonesia) and andaramang (Philippines) (Md Adnan, 1984). Fermented fish solid or also known as lacto-fermented fish is prepared by fermenting fish with either rice or other grains (Tadashi et al. 1991). Lacto-fermented fish is known as jeotkals in Korea (Mah et al. 2002) and plaa-som in Thailand (Palludan-Miller et al. 2002). Other ingredients usually added in making fermented fish products, depending on the country tradition. The common ingredients are garlic, boiled rice and other spices (Palludan-Miller et al. 2002). Almost all fermented fish products possess the deep and strong umami taste. This unique taste has driven the interest of food chemist to investigate and characterize the metabolites that contain in a wide variety of fermented fish products (Park et al. 2001). Metabolites are defined as any substance that is formed during chemical reaction either in human body or food (Pico et al. 2006). Meanwhile, metabolomic analysis is a study about metabolites and very popular as analytical approach in plant and food science. Metabolomics play significant roles in food science where it has become an efficient way to measure the quality, processing and safety of raw materials and end products (Cevallos-cevallos et al. 2009).

Some general reviews of fermented fish products have been published which most of them outline the processing techniques and describe the chemical compositions of the products. However, those publications are already outdated and only summarized the early research works. Thus, in this review, we summarize the current studies of various fermented fish products and it focuses on the manufacturing process, technological aspect, sensory and nutritional qualities and metabolites profiles of fermented fish products.

## 2.0 MANUFACTURING PROCESS OF FERMENTED FISH PRODUCTS

The basic raw materials for production of fermented fish products are fish and salt. Meanwhile, the techniques used to manufacture these products are different among countries since each country has its own traditional methods. In fish sauce production, fish and salt are mixed at certain ratio and being fermented for 9 to 12 months at ambient temperature (Lopetcharat et al. 2001). The ratio of fish to salt in budu manufacturing is 2:1 or 3:1. The salted fish are then allowed to be fermented in a closed tank at temperature of 30 – 40 °C for 6 to 12 months (Sim et al. 2009). Besides fish and salt mixture, the other ingredients that are added during production of budu are tamarind, palm sugar, monosodium glutamate and flavoring compounds (Rosma et al. 2009). *Nampla*, a Thai fish sauce is also produced using the same fish/salt ratio; however, the fermentation period is longer (12 to 18 months) compared to budu. On the other hand, in processing of *aekjeot*, a Korean fish sauce, fish (usually anchovies) and salt are placed in alternating layers. Normally, the amount of salt used is reaching 20 – 30%. The storage temperature, fat content and freshness of the fish are the factors that influence the amount of salt added. The duration of fermentation is 90 days and the fermentation temperature is quite low, nearly 20°C (Lopetcharat et al. 2001). Table 1 summarizes the fish sauce production methods and types of fish used in different countries.

Fermented shrimp pastes are produced from fish or shrimp which is blend with salt (20 – 25%) and goes through fermentation process under ambient temperature (Lopetcharat et al. 2001). Besides become the major producer of fish sauce, Thailand also produces some of fermented fish paste including *kapi*, *jalo* and *koong som*. *Kapi* is an indigeneous fermented shrimp paste which is usually produced from small shrimp or krill and salted at ratio of 3-5:1. Before fermentation, it underwent drying and blending processes. The minimum period for fermentation is 2 months until the essential aroma has generated (Beddow, 1985). On the other

hand, *jaloo* is fermented krill which is generally being fermented anaerobically for only 2 to 3 days. In *koong-som* production, small shrimp and palm-sap-sugar are mixed together and fermented by lactic acid bacteria in anaerobic jar (Pithakpol, 1993).

Table 1: Summary of fish sauce production methods and types of fish used in different countries (Beddow, 1985)

Country	Name	Fish species commercially used	Method Fish: Salt/Fermentation time
Cambodia	<i>Nouc-mam</i>	<i>Stolephorus spp.</i> <i>Ristrelliger spp.</i> <i>Engraulis spp.</i>	3:1-3:2/2-3 months
France	<i>Gau-ca</i>	<i>Ophicephalus spp.</i>	4:1/2-8 weeks
	<i>Pissala</i>	<i>Ahyapellucida spp.</i> <i>Gobius spp.</i> <i>Engraulis spp.</i>	
	Anchovy	<i>Engraulisencrasicholus spp.</i>	2:1/6-7 weeks
Hong Kong	<i>Yeesui</i>	<i>Sardinella spp.</i> <i>Engraulispupapa spp.</i>	4:1/3-12 months
India & Pakistan	<i>Colombo-cure</i>	<i>Ristrelliger spp.</i>	Gutted fish with gills removed and tamarind added 6:1/12 months
Indonesia	<i>Ketjap-ikan</i>	<i>Cybiium spp.</i>	6:1/6 months
		<i>Clupea spp.</i>	
		<i>Stolephorus spp.</i>	
		<i>Clupea spp.</i>	
		<i>Leiagnathus</i> <i>Osteochilus spp.</i> (fresh water fish)	
Japan	<i>Shotturu</i>	<i>Astroscoopusjaponicus</i> <i>Clupeapilchardus</i>	5:1/6 months, malt added
Korea	<i>Aekjeot</i>	<i>Astroscoopusjaponicus</i> <i>Engraulis japonica</i>	3-4:1/12 months
Malaysia	<i>Budu</i>	<i>Stolephorus spp.</i>	3-5:1/3-12 months, palm sugar/tamarind added
Phillipines	<i>Patis</i>	<i>Stolephorus spp.</i> <i>Clupea spp.</i> <i>Decapterus spp.</i> <i>Leionathus spp.</i>	3-4:1/3-12 months
Thailand	<i>Nampla</i>	<i>Stolephorus spp.</i> <i>Ristrelliger spp.</i>	1-5:1/5-12 months

On the contrary, lacto-fermented fish is usually a fermented fish product which composed of solid fermented fish and other solid ingredients such as rice and garlic (Tadashi et al. 1991). However, there is solid fermented fish product that is produced as fermented fish alone without other ingredients. For example, *feseekh*, an Egyptian salted-fermented fish. *Feseekh* is prepared in a glass jar where the fish, *Mugil cephalus*, is mixed with abundance of salt in alternating layers. The jar is then sealed and allowed to ferment for 60 days at room temperature (TCPS, 2005). Other examples of lacto-fermented fish are *plaa-som* and *som-fug* from Thailand, and *Myeolchi-jeotgal* from Korea. *Plaa-som* and *som fug* generally composed of fermented fish, rice and garlic. However, the way they are prepared is differ from each other. *Plaa-som* is produced from freshwater fish (*Channa striatus*) and the whole fish is mixed thoroughly with the sea salt and palm syrup. Then, roasted rice, in the form of powder is added to the salted fish. The product is packed in plastic bag and left for 8 – 12 days at 30 – 38°C (Palludan-Miller et al. 2002). Meanwhile, *som-fug* is consisting of chopped salted fish mixed with steamed rice and minced garlic. Typically, banana leaves or plastic bags are used to pack the product. The fermentation period and temperature of *som-fug* is shorter compared to *plaa-som*, that is only 2 – 5 days and 30°C, respectively (Rabie et al. 2009).

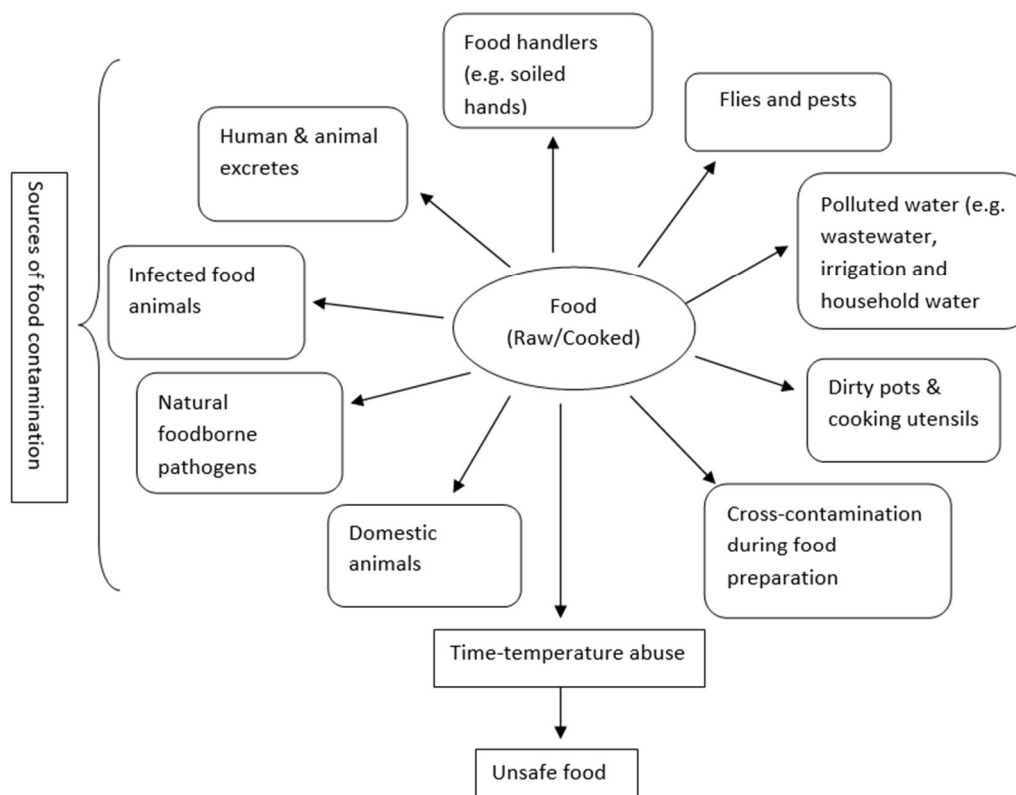
### 3.0 TECHNOLOGICAL ASPECT OF FERMENTED FISH PRODUCTS

Either raw or cooked foods may be a cause of foodborne disease which it becomes a crucial health problem around the world. However, developing countries are facing the most impact of this crisis. Figure 1 illustrates the factors that can lead to food contamination during food preparation (Rieybroy et al. 2004). As shown, the reasons that contribute to food contamination are diverse. In addition, there is a wide range of etiological agents that can cause foodborne illnesses such as bacteria, parasites and viruses. Fermentation is an imperative way to increase the food safety and public health especially in developing countries because it has the ability to inhibit the growth of most microorganisms (Nout et al. 1997).

Fermentation can be expressed as a biochemical process by microorganisms and their enzymes in modifying the raw materials. For the past centuries, fermentation has renowned as an excellent approach for food preservation and it has encountered evolution where it has been expanded and improved. As a technology, food fermentation has a very long history and nowadays, many developing countries have started to use this technology in small-scale food industry as well as at the household level. Numerous primary food products have been transformed into fermented products including fish. Technology of fermentation has altered the raw fish into new products in which their quality has been enhanced. By fermentation, the shelf life of the products has been prolonged compared to their original substrate and the nutritional values of the product also has been enriched. In addition, it also improves the digestibility (Nout et al. 1997).

Lactic acid fermentation is a well-known approach to prepare fermented fish products in East-Asia. The special characteristic of this method is the ability to preserve short-lived vegetables and fishes in hygienic and safe conditions. This is due to the presence of powerful anti-microbial agents, usually acetic acid and lactic acid which are produced by lactic acid bacteria. These compounds have improved the food safety of the product by lowering the pH and thus prevent the development of dangerous food pathogens (Motarjemi et al. 2002). In other words, lactic acid fermentation enables to reduce growth, survival and toxic production of bad bacteria (Rieybroy et al. 2004). *Sikhae* is a Korean lactic acid fermented fish product that has pH range of 4-5 and consist of 6-8% salt content. This salt content is relatively lower than other types of fermented fish products which usually reaching 20–25% of salt content. Fermentation of *sikhae* is a tremendous approach to preserve raw fish since the easily spoiled fishes can be kept for 1 – 2 months without being rotten (Lee, 1984).

Figure 1: Sources of food contamination during food preparation (Nout and Motarjemi, 1997)



#### 4.0 SENSORY ASPECTS OF FERMENTED FISH PRODUCTS

The most significant attribute of fermented fish products is their special, fragrant aroma and taste. Fermentation process induces the taste development in fermented fish products where enzymes and microbes degrade the fish protein into amino acids (Rabie et al. 2009). Dougan and Dougan and Howard (1975) reported that the odor of the fish sauce is the combination of cheesy, ammoniacal and meaty notes. The cheesy note is produced by low molecular weight fatty acid which is formed during from microorganisms' action. Meanwhile, the contributory factors to ammoniacal note are including ammonia, a wide range of amines and other compounds that contain nitrogen. Meaty note can be contributed by oxidation process.

Enzymes that are present in fish together with some halotolerant and halophile microorganisms have induced the fermentation process and resulting in hydrolysis of the fish protein. Free amino acids, peptides and ammonia are then produced from the hydrolysis. Since fermented food products have high salt concentration, the growth of pathogenic bacteria is under controlled and the salt results in 'umami' taste and aroma (Dougan and Howard, 1975). Nevertheless, salt also could give negative impacts to human health in which the excessive salt content in food could raise blood pressure and develop critical hypertension (Catharina et al. 1999). Besides sweet, sour, bitter and salty, 'umami' taste is also one of the fundamental taste types (MacGregor and Wardener, 2002). 'Umami' is the taste of glutamic acid which is identified by Japanese (Fuke and Shimizu, 1993). Peralta and others (2008) have discovered some of the

major free amino acid in shrimp paste and they are consisting of taurine, glycine, alanine, leucine and arginine. The amount of these compounds is increasing during fermentation and enhances the flavor quality of the shrimp paste.

Jiang and others (2007) have reported the sensory evaluation of *Yu-Lu*, a traditional fermented fish sauce from China during fermentation process. *Yu-Lu* contains a variety of amino acids that may contribute to the enhancement of flavor. Previous study has demonstrated the evaluation of sensory of aerobically and anaerobically fermented fish sauces using same rating test and hedonic rating test. *Patis*, which is produced anaerobically is proven more rancid, highly flavored and cheesy. In contrast, the aerobically produced fermented fish had a milder odor which is slightly sweet and slightly burn (Peralta et al. 2008).

## 5.0 NUTRITIONAL ASPECTS OF FERMENTED FISH PRODUCTS

As well as preventing food contamination by being fermented, fermented fish products also play a significant role to human nutrition since they are enriched with peptides, essential amino acids and other nitrogenous compounds. Microorganisms and enzymes are responsible for production of these beneficial compounds through their activity during fermentation. Moreover, a number of vitamins and minerals are also detected in these products. Vitamin A, vitamin B1 (thiamin), vitamin B2 (riboflavin), vitamin B6 (pyridoxine) and niacin are aqueous soluble vitamins that are found in fish sauce. In addition, fish products are also enriched with various minerals including manganese (Mn), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), and iron (Fe) [14]. Besides play an important role as flavor and aroma enhancer (Raksakulthai and Haard, 1992), peptides and amino acids which are naturally occurring during fermentation of salted fishery products also known as antioxidants. A lot of fermented fish products exhibit antioxidant activity such as fermented blue mussel (Jung et al. 2005), fish sauces (Harada et al. 2003) and fermented fish shrimp (Peralta et al. 2005).

Biogenic amines are found in fish product and mainly generated by microbial decarboxylation of amino acids (Halász et al. 1994). Since biogenic amines are the sources of nitrogen and could result in synthesis of hormones, nucleic acids, alkaloids and proteins, they are very essential to exert positive effect on human health (Silla-Santos, 1996). Moreover, they are also enable to control the processes in human system for example; body temperature regulation, increase or decrease of blood pressure and nutrition intake (Greif et al. 1997).

## 6.0 METABOLITE PROFILES OF FERMENTED FISH PRODUCTS

### Fermented Fish Sauce

Park and others (2001) have reported the chemical composition of 61 fish sauces that are collected from several Southeast and Asian countries. They are analyzed for free amino acids, nucleosides, nucleic acid bases, organic acids, creatine and creatinine. From the findings, the fish sauces that originally from Vietnam, Japan and Thailand have high content of nucleosides and nucleic acid bases but low concentration of organic acids. They showed almost similar amino acid pattern meanwhile those of Myanmar and Laos demonstrated different amino acid pattern and the lowest content of nucleosides and nucleic acid bases. Some of nucleic acid bases that have been detected in the samples are cytosine, uracil, thymine, guanine, adenine, hypoxanthine and xanthine. These nucleic acid bases are hydrolyzed into their respective nucleosides; cytidine, uridine, thymidine, guanosine, adenosine, inosine and xanthosine. On the other hand, fish sauce of Korea and China exhibited intermediate contents and similar amino acid patterns.

Among all organic acids that have been determined in the samples, acetate showed high amount in Myanmar and Chinese fish sauces. This result indicates acetic acid fermentation is the major type of fermentation that being applied in those countries. Normally, fish muscle accumulates a high concentration of creatine and traces of creatinine, which are produced from phosphocreatine. Phosphocreatine acts as primary energy storage in vertebrate muscle. From the

results, the fish sauces from Japan, Vietnam and Thailand showed the highest creatine and creatinine content in which the creatinine content was nearly half of creatine amount. Meanwhile, the values of creatine and creatinine that existed in the fish sauce of China and Korea took place in the middle range. On the other hand, fish sauce from Myanmar and Laos has the lowest concentration of creatine and creatinine.

In Malaysia, the most famous fermented fish sauce is known as budu. Budu is mainly produced in Kelantan and consumed by Malaysian especially people from East Coast state of West Malaysia (Kelantan and Terengganu). Rosma and others (2009) have reported the analysis of histamine and 3-MCPD (3-monochloropropane-1,2-diol) contents in 12 *budu* samples with different fermentation period. She concluded that 58% of budu samples had hazardous level of histamine but fortunately, none of them exceed the detection level of 2 ppb of 3-MCPD. Table 2 shows the data of histamine and 2-MCPD in 12 *budu* samples (Rosma et al., 2009).

Table 2: The histamine and 3-MCPD data of *budu* samples (Rosma et al., 2009)

Sample	Fermentation period (month)	Histamine* (mg/100g)	3-MCPD (ppb)
1	6	71.85 ± 0.49 <sup>h</sup>	<2
2	5	81.50 ± 0.14 <sup>i</sup>	<2
3	8	106.40 ± 0.42 <sup>k</sup>	<2
4	8	41.90 ± 0.42 <sup>d</sup>	<2
5	12	23.87 ± 0.31 <sup>b</sup>	<2
6	3	26.86 ± 0.23 <sup>c</sup>	<2
7	3	22.21 ± 0.16 <sup>a</sup>	<2
8	12	23.65 ± 0.39 <sup>b</sup>	<2
9	8	57.15 ± 0.21 <sup>e</sup>	<2
10	8	102.25 ± 0.07 <sup>j</sup>	<2
11	5	65.00 ± 0.14 <sup>g</sup>	<2
12	8	63.40 ± 0.71 <sup>f</sup>	<2

\*Results are expressed as means ± SD; values are means of duplicates from three independent samples. Means values in the same column with different lowercase letters are significantly different ( $p < 0.05$ ).

Histamine is a type of biogenic amines and its content can be a parameter to evaluate the quality of fish sauce. Histamine could impart the negative effect on human health by resulting histamine fish poisoning (Scombroid poisoning) if it is consumed more than 50 mg/100g. Scombroid poisoning is a food intoxication which has various symptoms including urticaria, rash,

vomiting, nausea, flushing and tingling and itching of the skin (Taylor, 1986). Generally, histamine is derived from free histidine. Fish tissues contain a large amount of free histidine which can be converted into histamine through decarboxylation when the fish is not well processed and stored (spoiled fish) (Taylor, 1986; Sanceda et al. 1996). Decarboxylation of histidine can be induced by numerous halophiles including *Photobacterium histaminum* sp. Nov, *Enterobacteriaceae*, *Proteus morgani*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae* and *Hafnia alvei* (Fujii et al. 1994). The term 'Scombroid poisoning' is used because most of the histamine poisoning cases involves scombroid fish such as mackerel, tuna, bonito and saury. This is due to the high level of free histidine in the muscle of those types of fish and different species of fish results in different histamine content (Mietzz and Karmas, 1977). Histamine is hard to destroy since it is stable when heated (Tapingkae et al. 2010). On the other hand, 3-MCPD is a carcinogen which is produced when chloride react with lipid in food. It is not naturally occurring in budu, but it is a contaminant that is normally occurring in sauce which comes from acid-hydrolyzed vegetable protein (acid-HVP) (JFSSG, 1999). Acid-HVP is usually added into sauce as savory ingredients (Wong et al. 2006). Since all the samples contain less than 2 ppb of 3-MCPD, it can be concluded that the preparation of studied budu did not involve acid hydrolysis and addition of acid-HVP (Rosma et al. 2009).

Korean fermented shrimp and anchovy sauces have been detected containing N-nitrosodimethylamine (NDMA) in the range of 1.3 – 3.4 µg/kg (Kim et al. 1985). N-nitrosamines are volatile compounds that are usually found in foods, drinks and cigarette. They are carcinogens which have great potential to induce tumors in important human organs such as kidney, liver, bladder, lung, esophagus, tongue and pancreas. Thicker (2000) reported that salting is one of the methods that allow the formation of N-nitrosamines in foods. Therefore, the implication of salted fish fermentation for a long period resulted in the accumulation of N-nitrosamines and their precursors (Ahn et al. 2003). Moreover, some of biogenic amines such as putrescine, cadaverine, spermine and spermidine also could develop nitrosoamine through reaction with nitrite in foods (Shalaby, 1996). N-nitrosamines are formed under the low pH condition, in the range of pH 2 – 6, thus the pH of salted shrimp and anchovy sauces are very suitable for N-nitrosamines formation (Thicker, 2000).

Ijong and Ohta (1995) had studied the amino acid compositions of *bakasang*, a traditional Indonesian fermented fish sauce. *Bakasang* is very popular among Manadonese people who live in the North Celebes Island. They usually use *bakasang* as flavoring in variety of local dishes and sometimes it is mixed with tomato, garlic, chillies and red onion. The amino acids that have been detected in *bakasang* were aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine. Amongst the amino acids detected, glutamic acid and lysine were prominent and the total amino acids in *bakasang* were in the range of 42.36 to 46.64 mg/mL. This amount indicates that *bakasang* is the good source of essential amino acids in the diet.

*Yu-Lu*, the fermented fish sauce from China has been analyzed for its free amino acid and histamine content for the sensory evaluation purpose. A total of 17 amino acids found in *Yu-Lu* as shown in Table 3. The content of amino acids changed prior to fermentation periods. According to Table 3, glutamic acid, leucine, lysine, valine and alanine were major amino acids in *Yu-Lu*. It was reported that the amount of each free acid was different among the samples. This result was due to the unbalance production of amino acids by autolysis and microbial action, respectively (Jiang et al. 2007). It is well-known that amino acids are important compounds that give huge contribution to the taste of fish sauce (Lopetcharat et al. 2001). For instance, the glutamic acid has meaty aroma. Glycine, serine, alanine and threonine give a sweet taste and in contrast, histidine, valine and phenylalanine have a bitter taste (Liu, 1989). For histamine content in *Yu-Lu*, the amount was increased only at the first two month of fermentation, and then it decreased until the final period of fermentation. This is due to the inhibition of proteolytic enzymes in the fish and growth of halophilic histamine-forming bacteria by high salt condition (Jiang et al. 2007). Both

proteolytic enzymes and halophilic bacteria play a significant role in histamine production. Halophilic bacteria may change free histidine from fish muscle into histamine through decarboxylation while proteolytic enzymes that produced by halophilic bacteria could release additional histidine from muscle protein and increase histamine production (Sapin-Jaloustre and Sapin-Jaloustre, 1957)

Table 3: Amino acid content in Yu-Lu (Jiang et al., 2007)

Amino acid	Ferment 30 days (mg/100g)	Ferment 60 days (mg/100g)	Ferment 90 days (mg/100g)	Ferment 120 days (mg/100g)	Ferment 150 days (mg/100g)	Ferment 180 days (mg/100g)
Aspartate	39.46	33.81	19.68	17.92	71.08	74.60
Glutamate	177.68	180.55	232.00	245.71	409.29	472.61
Serine	102.98	64.85	68.9	71.37	276.81	317.98
Histidine	154.51	138.59	150.98	157.5	277.20	291.37
Arginine	222.06	236.42	255.04	274.93	239.29	275.94
Threonine	129.81	123.99	156.82	157.66	185.42	219.97
Alanine	157.20	161.08	218.18	274.93	355.83	400.62
Tyrosine	77.82	58.47	45.6	110.85	57.55	78.33
Valine	149.68	162.83	230.86	297.94	354.26	422.94
Methionine	81.38	80.08	132.69	152.04	168.77	206.04
Cysteine	5.09	14.76	21.79	21.84	4.61	8.84
Isoleucine	102.6	122.30	164.52	218.32	271.84	309.01
Leucine	200.94	228.48	318.91	397.48	444.45	530.48
Tryptophan	191.99	414.95	745.49	912.2	350.78	469.51
Phenylalanine	89.23	112.30	156.73	189.17	200.75	247.19
Lysine	226.86	329.67	366.28	553.64	476.01	569.50
Glycine	77.78	54.97	61.58	59.14	82.50	121.73
Total	2187.10	2518.07	3346.07	4077.9	4226.46	5016.68

In 2008, Yang and others (2008) have reported the analysis of volatile flavour compounds in *Yu-Lu* by using gas chromatography. There were 22 of volatile compounds identified in the samples which consist of ketones, aldehydes, organic acids, esters, sulfur-containing compounds and nitrogen-containing compounds. No alcohol group was detected in *Yu-Lu* and this result was almost similar with the previous study which reported only a trace amount of alcohol detected in fish sauce (Lopetcharat et al. 2001). Yang and others (2008) have enlightened that *Yu-Lu* samples contained a large amount of dimethyl disulfides, pyrazine derivative and volatile organic acids. Only a few types of aldehydes and ketones found in *Yu-Lu* and most of them have simple structures such as acetone, 2-heptanone, acetophenone and benzaldehyde.

### Fermented fish/shrimp paste

Fermented shrimp paste in Malaysia is known as *belacan*. It is widely used as a condiment in many types of dishes such as fried rice (*nasi goreng belacan*), spicy noodle soup (*laksa*), chilli *belacan* (*sambal belacan*) and Indian fried rice (Yang et al. 2008). Jinap and others (2010) have reported the determination of free glutamic acid and 5'-nucleotides concentration in *belacan*. 5'-nucleotides content that are being determined in this study were 5'-inosine monophosphate (5'-IMP) and 5'-guanosine monophosphate (5'-GMP). The amount of free glutamic acid in different brands of *belacan* was 601 - 4207 mg/100g while the concentration of 5'-nucleotides in *belacan* samples were between 0.85 and 42.25 µg/g. The data is shown in Table 4.

Table 4: The concentration of free glutamic acid and 5'-nucleotides in *belacan* samples (Jinap et al. 2010)

Brand no.	Free glutamic acid (mg/100g)	5'-nucleotides (µg/g)		
		GMP	IMP	Total
1	1695	25.78±14.22	16.47±5.03	42.25±8.21
2	1987	ND	1.55±0.21	1.55±0.21
3	1332	ND	0.85±0.08	0.85±0.08
4	4207	0.49±0.11	1.75±0.25	2.24±0.29
5	3117	0.73±0.5	3.15±1.52	3.88±1.38
6	714	3.90±1.88	ND	3.90±1.88
7	1116	0.64±0.07	2.32±0.13	2.63±0.35
8	1143	0.57±0.11	0.85±0.06	1.41±0.06
9	3553	0.55±0.32	3.05±0.32	3.59±0.5
10	1231	7.42±3.55	0.63±0.21	8.04±2.26b
11	601	3.14±3.24	10.76±2.61	13.90±4.14

The factors that affect the variety of glutamate content in various brands are including the shrimp used, the fermentation period, product formulation as well as the moisture content of paste mixture. As for 5'-nucleotides content, from eleven samples that have been tested, ten of them contained GMP. On the other hand, IMP existed in nine samples but not all the samples contained GMP. There were seven brands that showed both of GMP and IMP content (Table 4). Glutamic acid and 5'-nucleotides are responsible for umami taste in foods in which the strength of umami taste is usually generated all the way through the synergistic bonding of free glutamate and 5'-ribonucleotides. In this case, 5'-ribonucleotides could develop the umami taste of L-glutamate (Zhang et al. 2008).

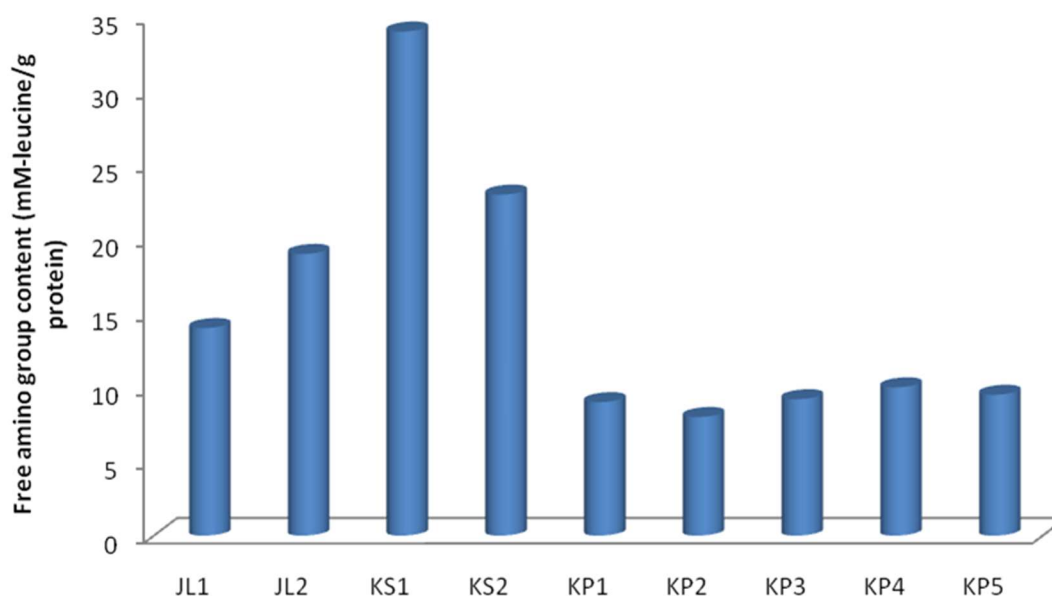
Besides fermented fish sauce, fermented fish paste has also been proven to contain numerous biogenic amines since the compounds occur naturally in fish (Fardiaz and Markakis, 1979). This finding has been confirmed by Naila and others (2011a) who reported a number of biogenic amines were found in *Rihaakuru*, a Maldives fish paste. In this study, ten biogenic amines have been detected in the samples including agmatine, cadaverine, histamine, putrescine, phenylethylamine, spermine, spermidine, serotonin, tyramine and tryptamine. Among all the biogenic amines, histamine is the most toxic and for health safety, its content must not exceed 50 ppm in a product (Greif et al. 1997). For other biogenic amines, the allowable limits are much higher; putrescine (2000 ppm), cadaverine (2000 ppm), tryptamine (2000 ppm), spermidine (600 ppm) and spermine (600 ppm) (Til et al. 1997). Fifteen samples (out of 28) showed the level of histamine that exceeds the permissible limit (>50 ppm). Meanwhile, the other seven samples contained very high histamine contents which exceeded 5000 ppm and could cause histamine poisoning. This result possibly related to the type of fish used as raw material. *Rihaakuru* is made from raw tuna, namely *Katsuwonus pelamis*, *Thunnus albacores*, *Thunnus obesus*, *Auxis thazard* and *Euthynnus affinis* (Naila et al. 2011b). Free histidine is found abundantly in tuna loin where the histamine forming bacteria decarboxylate the free histidine into histamine (Yoshinaga and Frank, 1982). Moreover, the existence of putrescine and cadaverine could enhance the histamine poisoning through synergistic effects (Stratton et al. 1991).

The amino acid composition and antioxidant activity of Thai traditional fermented shrimp and krill products have been reported by Faithong and others (2010). The products that have been examined in this study were *kapi*, *jaloo* and *koong-som*. According to Sathivel and others (2003), fermented fish products are enriched with peptides and amino acids, which demonstrate the antioxidant characteristic. *Kapi*, *jaloo* and *koong-som* were found to have different free amino group contents which possibly due to the partial proteolysis that occurred during fermentation. Even for the same product, the free amino group contents also varied. This is probably because the raw materials used were different, besides they underwent different processes and different fermentation periods. As shown in the Figure 2, the free amino acid content in *kapi* is much lower compared to *jaloo* and *koong-som*. This is because *kapi* has the longest period of fermentation and during this period, the free amino acids are decomposed through deamidation process or used by microorganisms. In this study, the free amino acids act as antioxidants which they exhibited antioxidant activity in those products. However, this study does not demonstrate the isolation and characterization of the antioxidant components (free amino acids).

The similar study was carried out for Philippine fermented shrimp paste by Peralta et al. (2008) In this study, the antioxidant activity and nutritional components of shrimp paste were improved by extending the fermentation period (1 to 360 days). It has been reported that the amount of free amino acids in Philippine fermented shrimp paste increased at 90 days but did not increase from 90 to 180 days ( $p > 0.05$ ). Then it drastically reduced at 360 days in contrast to 90 days ( $p < 0.05$ ). This reduction probably resulted from the degradation of amino acids to amines, volatile acids and other nitrogenous compounds. Arginine, taurine, glycine, alanine, leucine and lysine are predominant amino acids in the fermented shrimp paste and most of them showed significant increase in amount during fermentation (Peralta et al. 2008). A number of amino acids such as

methionine, tyrosine, lysine, histidine and tryptophan are recognized as antioxidant exhibitor (Kitts and Weiller, 2003). Antioxidant compounds are very important to human body because they are capable to avoid or delay the oxidative damages that caused by free radicals. Normally, free radicals are very reactive therefore; they tend to strike other molecules around them including lipids, protein and sugar. As a result, oxidative damages such as deterioration of foods, modifications of protein and enzyme inactivation occur. By reducing the production of primary catalyst of lipid peroxidation, antioxidant could delay or prevent the damages. Besides this approach, antioxidant substances also could react with free radicals and interfere the propagation step of lipid oxidation (Peralta et al. 2008). Moreover, some of the amino acids such as lysine, proline, serine, glutamic acid, alanine, leucine and glycine also influence the taste of fermented shrimp (Raksakulthai and Haard, 1992).

Figure 2: The amino acid group content. JL: *Jaloo*, KS: *Koong-som*, KP: *kapi*. Bars stand for the standard deviation from triplicate determination (Faithong et al. 2010)



Besides amino acid compositions, the polyunsaturated fatty acid contents in Philippine fermented fish and shrimp paste also have been studied (Montano et al. 2001). The samples that were tested consist of *Dalagang bukid*, *dilis*, *padas*, *terong* and *alamang*. It was reported that numerous of fatty acid compositions have been detected in the samples, including all-cis-4,7,10,13,16,19-docosahexaenoic acid (DHA). Marine life especially fish are enriched with long-chain polyunsaturated fatty acids which are part of the  $\omega$ -3 or n-3 family. Uauy and others (2002) mentioned that this type of fatty acids is crucial for neural development in infant both during in utero and the first few years after birth. In this case, DHA as one of the important  $\omega$ -3 fatty acids is integrated in the brain and retina of the growing infant. The significant  $\omega$ -3 fatty acids that were identified in the samples are 18:3 $\omega$ 3, 20:5 $\omega$ 3, 22:5 $\omega$ 3 and 22:6 $\omega$ 3. The general formula for fatty acid is m:n $\omega$ x where m is the carbon chain length, n indicates the number of cis double bonds and  $\omega$ x shows the polyunsaturated fatty acid category. Amongst the samples, *alamang* had the highest proportion of  $\omega$ -3 fatty acids as well as DHA content. In contrast, *Dagang bukid* showed the lowest content of DHA while *padas* was intermediate (Uauy et al. 2002).

Fish miso is the first trial of Japanese fermented fish meat paste that produced from trash fishes. The trash fishes are referred to the small-sized fishes that are wasted every year since people do not know how to manage that valuable protein sources properly. Therefore, it is very economical if the trash fishes can be used as the raw material in making fermented fish paste. Giri, and others (2010) have reported the study on fish miso in term of identification and quantification of volatile compounds. The analysis was carried out on four fish miso samples, each is produced from different species of trash fish including spotted mackerel, lizard fish, horse mackerel and common squid. As a result, 107 volatile compounds have been detected in the four samples of fish miso. Those volatile compounds were consisting of 17 aldehydes, 31 alcohols, 13 esters, 7 ketones, 7 furans and 7 sulphurs, 5 nitrogenous compounds, 18 aromatic compounds, 1 acid and 1 hydrocarbon. Amongst all the volatile compounds detected, alcohol functional group was predominant in all the samples where ethanol showed the highest concentration. Generally, the odor of miso products is contributed by the long straight chain alcohols like n-pentanol, n-hexanol, n-heptanol, n-octanol and n-nonanol. Additionally, alcohols with branch chains also give important aroma to the miso products as they exhibit lower threshold value like those long straight chain alcohols.

Aldehydes are also important components where they are able to confer desired aroma in foods. However, aldehydes can contribute to rancid odor as well during breakdown of fat and fatty foods (Forss, 1972). Generally, grassy, herbaceous and pungent odors are closely related to straight and branched-chain aldehydes. Contrastingly, the unsaturated aldehydes are correlated to vegetal and fishy aromas. Ethyl ester of fatty acids and acetate of long chain alcohols play a major role in enhancing the aroma of miso products. The ethyl ester especially medium chain fatty acids provide a fruity essence. The amount of yeast and oxidative state of fermentation can influence the production of these volatile compounds since they are produced during the fermentation of alcohol of fish meat. As possesses low threshold value, the ester group is linked to sweet aroma in miso samples. They are mainly isoamyl acetate, 3-methylbutyl butanoate, ethyl pentanoate, ethyl hexanoate and 2-methylbutyl-2-methylbutanoate.

Besides ester, ketones as well play a major part in sensory of fish miso due to their low threshold value, particularly 2,3-butanedione (Giri et al. 2010). Meanwhile, Whistler and Daniel (1985) did mention about the capability of furan in providing aroma brunt to the fermented foods. Among 5 furans were identified in fish miso, 2-pentylfuran which have lower threshold value was counted as the main contributor in giving impact to the aroma of fish miso (Giri et al. 2010).

### **Lacto-fermented fish**

The amino acid and biogenic amines content in *feseekh* have been investigated by Rabie et al. (2009). The actual aim of the study was to investigate the changes in amino acid and biogenic amines production during ripening and storage. From the study, 21 amino acids have been identified in *feseekh*. The major free amino acids (68% of total concentration) detected after 60 days of storage were glutamic acid, leucine, lysine, valine, isoleucine, alanine, aspartic acid and citrulline. Meanwhile, 30% of total concentration of amino acids is represented by histidine, tyrosine, methionine, threonine, glycine, phenylalanine, proline and ornithine. The remaining of amino acids that showed very low concentration were included glutamine, serine, cystathionine, arginine and  $\gamma$ -amino butyric acid. These amino acids represent 2% of total concentration in *feseekh*. There are several amino acids that become the main precursors of biogenic amines and they are consisting of lysine, tyrosine and histidine.

Six biogenic amines have been discovered in *feseekh* and they were comprised of tyramine, putrescine, spermidine, cadaverine, histamine and spermine. Cadaverine represented 61% of total amount of biogenic amines and makes it become the major biogenic amine in *feseekh*. The second most abundant biogenic amine was putrescine (Rabie et al. 2009). Biogenic amines exhibit special characteristic in which they have capacity to determine the quality of foods (Halász et al. 1994). In the previous study, in order to detect the spoilage in raw materials such as fresh fish,

meat and vegetables, putrescine, histamine and cadaverine have been proposed as indicators (Rabie et al. 2009).

Riebroy et al. (2004) have reported the characteristics of *som-fug*, a Thai traditional fermented fish product. *Som-fug* has been analyzed for pH, salt content, protein, lipid, moisture, ash, organic acid and biogenic amines content. Table 5 shows the summary of the results. As shown in Table 5, lactic acid was the most significant organic acid in *som-fug* as it is the key determination of flavor. Saisithi et al. (1986) reported that the most acceptable amount of lactic acid for consumer is in the range of 2.4 – 2.5%. In addition, *som-fug* that contain of lactic acid within this range has the extraordinary sensory attribution (Østergaard et al. 1998). The salt content controls the viability of lactic acid bacteria and consequently the amount of lactic acid they produce (Awan et al. 2008). At the same time, acetic acid was also an important organic acid since it was detected in all samples of *som-fug* even at lower value. Hence, lactic as well as acetic acid are crucial for contributing highly seasoned nature and increase the saltiness, in some way by shielding other flavors (Visessanguan et al. 2001).

Table 5: Total acidity and organic acid in *som-fug* (Rieybroy et al. 2004)

Brands	Total	Organic acids (%)				
	acidity (% lactic acid)	Lactic acid	Acetic acid	Citric acid	Succinic acid	Pyruvic acid
A	2.00 ± 0.04 <sup>d</sup>	1.89 ± 0.03 <sup>d</sup>	0.10 ± 0.00 <sup>d</sup>	ND <sup>c</sup>	ND	ND
B	2.02 ± 0.02 <sup>d</sup>	1.75 ± 0.01 <sup>c</sup>	0.10 ± 0.00 <sup>d</sup>	0.01 ± 0.00	ND	ND
C	2.07 ± 0.06 <sup>d</sup>	2.03 ± 0.06 <sup>e</sup>	0.05 ± 0.00 <sup>b</sup>	0.01 ± 0.00	0.01 ± 0.00	ND
D	1.79 ± 0.04 <sup>c</sup>	1.70 ± 0.02 <sup>c</sup>	0.06 ± 0.00 <sup>b,c</sup>	ND	ND	ND
E	1.42 ± 0.03 <sup>b</sup>	1.21 ± 0.04 <sup>b</sup>	0.06 ± 0.00 <sup>b,c</sup>	ND	ND	0.02 ± 0.00
F	2.35 ± 0.03 <sup>f</sup>	2.17 ± 0.07 <sup>f</sup>	0.07 ± 0.00 <sup>c</sup>	ND	ND	ND
G	2.11 ± 0.08 <sup>e</sup>	2.15 ± 0.08 <sup>f</sup>	0.10 ± 0.00 <sup>d</sup>	ND	0.01 ± 0.00	ND

\*Different superscripts in the same column indicate significant differences ( $p < 0.05$ ).

<sup>b</sup>Means ± SD from six determinations.

<sup>c</sup> ND: not detectable.

Several biogenic amines have been detected in *som-fug*, including  $\beta$ -phenylethylamine, tryptamine, cadaverine, histamine, putrescine and tyramine. Putrescine and cadaverine showed the highest concentration in the samples. Koutsoumanis and others (1999) stated that both of these biogenic amines can be employed as spoilage indicator in fish. However, putrescine and cadaverine may also contribute to histamine poisoning by inhibiting the metabolizing enzymes of histamine (Halász et al. 1994; Shalaby, 1996). In the meantime, the histamine content was varied in seven samples of *som-fug*. The highest level of histamine was recorded in brand G (291 mg/kg). *Som-fug* samples contained tyramine that range from 19.3 to 225 mg/kg and this amount was still below toxic level. Shalaby (1996) indicated that the permissible maximum level of tyramine in

foods is 100 – 800 mg/kg. Therefore, foods that contain tyramine with a level of 1080 mg/kg are poisonous.

The biogenic amines content also was studied for *jeotkals*, the famous Korean fermented fish product [9]. *Jeotkals* is usually consumed by Korean people as side dish and also as ingredient in preparing kimchi (Korean traditional food). Eleven samples of *jeotkals* were investigated their biogenic amines content and *myeolchi-jeotkals* demonstrate the highest amount of biogenic amines particularly cadaverine (665 mg/kg). The result was summarized in Table 6. However, other biogenic amines such as histamine, tyramine and putrescine were also high which approaching 155 – 579 mg/kg, 63 – 224 mg/kg and 92 – 241 mg/kg respectively. However, spermine and cadaverine showed low content, less than 100 mg. According to Taylor (1986), the level of histamine should not exceed 50 mg/100 g for human health, otherwise it will be toxic. Putrescine and cadaverine possibly raise the histamine content in foods. Since the concentration of histamine in *myeolchi-jeotkals* was in the range of 155 to 579 mg/kg and the amount of cadaverine and putrescine were also high, it therefore concluded that *myeolchi-jeotkals* might be hazardous for consumption under certain circumstances at high temperature (Mah et al. 2002).

Table 6: Biogenic amine contents of *Jeotkals*, Korean fermented fish product (Mah et al., 2002)

<i>Jeotkals</i>	Amines <sup>b</sup>					
	Put	Cad	His	Tyr	Spd	Spm
<i>Ojingeo-jeot</i>	ND-30	ND	ND	ND	ND	ND
<i>Myeolchi-jeot</i>	92-241	ND-665	155-579	63-244	ND-43	ND-77
<i>Changran-jeot</i>	ND-20	ND	ND	ND	ND	ND-51
<i>Myeongran-jeot</i>	15-136	ND-85	ND	22-171	ND	26-58
<i>Saeu-jeot</i>	ND	ND	ND	ND	ND	33-62
<i>Toha-jeot</i>	ND-30	ND	ND	ND	ND	ND-26
<i>Jogae-jeot</i>	ND	ND	ND	ND	ND	44-61
<i>Baendaengi-jeot</i>	ND22	ND	ND-11	ND	ND	ND-18
<i>Eorigul-jeot</i>	ND-20	ND-29	ND	ND-31	ND-12	ND-42
<i>Kkolttugi-jeot</i>	ND-32	ND	ND	ND	ND	ND
<i>Agami-jeot</i>	ND-46	ND	ND	ND-14	ND	ND-43

<sup>b</sup>Put, putrescine; Cad, cadaverine; His, histamine; Tyr, tyramine; Spd, spermidine; Spm, spermine. ND, not detected. *Ojingeo-jeot* (sliced squid), *Myeolchi-jeot* (anchovy), *Changran-jeot* (Pollock entrails), *Myeongran-jeot* (pollock roe), *Saeu-jeot* (shrimp), *Toha-jeot* (toha shrimp), *Jogae-jeot* (clam), *Baendaengi-jeot* (big eyed herring), *Eorigul-jeot* (oyster), *Kkolttugi-jeot* (small squid) and *Agami-jeot* (pacific cod gills).

## 7.0 CONCLUSION

The method of manufacturing and the fish species used for fermented fish products are differ from country to country. This is depending on the culture and temperature/weather of the country. However, in general, all fermented fish products are produced by mixing fishes with salt, and undergo fermentation for certain period, depending on the types of the products. For fermented fish sauce, the fermentation process takes a longer time which can reach up to 18 months. On the other hand, fermented fish paste and lacto-fermented fish are fermented for about 2 months, but in some countries, the fermentation period can be only 5 - 12 days or shorter.

In term of food safety, fermented fish products have a low risk of foodborne diseases since fermentation process could control the growth of spoilage microorganisms in the products. Thus, fermented fish products have made an important contribution to food industry, particularly in developing countries, where the food safety becomes a crucial issue due to the economic problem.

Another attraction of fermented fish product is their special characteristic of taste and aroma, which make them popular as condiment and side dishes. At present, a wide variety of fermented fish products exist in the world and the demand for these products is very great and kept increasing. Moreover, with the low-cost production, the business in fermented fish products becomes a very profitable business and has a huge potential in food industry.

Today, health benefit is the top consideration in choosing food by consumers around the world. Healthier food option becomes a trend which contributes to the rise of demand for the good qualities and healthy foods. As a good source of protein, amino acids and vitamins, fermented fish products can be considered as healthy foods and they play a significant role in the human diet.

Fermented fish products contain a wide range of metabolites which may contribute a lot of benefits to human. Almost all the fermented fish products consist of amino acids, biogenic amines, organic acids and volatile compounds. Each of these compounds has its special own functions. For instance, amino acids are excellent contributors to sensory and antioxidative activity. As biogenic amines could confer positive effect on human body, organic acids and volatiles compounds may enhance the aroma and taste of fermented fish products. Unfortunately, some metabolites also could give negative effects such as histamine and N-nitrosoamine if they are consumed more than permitted level. Further investigation of increasing fermented fish products production by using modern and more hygienic technique should be carried out in the future. In addition, the study that includes the complete profiling of all metabolites present in the fermented fish products and their characterizations also should be conducted. These researches will provide thorough information which can be used as an important reference in improving the quality of fermented fish products.

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