Chapter

Fatty Acid Compositions in Fermented Fish Products

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Abstract

Fish fermentation differs from one region to another in the world. Different types of fish, different fermentation conditions, and different fermentation processes are used, thus resulting in different fermented fish products. The most investigated fermented fish products in regard to the fatty acid contents are Kejeik from Sudan, Fseekh from Egypt, Hatahata-zushi from Japan, and Tareeh and Mehiawah from the Middle East. The results of those studies were not consistent regarding the effect of the fermentation process on the contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Some of those studies reported an increase in the level of SFAs and a decrease in the PUFAs contents, while other studies reported the opposite. The fermentation process itself was attributed to different microorganisms such as lactic acid bacteria (LAB), halophilic bacteria, the bacterial flora of *Micrococcus* and *Bacillus* species, and a new bacillus strain named *Bacillus mojavensis-ASK*. Autolytic enzymes from the fish were also reported to be responsible for the fermentation process.

Keywords: fatty acids, fish, fermentation, PUFAs

1. Introduction

Lipids are considered as one major group of the naturally occurring organic molecules, along with carbohydrates, proteins, and nucleic acids [1]. Lipids are characterized according to their solubility (physical property) rather than their structure, in which they are insoluble in water, but soluble in nonpolar organic solvents, such as chloroform and benzene [1, 2]. All lipids are composed of carbon, hydrogen, and oxygen atoms; however, some lipids contain phosphorus, sulfur, nitrogen, or other elements [3]. Lipids are divided into three classes, (a) triacylglycerol's (TGs), which are used as long-term energy stores such as fats and oils; (b) phospholipids (PLs), which function primarily in cell membranes; and (c) steroids, like cholesterol which is a component of animal cell membranes and a precursor in the synthesis of various steroid hormones [1, 3]. Lipids play a structural role in the cell membranes in combination with proteins to give the membranes their semipermeable property [1]. In addition, lipids give the membranes their shape and protect them from the external environment [3].

1.1 Fatty acids (FAs)

Fatty acids are the building blocks for the majority of lipids especially TGs and PLs [3, 4]. FAs are composed of a long hydrocarbon chain (nonpolar) that is

conjugated to a carboxyl group (polar) which is an acidic functional group [5]. FAs hydrocarbon chains range in length from 2 to 80 but commonly from 12 up to 24. Chain length from 2 to 6 are called short-chain, from 6 to 10 are called medium-chain, and 12 up to 24 are called long-chain [6]. FAs containing 16 and 18 carbons are the most prevalent. The majority of FAs have an even number of carbon atoms because they are synthesized by combining the C_2 units of acetyl CoA [7, 8]. FAs are usually synthesized by the enzyme fatty acid synthase that is responsible to convert acetyl CoA into fatty acid [5]. The hydrocarbon chains of FAs are usually unbranched and can be divided into saturated and unsaturated [9]. Saturated fatty acids (SFAs) have no double bond in their hydrocarbon chain, while unsaturated fatty acids (UFAs) have one or more double bonds in their chain. These double bonds cause the formation of bents or "kinks" in the fatty acid chains making them liquid at room temperature [3].

UFAs are divided into monounsaturated fatty acids (MUFAs) which have one double bond in their chain and polyunsaturated fatty acids (PUFAs) which have two or more double bonds [10]. The double bonds locations follow a unique pattern; the MUFAs usually have the double bond between carbons 9 and 10 (Δ^9) where C1 is the carboxyl carbon. The second double bond in PUFAs is mostly between carbons 12 and 13 (Δ^{12}). PUFAs do not normally have conjugated double bonds (—CH═CH—CH═CH—), instead their double bonds are usually separated by at least one methylene group (—CH═CH—CH2—CH═CH—) [7–10]. The stereochemistry of the double bond in the naturally occurring UFAs is usually *cis*, i.e., the two hydrogens on the carbon atoms of the double bond are on the same side of the molecule [9, 10]. *Trans* FAs are formed during the hydrogenation process of UFAs to produce SFAs [2]. In the *trans*isomers, the two hydrogens on the carbon atoms of the double bond are on the opposite sides of the molecule [10].

1.2 Nomenclature of fatty acids

Fatty acids can be named using (a) systematic naming addressed in detail by the International Union of Pure and Applied Chemists and the International Union of Biochemistry and Molecular Biology (IUPAC-IUBMB) and (b) common naming [4]. The systematic naming of FAs ends with the suffix "oic acid" on to the name of the parent hydrocarbon. However, the ionized form of the fatty acid at physiological pH ends with the suffix "ate" rather than "oic acid." FAs are named according to the total number of carbon atoms, in addition to the number and position of double bonds, if present. The carbon atoms in FAs are numbered from the carboxylic acid residue (C1), and the position of the double bond is described by the symbol delta (Δ) followed by the number of the first carbon involved in the bond. For example, the full systematic name for palmitoleic acid (common name) is *cis*-Δ^9hexadecenoic acid, and it is written as 16:1(Δ^9) in which it consists of 16 carbons in its hydrocarbon chain with a double bond positioned between carbons 9 and 10 [10].

The common names for FAs reflect their prominent food sources such as palmitic acid (C16:0) found in palm oil and *arachidonic* acid (*cis*5, *cis*8, *cis*11, *cis*14–20:4; from *arachis*, meaning legume or peanut) found in peanut butter [11]. The common names are much simpler than the systematic names; however, they do not give any information about the structure of the fatty acids.

Omega (ω, n) system is an alternative system of naming fatty acids. In this system, carbon atoms are numbered relative to the methyl end of the molecule. Omega system can be distinguished from the IUPAC naming system in the bases of the following: (a) omega nomenclature is only applied to unsaturated fatty acids, (b) omega system does not identify whether the double bond have *cis* or *trans*

configuration, and (c) omega system does not identify the location of other double bonds in the molecule. For example, linoleic acid C18:2n-6($\Delta^{9,12}$) can be written as ω-6 fatty acid in omega system [11].

1.3 Essential fatty acids (EFAs)

Fatty acids that the body needs but cannot synthesize in sufficient amounts to meet physiological needs due to the absence of the required enzymes are called essential fatty acids. The body cannot synthesize two polyunsaturated fatty acids: linoleic acid (C18:2n6, LA) and alpha-linolenic acid (C18:3n-3, ALA); therefore, they must be supplied by the diet [12]. Animal cells are unable to introduce double bonds in the n-3 and n-6 positions because they are deficient in certain required desaturase enzymes. However, these cells have the ability to introduce double bonds into all other positions in fatty acid hydrocarbon chain [13]. Dietary EFAs are used to produce long-chain polyunsaturated fatty acids [14].

1.3.1 Omega-3 and omega-6 fatty acids

Although omega-6 FAs were the first to be described as an essential fatty acids in the 1920s, omega-3 FAs take more attention than omega-6 [13]. Linolenic acid (ω-3) is the parent of the omega-3 FA family in which it can be used to produce other members of the family (**Figure 1**) [12, 15].

Alpha-linolenic acid is the precursor for linolenic acid that is used to synthesize two active biological components: eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) [12, 14, 16]. ALA is found in plant oils, nuts, and seeds such as flaxseeds, walnuts, soybeans [12].

Aquatic ecosystems are the principal sources of DHA and EPA in the biosphere provided by the fish and fish oils in human diet [12, 16]. On the other hand, linoleic acid can be used to produce other members of the omega-6 family like arachidonic acid (C20:4n-6, AA) that acts as a starting material for a number of eicosanoids, i.e., biologically active molecules that regulate body functions [12].

Omega-6 FAs are found in seeds, nuts, and vegetable oils of corn, sesame, and sunflower [12]. Before the industrialization, the ratio of omega-6 to omega-3 (n-6/n-3) was around 1:1 to 2:1 due the abundant consumption of vegetables and seafood high in omega-3 fatty acids. However, there is a gradual change in this ratio with the industrialization, mainly due to the consumption of refined oils and seeds with a high content of omega-6 fatty acids in which the (n-6/n-3) average ratio has been around 10:1 to 20:1 [17].

This imbalanced n-6/n-3 ratio can even be worsened by the overnutrition habit associated with the Western diet around the world. Overnutrition, associated with an increased amount of FAs made available for oxidation in the liver, favors the pro-inflammatory state due to the depletion of n-3 long-chain PUFA (n-3 LCPUFA) such as EPA and DHA, elevated n-6/n-3 ratio, hyperinsulinemia, and insulin resistance (IR). Such changes may result in the development of nonalcoholic fatty liver disease (NAFLD), steatosis [hepatocyte triacylglycerol accumulation, and cirrhosis (steatohepatitis)] [18].

1.3.2 The role of omega-3 and omega-6 fatty acids

The consumption of omega-3, omega-6 PUFAs, and their derivatives has various beneficial effects, ranging from fetal development to cancer prevention [19]. PUFAs have a preventive effect against arterial hypertension, asthma, inflammatory diseases, human breast cancer, and disorders of the immune system [20].

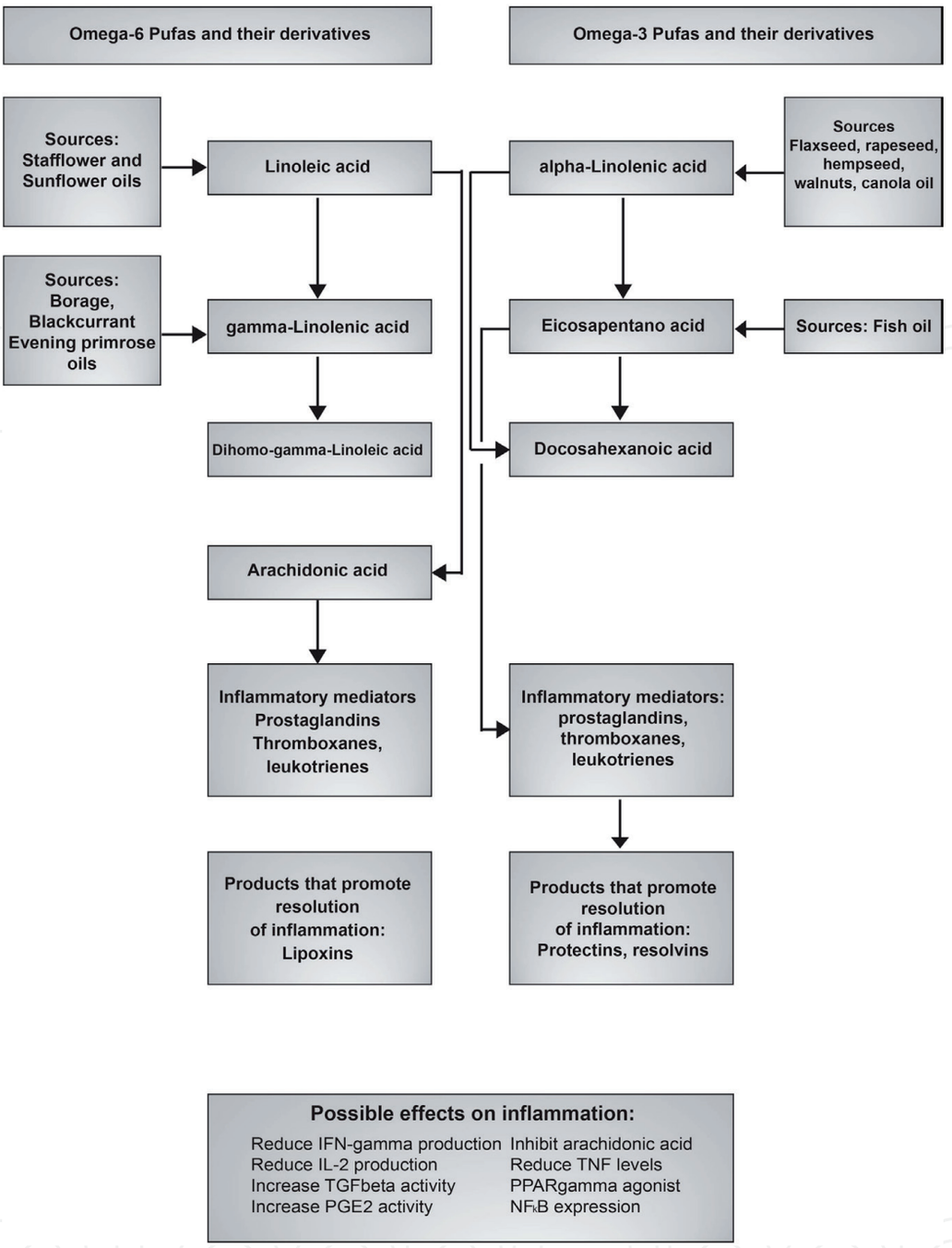

Figure 1.
Omega-6 and omega-3 PUFAs and their respective sources and metabolic derivatives [15].

n-3 FAs protect against several types of cardiovascular diseases such as myocardial infarction, atherosclerosis, arrhythmia, hypertension, and human coronary artery disease [19, 21]. Moreover, consumption of n-3 FAs interferes with prostaglandin metabolism, which can reduce the platelet aggregation and adhesion to blood vessels, which reduce the blood pressure [22]. Omega-3 PUFAs can reduce the incidence of high cholesterol level in the blood, psoriasis, cancer, and arthritis [23].

The n-3 LCPUFA DHA plays a major role in the development of the nervous system during the life of fetus and neonates [24]. The study of Barrera et al. (2018) has shown that a low dietary intake of n-3 LCPUFA in pregnant Chilean women has resulted in a significant decline in their erythrocytes and breast milk DHA levels. Therefore, the improvement of the quality of FAs intake specifically DHA was recommended during pregnancy and lactation periods in order to supply adequate amount of DHA to embryos and neonates [25]. In addition, n-3 supplements intake

was suggested to improve the low level of n-3 LCPUFA associated with NAFLD patients in order to lower their n-6/n-3 ratio and thus reduce the inflammatory status as a treatment for the nutritional hepatic steatosis in adults [24].

Omega-6 PUFAs are converted to other important compounds through various enzymatic reactions to the key intermediate arachidonic acid, which is subsequently converted to eicosanoids that act somewhat like hormones such as prostaglandins, thromboxanes, and leukotrienes [15]. Eicosanoids play an important role in muscle relaxation and contraction, blood clot formation, blood vessel contraction and dilution, blood lipid regulation, and immune response to injury and infections [12].

Omega-3 PUFAs are considered as more potent anti-inflammatory agents than n-6 PUFAs, but the effects of n-6 PUFAs are more dominant due to the abundance of these compounds in the diet [15].

1.3.3 EPA and DHA

Alpha-linolenic acid can be used in the body as a precursor to produce EPA; however, EPA can be directly obtained through the consumption of fish oils. Moreover, EPA can be converted to DHA and also to eicosanoids that are essential for inflammatory signaling, such as prostaglandins, thromboxanes, and leukotrienes. DHA can also be converted to eicosanoids to produce anti-inflammatory mediators such as protectins and resolvins [15]. In 2004, The International Society for the Study of Fatty Acids and Lipids (ISSFAL) has recommended the minimum intake of 0.5 g/day of EPA and DHA for the prevention of cardiovascular disease [26].

1.4 Metabolism of long-chain PUFAs

ALA and LA obtained from diet can be converted into longer chains of PUFAs by the help of two important enzymes, desaturase and elongase, that work to increase the degree of unsaturation. Elongase works by adding carbon atoms into the chain, while desaturase works to introduce double bonds by removing hydrogen atoms [27]. The n-3 and n-6 FA families compete especially at the rate-limiting Δ6 desaturase enzyme that both pathways start with. Usually desaturase enzymes display highest affinity to ALA (n-3 family) more than LA (n-6 family) [28]. The n-3 pathway starts with ALA (C18:3n-3) and ends with DHA (C22:6n-3), while the n-6 pathway starts with LA (C18:2n-6) and ends usually with AA (C20:4n-6) [27, 29].

The major difference between n-3 and n-6 pathways is that n-6 pathway usually does not proceed beyond AA; however, the n-3 pathway involves more complex steps. In the n-3 pathway, there are two elongation steps after the formation of EPA (C20:5n-3), leading to the formation of tetracosapentaenoic acid (C24:5n-3), followed by the reduction by Δ6 desaturase enzyme to produce tetracosahexaenoic acid (Nisinic acid) (C24:6n-3). The final step in n-3 pathway yields DHA (22:6n-3) by the retroconversion of (24:6n-3) to (22:6n-3) which involves peroxisomal ß-oxidation step (**Figure 2**) [27, 30]. In the mammalian cells, the FAs from (n-3) and (n-6) families cannot be interconverted because of lack of Δ12 or Δ15 desaturase enzymes, while the interconversion step takes place in plants [28].

1.5 Sources of omega-3 PUFAs

Fish is a major source of animal protein diet in many countries that is easily digestible than red meat because fish flesh contains long muscle fibers [18]. Consumption of fish have many benefits for human health due to its high content of essential n-3 PUFAs, namely, EPA and DHA [14, 31, 32].The FAs content of fish varies according to diet, i.e., availability of planktons [33], environmental conditions

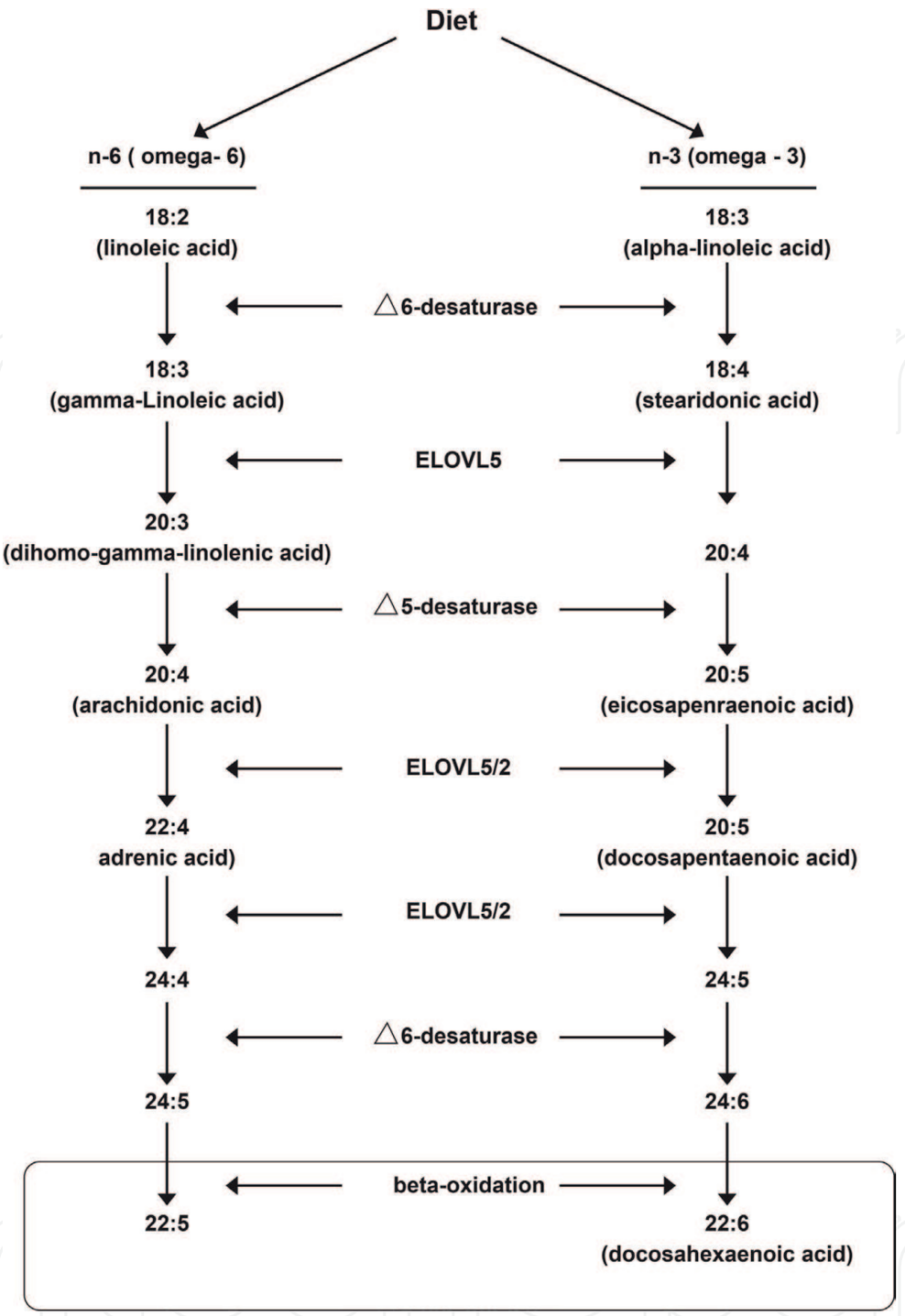

Figure 2.
The elongation-desaturation pathway for the metabolism of n-6 and n-3 polyunsaturated fatty acids [30].

[15], and seasonal variation [34]. A regular consumption of fatty fish prevents cardiovascular disease and neural disorders [35].

PUFAs are important for brain and retina development since studies with animals have shown that deficiencies in n-3 FAs decrease the concentration of DHA in the brain and retina tissues [16]. Fish and terrestrial animals are not able to synthesize n-3 or n-6 PUFAs. The primary producers for PUFAs are marine photosynthetic organisms including phytoplankton, macroalgae, and seaweeds [36].

Only some microalgae species are effective producers for EPA and DHA; therefore, aquatic ecosystems are the principal sources of these two essential PUFAs in the biosphere, in which humans obtain these FAs through the consumption of fish and other marine products (**Figure 2**) [30, 37]. Fish need PUFAs to tolerate low

water temperature; thus, low amounts are expected in wormer water like tropical waters [38]. Fish oil become very popular and plays an important role in the prevention of cardiovascular disease and some type of cancers like breast, colon, and prostate [39]. It was observed that there are lower incidence of cardiovascular diseases, hypertension, and autoimmune disorders in populations that consume diet rich in marine fish like Eskimos and Japanese [40].

2. Fish fermentation in relation to fatty acids

Fresh fish is prone to spoilage which is caused by both microbiological and chemical reactions [41]. Lipid deterioration limits the shelf life of oily fish during storage [42]. Lipid hydrolysis induced by lipases and phospholipases produce free FAs that are susceptible to further oxidation to produce low molecular weight compounds responsible for the rancid of fish products [43]. Fish quality may decline during processing and storage mainly due to the oxidation of the PUFAs which is related to the production of unpleasant flavors and odors [44, 45].EPA and DHA are especially susceptible to oxidation during heating or other culinary treatments [46].

A long time ago, people used to preserve food either by sun drying or salting methods [47]. Salting of fish is an old-age technology that is still in use nowadays even in developing countries due to its simplicity and low cost [47, 48]. Salting fatty fish involves a certain degree of fermentation which is brought by autolytic enzymes from the fish and microorganisms in the presence of high concentrations of salt [47]. Fish fermentation is the transformation of organic substances into simpler compounds like peptides and amino acids by the action of endogenous enzymes or microorganisms, normally in the presence of salt [48–50].

Lactic acid bacteria (LAB) are used to ferment fish along with other food materials like dairy, meat, vegetables, and beverage products [51] resulting in shelf life extension and the addition of new aromas and consistencies [32]. Fermentation by lactic acid bacteria preserves food by the production of lactic acid and other organic acids, which help to reduce pH of the food and inhibit the growth of pathogenic and spoilage organisms [52].

2.1 Fish fermentation all over the world

2.1.1 Asian fermented fish products

Many types of fish sauce and paste are famous in Japan, Southeast Asia, and India. Traditional fermented Japanese seafood is Hatahata-zushi which is processed with boiled rice. Hatahata-zushi is prepared by soaking the sandfish (*Arctoscopus japonicas*) in water, salt, and rice vinegar, which is then fermented with boiled rice and some vegetables [53]. Bagoong is a fish paste produced in the Philippines through the neutral fermentation process of whole fish or shrimp in the presence of 20–25% salt, while Patis is a Philippine yellowish fish sauce prepared from sardines, anchovies, and shrimps [33, 50]. “Suanyu” is a Chinese low-salt fermented whole fish snack which is prepared from fresh fish mixed with cooked carbohydrates, salt, and spices [54]. Lona ilish is a salt fermented fish from India which has strong aroma mixed with some sweet, fruity, and acidic flavors with some saltiness [47].

2.1.2 European fermented fish products

Scandinavia is the main producer of fermented fish products in Europe. Surstromming is produced in Sweden and rakefish in Norway. These fermented fish

are made by immersing whole herring and trout in brine (salty water) for 1–2 days, eviscerating, and retaining the roe or milt in barrels with fresh brine. The final fermented product is packed in cans and usually consumed on special occasions [49]. "Tidbits" is another Scandinavian product that is canned with vinegar, sugar, and spices after maturation. In France, the fish *Engraulis encrasicholus*is is salted to prepare anchovies, while in southern France, a fish sauce called "Pissala" is prepared from small fish of the *Engraulis* sp., *Aphya* sp., and *Gobius* sp. [49].

2.1.3 Middle East, African, and Asian fermented fish products

Many types of fermented fish are prepared in the Middle East. "Fseekh" is a famous fermented fish prepared in Egypt and Sudan from different types of fish [49]. Kejeik is a fermented dried fish produce that is common in Sudan and central Africa which is powdered and thickened with okra after boiling [48]. The FA content of Bouri fish varies with fish size; in which it was much greater in small size than large size fish because of the higher activity of lipolytic enzymes in small fish as stated by the authors [48]. However, the fresh and fermented product Fseekh of both fish sizes had the same FA profile. The high salt content was found to have no effect on those enzymes responsible for the liberation of free fatty acids from the lipids [48]. The ratio of UFAs/SFAs decreased as the amount of UFAs decreased significantly after the salting and fermenting process. Moreover, all SFAs except palmitic acid (C16:0) increased significantly. The major SFA was C16:0, and the major UFAs were palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9). Appreciable amounts of PUFAs such as C18:2n-6, C18:3n-3, stearidonic acid (C18:4n-3, SDA), C20:5n-3, docosapentaenoic acid (C22:5n-3, DPA), and C22:6n-3 were also present. The presence of the odd-chain pentadecylic acid (C15:0) and heptadecanoic acid (C17:0) FAs is considered a unique characteristic of the Bouri fish oil [48].

The analysis of fatty acid compositions was done for different fermented seafoods all over the world. For example, the analysis of Hatahata-zushi which is a Japanese fermented fish product of sandfish has shown no change in the fatty acid compositions throughout the fermentation process; however, the content of SFAs and MUFAs increased, while the content of PUFAs decreased markedly during the process of fermentation. The highest concentrations of the FAs in "Hatahata-zushi" were C18:1n-9, C16:0, C22:6n-3, C20:5n-3, and C16:1n-7, respectively [53].

It has been noticed that bacterial enzymes play an important role in fish fermentation in which the aroma in fermented fish products is claimed to be derived from the activity of halophilic bacteria [47]. The enzymes that participate in the production of PUFAs are thought to be either fish tissue enzymes or microbial enzymes [47]. The traditional fermented fish product Lona ilish of Northeast India has shown that salting plays an important role in the fermentation and preservation of food. Salt preserves the fermented products through the reduction of water activity (aw) of the system, thus rendering a condition less suitable (low moisture) for the microbial growth. Generally, food pathogenic bacteria are inhibited when the water activity is 0.92 or less which is equivalent to NaCl concentration of 13% (w/v). It was reported that halophilic bacteria were responsible for the fermentation process of fish product Lona ilish since these bacteria can tolerate high salt conditions. The bacterial flora of *Micrococcus* and *Bacillus* species were reported to be involved in the processing of'Lona ilish [47]. Other microorganisms are found in the spontaneously fermented Chinese fish product Suanyu such as lactic acid bacteria, *Staphylococcus*, and yeast [54].

The two main products of fish fermentation in the Arabian Gulf region are fish paste and sauce. It is difficult to classify these products due to the lack of standardization throughout the world. Generally, fish paste is a thick product

with whole fermented fish, while fish sauce is a thinner product with additives such as spices and cereals. The color of fish paste or sauce is usually brown resembling soy sauce. Fish paste is more nutritious than fish sauce [49]. Tareeh is a salt fermented fish paste in the Arabian Gulf countries prepared from a high-fat fish *Sardinella* spp. [33, 49]. The famous fish sauce in the Arabian Gulf Countries is called Mehiawah that is prepared by the addition of spices to the fermented fish Tareeh [49, 55].

The fatty acid contents of the fish product Tareeh and Mehiawah of white sardinella (Oom) were recently investigated [56, 57]. The study of Freije et al. (2018) was conducted in order to determine the effect of fermentation under high salting conditions (20%) for 8 weeks on weekly basis on the fatty acid compositions of white sardinella (Sardinella albella) [57]. The fatty acid compositions of Tareeh have shown a great deal of variation as the fermentation process proceeded. The concentrations of SFAs and MUFAs significantly declined in Tareeh samples compared to raw fish, whereas the concentrations of UFAs and PUFAs were significantly increased starting from week 4 of fermentation. The amount of n-6 FAs as well as n-3 FAs increased during the fermentation process, and the enzymatic activities of the elongases and desaturases were found to have higher affinity to n-3 FAs than n-6 FAs. The ratio of n-6/n-3 was decreased after 8 weeks of fermentation, while the proportions of EPA + DHA were increased. This unique fermentation process might have a great application in the food industry [56]. It was also concluded that the elongation and desaturation process was carried out by single new bacterial strain from the *Bacillus* species named *Bacillus mojavensis-ASK* [58, 59].

3. Conclusion

The studies that investigated fatty acid compositions in fermented fish products all over the world are limited. Each of those studies has given different results without any common consistency among them. Some of those studies have reported a decrease in UFAs and an increase in SFAs, while others reported increased SFAs and MUFAs but decreased PUFAs during fermentations. On the contrary, the most recent studies have reported declined levels of SFAs and MUFAs and a rapid increase in PUFAs. Such contradicted results can be attributed to the difference in the type of fermented fish, fermentation conditions, and the addition of different additives. Therefore, this process requires thorough investigations in order to reveal the mystery behind such contradictions.

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