Off-Flavors in Fish: A Review of Potential Development Mechanisms, Identification and Prevention Methods

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ABSTRACT

Off-flavor, unpleasant and unacceptable tastes and odors, is a global problem in the fishery industries which causes many economic losses and health hazards. Off-flavors can be developed in the fish during the pre- or post-hunting phase. Pre-hunting off-flavors can be caused by environmental factors, feeding, and contaminants. However, post-hunting spoilage creates different off-flavors in the fish due to the growth of microorganisms, activity of enzymes, and oxidative processes. Identifying the sources of off-flavors can sometimes help the processors to perform corrective actions. Therefore, this study aims to discuss the different types of off-flavors, mechanisms, and effective compounds in their development. Further, the methods for identifying and reducing the off-flavors are introduced.

1. Introduction

Fish flavor consists of non-volatile taste compounds and volatile odor compounds. Taste compounds include low molecular weight peptides, free amino acids, organic acids and bases, nucleotides and related compounds, carbohydrates, and inorganic salts [1, 2]. Alcohols and carbonyls, sulfur compounds, bromophenols, and hydrocarbons are also the main ingredients of fish aroma [3-6]. Moreover, fish species (salmon, cod, etc.), feeding sources, physical and chemical properties of fish, storage conditions, and processing technologies, can affect fish flavor [5, 7-10].

Fish and edible crustaceans are sometimes off-flavored which their characterization is often difficult since tricky chemical compounds and mechanisms are involved in developing the off-flavors, similar compounds may sense differently by different people, several flavors may be developed in the presence of more than one chemical compound, changes in the concentration of odor compounds may cause changes in flavor characteristics, and a compound...
may create different flavors in various fish species [11]. Pre-hunting off-flavors can be induced by environmental pollutants, compounds produced in the water by aquatic organisms and microorganisms, and the presence of off-flavors and their precursors in the water in which the fish lives. These off-flavor-generating compounds can be entered the fish body through feeding, skin absorption, and/or gills [11, 12]. Post-hunting off-flavors are formed through microbial spoilage, enzymatic activities, and/or oxidation of lipids.

2. Discussion

2.1. Pre-Hunting Off-flavors

2.1.1. Earthy and muddy flavors

Musty, muddy, earthy, and moldy flavors are the common off-flavors in freshwater fish. Geosmin (GSM) and 2-methyl isoborneol (MB), the secondary metabolites produced by various types of actinomycetes (such as Streptomyces and Actinomycetes) and cyanobacteria (blue-green algae), are known as two main chemical compounds responsible for these off-flavors [13-15]. Moreover, 2-methoxy-3-isopropylpyrazine produced by Streptomyces has also been considered as off-flavor contributors [16]. These compounds can be absorbed through the skin, gills, small intestine, and/or stomach of the fish [17]. Regarding the higher fat solubility of GSM and MIB compared to water, their concentration is much higher in the fat layers, and consequently, their perception threshold differ in fishes with different fat contents [18].

2.1.2. Iodoform flavor

The most common off-flavor that is frequently developed in prawns, shrimp, and certain fish species in the sea is iodoform- or iodine-like or medicinal flavor which is supposed to be induced by 2,6-dibromophenol compound. This off-flavor is considered an inevitable feature of some crustaceans and lowers the consumer acceptance of particular fish species [19]. Considering the presence of 2,6-dibromophenol in the digestive tract of fishes, it has been hypothesized that animals’ diet is the most likely source of this off-flavor. Therefore, promptly separating the guts after death and before cooking fish is considered the primary strategy to reduce the severity of the problem [20]. The potential pathway of bromophenols biosynthesis from tyrosine and 4-hydroxybenzoic acid in marine algae and polychaete worms is shown in Figure 1. It has been shown that when phenols, H₂O₂, and sodium bromide react in the presence of bromo-peroxidases, the bromophenols are produced by bromination catalyzed by the peroxidase and then removing the carboxylic acid or alanine, which are sensed in iodine-like or medicinal flavors at high concentrations [21].

2.1.3. Garlic flavor

Different prawns and sand lobsters are frequently affected by this type of off-flavor created by the bis-(methylthio)-methane (BMTM) compound [22]. Since BMTM is mainly present in the gastrointestinal tract, its potential source is the diet and removing the guts before cooking is considered helpful in decreasing the severity of this problem [23]. In the proposed route for forming BMTM, the enzymes or the bacteria convert prime reactants, methanethiol derived from cysteine and formaldehyde derived from trimethylamine oxide (TMAO), into BMTM (Figure 2). It is not currently clear whether the compound is eaten in this form or is a metabolite formed in the animals’ digestive tract.

Figure 1: Possible route of bromophenols biosynthesis from tyrosine or 4-hydroxybenzoic acid in algae and polychaete worms

Figure 2: Possible route of bis-(methylthio)-methane (BMTM) production from cysteine and trimethylamine oxide (TMAO) in marine organisms [24]
2.1.4. Blackberry flavor

Dimethyl sulfide (DMS) is known as the main compound of this off-flavor. It has been suggested that DMS originates from algae, enters the invertebrates from their algal diet, and when the fish eats the invertebrates it causes the fish fillets to take the blackberry off-odor [25]. DMS is created by cleavage of its precursor, dimethyl-β-propiothetine (DMPT), either by algal enzymes or through the cooking process of the contaminated muscle. DMS has also been reported to be responsible for the petroleum-like odor in canned salmon [23].

2.1.5. Off-Flavors induced by environmental contaminants

Fish flavor may also be affected by environmental contaminants. Entrance of untreated industrial effluents into the water imports a wide range of pollutants that can cause various off-flavors such as petroleum, phenolic, and sulfurous flavors which are created by petroleum and mineral oils, alkylphenols (2- / 3- / 4- isopropyl, 2,4-diisopropyl, 2,5-diisopropyl, 3,5-diisopropyl, 5-methyl-2-isopropyl, and 2-methyl-5-isopropyl) and thiophenols, respectively [27]. Adverse effects can be induced by mineral oils and petroleum substances on various off-flavors such as petroleum, phenolic, and sulfurous flavors which are created by petroleum and mineral oils, alkylphenols (2- / 3- / 4- isopropyl, 2,4-diisopropyl, 2,5-diisopropyl, 3,5-diisopropyl, 5-methyl-2-isopropyl, and 2-methyl-5-isopropyl) and thiophenols, respectively [27]. Adverse effects can be induced by mineral oils and petroleum substances on fish flavor after hours to days of exposure depending on their concentrations. Oil products are highly lipophilic; thus, they quickly enter and accumulate in the fish body and then are excreted very slowly [28].

Table 1: Some off-flavor precursors and their threshold limits [23, 26]

| Compound          | Off-flavor            | Lowest olfactory threshold (µg/l water) |
|-------------------|-----------------------|----------------------------------------|
| 2-Bromophenol     | iodine-like, medicinal| 0.1                                    |
| 3-Bromophenol     | iodine-like, medicinal| 1.0                                    |
| 4-Bromophenol     | iodine-like, medicinal| 4.0                                    |
| 2-Chlorophenol    | iodine-like, medicinal| 2.0                                    |
| 3-Chlorophenol    | iodine-like, medicinal| 50.0                                   |
| 4-Chlorophenol    | iodine-like, medicinal| 60.0                                   |
| 2,4-Dibromophenol | iodine-like, medicinal| 0.5                                    |
| 2,6-Dibromophenol | iodine-like, medicinal| 0.1                                    |
| 2,4-Dichlorophenol| iodine-like, medicinal| 0.4                                    |
| 2,6-Dichlorophenol| iodine-like, medicinal| 3.5                                    |
| 2,4,6-Tribromophenol| iodine-like, medicinal| 0.1                                   |
| Geosmin           | earthy, moldy         | 0.01                                   |
| 2-Isopropyl methoxy pyrazine | earthy, moldy | 0.002                               |
| 2-Isobutyl methoxy pyrazine | earthy, moldy | 0.002                               |
| 2-Methylisoborneol| Earthy and camphor-like| 0.01                               |
| 2,3,6-Trichloroanisole | Moldy             | 0.007                                  |
| Bis-(methylthio)-methane | garlic          | 0.3                                    |
| Dimethyl sulfide  | blackberry            | 0.33                                   |

The entrance of chlorine bleaching wastewaters into the water or the control of algae and potential pathogens by water chlorination results in off-flavors in the fish and typical aseptic chlorination taste in water by chlorophenols, methoxy, and dimethoxybenzenes [29].

It should be noted that factors such as age, fat content and feeding habits of fish, salinity and precipitation degree of water, water streams, dilution, fishing area, water temperature, the concentration of odorous compounds and contaminants, exposure period, physicochemical properties of the substances involved, season and several other factors can affect the severity of different pre-hunting off-flavors in fish [11, 30]. The fish with higher fat content usually accumulates higher concentrations of off-flavor precursors. The removal of off-flavor compounds from lipid-rich tissues is also relatively slow. Some off-flavors such as iodine-like one are only observed in marine fish, while some other off-flavors such as the earthy-muddy ones are more common in the freshwater fish [11]. Oil-like flavors are observed in the fish flesh in areas where oil extraction or deposition is extensively performed. In some cases, the pre-hunting off-flavors can be reduced by lowering the water temperature due to decreased organisms’ growth with the potential creation of off-flavor precursors. In addition, the absorption and elimination of the off-flavors decrease with decreasing the temperature. However, off-flavor creation is increased with increased concentrations of pollutants and exposure time. There are also differences in absorbing and eliminating the off-flavors for various sizes and species of fish [18].

2.2. Post-Hunting off-flavors

2.2.1. Microbial off-flavors

Different microorganisms can grow on fish during storage depending on the conditions (temperature, gaseous atmosphere, etc.) and make characteristic spoilage flavors. Some important microbial species which cause such off-flavors are discussed below.

2.2.1.1. Alcaligenes (achromobacter) spp.

The compounds produced by the species of this microorganism are methanethiol, dimethyl disulfide, 1-penten-3-ol, 3-methyl-butanal, and trimethylamine (TMA) which produce specific spoilage flavors. The primary sources of these compounds are known to be amino acids and TMAO. Some lipid decomposition also occurs in the presence of this bacterium [31].

2.2.1.2. Pseudomonas fluorescens

These organisms are commonly involved in fish spoilage. Blue-green fluorescence and putrid odors induced by methanethiol and dimethyl disulfide indicate their presence [31].
2.2.1.3. Pseudomonas fragi

Pseudomonas fragi strains can produce fruity and onion-like flavors during cold storage [32]. Fruity odors are found in the early stages of spoilage and are related to the formation of ethanol, ethyl acetate, ethyl butanoate, and ethyl hexanoate. The strong sulfide or onion-like odor in the continued storage is related to the methanethiol, DMS, and dimethyl disulfide formation [31].

2.2.1.4. Pseudomonas perolens

The musty and potato-like odors in fish attributed to 2-methoxy-3-isopropylpyrazine and possibly 2-methoxy-3-sec-butylpyrazine are generally induced by Pseudomonas perolens growth [33]. Other compounds produced by this organism are methanethiol, dimethyl disulfide, dimethyl trisulfide, 3-methylbutanol, and 2-butanone. It has been suggested that the endogenous valine, glycine, and methionine are the probable precursors of 2-methoxy-3-isopropylpyrazine produced by this bacterium grown in pyruvate-containing media as the only source of carbon [34].

2.2.1.5. Shewanella putrefaciens

The presence of this organism is characterized by ammoniacal, rotten and hydrogen sulfide odors due to the formation of sulfurous compounds, 3-methylbutanol, 1-penten-3-ol, and TMA. This bacterium is the main producer of hydrogen sulfide. It has been considered that the metabolism of methionine, cysteine, cystine, and possibly glutathione is the probable source of sulfurous compounds produced by all these organisms [31].

Frequent off-flavors created by the microorganisms in fish and the metabolites responsible for these off-flavors are shown in Table 2.

2.2.2. Lipid oxidation off-flavors

Autoxidation of fish lipids has long been associated with the production of fishy and rancid flavors in the fishes stored under chilled and frozen states [12]. Initially, aromas such as cucumber-like or green (a mild plant-like odor mainly induced by carboxyls) are created by short-chain unsaturated and saturated aldehydes mainly including hexanal and (E)-2-hexenal [37]. With the progress of oxidation, odors described as fishy, rancid, cod liver oil-like or burnt are developed due to the production of volatile secondary metabolites of the oxidation by the breakdown of lipid hydroperoxides, especially (E,E,Z) and (E,Z,Z)-2,4,7-decatrienal derived from oxidation of long-chain polyunsaturated omega-3 fatty acids which are abundant in fish lipids [38]. This compound and other aldehydes are produced through the \( \beta \)-omission of alkoxy radicals created by the hemolytic breakdown of hydroperoxides (Figure 3).

2.2.3. Some of Off-Flavor-Generating Compounds Produced During Storage

2.2.3.1. Trimethylamine and related compounds

TMAO, a quaternary ammonium compound, plays an important role in regulating osmotic pressure in saltwater fish. Therefore, it has not been observed in freshwater species [40]. TMAO does not have any odors or tastes; however, its breakdown metabolites participate in spoilage odors. The odor of TMA is described as ‘old-fishy’ or ‘fish house-like’ (threshold 300-600 ppb). It is produced by the TMAO reduction during microbial spoilage at the temperatures above 0 °C [41]. Since TMA has an ammoniacal odor in the pure state, it is not responsible for the fishy odor alone, but its reaction with fat in the fish tissue creates the fishy odor [42]. DMA produced through the enzymatic breakdown of TMAO in fish muscles has an ammoniacal aroma and exhibits less fishy aroma than TMA [38, 43].

2.2.3.2. Urea and related compounds

Urea exists at low concentrations in the tissues of all fish species; although marine elasmobranchs contain urea at relatively high concentrations (1-2.5%) to regulate the osmotic pressure [44]. Urea has no flavors, but it is quickly decomposed into ammonia and CO\(_2\) by bacterial urease (or, in sharks, by the urease in the blood). The pungent odor of ammonia may result in an unacceptable fish quality [45].

2.2.3.3. Sulfurous compounds

In the later spoilage stages of the chill stored cod, off-odors described as stale cabbage, mercaptan-like, sulfidy and hydrogen sulfide are developed due to the production of methyl mercaptan, hydrogen sulfide and DMS. These volatile sulfides are formed through microbial decomposition of free cysteine and methionine in the fish muscle [46].

2.2.3.4. Biogenic amines (BAs)

BAs are nitrogenous compounds mainly produced through bacterial decarboxylation of amino acids during storage. Among them, putrescine and cadaverine have a putrid flavor, histamine has a pungent flavor, and phenylethylamine has a fishy flavor. These compounds are heat-resistant and therefore are used as indices to determine fish freshness [12].

2.2.3.5. Volatile acids

Different volatile acids formed in the fish flesh during storage which create undesirable and extreme sweaty odors. These compounds are considered essential indices of fish oil quality; however, a study indicated that the concentration of short-chain fatty acids found in the fish during oxidation was not significant for burnt/fishy flavors [47].
2.2.3.6. Carboxyls

A study on variations of the odor compounds in boiled trout and cod during storage indicated that the increase in TMA, methyl propanal, 2- and 3- methylbutanal, and butane-2,3-dione in cod, and the acetaldehyde, propionaldehyde, butane-2,3-dione, pentane-2,3-dione and C6, C8, and C9 carboxyl compounds in trout resulted in off-flavor formation [49].

The deteriorated aromas of cod and related species have also been related to (Z)-4-heptenal. This compound is characterized by a cardboardy character at low concentrations in water, but paint-, putty- or linseed oil-like flavors at higher concentrations [50]. The aroma of (Z)-4-heptenal is also like the cold-boiled potato [51].
Table 3: Volatile fatty acids identified in oxidized sardine oil and their odor thresholds [48]

| Name of acid     | Odor threshold (ppm) |
|------------------|----------------------|
| Acetic acid      | 34.2                 |
| Propionic acid   | 32.8                 |
| n-Butyric acid  | 3                    |
| Isobutyric acid | 9.2                  |
| n-Valeric acid  | 1.1                  |
| Isovaleric acid | 1.7                  |
| n-Caproic acid  | 7.5                  |
| Isocaproic acid | -                    |

It does not create distinctive fishy flavors, but increases the burnt, fishy, stale, and cod liver oil-like flavors created by 2,4,7-decatrienal [52]. This compound is produced by retroaldol condensation of (E,Z)-2,6-nonadienal by water, and the suggested mechanism is shown in Figure 4. (Z)-4-heptenal production is accelerated at high pH and temperatures; therefore, is usually found in cooked and stored seafood [5].

2.3. Identification of the Off-Flavors in Fish

Several methods have been developed to detect off-flavors in fish including sensory analysis and instrumental methods such as the ELISA, colorimetric methods, chromatography, and the electronic nose [54].

Colorimetric methods, the most common methods used to measure the off-flavors, are simple, low-cost, and fast. ELISA provides a quick test, although its low sensitivity makes it inappropriate for detecting the off-flavors in aqueous cultures. Chromatography requires a precise and tedious sample preparation which depends on the off-flavor-generating compound and the sample matrix. Since these compounds exist in fish samples at low concentrations, analysis can be performed on the sample extracts [55]. The electronic nose is responsive to a specific or a group of volatile compounds and can be used to evaluate spoilage. These measurements require only a little sample preparation and relatively short analysis time. Nevertheless, the stability of correlation between the electronic nose and the sensory data is still an obstacle for the practical application of these sensors in the evaluation of seafood shelf life [54].

Overall, the instrumental methods to evaluate the fish flavor quality are costly and time-consuming and can detect only a few numbers of odorous compounds. Sensory analysis by well-trained panels is more effective for routine assessment of the fish flavor, since they can identify odorous compounds at low concentrations and distinguish between different off-flavors and their intensities according to various scales depending on the country, species, etc. [11]. In the sensory analysis ethical issues must be respected; when selecting and training assessors for sensory analysis, it is imperative to know that some people cannot taste specific flavors, some are not responsive to some flavors properly, and some are allergic to different fish proteins or histamine.

The interpreters of stimuli and responses must also be carefully trained to receive genuine responses. Fish should be cooked by microwave or steam above the boiling water for an appropriate time depending on the method used, the size, and the number of fish being analyzed. The evaluation must be performed under stable conditions (light, temperature, etc.) and in an environment without odorous compounds that can interlope with sensory analysis. The testers should take a break between tasting the samples to wash their mouth with water and eat a piece of bread or anything else with a neutral taste to remove contamination caused by any of the samples [16]. It should be noted that trained dogs can detect some off-flavors like the muddy flavor in catfish. The limitation of the sensory evaluation is its low repeatability [56].

Gas chromatography can be combined with sensory evaluation using special equipment. In this method, which is described as gas chromatography olfactometry (GCO), the emerging eluent of the GC column is divided by the outlet divider. Then, the off-flavor compounds are detected by the single-sniff analysis and the separation is also performed on a packed analytical column with an oven temperature programming. Finally, a skilled tester could identify the peaks of the off-flavor compounds [11].

Figure 4: Suggested mechanism for the (Z)-4-heptenal formation from (E,Z) 2,6-nonadienal [53]
2.4. Reducing and preventing off-flavors in fish

A number of methods have been developed to prevent, eliminate or reduce off-flavors in fish. Off-flavors management methods should be environmentally- and user-friendly, efficient, and cost-effective [11].

1. In trying to reduce or prevent the off-flavors occurrence in fish culture systems or lakes, several methods have been proposed:
   - Reducing the water temperature can decrease off-flavors occurrence by decreasing the growth of species which produce off-flavor-generating compounds. It has been reported that by decreasing the water temperature to lower than 15 °C no off-flavor-generating microorganisms can grow in the water of fish culture systems. Further, as mentioned before, absorption and removal of off-flavors decrease by reducing the temperature [57].
   - Killing the microorganisms that produce the off-flavor-generating compounds; for example, treatment with copper sulfate and other copper-based algicide products has been proposed to kill the blue-green algae which produce earthy-muddy off-flavors.
   - Water refinement in fish farming systems using methods such as filtering with active carbon to control the concentration of suspended particles and reduce the growth of off-flavor-generating microorganisms, ozonation, changes in feeding operations in fish culture to reduce the organic load and inhibit the growth of off-flavor-generating microorganisms and plants, a photocatalytic process to destroy off-flavor compounds, for example, using titanium dioxide, etc. [16, 58, 59].
   - Education and raising the awareness of fish farmers to improve their knowledge about the nutritional habits of fish, management, and measurement of off-flavors [11].
   - Transportation of mineral oils and other hazardous liquid chemicals in safe tanks and prohibition of cleaning them at sea [26].

2. Attempt to remove off-flavors after their development: Off-flavors are removed from the fish only when they are no longer exposed to odorous compounds; for example, transferring them from the polluted medium to clean water for a suitable period of time, completely remove some off-flavor compounds from their body. However, this method cannot effectively eliminate the petroleum off-flavors caused by oil contaminants [26].

3. Masking the off-flavor-generating compounds: Studies have shown that MIB is perceived less or not perceived in the catfish in the presence of lemon-pepper as the masking agents. In addition, the earthy-muddy off-flavors in common carp can be masked using garlic along with roasting [11]. Since MIB and GSM are semi-volatile alcoholic cyclic compounds, food-grade acids such as citric acid together with tumbling under vacuum may also be beneficial in the processing of off-flavored fish [60]. Moreover, it has been reported that fishy aromas can be suppressed by adding sodium bisulfite (100-500 ppm) to fresh and oxidized fish through reacting with aldehydes and many oxidation ketones to form non-volatile compounds, and adding dl- [3-amino-3-carboxypropyl] dimethyl sulfonium chloride (also known as the vitamin U-chloride), through reacting with TMA [61, 62]. Thus, it is possible to mask the off-flavors by adding the masking agents; however, it should be studied how these agents are perceived when used in the food.

4. Treatment with ozone: Ozone may provide a mean to reduce or eliminate the off-flavor resulting from GSM and MIB in fresh fish fillets; although the effectiveness of this method depends on the concentration of off-flavor-generating compounds [63].

5. Removing the guts immediately after hunting and before processing: It can be an excellent method to reduce or eliminate some off-flavors created before hunting and prevent or decrease the intensity of some off-flavors after hunting [11].

6. Improving storage methods: Storage under suitable conditions (temperature, humidity, light, etc.) can prevent some off-flavors by retarding oxidation, bacterial growth, and enzymatic activities.

3. Conclusion

Fish flavor is significant in the consumers’ acceptability. Regarding the high nutritional value of fish in the human diet, investigating the off-flavor-generating factors and the methods for their prevention and elimination are of great importance. Fresh fish flavors are unstable and several off-flavors may be developed under inappropriate conditions. Different contaminants and off-flavor-generating compounds can enter the fish body through feeding, skin, or gills and induce various off-flavors. In addition, the fish provide a suitable environment for microbial growth due to its nutrient-dense nature. Therefore, a variety of pathogens and spoilage microorganisms can grow on it. Fish lipids are also predominantly unsaturated and sensitive to oxidation, which again increases the loss of this product. Microbial growth, lipid oxidation and enzymatic activities produce undesirable metabolites and change the product’s texture, flavor, appearance, and safety. Different methods can identify the off-flavors and suitable measures can be taken to prevent, reduce or remove them.

Authors’ Contributions

M.M., designed the study, administrated the project, collected the data, drafted the manuscript, and revised the manuscript. A.M.A., and N.M.M., contributed to data collection and manuscript revision. All the authors approved the final manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest.
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