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Changes in Fish During Traditional Smoking Process

Adel A El-Lahamy[1]*, Khalil I Khalil[2], Shaban A El-Sherif[1], Abdelazim SAA[3], Awad A mahmud[2] and Hassan R Mohamed[1]

[1]*Laboratory Fish Processing Technology, National Institute of Oceanography and Fisheries, Egypt*

[2]*Faculty of Agriculture, Department of Food Science and Technology, Fayoum University, Egypt.*

[3]*Food Technology Research Institute, Agricultural Research Center, Egypt*

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***Corresponding author:** Adel A El-Lahamy, Laboratory Fish Processing Technology, National Institute of Oceanography and Fisheries, Egypt

Abstract

Fish smoking is particularly relevant in the artisanal fisheries sector that prolongs the shelf life of fish, enhances flavor and increases utilization of the fish in addition to reducing waste as well as increasing protein availability to people. The review purpose was to give an overview of the effect of smoking process on the chemical composition, quality attributes and microbiological quality of smoked fish. Meanwhile, potential health hazards associated with the smoked fish such as poly cyclic aromatic hydrocarbon (PAHs). Also, changes in quality attributes and microbiological quality of smoked fish during storage periods.

Keywords: Fish smoking; Chemical composition; Quality attributes; PAHs

Introduction

Smoking method is one of the oldest methods that have been used to process and preserve food. Smoking is a traditional preservation technology that combines the effect of salting, deposition of smoke components and drying. It produces the characteristic taste and color that is much appreciated by consumers. Smoke contains many different components, such as aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols, ethers, etc. [1]. The drying effect during smoking, along with the antioxidant and antimicrobial effects of the smoke, allow smoked products to have extended shelf-life [2]. Smoking has become a mean of offering diversified, high value-added products as an additional marketing option for certain fish species where fresh consumption becomes limited [3]. Meanwhile, potential health hazards associated with the smoked foods may be caused by carcinogenic components of wood smoke. These components are mainly poly cyclic aromatic hydrocarbons (PAHs), derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amine [4]. Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. They are primarily formed by incomplete combustion or pyrolysis of organic matter and during various industrial processes [5]. Food can become contaminated by PAHs during thermal processing and cooking operations such as smoking, roasting, baking, grilling and frying [6]. Among PAHs, the benzo[a] pyrene (BaP) concentration has received particular attention due to its higher contribution to overall burden of cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food [7].

Smoking Process

Smoking is a method that utilizes smoke to introduce flavor, taste, and preservative compounds into the food. Depending upon smoking temperature; two basic types of smoking can be defined: hot smoking and cold smoking [8]. Hot smoking is the traditional smoking method using both heat and smoke, which usually occurs at temperatures above 70 °C. For smoked fish and fisheries products, a minimum thermal process at or above 145 °F (62.8 °C) is required by FDA [9]. The cold smoking that is used in the developed country only applies smoke to the product at temperature less than 90 ºF (32 °C). The protein content in this fish will not coagulate at this condition [10]. Muratore & Licciardello [11] reported that the preservative effect of smoking process is due to the presence of some antimicrobial compounds in smoke such as phenols and formaldehyde. The smoking process is often coupled with other treatments, such as salting, addition of spices, packaging techniques and chilled storage, to produce an antimicrobial effect on spoilage microorganisms and to increase shelf life [12].

Effect of Smoking Method on Chemical Composition of Fish

The moisture content of Silver Carp fillets decreased from 76.95% in raw fillets to 63.63% and 59.44% in cold and hot smoked samples, respectively. While protein, fat, ash, and carbohydrates increased from 19.44, 2.02, 1.17 and 0.23% in raw Silver carp to 26.98, 2.80, 6.36 and 0.32%, respectively in cold smoked samples, and to 29.46, 3.09, 7.64 and 0.36% (on wet weight basis), respectively in hot smoked samples [13]. Ünlüsayın et al.,

[14] investigated the effects of smoking method on the chemical compositions of several species of fish. They found that moisture content of raw European eel (*Anguilla L* 1766) decreased from 65.25% in raw samples to 56.45% after hot smoking process while protein, fat and ash contents increased from 15.16%, 16.82% and 1.25 % to 17.77%, 21.36 % and 2.30%, respectively. They also observed that moisture content of raw Pike perch (*Sander lucioperca* L kottelat 1997) decreased from 78.16% to 56.28%in hot smoked samples, while protein, fat and ash contents increased from 16.25%, 1.72% and 2.02% to 28.92%, 3.03 % and 4.47% in smoked samples, respectively. Also, the moisture content of raw Rainbow trout (*oncorhrynchus mykiss* WALBUM 1792) decreased from 74.86% to 61.20% after hot smoking process while, protein, fat and ash contents increased from 16.45%, 4.46% and 1.80% to 22.21%, 7.42% and 3.52%±0.11, respectively in smoked fish as reported by [14]. Yanar [15] found that the hot smoking method affected the chemical composition of Catfish (*Clarias gariepinus*) fillets. Protein, fat and ash contents of fresh fillets increased from 17.85%±0.12, 3.64%±0.03 and 0.68%±0.02 to 22.54%± 0.21, 7.90%± 0.03 and 4.39%±0.12, respectively in hot smoked Catfish fish fillets, while moisture content decreased from 77.89±0.17 to 65.15±0.03 in the smoked samples. Bilgin et al., [16] found that moisture, protein, fat and ash contents of Gilthead Seabream (*Sparus aurata* L., 1758) changed from 72.93%±0.28, 20.40%±0.37, 2.98%±0.12 and 1.39%±0.02%, respectively in raw sample to 60.47±0.7, 26.40±0.50, 4.54±0.40 and 3.78±0.17%%, respectively in the hot smoked sample. While, in cold smoked samples the values changed to 64.53%±1.12, 22.88% ±0.33, 3.85%±0.30 and 4.60%±0.26%, respectively. Similarly, Koral et al., [17] reported that hot smoking process resulted in decreasing moisture content of Garfish from 72.05%±0.47 in fresh sample to 60.56% ±0.09 in the hot smoked sample. On the other hand, protein, fat and ash contents increased from 21.53% ±0.33, 2.96%±0.32 and 2.37% ±0.1947 to 26.29±0.42, 5.08±0.53 and 4.02±0.32 in the hot smoked sample, respectively. El-Lahamy et al., [18] found that the gross chemical composition of fresh Catfish was affected by smoking operation, the results showed that moisture, protein, fat and ash contents of fresh catfish were 75.95±0.259, 16.90±0.173, 5.65±0.086, 1.22±0.069 and 0.28±0.202 %, respectively. These values changed to 54.60±.086, 28.60±.057, 9.62±.040, 6.83±.132 and 0.35±.028 in hot smoked fillets, respectively while their contents in cold smoked samples were 58.86±.577, 26.00±.051, 8.55±.086, 6.15±.086 and 0.44±.115, respectively. These data could be due to the loss of water during smoking process, consequently dry matters were increased.

Effect of Smoking Method on Physicochemical Quality Criteria of Fish

Total volatile basic nitrogen (TVB-N)

TVB-N content of smoked products may be changed according to the quality of raw material, storage conditions, brine concentration, smoking conditions and packaging material [19]. Abd El-Mageed [13] observed that total volatile basic nitrogen (TVB-N) content of Silver Carp fillets decreased from 36.21mg/100g in raw fillet to 30.79 and 21.52mg/100g (on dry weight basis) in cold and hot smoked fish products, respectively. On the contrary of this observation, several studies indicated that smoking process resulted in increasing the total volatile basic nitrogen content of fish. Yanar [15] found that the total volatile basic nitrogen (TVB-N) of raw Catfish *(Clarias gariepinus)* fillets increased from 15.47±0.22mg/100g to 17.67±0.81mg/100g in the hot smoked product. Bilgin et al., [16] reported that the total volatile basic nitrogen (TVB-N) of raw Gilthead Seabream (*Sparus aurata L.*, 1758) increased from 14.28±0.57 mg/100g to 16.307±0.56 and 19.807 ± 0.41mg/100g in the hot and cold smoked products, respectively. Koral et al., [17] studied the effect of smoking process on the quality criteria of Garfish and stated that the total volatile basic nitrogen (TVB-N) of raw fish increased from 9.81±0.12mg/100g to 10.48±0.07mg/100g in the hot smoked sample. Recently, Abo-Taleb et al., [20] reported that the total volatile basic nitrogen (TVB-N) of the hot and cold smoked Silver Carp fillets after smoking process immediately were 13.81 and 15.56 mg/100g sample respectively. More recently, Huong [21] studied the effect of smoking methods (liquid and cold) on the TVB-N of Mackerel fish fillets and observed that the TVB-N in the liquid smoked group significantly decreased from the initial value of 15.7±0.57mg/100g to 9.5±0.99mg/100g while, in the cold smoked sample, TVB-N was slightly decreased to 15±0.57mg/100g. El-Lahamy [22] indicated that TVB-N significantly ($P<0.05$) increased in the smoked Catfish samples in comparison with fresh unsmoked fish. It was found that TVB-N values in fresh, hot and cold smoked samples were 13.77±0.098, 17.80±0.173 and 18.95±0.202mg/100g, respectively.

Thiobarbituric acid (TBA)

The thiobarbituric acid (TBA) increased from 0.56 in raw Silver Carp fillet to 0.65 and 0.73mg/kg (on dry weight basis) in the cold and hot smoked fillets, respectively [13]. Thiobarbituric acid (TBA) value of raw Catfish (*Clarias gariepinus*) flesh determined by 0.45±0.04 mg malonaldehyde/kg increased to 0.84± 0.03 after hot smoking process [15]. Bilgin et al., [16] reported that the tiobarbituric acid (TBA) of raw Gilthead Seabream (*Sparusaurata* L., 1758) was 0.594±0.04mg malonaldehyde/kg and this value increased to 1.027±0.11 and 0.834±0.031mg malonaldehyde/kg in the hot and cold smoked samples, respectively. Koral et al., [17] found that the thiobarbituric acid (TBA) value of raw Garfish increased from 0.66±0.04 mg malonaldehyde /kg to 0.84±0.04 in the smoked product. Abo-Taleb et al., [20] found that thiobarbituric acid (TBA) increased after smoking and the TBA values determined by 0.63 and 0.60 mg malonaldehyde /kg for the hot and cold smoked Silver carp fillets. The thiobarbituric (TBA) values for raw Catfish fillets significantly ($P<0.05$) increased from 0.23±0.017 to 0.44±0.023 and 0.29±0.011 mg malonaldehyde/kg in hot and cold smoked samples, respectively [22].

pH value

Etman [23] indicated that there was negligible and slight decrease in pH value of Mirror carp fish flesh after cold smoking

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resulted from absorption of some organic acids from the smoke by fish flesh during smoking process. Also, Hammad reported that hot smoking of Eel fish led to a decrease of pH value by absorption of some acid constituents from the wood smoke during hot smoking process. Abd El- Mageed [13] studied the effect of smoking process on the quality attributes of Silver carp fillets and observed that pH value of fresh samples decreased from 6.70 in fresh sample to 5.52 and 5.64 in the hot and cold smoked fillets, respectively. Yanar [15] reported that pH value of fresh Catfish flesh (*Clarias gariepinus*) decreased from 6.78±0.01 to 6.74±0.19 in the smoked Catfish. Bilgin et al., [16] observed that pH value of raw Gilthead Seabream fish (*Sparus aurata* L., 1758) slightly increased from 6.19±0.04 to 6.39±0.01and 6.34±0.16 in the hot and cold smoked products, respectively. Abo-Taleb et al., [20] reported that pH value of Silver carp fillets decreased immediately after smoking process and the decreasing was much higher in cold smoked samples than in hot smoked samples. This decrease of pH value had been attributed to absorption of high amount of some organic acids from smoke during the long time of cold smoking process. Physiochemical analysis indicated that pH value of raw Catfish fillets was 6.40±0.086 while hot and cold Catfish fillets showed pH values of 6.10±0.046 and 6.20±0.011, respectively [18].

Microbiological Aspects of Smoked Fish

Effect of smoking method on the total bacterial count (TBC) in fish was studied. Abd El-Mageed [13] observed that the total bacterial count (TBC) of fresh Silver carp fillets was 12 ×103cell/g (4.07 log10cfu/g) and decreased to 6×103cell/g (3.77 log10 cfu/g) and 4×103cell/g (3.60 log10 cfu/g) in the cold and hot smoked fillets, respectively. Yanar [15] found that the total viable count (TVC) of fresh Catfish (*Clarias gariepinus*) decreased from 5.00±0.42 log10cfu/g, to1.20±0.27 log10cfu/g immediately after hot smoking. Bilgin et al., [16] reported that the total bacterial count (TBC) of raw Gilthead Seabream (*Sparus aurata* L., 1758) decreased from 9.033±0.12 (log cfu/g), to 2.283±0.03 and 3.287 ±0.02 in the hot and cold smoked samples, respectively. Omojowo et al., [24] found that smoking process resulted in decreasing the TVC of raw Catfish (*Clarias gariepinus*) fillets from 5.50 log10cfu/g to 3.48 log10cfu/g. Abo-Taleb et al., [20] observed that total bacterial count (TBC) of hot smoked Silver carp fillets was lower as compared with cold smoked samples and attributed this observation to the effect of smoke constituents, which had an antimicrobial effect, in addition to heating and dehydration through hot smoking process. Huong [21] studied the effect of smoking methods (liquid and cold) on the total bacterial count (TBC) of Mackerel fish and reported that the initial TBC of raw material decreased from 3.65 (log10 cfu/g) to 2. 23 log10 cfu/g after liquid smoking and to 3.33 log cfu/g for the cold smoked samples. The effect of smoking process of fish on yeast and mold counts was also reported. Abd El-Mageed [13] investigated the effect of smoking process (hot and cold) on the total count of yeast and mold of Sliver carp fillets and noticed that the yeast and mold count of fresh silver carp fillets was 2.6×103 cell/g (2.41 log10 cfu/g) while, after hot and cold smoking process yeasts and molds were not detected in the smoked fish samples. Bilgin et al., [16] observed that the yeast and mold were not detected in raw and smoked Gilthead Seabream (*Sparus aurata* L., 1758). Idris et al., [25] studied the effect of smoking process on the fungi count of Catfish (*Clarias gariepinus*) and reported that fungi count of fresh Catfish ranged between 1.65 to 2.28 Cfu/g log10 and it was decreased in the smoked samples to the range of log 0.70 to 1.35cfu/g sample. Omojowo et al., [24] revealed that the fungi count of Catfish (*Clarias gariepinus*) reduced from 4.21 log10 cfu/g of raw sample to 2.0 log10cfu/g after smoking process. Abo-Taleb et al., [20] determined the yeast and mold counts after smoking process immediately and found that the molds and yeasts were not detected in hot and cold smoked Carp fillet. Microbiological examination showed that TBC of fresh raw Catfish samples decreased from 4.49±0.051 to 3.07±0.040 and 3.23±0.161 logcfu/g, while yeast and mold count decreased from 2.30±0.069 to 1.0±0.040 and 1.11± 0.057 for hot and cold smoked, respectively [26].

Polycyclic Aromatic Hydrocarbons (PAHs) in Smoked Fish

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. PAHs are generally occurred in complex mixtures of hundreds of compounds. PAHs may be formed in three ways: processing at high temperature (700 °C), pyrolysis of organic materials at low to moderate temperature (for example, 100 to 150 °C) and digenesis of organic materials by microorganisms [27]. Diet is the major source of human exposure to PAHs as it accounts about 88 to 98% of such contamination [28]. PAHs are primarily formed in food by incomplete combustion or pyrolysis of the organic matter during food processing and cooking operations that are carried out at high temperatures such as drying, smoking, roasting, grilling, frying and baking [5,6,29]. The amount of PAHs formed during smoking fish depends mostly on the conditions of smoking process such as; heat source (coal, wood, gas), the type and composition of wood, the type of generator (internal or external), the oxygen accessibility, the temperature of smoke generation, the flame intensity in flame combustion and the time and temperature of smoking [30,31]. In the traditional smoking, smoke is generated at the bottom of an oven and the food is placed directly over the smoking wood which results in the contamination with PAHs if the process is not adequately controlled or if very intense smoke is used. However, in the modern industrial smoking ovens, smoke is generated in a separate chamber and fed into the smoking chamber where the products are placed; therefore, better control of the smoking process is achieved [32,33].

Chemical Structure, Physicochemical Properties and Toxicology of Polycyclic Aromatic Hydrocarbons

According to the United States Environmental Protection Agency [34], sixteen compounds of the PAHs have been identified as priority pollutants due to to their mutagenic and carcinogenic properties. They include: naphthalene, acenaphthylene, benzo[b]

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fluoranthene, phenanthrene, dibenzo[a,h]anthracene, chrysene, benzo[a]pyrene, acenaphthene, benzo[k]fluoranthene, fluorene, pyrene, benzo[a]anthracene, anthracene, fluoranthene, indeno [1,2,3-cd]pyrene, and benzo[g,h,i]perylene. The PAHs that contain up to four fused benzene rings are known as light PAHs and those containing more than four benzene rings are called heavy PAHs. The heavy PAHs are more stable and more toxic than light ones [35]. Also, the light PAHs are more volatile, water soluble, and less lipophilic than the heavy PAHs. Consequently, PAHs migrate through the food product into the hydrophobic compartments and thus accumulate in the lipid components due to their lipophilic nature [36]. Neff [27] reported that PAHs contained four, five and six rings are more carcinogenic than PAHs with the smaller or larger ring systems and the highly angular configurations tend to be more carcinogenic than the linear ring systems. PAH4 are the sum of Benzo(a) Pyrene, chrysene, benz [a] anthracene and benzo [b] fluoranthene, and PAH8 are the sum of Benzo (a) anthracene, Chrysene, Dibenzo (a, h) anthracene, Benzo (g, h, i) perylene, Benzo (b) Fluoranthene, Benzo (k) fluoranthene, Benzo(a) Pyrene and Indeno (1, 2, 3-c, d) Pyrene) [5]. Among the PAHs, benzo[a] pyrene (BaP) has received a particular attention due to its higher contribution to the overall burden of cancer in human and is used as a marker for the occurrence of carcinogenic PAHs in food [5,7,37,38]. Meanwhile, the European Food Safety Authority [5] concluded that (Bap) alone is not a suitable indicator for the toxicity of PAHs in food and that eight specified PAHs (PAH8), for which oral carcinogenicity data are available, and/or a subgroup of these, PAH4, are more suitable markers. Recently, OJEU [39] reported that the maximum levels for the sum of four substances included; benzo (a) pyrene, benz (a) anthracene, benzo (b) fluoranthene and chrysene should be introduced, whilst maintaining a separate maximum level for benzo (a) pyrene. Moreover, Silva et al., [29] stated that the low molecular weight PAHs such as naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene are not regarded as very carcinogenic. PAHs are present in the environment; in water, air, soil and traces of these substances have been found in the various food products [5,29]. El-Lahamy et al., [40] found that Benz (a) anthracene and chrysene (PAH4) were found in the cold smoked fish sample with concentration as low as only 10.7µg/kg, while these compounds could not detected in the hot smoked fillets. This level (10.7µg/kg) is below than the maximum tolerable risk limit (12µg/kg). Also, he stated that Sum of toxic equivalency BaPeq (TEQ) was recorded a significant higher ($P \leq 0.05$) value for cold smoked Catfish fillets than hot smoked fillets.

Levels of (PAHs) in Smoked Fish Products

The potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke; mainly PAHs, derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amine [4]. The maximum tolerable risk limits of (PAH4) and (benzo(a)pyrene were 30.0 and 5.0µg/kg respectively, for muscle meat of smoked fish [41]. Meanwhile, lower maximum tolerable risk limits of (PAH4) and (benzo (a) pyrene) determined by 12.0 and 2.0µg/kg, respectively, for muscle meat of smoked fish were recently reported by OJEU [39]. Basak et al., [31] determined the PAHs in smoked Salmon, Rainbow trout and found that the amounts of the low molecular weight PAHs were higher than the high molecular weight PAHs in the samples. However, as the high molecular weight PAHs are more carcinogenic at lower levels, the tolerable limits of these compounds should also be established by food codex regulations. Silva et al., [29] found that the concentrations of total PAHs in smoked Catfish (Arius heude loti), Sole (*Cynoglossus senegalensis*) and Haake were 2058.1, 1395.2 and 856.2µg/kg, respectively when the sawdust was used as a source of fuel and were 1320.9, 1257.2 and 780.8µg/kg, respectively for the fire wood as a source of fuel. They also observed that Benzo (a) pyrene was not detected in both the oven dried and in the smoked Catfish (Arius heude loti) for the sawdust as a source of fuel. Meanwhile, Mihalca et al., [42] reported that the levels of benzo[a]pyrene of smoked Rainbow trout and Brook trout fish were 8.4 and 0.4 µg/kg, respectively. Mičulis et al., [43] determined the levels of PAH4 in industrial and traditional smoked fish products and found that the PAH4 contents in Herring, Sprats in oil, Scomber, Hake, Smelt, Cod, Salmon and Trout were 20.20, 18.14, 16.11, 15.30, 8.40, 7.05, 4.34 and 3.80µg/kg, respectively. Ongwech et al., [44] determined that the levels of PAHs in three smoked samples of (*Lates niloticus*) fish and found that 9 compounds of the PAHs were detected in the smoked fish samples and their total concentrations ranged between 23.40 to 58.10µg/kg. Benzo (a) Pyrene equivalency (BaPeq) and toxic equivalency (TEQ) concentrations in three samples of smoked fish (*Lates niloticus*) from three markets ranged between 1.731 and 3.86ug/kg while the concentrations of PAH4 in the same samples were 8.14 - 11.00µg/kg. Polycyclic aromatic hydrocarbon (PAHs) detection of smoked Catfish fillets indicated that 7 compounds of PAHs; acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene were detected in both cold and hot smoked fish samples, while benzo (a) anthracene and chrysene were detected only in cold smoked samples. Meanwhile the total PAHs in the smoked fillets were determined by 606.1 and 180.69µg/kg for the cold and hot smoked samples, respectively [40]. Recently, Hafez et al., [45] investigated the effect of cold smoking method on the levels of polycyclic aromatic hydrocarbon in Mullet fish obtained from two farms in Qarun Lake and they revealed that the Benzo (a) pyrene compound that is carcinogenic indicator was not detected.

Storage Stability of Smoked Fish Products During Cold Storage

Physiochemical quality attributes

Total volatile basic nitrogen (TVB-N): Total volatile basic nitrogen (TVB-N) is one of the most important criteria in the quality evaluation of fish products. Several studies were carried out to follow up quality changes in smokes fish products during storage. Abd El- Mageed [13] observed that the total volatile basic nitrogen (TVB-N) content of hot and cold smoked Silver carp fillets gradually increased during refrigeration storage. Kolsarici

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& Özkaya [46] found that the initial TVB-N value of hot smoked Rainbow trout was 18.55mg/100g; and this value increased to 32.72mg/100g after 48 days of refrigerated storage. Also, Yanar [15] reported that the initial TVB-N value of hot smoked Catfish increased from 17.67mg/100 g to 29.16 mg/100 g after refrigerated storage for 24 days. Bilgin et al., [16] determined the total volatile basic nitrogen (TVB-N) of hot and cold smoked Sea bream fish during cold storage at 4.0 ˚C. The results showed that TVB-N of cold smoked samples increased from 16.307±0.56 at zero time to 33.307±0.47 after 60 days of storage while in hot smoked samples TVB-N increased from 19.807±0.41mg/100g to 40.790±0.51mg/100g under the same storage conditions. Koral et al., [17] studied the quality criteria of smoked Garfish and stated that the initial value of TVB-N increased from 11.21±0.40 mg/100 g to 37.47±0.35 after refrigerated storage for 25 days. Abo-Taleb et al., [20] followed the changes in quality criteria in cold and hot smoked Carp fish fillets during cold storage at 4.0±1.0 ºC by determining TVB-N values. The results indicated that TVB-N values of cold and hot smoked samples determined by 15.56 and 13.81mg/100g (on wet weight basis), respectively increased up to 29.21 and 27.84mg/100g, respectively at the end of 60 days storage. This increase might be attributed to the effect of microorganisms as well as autolysis processes. Daramola et al., [47] found that the total volatile basic nitrogen in hot-smoked *Clarias gariepinus* samples increased with storage time for 6 week from13.65 to 31.52mg/100g. Frank et al., [48] studied the quality criteria of smoked Silver carp (*Hypophthalmichthys* molitrix) fillets during chilled storage at 4.0 ˚C and found that the TVB-N increased from 7.22mg/100g at day 0 to 22.8mg/100 at the end of storage period of 24 day. El-Lahamy [22] indicated that the initial values of TVB-N of hot and cold smoked Catfish fillets were determined by 16.8±0.173 and 18.95±0.202mg/100g, respectively. At the end of 40 days storage, TVB-N values of hot and cold smoked samples were determined by 30.17±0.479 and 32.2±0.173 mg/100g, respectively.

Thiobarbituric acid values (TBA): Thiobarbituric acid (TBA) is widely used as an indicator of lipid oxidation, and the presence of TBA reactive substances is due to the second stage of autooxidation [15,49]. Thiobarbituric acid (TBA) contents of hot and cold smoked Silver carp fillets brined in 10% NaCl solution was elevated during refrigeration storage [13]. Yanar [15] found that the TBA value of hot smoked Catfish (*Clarias gariepinus*) increased from 0.84±0.03mg malonaldehyde /kg at zero time of refrigerated storage to 2.67±0.62 at the end of 24 days storage. Bilgin et al., [16] reported that the initial value of tiobarbituric acid of hot and cold smoked Gilthead Seabream (*Sparusaurata* L., 1758) were 1.027±0.11 and 0.834±0.031 malonaldehyde/kg, respectively and these values increased to 2.517±0.44 and 3.883 ±0.08mg malonaldehyde /kg after 60 days of storage at 4.0 ˚C. Koral et al., [17] studied the effect of storage on the quality criteria of Garfish during refrigerated storage and stated that the initial value of TBA increased from 0.90±0.02mg malonaldehyde/kg to 2.98±0.05mg malonaldehyde /kg at the end of 25 days storage. Abo-Taleb et al., [20] followed changes in the quality attributes in cold and hot smoked Carp fillets during cold storage. TBA values increased gradually as the time of cold storage increased. The increasing rate of TBA value was higher in the hot smoked fish products than in the cold smoked samples. TBA values increased from 0.63 and 0.60mg malonaldehyde /kg before storage to 2.12 and 1.92mg malonaldehyde /kg after 60 days in cold and hot smoked fillets, respectively. Frank et al., [48] studied the quality criteria of smoked Silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage at 4.0 ˚C and found that the TBA value increased from 0.23mg malonaldehyde /kg at day zero to 1.56mg malonaldehyde /kg at the end of storage period for 24 day. The initial TBA values of hot and cold smoked Catfish fillets samples were 0.44±0.023 and 0.29±0.011mg malonaldhyde /kg, respectively. At the end of storage period (40 days), TBA values of hot and cold smoked fillets increased up to 2.41±0.173 and 2.26±0.115mg malonaldhyde /kg, respectively [22].

pH value: pH value has been used for the evaluation of fish freshness and as an index of the quality of fish products [50]. The increase in pH value during storage could be used as a sign for the deterioration and spoilage of fish and fish products [51]. Abd El-Mageed [13] mentioned that the pH value of hot and cold smoked Silver carp fillets showed continues but slight increase during refrigeration storage. Yanar [15] studied the changes in pH value of hot smoked Catfish (*Clarias gariepinus*) during refrigerated storage and stated that the pH changed from the initial value of 6.74 to 6.65 and 6.81 after 19 and 24 days of storage, respectively. Bilgin et al., [17] followed the changes in pH value of hot and cold smoked Sea bream fish during cold storage at 4.0 °C and no significant differences could be observed during storage. The pH value of the smoked fish samples increased slightly from 6.39± 0.01and 6.34±0.16 at zero time to 6.56±0.01and 6.52±0.01 in hot and cold smoked samples, respectively after 60 days of storage. Abo-Taleb et al., [20] observed that pH value of smoked Carp fish fillets gradually and slightly increased during storage from 5.27 and 5.98 before storage to 6.85 and 6.55 in the cold and hot smoked fillet at the end of 60 days of cold storage, respectively. Daramola et al. [47] reported that the biochemical parameters increased with storage time in the hot-smoked *Clarias gariepinus* samples and the pH value ranged between 6.36 - 6.69 during cold storage of 6 weeks. Frank et al., [48] studied the quality criteria of smoked Silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage at 4.0 ˚C and found that the pH value was 6.07±0.5 at day zero and this value increased to 6.52 ± 0.12 at the end of storage period of 24 day. The initial pH values for hot and cold smoked samples were almost the same (6.10±0.046 and 6.20±0.011). These initial values slightly increased up to 6.22±0.034 and 6.44±0.046 for hot and cold smoked samples, respectively at the end of 40 days of refrigeration storage [18].

Microbiological Aspects

ICMSF [52] reported that the changes in the microbial population and storage instability depend on fish type, smoking method,

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duration of smoking and the post-storage conditions. The smoke generated during smoking process contains several antibacterial components like formaldehyde and phenols. Moreover, heat of smoking process results in reducing the water activity of fish resulting in microbial destruction and better preservation [53].

Total Bacterial Count (TBC)

Total bacterial count (TBC) is an important criterion for quality evaluation of processed fish products. The maximum recommended bacterial count for good quality products is 5.7 log-10cfu/g, while the maximum recommended bacterial count for marginally acceptable quality products is 7.0 log10cfu/g [52]. Kolodziejska et al., [54] reported that the initial TVC of hot smoked Mackerel fish increased from 1.6 log10cfu/g before storage to 4.7 log10cfu/g after 21 days of storage at 8.0 ºC. Yanar [15] studied the microbiological changes in hot smoked Catfish during refrigerated storage and reported that the total viable count (TVC) increased exponentially with storage time. At day 6 of storage, TVC was 3.11 log10cfu/g and increased to 4.36 log10cfu/g at day 12. The microbial load sharply increased on the 19th day of storage and reached to 7.25 log10cfu/g at the end of 24 days of storage. Bilgin et al., [16] reported that the initial total bacterial counts of hot and cold smoked Gilthead Sea bream fish (*Sparusaurata* L., 1758) were 2.283±0.03 and 3.287±0.02 log10cfu/g and increased to 6.363±0.07 and 6.903 ± 0.02 in after 60 days of storage at 4.0 °C, respectively. Abo-Taleb et al., [20] found that (TBC) of hot and cold smoked Silver carp fillet were 3.61 and 3.75 log10cfu/g at zero time, respectively. TBC of the hot and cold smoked samples increased during cold storage up to 3.78 and 4.90 log10cfu/g, respectively at the end of 60 days storage. Daramola et al., [55] showed that the total viable count (TVC) of smoked Catfish (with 10% salt) increased from 2.7 x 106 cfu/g at the beginning of storage to 4.9 x 106cfu/g at the end of 6 weeks of storage. Huong [21] studied the effect of cold storage on the total bacterial count of liquid and cold smoked Mackerel fish and found that after one week of storage at -1.0 °C, the difference was not significant between the two groups. However, when storage temperature increased to 4.0 - 5.0 °C, the total plate count of the liquid smoked group increased sharply to 7.33 logcfu/g after two weeks and continued higher than the wood smoked sample until four weeks of storage. Frank et al., [48] studied the microbiological aspects of smoked Silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage at 4.0 ˚C and found that the total viable count was 2.26 ±0.04 log10 cfu /g at day 0 and increased to 6.91±0.27 log10cfu/g at the end of storage period of 24 day. El-Lahamy et al., [26] found that the microbiological examination of smoked Catfish fillets indicated that the initial total bacterial counts for hot and cold smoked Catfish fillets were 3.07±0.040 and 3.38±0.161 logcfu/g, respectively. These initial values gradually increased ($p < 0.05$) up to 5.60±0.115 and 5.84±0.057 log10cfu/g, respectively at the end of 40 days of refrigeration storage.

Yeast and Mold Count

Bilgin et al., [16] observed that the yeast-mold count of hot and cold smoked Gilthead Sea bream (*Sparusaurata* L., 1758) were not detected during storage period at 4.0 °C over 60 days of storage. Afterward, the yeast and mold counts were 2.557±0.01 and 2.340±0.01 in hot and cold smoked Sea bream, respectively. Idris et al., [25] found that the fungi count of smoked Catfish was 2.30±0.03 log10cfu/g at before storage and this value increased to 6.02±0.06 log10cfu/g at the end of 8 weeks storage. During refrigeration storage at 4.0±1.0 ºC of hot and cold smoked Silver carp fish fillets, Abo-Taleb et al., [20] observed that mold and yeast appeared in the cold smoked products after 45 days of storage and remained up to the end of storage period while in the hot smoked samples mold and yeast were detected after 60 days of storage. Daramola et al., [55] showed that molds of smoked Catfish (with 10% salt) increased from 2.5 x 106Cfu/g at the beginning of storage to 4.9x 106cfu/g at the end of 6 week. The results showed that the initial counts of mold and yeast in the hot and cold smoked samples increased ($p < 0.05$) from 1.00± 0.040 and 1.11±0.057 log10cfu/g to 4.75±0.040 and 4.5±0.028 log10cfu/g, respectively at the end of 40 days of refrigeration storage [26].

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