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REVIEW

Biochemical and Microbiological Safety Risks in Salted Fish Products: A Review

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ABSTRACT

Salting is one of the most widely used fish processing methods, although fish prepared in such a way is exposed to many biochemical changes. There is still insufficient information on the mechanisms of reactions that occur during salting, so the process is still conducted on an experimental basis. Sodium chloride (NaCl) reduces water activity values and consequently has an antimicrobial effect on salted fish products. The main changes that happen during this process are: protein loss, lipid oxidation, enzymatic activity, and growth of halophilic bacteria. In this paper, an extensive literature review has been made on the topic of previously listed changes that occur during fish salting, and it is expected that it will broaden the picture of biochemical changes during fish salting with the aim of developing a safer and more effective procedure concerning the health of the final consumer. *Staphylococcus aureus*, officially classified as a high-priority bacterial pathogen, is very common in salted fish all around the world and should be taken into account, concerning the fact that it causes one million deaths annually and that it is also Methicillin-resistant (MRSA). Therefore, microbiological analysis of fish and salt regarding *S. aureus* should be recommended as obligatory in fish salting procedures by relevant authorities. Malondialdehyde (MDA) is a very reactive product of lipid oxidation, and it is very

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harmful because the newest researches suggest its engagement in various disease development and therefore should also be determined in all salted fish products.

Keywords: Fish Salting; Enzyme Activity; Halophilic Microorganisms; Lipid Oxidation; Protein Degradation; Ripening; Water Activity; Human Health

1. Introduction

Salting is one of the oldest and most used methods all around the world because it is cheap and very simple [1–4]. Preservation by salting is based upon the principle of osmosis. When food is salted (usually with sodium chloride), water diffuses out of microorganisms into the surrounding environment, towards the higher salt and lower water concentration. This flow of water, called osmosis, leaves the microorganisms dehydrated, and consequently, they die [5]. The inhibitory effects of salt are independent of pH [6]. Salt (sodium chloride, NaCl) has been used as a food preservative since ancient times. In 250 BC, camel caravans were used to transport various foods and items, including salted fish, between Egypt, Gaza, Galilee, and Sidon [7]. The early applications of salt were for the purpose of conserving meat and fish. This usage is based on the previously described fact that at high concentrations, salt causes a drying effect on both food and microorganisms. The greater the amount of salt, the better the preservation effect [6].

The initial phase of fish salting ends when equilibrium between salt concentration in tissue and surrounding brine is achieved [8]. There are several methods of salting used in the fish industry worldwide: dry salting, dry-wet salting, and brine salting. In dry salting, salt is used directly on the surface of fish, and the fish loses excessive amounts of water. This type of salting is frequently used for fish with high water content. In dry-wet salting, salt and brine are simultaneous in the procedure. In brine salting, fish are immersed directly in brine for a certain period of time. New brine must be prepared every day for the last-mentioned type of salting. In addition, small fish can be salted without evisceration [6,9,10]. In recent years, a lot of progress has been made in research on protein degradation during fish salting, especially regarding the impact of certain proteases [8,11,12]. The role of ions in salt has also been studied [3,6], but halophilic microorganisms and the crucial problem of lipid oxidation have seldom been studied, notwithstanding the fact that they may pose a variety of health and safety risks [13–16]. In addition, it is hard to find scientific studies about fish salting processes that document the complexity through all the biochemical aspects [17]. In view of the above, the aim of this paper is to clarify processes that occur during the salting of fish and their possible influence on human health.

2. Methodology

2.1. Literature Search Strategy

A comprehensive literature search was conducted across four academic databases: Taylor and Francis Online, Science Direct, Wiley Online Library, and PubMed. The search covered publications from January 1995 to May 2025 were limited to peer-reviewed articles in English.

Keywords and MeSH terms such as “lipid oxidation”, “fish salting”, “enzyme activity”, “water activity”, “enzymes”, “proteases”, “endopeptidases”, “exopeptidases”, “halophilic microorganisms”, “*Staphylococcus aureus*”, “salted fish”, “Maillard reactions”, and “food” were combined using the Boolean operator AND to refine the results. The exact search strings for each database are listed in **Appendix A**.

Studies were included if they met the following requirements:

- Studies addressing fish salting and food safety risks related to fish salting (mainly on *Staphylococcus aureus*).
- Studies focused on lipid oxidation, protein degradation, and halophilic microorganisms in salted fish products.

Studies were excluded if they met any of the following conditions:

- Conference abstracts
- Non-English language publications.
- Studies with failure to meet the scope of the review.

2.2. Study Selection Process

The study selection process was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [18].

A total of 18,300 records were identified throughout the above mentioned four database searches.

The selection process consists of two stages: first, screening titles and abstracts for eligibility, and second, reviewing full texts to assess relevance and extract key information.

Remaining 71 studies met the inclusion criteria and were included in this review.

All screening and eligibility assessments were performed by the author. The overall process of study identification, screening, eligibility assessment, and inclusion is illustrated in the PRISMA flow diagram (**Figure 1**) [18].

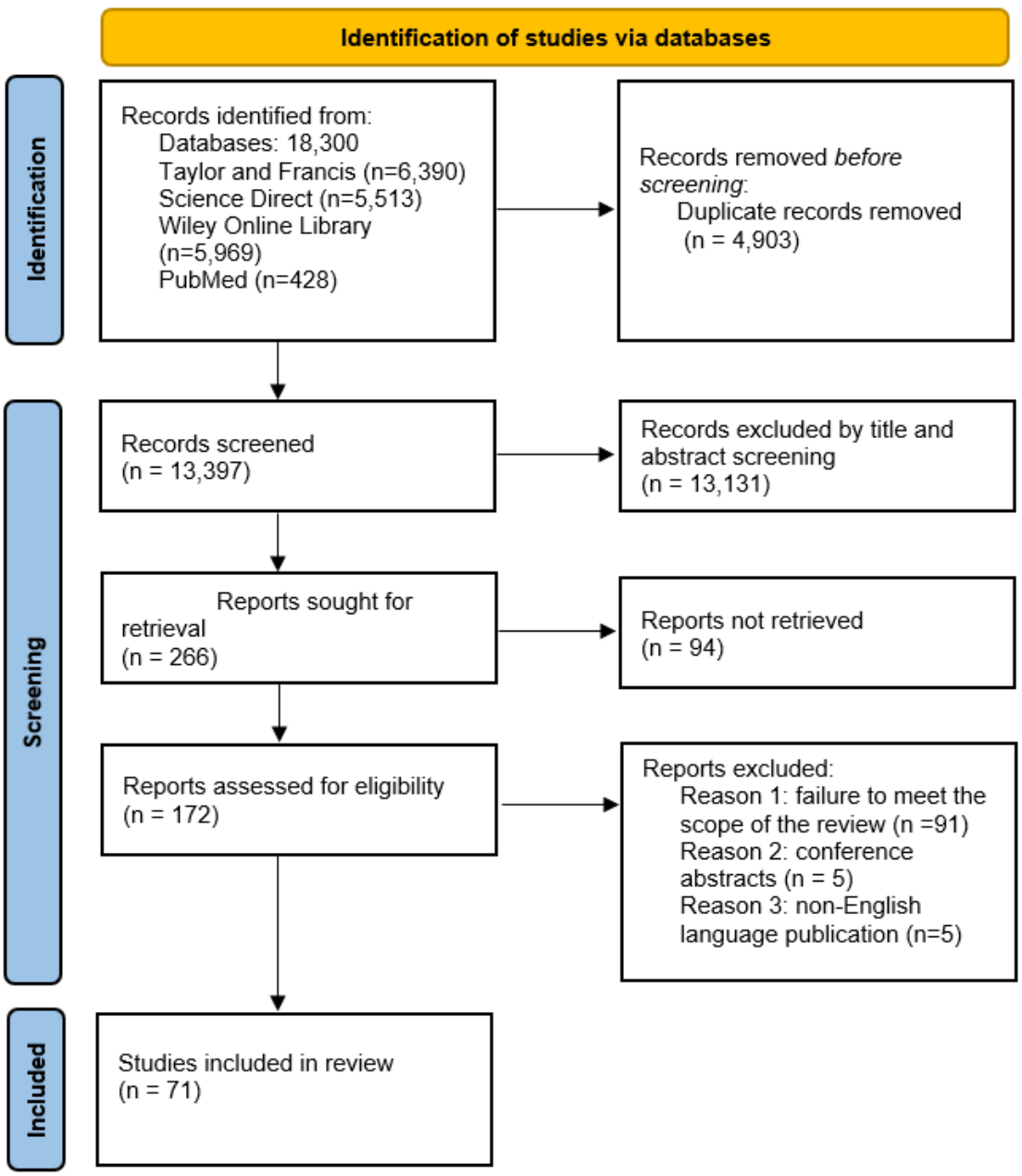

Figure 1. The PRISMA flow diagram.

Exact search strings used for each database (including Boolean operators) are provided to ensure full transparency and replicability of the used methodology (See **Appendix A**).

3. Water Activity

In different food systems, total water can be present as bound water and free water. Free water intensifies microbial growth of various types of microorganisms. Bound water inhibits microbial growth. Water activity (a_w) represents the amount of water that is available to microorganisms for their activity [17]. The aw of food can be formulated by the ratio of water vapour pressure on the surface of food in comparison to that of pure water. The a_w of food ranges from 0.1 to 0.99, and it can be reduced by removing water (desorption). Balance is directed towards the mould growth rather than the pathogen growth [17]. The a_w value of fish ranges from 0.98 to 0.99, while the a_w of salted fish ranges from 0.93 to 0.98 [19,20]. The stability of salted fish product is highly dependent on water activity (**Figure 2**) [21]. Each group of microorganisms has an optimal value of water activity for its growth. Most moulds are best suited for a water activity of about 0.8, and the lowest activity for moulds is about 0.6.

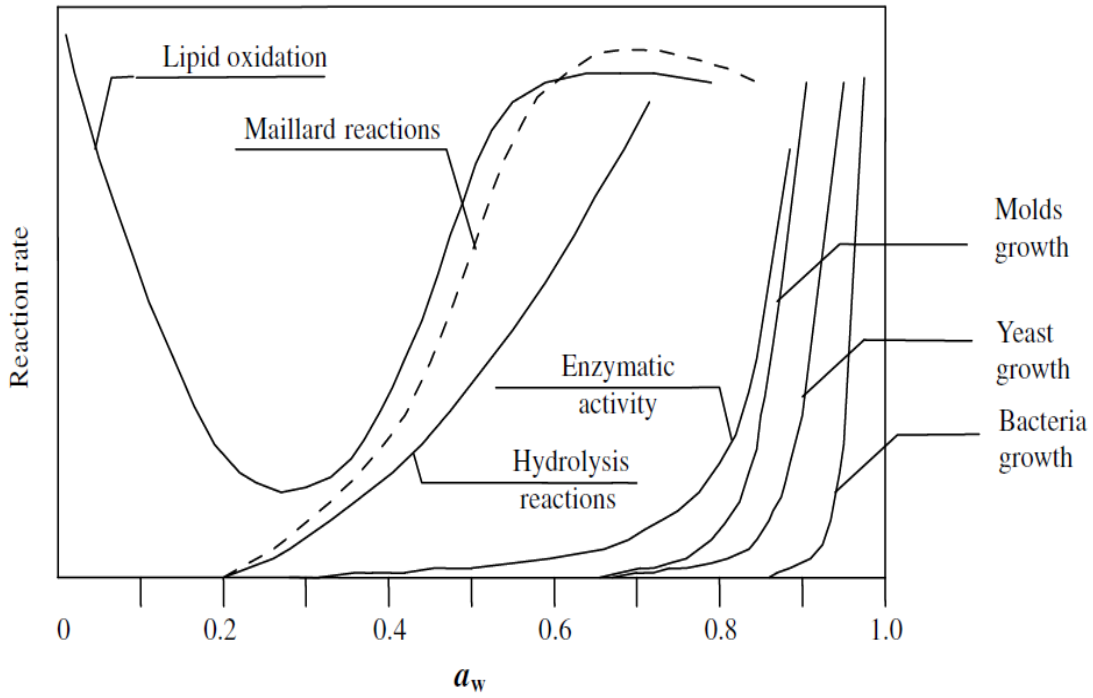

Figure 2. Influence of a product's water activity on types of reactions.

The most corresponding water activity for the majority of Gram-positive bacteria is 0.9, and for the Gram-negative bacteria, 0.93. *Staphylococcus aureus* can also grow in conditions where water activity is very low, down to 0.85. If the value of water activity significantly decreases, the growth ability of microorganisms in the beginning rapidly decreases and then the decrease slows down [17]. If fish are excessively wet salted, the growth of yeasts will be rapid, and in a dry way of salting, spoilage will be caused by moulds (**Figure 2**) [21]. Moulds of genera *Aspergillus* are well known for forming mycotoxins, which is a big health threat connected to salted fish. Therefore, propionic acid is recommended as a preservative during fish salting [21,22].

4. Salt Properties

Salt composition is one of the most important factors in fish salting. Purified salt is almost pure sodium chloride [3,6]. Huge concentrations of calcium and magnesium salts may cause solidification of cell walls and reduce diffusion of salt into fish tissue, which makes spoilage probable [3]. During fish salting, it is of utmost importance to have a

high-grade salt, as well as having good quality fish. Before use, salt is to be analysed in a laboratory because iron, copper and magnesium significantly influence the quality of fish salting products. Also, salt cannot be contaminated by microorganisms, which may influence the quality of fish during the fish salting process. Fine granulated salt accelerates osmotic processes in the fish tissue, and because of that, the surface of the fish meat becomes too dry and very salty. Agglutination of the fish also becomes obvious in such cases. On the other hand, coarse granulated salt has a less intensive influence on the fish tissue because it penetrates the tissue more slowly. As a result, fish spoilage could overcome the fish salting process. Due to that fact, it is very important to mix both granulations of the above-mentioned salts. A mixture of 1/3 of fine salt and 2/3 of granulated salt seems to be optimal [3,6].

High salt consumption has harmful effects on human health, causing high blood pressure, cardiovascular diseases, and other diseases (e.g., stomach cancer, metabolic syndrome, autoimmunity, kidney stones) [23]. Therefore, it is very important to avoid overconsumption. The usage of KCl can serve as a partial replacement for NaCl during fish salting [24].

5. Halophilic Microorganisms

Salt can't influence certain microorganisms, which are usually named halotolerant or halophilic microorganisms [2]. Salt can, in certain amounts, prevent the development of a great number of microorganisms. Some microorganisms cannot be altered by salt, e.g., halophilic microorganisms. Halophilic microorganisms are those that require an above-average amount of salt in their environment to survive, meaning that these microorganisms require salt concentrations higher than those in seawater for their growth. According to Bergey's Manual of Systematic Bacteriology (2003), the *extremely* halophilic genera are: *Staphylococcus, Halococcus, Halobacterium, Haloferax, Halomonas, Methanohalobium, Natrinema, Haloarcula, Halobaculum, Halorubrum, Haloterigena, Natrialba, Natronococcus, Natronomonas and Natronorubrum*. Depending on the optimum concentration of salts required for their growth, halophilic microorganisms are divided into halotolerant, halophilic and highly halophilic. The lowest concentration for the development of halotolerant microorganisms is approximately 0.3 M and the highest concentration is about 5.0 M. Halotolerant microorganisms can become facultative. Many strains of extremely halophilic bacteria can be found in salt pans [14,25]. Halophilic bacteria naturally occur in salts which can be used for fish salting, since they demand potassium and magnesium ions for their growth. Because of the presence of extreme halophiles, food can change colour to reddish, and therefore be rejected by buyers. Some fish (e.g., anchovies) which have a longer period of ripening can, because of their chemical composition, have the ability to degrade proteins and fats from their tissues. Therefore, determination of halophilic bacteria in salted fish products is extremely important [2,6]. There is a lack of reports about halophilic archaea found in salted foods [13] and the functions of these microorganisms are not completely understood [14]. In 2013, Japanese scientists found that halophilic bacteria are much more present in food than was previously suspected and the risk of coming in contact with humans is also very high, despite the fact that salt in salterns is dried for at least 10 days [13,26,27].

There are even reports showing that the halophilic archaea may be directly included in the crystallization of halite in salterns [28]. There is an assumption from the past that the possibility of existence of halophilic archaea in food is very low. The possibility of having a wide variety of halophilic archaea, which is higher in food products than would have been expected previously, needs to be taken into consideration [13]. Systematic studies on the occurrence of such microorganisms in food have rarely been performed [29]. Fermentations that involve low salt concentrations generally give rise to the development of species of *Tetragenococcus, Halomonas, Halobacillus, Lentibacillus* and other bacterial genera [28]. Fermented products of East and Southeast Asia are considered newly discovered sources of halophilic and halotolerant strains. Glutaminase activity, in the presence of high salt concentration, is the cause of the savoury taste of fermented fish products [8,14].

The most explored halophilic bacterium in salted fish is *Staphylococcus aureus*. Gram-positive, spherically shaped asporogenic bacteria is a member of the genus *Staphylococcus*. It causes approximately one million deaths annually and is classified as a high-priority bacterial pathogen according to the World Health Organization

(WHO) Bacterial Priority Pathogens List from 2024 [30]. *S. aureus* is a special concern because it is Methicillin-resistant (MRSA) and food poisoning is related to its enterotoxins, primarily formed in food samples of animal origin [31,32]. Contaminated water, possibly used in some phases from fishing to the final products, including water for ice preparation when cooling or freezing systems are not available, together with improper hygiene conditions, can cause the incidence of *S. aureus* in fish samples [33].

In the last five years, several studies have been conducted regarding the occurrence of *Staphylococcus aureus* in various species of salted fish throughout the world.

In Egypt, there was microbiological testing of *S. aureus* in salted fish "maloha" (25 samples) and "fesikh" (25 samples). In 76% of "moloha" samples and 92% of "fesikh" samples, *S. aureus* was found. The results showed that "moloha" (20%) and "fesikh" (36%) samples are sources of Methicillin and Vancomycin-resistant *S. aureus* [34].

In another research conducted in Egypt, 60 samples of salted fish were tested from three species (salted sardines, "molouha" and "fesikh"). *S. aureus* was determined in 30% salted sardine, 20% "feseikh" and 10% "molouha" samples. All positive *S. aureus* samples were positive for MRSA [35].

In Pakistan, 50 samples of salted fish were analysed in order to determine the presence of *S. aureus* and MRSA (Methicillin Resistant *Staphylococcus aureus*). In 26% of the samples, S. aureus was detected [36].

In the third research conducted in Egypt, 80 samples of four fish species (salted sardine, fesiekh, sahlia and salted herrings) were analysed (20 of each). *S. aureus* was detected in 15% of salted sardines, 40% of fesiekh, 30% of sahlia and 15% of salted herrings. *S. aureus* isolates, as in another researches, also showed multidrug resistance [37].

Research conducted in China on salted white herring (*Ilisha elongata*) divided samples into three groups. In the first group were traditionally salted white herrings, in the second group non-starter salted white herrings and in the third group starter culture salted herrings. During traditional salting, *Staphylococcus aureus* became dominant after 20 days of salting (38.91%), and after 40 days was dominant in 58.65% fish samples. Results also showed that during salting, *S. aureus* progressively became dominant in each of the three above-mentioned groups because it can tolerate high concentrations of salt [38].

Research conducted in South Korea showed that genus *Staphylococcus* was detected in salted Alaska pollock roe (during summer sampling) in an amount of 50.55% and *S. aureus* as well. The relative abundance of the genus *Staphylococcus* is significantly different between samples collected from the southwestern and northern parts of South Korea. This genus has also been detected in Damselfish (also during summer sampling) in an amount of approximately 10% [39].

Research conducted in Poland included 89 samples of various seafood, including fish, crustaceans and mollusks. Seafood originated from three fishing regions: 1. Baltic Sea and Polish domestic farms, 2. European Countries and 3. Asia and South America (including Bangladesh, India, Argentina, Chile, Vietnam, and China). Analysed fish were: hake (9), catfish (2), pollock (5), carp (5), perch (5), cod (6), salmon (15), halibut (1), flounder (2), trout (9), herring (2) and mackerel (1). Fish were frozen or fresh/raw, and none of them were salted. Researchers concluded that there is a great distribution of *S. aureus* in analysed samples, accompanied by the prevalence of antibiotic-resistant strains, and that therefore it is necessary to make a global monitoring, since *S. aureus* is a high-priority bacterial pathogen according to WHO [30,40]. Worldwide surveillance should have great importance because microorganisms in salted fish originate also from the fish itself and not only from later added salt. Also, it is necessary to implement the principles of Good Hygiene Practices (GHP) to reduce transfer of *S. aureus* during fish salting as well as in other phases in the production of it [41].

Besides halophilic bacteria, there are also halophilic moulds in salted fish. Representatives are: *Aspergillus*, *Rhizopus*, *Penicillium*, *Absidia* and *Mucor* [42]. One of the most dangerous mould species, also found in salted fish, from the genus *Aspergillus* is *Aspergillus flavus,* capable of producing aflatoxins. Aflatoxin B_1 is the most carcinogenic toxin, causing liver and kidney damage [43,44]. In accordance with the above, it is also necessary to implement principles of GHP regarding moulds.

6. Protein Degradation during Salting

Generally, the salting process is important not just for the preservation of fish but also for the production of a well-ripened product with a tender consistency and a pleasant taste and odour [8]. It is believed that the ripening which occurs during salting is mainly a proteolytic phenomenon involving the degradation of proteins (mostly in muscles) into taste-active peptides [11,12,45] (**Figure 2**) [21]. One of the major effects related to salting is the loss of nutrients. Salting leads to a reduction in water content that has been attributed to the denaturing effect of salt on proteins, since denaturation of muscle protein enables the acceleration of the diffusion of water from fish. Additionally, salting treatment decreases the protein content of the fermented fish product as a result of protein being dissolved in the brine, a phenomenon which could also happen to other nitrogenous substances, including free amino acids [46,47]. A decrease in protein content could also be attributed to protease activity. Due to protein degradation, proteins change their structure because some amino acids are modified or completely lost. Those changes can be modified into 3 categories: a) modification of the primary structure of proteins due to modification of certain amino acids, b) loss of particular amino acids, protein aggregation or protein fragmentation, c) modification of secondary and tertiary structure of proteins, which leads to changes of solubility and molecular charge. Changes in physical and chemical properties of proteins also result in a decrease in heat stability and changes in viscosity. Amino acid degradation leads to the release of Maillard reaction products and the occurrence of brown colour of salted fish product as well as intensive fluorescence [28,48]. When the analysis of "lakerda" (a salted fish product from the Mediterranean, produced from Atlantic bonito (*Sarda sarda*)) was made, it was confirmed that in comparison to raw samples, the amounts and ratios of total aromatic amino acids decreased, whereas total sulphur amino acids increased during salting [15].

The Maillard reaction is the reaction of non-enzymatic browning, which occurs due to condensation between an amino compound and reducing sugars in order to produce N-glycosylamine. Oxygen, light and temperature are the catalysts for the Maillard reaction. Reactants are the carbonyl group of reducing sugars and the free amino group from an amino compound. In most cases, the Maillard reaction causes the formation of unwanted aromas and colours of various types of food and odours as well. Maillard reactions (carbonyl-amino reactions) are divided into 3 stages (**Figure 3**) [49]. In the first stage, the reaction of condensation occurs between reducing sugars and free groups of amino acids, with elimination of water molecule and formation of N-glycosylamine. N-glycosylamine is very unstable and due to Amadori rearrangement, it builds up 1-amino-1-deoxyketone. In the second stage, 1-amino-1-deoxyketone is very reactive and can react in 3 different ways. In the first case, dehydration of 1-amino-1-deoxyketoze continues (with the loss of 2 water molecules) and reductons and dehydroreductones are formed. Those compounds can compose essential caramel products. This process is also called *mild dehydration*. The second type of reaction takes place only if the pH is higher than 7. Consequently, acetol, pyruvaldehyde and diacetyl are formed. The above-mentioned compounds go through the Strecker degradation and after that, they form aldehydes with one C-atom less. Such formed aldehyde products give an unpleasant odour to food. (e.g., fish). This process is also called *fission.* The third type of reaction takes place only if the pH is below 7. Through this procedure, the Schiff base types of products are formed. The Schiff base types of products are furfural aldehydes or HMF (hydroxymethylfurfural). This process is also called *strong dehydration*. Those types of aldehydes are undesirable. In the third stage, brown nitrogenous polymers (pigments) called melanoidins are formed [4,31–34,50–52].

AGEs (Advanced Glycation End Products) are in a cycle of Maillard reactions and refer to non-enzymatic glycation between reduced carbohydrates, free amino acids, fatty acids and nucleic acids. The reactions can appear as endogen or exogen and therefore AGEs appear as Edogen or Exogen AGEs. Endogen AGEs are produced slowly in the body during oxidative stress, infllamations and hyperglycemia. Exogenous AGEs, which are highly important for this review, are found in food and beverages (e.g., salted fish, soft drinks). The amount of AGEs from exogenous sources is usually much higher than from endogenous sources [53]. The food abundant with AGEs usually have

very intense aroma, colour and taste. Although humans have enzymes capable of reducing AGEs that mechanism is not functioning properly in case of great food intake of AGEs or with individuals suffering from certain diseases, e.g., diabetes mellitus or kidney disease. AGEs can modify protein structure in organism especially collagen and low-density lipoprotein (LDL). Through protein modification, e.g., LDL is not recognized anymore by LDL receptors and dysfunctional collagen builds plaque on arterial cell walls. Therefore, there is a great risk of developing cardiovascular diseases like atherosclerosis or hypertension. On the other side, AGEs can activate various cell signals, e.g., through binding of AGEs to Receptors for Advanced Glycation End Products (RAGE) and this interaction consequently leads to intracellular oxidative stress and inflammation. Therefore, the risk of a lot of chronic diseases, e.g., diabetes, neurodegenerative diseases or kidney diseases, is increased [54].

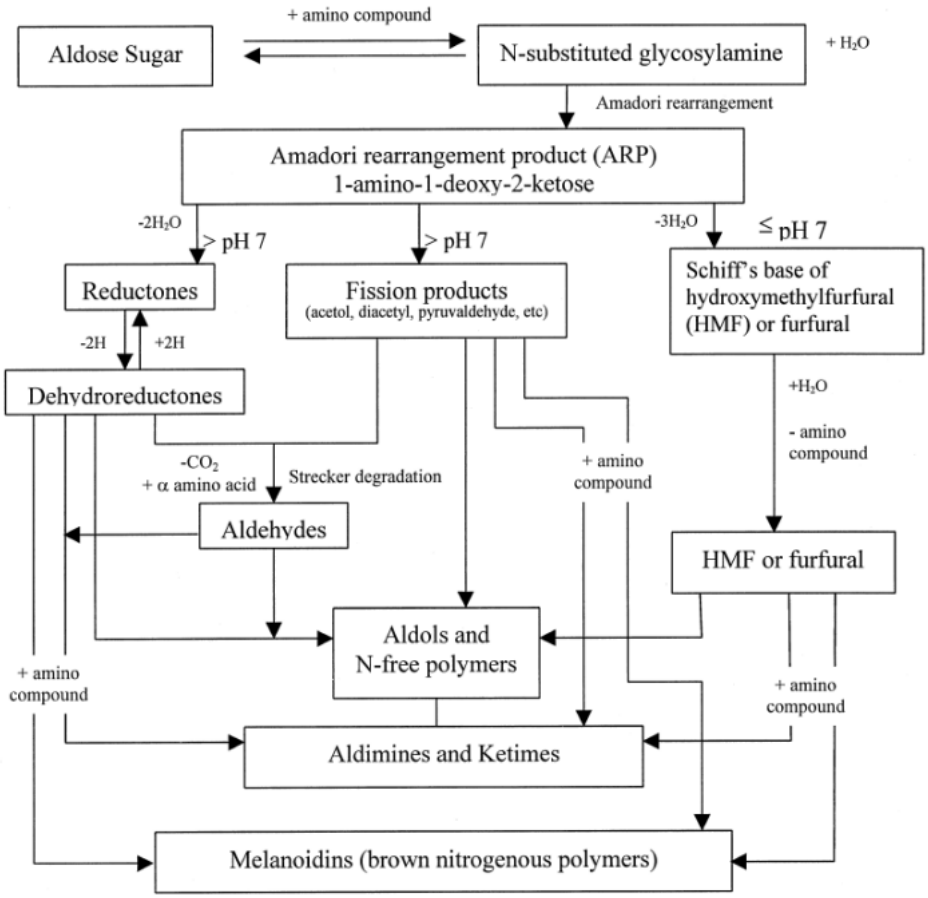

Figure 3. Scheme of Maillard reactions.

7. Lipid Oxidation during Salting

Lipid oxidation is one of the major causes of quality degradation of some food products, especially of undesired colour and taste modifications. Salting can cause oxidation of the fish products [15]. Unsaturated fats can be oxidized with the help of free radicals. Intensity of oxidation depends on the number of double bonds [46,49,55–59]. Consequently, polyunsaturated fatty acids are more oxidative than saturated fatty acids. Lipid oxidation is a chain reaction, and a well-known characteristic of all chain reactions is that they are performed in several steps. Free radicals are atoms or molecules with an unpaired electron, and because of this, they are very reactive [15,54,60]. Initiation is the beginning of lipid oxidation because oxygen has a direct impact on fatty acids and brings free radicals to the reaction. Likewise, the formation of free radicals can also be caused by the presence of, e.g., metal, light, heat and radiation. This step involves the formation of lipid-free radicals. During *propagation*, peroxyl radicals react with other lipid

molecules to form hydroperoxides and a new free radical. In the third phase—*termination*, radicals formed in propagation can react with each other, forming new molecules. That way, they are slowing and eventually closing reactions in the process of lipid oxidation. When there are no more free radicals for the continuation of lipid oxidation, it is necessary to begin new reactions of initiation [40]. During termination, free radicals react with oxygen, and that leads to the release of peroxyl radicals. Peroxyl radicals are taking hydrogen from molecules of fatty acids and releasing new free radicals and hydroperoxides. Hydroperoxides are highly unstable and fall apart into two new radicals. Both of them are separately taking off a hydrogen atom from every molecule of fatty acids and new free radicals are formed. Those new radicals are initiators of the new chain reactions [51,59]. The colour changes that occur during salting take place due to lipid oxidation. Haemoglobin (Hb) and myoglobin (Mb) contain iron in their structures and have the ability to make lipid oxidation faster. Due to the strong prooxidative activity of haemoglobin (Hb), lipid oxidation can also occur in fish muscles with low levels of lipids (down to 0.1 % wet weight) [41–43,57,61–63]. The decrease in peroxide value (POV) in the last stages of salting indicates that more hydroperoxides break down to carbonyls or other degradation products. TBARS (Thiobarbituric Acid Reactive Substances) are secondary products of lipid oxidation. In this method, the reaction between thiobarbituric acid and malondialdehyde (MDA), which is the secondary product of oxidation of PUFAs (Polyunsaturated Fatty Acids), is crucial. MDA reacts with the active methylene groups of thiobarbituric acid. The concentration of MDA is in strong correlation with lipid oxidation. This is a very old and most frequently used method for quality control in fish industry. These observations have been made during the salting of White Chinese Croaker fish (*Argyrosomus amoyensis*) [16,64]. EDTA (ethylenediaminetetraacetic acid) and phenolic antioxidants can be used as good inhibitors of lipid oxidation mechanisms [46].

There is a hypothesis that products of lipid oxidation affect protein oxidation. Malondialdehyde (MDA), as a product of lipid oxidation, is electrophilic and can react with nucleophilic amino acid groups in protein side chains, especially those originating from threonine, lysine, arginine, and proline. Aggregated proteins can also be formed, which affects their solubility. This hypothesis needs to be thoroughly explored in fish samples and under laboratory conditions as well [65,66].

Because of its high reactivity, MDA can react with a great amount of biomolecules (e.g., proteins, DNA and phospholipids) and cause a variety of diseases (e.g., cancer, cardiovascular diseases, diabetes). There is a progressive awakening about its engagement in disease development and those efforts should be continued in the future [67–69].

8. Enzyme Activity during Salting

Enzymes, proteases (peptidases), are usually found in muscles and the intestine after fishing and contribute to degradation during manufacturing. Also, changes in odour or product degradation can be caused by proteolytic enzymes [46,67]. Protein hydrolysis can contribute to advancement or deficiency in seafood quality. Hydrolases catalyse the hydrolysis of various chemical bonds of substrates with the help of water molecules. Namely, proteases (peptidases) are an example of those enzymes that degrade molecules into two or more molecules by adding water [46]. Endopeptidases (proteinases) show specificity for intact proteins and they degrade the proteins into large peptide fragments, which serve as substrates for exopeptidases, as these enzymes degrade amino acids or small peptides (2–3 amino acids) from the terminals of the large peptides. Different types of endo- and exopeptidases exist in many fish_species, and a complete degradation of large muscle proteins is only possible by a combined action of endopeptidases and exopeptidases [45]. NaCl can control autolytic spoilage because it can slow down autolytic enzymes in maritime species. Salt (NaCl) renders the enzyme cathepsin (which belongs to proteases) inactive [46]. Siringan has conducted extensive research on Indian anchovies and determined that autolytic endopeptidase activity decreased in the presence of 25% of NaCl in the solution. In addition, it was observed that autolytic activity decreased as the NaCl concentration increased so that no activity was observed at the 30% NaCl concentration. All of this was observed at a temperature of 55 °C and at a basic pH of about 9 [46]. Proteinases (endopeptidases) from the herring intestines play a major role, but enzymes from the muscles also have an important role during salting [8,68]. Proteinases have been

thoroughly studied among a few fish species [69]. Major proteinases in *pyloric caeca* and intestines are trypsin, chymotrypsin, elastase, and aminopeptidase. It has been determined that endopeptidase, isolated from Indian anchovies, in the presence of a very high concentration of salt, significantly reduces proteins. The measurements have been conducted at 60 °C [70].

The activity proved very low for the gutted herring in comparison with the nobbed herring, although it was not determined exactly how much of the intestines were removed by the nobbing procedure. The finding that a completely gutted herring ripens, as determined by sensory analysis, may suggest that intestinal enzymes are only one of several factors that contribute to the ripening phenomenon and that other factors such as muscle enzymes and salting procedures, are not of minor importance. Because of that, we can state that it is not always necessary for the ripening of salted herring to include parts of the intestine such as *pyloric caeca* [45]. It has been shown again that endopeptidase activity decreases as the salt concentration is higher and vice versa. In the range of 1 to 5% of NaCl, type-II proteinase kept considerably higher activity than type-I proteinase. Type I and type II proteinases (endopeptidases) belong to muscles but not to the intestine and they are called *latent* proteinases. They have been found in anchovies (*Engraulis japonica*). Those enzymes can degrade some muscle proteins in the temperature range of 50–65 °C [68,71]. Both kinds of endopeptidases were active in extremely high concentrations of salt (16 to 17%) [70]. Research conducted with tuna samples has shown a greater activity of splenic proteinase at yellowfin tuna (*Thunnus albacares*), than at skipjack tuna (*Katsuwonus pelamis*) and tongol tuna (*Thunnus tonggo*). The range of NaCl was between 1 to 10%. Enzyme activity was not determined at a NaCl concentration of 30%. The type of tissue also plays an important role in decreasing the proteinase activity in the following order: spleen, pancreas, stomach and liver [69].

9. Conclusions

There are two existing theories about biochemical changes during fish salting. The first theory is directed towards the intestinal microbiota and towards its influence on the types of changes which are possible during fish salting. The other theory is the autolytic theory, and according to it, enzymes play a key role in fish salting. It is necessary to combine both theories in order to understand the complexity of the changes taking place during fish salting and ripening. There is also a need to conduct more research about halophilic bacteria, which are able to survive in gut microbiota and about the specific metabolism of those microorganisms, because there is not enough evidence for the food safety of halophilic archaea itself. That is very important because *Staphylococcus aureus* is isolated throughout the world in many salted fish (and raw fish) samples and it is well known as a high-priority (halophilic) bacterial pathogen and MRSA and therefore is a serious threat to human health. This review contributes to addressing food safety risks regarding salted fish and highlights the importance of global monitoring before and during salted fish production. It could be very useful in fish processing technology as well as for researchers in the field of food safety. Future researches should include development of effective preventive methods against contamination of salted fish with *Staphylococcus aureus*, and other high-priority pathogens, as well as outbreak reporting.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of interest

The author declares no conflict of interest.

Appendix A

Table A1. Search Strings, Databases and Reports Assessed for Eligibility.

Search Strings/Keyword	Database	Reports Assessed for Eligibility
lipid oxidation AND fish salting	Taylor and Francis Online	14
	ScienceDirect	5
	Wiley Online Library	12
	PubMed	12
protein degradation AND fish salting	Taylor and Francis Online	3
	Science Direct	7
	Wiley Online Library	4
	PubMed	1
enzyme activity AND fish salting	Taylor and Francis Online	4
	Science Direct	2
	Wiley Online Library	5
	PubMed	2
fish salting	Taylor and Francis Online	2
	Science Direct	4
	Wiley Online Library	8
	PubMed	4
water activity AND fish salting	Taylor and Francis Online	0
	Science Direct	0
	Wiley Online Library	2
	PubMed	0
enzymes AND fish salting	Taylor and Francis Online	1
	Science Direct	1
	Wiley Online Library	2
	PubMed	1
proteases AND fish salting	Taylor and Francis Online	1
	Science Direct	0
	Wiley Online Library	3
	PubMed	2
endopeptidases AND fish salting	Taylor and Francis Online	2
	Science Direct	1
	Wiley Online Library	7
	PubMed	0
halophilic microorganisms AND fish salting	Taylor and Francis Online	4
	Science Direct	3
	Wiley Online Library	5
	PubMed	1
Staphylococcus aureus AND salted fish	Taylor and Francis Online	2
	Science Direct	1
	Wiley Online Library	5
	PubMed	6
maillard reactions AND salted fish	Taylor and Francis Online	2
	Science Direct	1
	Wiley Online Library	4
	PubMed	0

Table A1. *Cont.*

Search Strings/Keyword	Database	Reports Assessed for Eligibility
maillard reactions AND food	Taylor and Francis Online	4
	Science Direct	1
	Wiley Online Library	5
	PubMed	1
maillard reactions	Taylor and Francis Online	3
	Science Direct	1
	Wiley Online Library	5
	PubMed	6
	Total Number of Reports	172

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