

Quality and Stability of Liquid Smoked Kapreeta Fish During Refrigerated Storage

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ABSTRACT: In Egypt, Kapreeta fish (like tuna) as marine fish, is not appreciated among Egyptian consumers because it is bloody dark flesh and presence of many blood vessels. Therefore, this kind of fish must be utilized as fish products through different preservation methods as canning and smoking.

The aim of this study is to investigate the effects of liquid smoking and essential oils prior smoking on the quality of smoked kapreeta fish fillets just after smoking and during refrigerated storage up to 90 days. Proximate composition was determined in fresh and smoked fish fillets. Chemical, microbiological and sensory analyses of the samples as well as fatty acids composition of fresh and smoked kapreeta fish fillets were carried out during the storage to test their quality and lipid stability. Moisture content decreased while protein, lipid and ash increased after smoking. The pH values were slightly increased by storage time. Total volatile basic nitrogen, trimethyl amine nitrogen, peroxide values, thiobarbituric acid, free fatty acids and total viable count values were increased more in untreated fish samples than smoked fish samples treated with thyme or sage essential oils, while sensory scores decreased during storage. The total polyunsaturated fatty acids content was 35.14% of total fatty acids in untreated smoked fish and 35.80% in treated samples with 5% thyme oil and 36.14% in treated samples with 5% sage oil prior to smoking, with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) being the prominent polyunsaturated fatty acids. The decrease of PUFAs may indicate the oxidation of these unsaturated fatty acids during storage. Such changes were not observed in sage or thyme extracts treated lots.

According to the results of physicochemical, microbiological and sensory analyses, it was found that untreated smoked kapreeta fish samples were in high quality and lipid stability for 30 days under refrigerated storage and smoked kapreeta fish samples treated with 5% thyme oil or sage oil were in high quality till 60 and 75 days under refrigerated storage. It can be concluded that kapreeta fish fillets treated with sage and thyme essential oils prior to liquid smoking have positive effect on quality and shelf life of smoked fish as well as induced the stability of fatty acids profiles without altering their composition and minimize oxidation.

Key words: Kapreeta fish, quality, shelf life, smoked fish, essential oils, Chemical and microbiological analyses, sensory evaluation

INTRODUCTION

Fish constitutes a very important component of diet for many people, and often provides much needed nutrients for human health. Fish serves as a principal source of dietary protein, which is very inexpensive in relation to other protein foods (Fawole *et al.*, 2007).

Fish is known to contain high quality of lipids. The long chain polyunsaturated fatty acids (PUFAs), especially the n-3 PUFAs family including eicosapentaenoic acid (EPA or 20:5 n-3), docosapentaenoic acid (DHA or 22:6 n-3) present in marine lipids have beneficial health effects (Osman *et al.*, 2001, Boran *et al.*, 2006, Stołyhwo *et al.*, 2006 and Karlsdottir *et al.*, 2014).

Fresh fish have soft tissues and high amount of water and this enhances its susceptibility to microbial contamination (Olayemi *et al.*, 2012). So that fresh fish is highly perishable and various preservation techniques such as chilling, freezing, drying, salting, and smoking have been used universally to extend

shelf life. In developing countries, the most affordable and widely used fish preservation methods are drying and smoking (Oduor-Odote *et al.*, 2010a,b and Darvishi *et al.*, 2013).

Smoking of fish is one of the most ancient processing technology and one of many different preservation methods. Smoking is commonly carried out at temperatures of 70–80°C (Marc *et al.*, 1997 and Erkan *et al.*, 2011). In contrast, cold smoking is achieved without thermal treatment usually at temperature $\leq 30^{\circ}\text{C}$ (Goulas and Kontominas, 2005). Smoked fish products are commonly salted. The use of salt is essential to complement the bacterial inhibitory effect of smoke by reducing water activity. For health and acceptability reasons, the practice is to have products with low salt content. Nevertheless, one of the problems faced in smoking process is no standard process implemented yet so that the quality of smoked fish produced can change. Different smoking temperature, different smoking duration, different number of smoking materials, different quality of smoking material, and different water content have caused different quality of smoked fish produced affecting the consumer's demand level (Oduor-Odote *et al.*, 2010a,b and Salindeho and Mamujaja, 2015)

Liquid smoke is a natural smoke from plant based material which has been condensed into a liquid and then refined to remove certain toxic compounds from it. Liquid smoke is used in several applications on meat and fish to impart the flavour, colour and preservative characteristics of natural smoke devoid of the toxic tar compounds (Varlet *et al.*, 2010). These may be used to preserve quality and ensure safety of foods (Martin *et al.*, 2010). The use of liquid smoke has several advantages over traditional smoking procedures having no detectable levels of benzo α -pyrene and no mutagenic activity. Liquid smoke performs all the desired functions, allows more rigid flavor control and has the added advantages of lowering costs, less environmental damage and greater availability and variety of application methods (Dillon *et al.*, 1994). The liquid smoke is applied on the food by either marinating, drenching, spraying or injecting the food with the liquid smoke before heat processing. The liquid smoke can also be applied to the food through liquid smoke coatings or liquid smoke nets (Jenkins, 2010). The use of liquid smoke has been shown to be four to five times more efficient in converting wood biomass to useable smoke than the traditional smoking process (Red Arrow, 2014).

Although smoking increases shelf life of the fish products, hygienic standards of the fish products before, during and after smoking are suspects. However, investigations have shown the presence of microbial contaminants even on smoked fish (Nyarko *et al.*, 2011). Most of the post processing microbial contaminants such as bacteria and fungi originate from poor handling practices while some could be from the air, the source of the fish, or from other degrading substances.

Oxidation of lipids that occurs in fish during processing, heat treatment, and in the final products during subsequent storage, is one of the basic processes causing rancidity in fish products (Donelli and Robinson 1995). Such oxidative deterioration may affect on the quality and the organoleptic

characteristics, including taste and aroma making the final product unacceptable for consumption. Therefore, several investigations have been undertaken with the aim to enhance the shelf-life extension, the stability of lipid containing products and food quality.

The use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has been very effective in controlling rancidity (Frankel, 1993 and Karpinska *et al.* 2001). However, synthetic antioxidants have frequently been associated with certain health problems (Thompson and Trush, 1986 and Siripongvutikorn *et al.*, 2009). This has necessitated the use of natural antioxidants, such as spices, herbs, and vegetable extracts in the prevention of rancidity in chilled and smoked fish (Kahkonen *et al.*, 1999 and Patrick-Iwuanyanwu *et al.*, 2007) due to their antioxidant and antimicrobial properties (Akhtar *et al.* 1998 and Abdel-Hamied *et al.*, 2009). The spices, notably the Lamiaceae family, are well known for their antioxidative properties, especially rosemary, sage and thyme, which have been reported to have strong such characteristics (Aruoma *et al.*, 1992, Al-Flailih, 2009).

Thyme has been commonly used as a spice to add flavor to food (Maksimovic *et al.*, 2008). The phenolics monoterpenes, thymol and carvacrol are the main compounds identified with significant antioxidant and antibacterial activities (Lacroix *et al.* 1997 and Viuda-Martos *et al.*, 2008). Sage extracts have exhibited potent antioxidant activity and are widely used in the food industry. The antioxidant activity of sage extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol and rosmarinic acid which break free radical chain reactions by hydrogen atom donation (Basaga *et al.*, 1997). A number of researchers have reported the effectiveness of sage extracts for achieving higher sensory scores and retarding lipid oxidation in various foods (Stoick *et al.*, 1991 and Zaki, 2010).

In Egypt, Kapreeta fish (like tuna) as marine fish, is not appreciated among Egyptian consumers because it is bloody dark flesh and presence of many blood vessels. Therefore, this kind of fish must be utilized as fish products through different preservation methods such as canning and smoking (Abou Tor, 2002 a,b and Korish *et al.*, 2008).

The objective of the present work was to study the effect of liquid smoking on the quality and lipid stability of Kapreeta fish fillets treated with essential oils of sage and thyme as natural antioxidants during refrigerated storage.

MATERIALS AND METHODS

Materials

Kapreeta fish (*Scombromous* spp.) like Tuna was purchased from the Max Bay, Alexandria during the summer season of 2015. Fish samples (six fish with 30 kg) transported in iceboxes to fish Processing and Technology laboratory, National Institute of Oceanography and fisheries, Alexandria.

Sensory Evaluation of raw fish included eye, gill, belly and texture were carried out.

Liquid smoke composition was 90% water, 2% phenols, 4% acids, 3% carbonyl and 1% tar.

All chemicals used for analysis were of analytical grade.

Methods

1. Preparation of fish samples

Fish samples were washed with tap water, beheaded, eviscerated, skinned and filleted. After traces of blood were removed by tap water, one hundred and twenty (120) fillet samples were prepared as steaks with the dimension of 10 cm (length), 5 cm (width) and 1.5 cm (thickness). The weight of each fillet sample was 135 ± 5 g.

2. Extraction of essential oils

Fresh thyme and sage obtained from Siwa Oasis were properly cleaned, washed and ground. Essential oils were extracted by hydrodistillation procedure as described by Balbaa *et al.* (1981) and modified by Iheagwara, (2013). The essential oils were dried over anhydrous sodium sulphate and stored at 5 °C for further analysis.

3. Liquid smoking of fish fillet samples and storage

The cleaned, brined and spiced fish samples were soaked for 30 min in 1000 ml of diluted liquid smoking solution (300 ml concentrated liquid smoke + 700 ml water in rectangle glass Jar) containing 0.0% (control), 5.0% and 10.0% of thyme essential oil (60 fish fillet samples) or sage oil at the same concentrations (60 fish fillet samples) with 10% salt. Hot smoking was carried out with liquid smoke, essential oils and salt at a temperature of 80 ± 2 °C for 3 hours. Samples were stored at refrigerated temperature (5 ± 1 °C) for three months and samples were analyzed every 15 days. Chemical and microbial analysis and organoleptic evaluation were determined.

4. Physico-Chemical Analysis

1. Salt Content

Sodium chloride content in smoked fish samples was determined by volumetric method of Volhard (AOAC, 1990).

2. Determination of pH

pH value was estimated according to Goulas and Kontominas (2005) as follows. Ten g of sample was homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a pH meter (HANNA pH213) at ambient temperature.

3. Proximate Composition

Proximate composition included moisture, protein, fat and ash contents of the fresh and smoked fish samples were determined using the standard methods of AOAC (2007).

4. Determination of Total Volatile Basic Nitrogen (TVB-N)

Total Volatile Base Nitrogen (TVB-N) value was estimated by the semi-micro distillation procedure (AMC, 1979; Kirk and Sawyer, 1991). The bases are steam distilled into standard acid and back-titration with standard alkali.

5. Determination of Trimethylamine Nitrogen

Trimethylamine Nitrogen (TMAN) was determined using the above mentioned TVBN method after appropriate modification: formaldehyde was used to block the primary and secondary amines (AMC, 1979).

6. Extraction of Lipids

Lipid was extracted from the mixed smoked fish samples with a mixture of chloroform / methanol (2: 1 v/v) according to the method described by Folch *et al.* (1957).

7. Determination of 2-Thiobarbituric Acid (TBA)

2-Thiobarbituric acid (TBA) value of smoked fish samples was determined colorimetrically by using the method published by Kirk and Sawyer (1991).

8. Determination of Peroxide Value

Peroxide value (PV) was expressed in unit's meq / kg lipid was determined by the titration method (Kirk and Sawyer, 1991).

9. Free fatty acids

Free fatty acid content of the fish samples was determined according to the method of Kirk and Sawyer (1991). A mixture of diethyl ether (25 mL), ethanol (70% v/v) (25 mL) and 1% phenolphthalein solution (1 mL) was prepared then neutralized with 0.1 M NaOH solution. Two grams quantities of fish samples were blended in the neutral solvent prepared above for about 20 min, and then titrated with 0.1 M NaOH with constant shaking until a pink color was formed which persisted for about 15 s. All samples were analyzed in triplicate and the free fatty acid content was expressed as oleic acid equivalent.

10. Fatty acid composition

Fatty acids Methyl Esters (FAMES) were obtained by the method described by Metcalfe *et al.* (1966) and modified by Selmi and Sadok (2008). Ten gram of the lipid extract was saponified with 0.5 mol/L NaOH in methanol followed by a methylation in 12% boron trifluoride in methanol (BF₃/MeOH). The methylated sample was then extracted with n-hexane. All of these reactions were performed in quadruplicate for each sample. The resulting methyl esters were analysed by gas chromatography (GC) using an Agilent Technologies chromatograph 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30 M silica capillary column (0.25 mm i.d. & 0.25 µm film thickness). The temperature of the injector and detector were 220 °C and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/min. Peaks were identified by comparison of their retention times with FAMES standards (SUPELCO). The sequences of fatty acids were ranged according to their

chromatographic retention times and the values are given as percentages of the total fatty acids methyl esters.

Microbial analysis

Total bacterial count (TBC) was determined by using Nutrient agar, MacConkey agar, and Potato Dextrose agar media according to the procedures described by APHA (1976). The mould counts in the smoked fish samples were determined, according to the method described by Fawole and Oso (1995).

Sensory quality assessment

Organoleptic attributes of appearance, juiciness, saltiness, rancidity, flavour and general acceptability of the smoked fish samples were evaluated by a 10 selected members. A 9-point hedonic scale was used with 9 for like extremely, down to 1 for dislike extremely (Carbonell *et al.*, 2002). For the evaluation, the samples were rinsed with water for 1 min, covered with aluminum foil, heated in an oven at 80°C for 30 min and allowed to cool at ambient temperature, before presentation to the panelists.

RESULTS AND DISCUSSION

1. Sensory Evaluation of Raw Fish

Score sheet of sensory evaluation used was based on Egyptian Organization for Standardization (2005). Eye, gill, belly and texture of fresh kapreeta (like tuna) fish were tested by five panelists with 10 for highest score. The results are present in Table (1). Samples of raw materials used for study were generally of high organoleptic value with the characteristics of eyes perfectly fresh, convex black pupil, translucent cornea, bright red gills, no bacterial slime, outer slime water-white or transparent, bright opalescent sheen. No reddening along the backbone and no discoloration of the belly flaps. The fish was fresh 'seaweedy' odours with firm, elastic to the finger touch.

2. Proximate composition

Preliminary experiments were carried out on pretreatments with concentrations from 1- 5% sage or thyme extracts prior fish filets liquid smoking. Sensory evaluation recorded that adding 5% of essential thyme oil or essential sage oil to kapreeta fish fillets for 30 min prior smoking were generally of highest organoleptic values. The results of proximate composition are presented in Table (2). Moisture content decreased from 71.75% in fresh fish to 53.28% in liquid smoked kapreeta fish fillet without pretreatment, while decreased to 54.50% in smoked fish fillet pretreated with 5% thyme oil and to 54.74% in smoked fish fillet pretreated with 5% sage oil. The lower moisture content values with the sample might be due to the loss of water during smoking (Asiedu *et al.*, 1991). Industrial specifications for smoked finished products generally are recommended with water content in the fish flesh of less than 65% (Cardinal *et al.*, 2001). Kolodziejska *et al.* (2002) also reported that moisture content of smoked Mackerel was 56.7% while Goulas and Kontominos (2005) reported that the moisture content of smoked chum Mackerel samples varied from 58.1% to 59%.

Moisture content of treated smoked fish fillet was 54.50% and 54.74% for smoked fish pretreated with 5% thyme oil or sage oil, respectively. These results are in agreement with Iheagwara (2013) who found that pretreatment with ginger extract prior smoking effect on moisture content of mackerel fish.

The protein, lipid and ash contents of fresh Kapreeta fish samples were 24.54%, 2.11% and 1.60%, respectively increased after liquid smoking in untreated smoked fish to be 36.15%, 4.13% and 5.84%, respectively. Decrease of moisture content and increase of protein, fats and ash contents due to reduction of moisture were the most prominent changes after smoking (Daramola *et al.*, 2007, Bilgini *et al.*, 2008 and Al-Reza *et al.*, 2015).

Protein content of smoked fish treated with 5% thyme oil (36.90%) or 5% sage oil (37.03%) were slightly increased than untreated samples, while lipid content decreased to 3.40% in smoked fish treated with 5% thyme oil and to 3.02% in smoked fish treated with 5% sage oil. These results are in agreement with Iheagwara (2013) who found that pretreatment with ginger extract prior smoking effect on the increasing of protein and decreasing of lipid content.

3. Quality and lipid oxidation parameters of kapreeta fish fillets

The quality parameters of raw and smoked kapreeta fish fillets are given in Table (3).

1. Salt content

Salt content of the Kapreeta smoked fish sample was analyzed to be 1.75%. Birkeland and Bjerkeng (2005) reported that acceptable salt content of smoked fish was 1.80g and it was increased in experimental sample by increasing the time of salting. Jittinandana *et al.* (2002) found that salt content of products soaked in higher brine concentration was greater than of those from the lower brine concentration for the same brining time. In this study, the salt content increased during storage of untreated smoked fish and slightly increased in kapreeta fish treated with essential oils prior smoking during storage at $5 \pm 1^\circ$. This increase in sodium chloride content was oftentimes accompanied by partial dehydration and clear shortage of free water (Dessoiki, 1971 and El-Akeel, 1988).

2. pH

The pH of fresh kapreeta (like tuna) fish flesh was approximately neutral (6.4) which decreased to 6.1 after smoking (Table 3). This decrease could be due to the presence of different smoke components like acids which get deposited on the fish during the smoking process. These values are partially in agreement with that of Goulas and Kontominas (2005) who found that a pH value of 6.22 for smoked chub mackerel. pH slightly increased during storage. The increase in pH may be attributed to the decomposition of nitrogenous compounds and the production of volatile basic components such as ammonia, trimethylamine and total volatile nitrogen by fish spoilage bacteria which indicates a loss of quality (Ruiz-Capillas and Moral, 2005, Can. 2011 and Topuz *et al.*, 2014).

3. Total volatile base nitrogen (TVB-N)

Total volatile base nitrogen (TVB-N) is widely used as an indicator of fish spoilage; its increase is related to the activity of spoilage bacteria and endogenous enzymes and TVB-N levels are often used as an index to assess the quality and shelf life of products (Ruiz-Capillas and Moral 2005, Ozogul *et al.*, 2006 and Ucak *et al.*, 2011).

In the present study, TVB-N of Kapreeta fresh fish was 14.1 mgN/100g and decreased after liquid smoking to be 11.2 mgN/100g in untreated smoked fish fillets and to 9.4 and 8.1 mgN/100g in smoked kapreeta fish fillet treated with 5% thyme oil or 5% sage oil, respectively, prior smoking. This could be associated with lower moisture content and higher salt level which reducing spoilage bacteria growth and activity of endogenous enzymes. Results of current findings showed that on day 0, smoked fish fillets were within the accepted TVBN limits for raw and smoked fish samples, because fish samples had values less than 30 mgN/100 g (Daramola *et al.*, 2007). Pearson (1982) and Connell (1995) reported and also recommended that the limit of acceptability of fish is 20 to 30 mgN/100 g, while Huss (1988) and Kirk and Sawyer (1991) suggested a value of 30 to 40 mgN/100 g as the upper limit. The TVB-N content of fresh chela was found 7.10 mgN/100 g of sample, which is below the level of 35 mgN/100 g, has been suggested as border line for various fish and fish products (Ghaly *et al.*, 2010). Values similar to our TVB-N data have been reported for smoked fish (Gokoglu *et al.*, 2004, Kilinc and Cakli, 2005, Can and Ersan, 2013 and Topuz *et al.*, 2014).

During storage period at 5 ± 1 °C, TVB-N values were increased. Untreated smoked Kapreeta fish fillets increased to 23.3 after 15 days and to 32.2 after 30 days and to 48.1 mgN/100 g after 45 days, while smoked kapreeta fish fillet treated with 5% thyme oil increased to 25.3 after 45 days and to 32.1 mgN/100 g after 60 days. On the other hand, smoked kapreeta fish fillets treated with 5% sage oil increased to 27.3 after 60 days and to 33.2 after 75 days and reach to 38.1 mgN/100 g after 90 days storage. The highest TVB-N values were recorded in untreated fish fillets, while the lowest were shown in treated with sage followed by thyme essential oils.

Treated fish fillets with 5% sage oil recorded lowest TVB-N during storage because of the lower bacteria count (Erkan *et al.*, 2011). Sage essential oil has stronger antibacterial effect than thyme essential oil (Mejlholm and Dalgaard, 2002). In the present study, the results establish the effectiveness of thyme and sage essential oils as antioxidants and antimicrobials due to reduction in TVB-N on treated samples as observed in Table (3).

4. Trimethylamine Nitrogen (TMA-N)

Trimethylamine Nitrogen (TMA-N) is produced from Trimethylamine Oxide (TMAO) possible partly by action of intrinsic enzymes but certainly through bacterial action, is the main component responsible for a pleasant "fishy" odor (Rodriguez *et al.*, 1999 and Shakila *et al.*, 2003). In the present study TMA-N content of fresh fish was 5.5 mg N/100 g and decreased after liquid smoking to be 4.2 mg N/100 g in untreated smoked kapreeta fish fillet but increased to 9.8 after 30 days and to 11.4 after 45 days. Treated fish fillets with

5% thyme or sage oils recorded lowest TMA-N during storage at 5 ± 1 °C. Treated samples with 5% thyme oil reached to 8.5 after 45 days and to 9.3 mg N/100 g after 60 days storage and in treated samples with 5% sage oil, TMA-N decreased to 7.1 after 45 days and to 9.4 mg N/100 g after 75 days refrigerated storage. According to the Egyptian Organization for Standardization (2005). for TMN-A values of smoked fish (10 mg N/100 g), smoked kapreeta fish fillets treated with sage oil had higher shelf life followed by thyme oil. Lower production of TMA-N in smoked kapreeta fish samples may be due to the antibacterial properties of thyme and sage essential oils (Erkan, 2012 and Yildiz, 2016).

5. Lipid oxidation parameters

Lipid oxidation is a major quality problem especially in fatty marine species. The highly unsaturated fatty acids found in fish lipids are very susceptible to oxidation. It leads to the development of off odours and off-tastes in edible oils and fat containing foods, known as oxidative rancidity. To evaluate the degree of lipid oxidation, the Thiobarbituric Acid (TBA) and Peroxide Value (PV) were determined. The TBA index value is an index of lipid oxidation measuring MDA content. MDA is formed through hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen (Rezaei *et al.*, 2008).

In the present study, PV and TBA of fresh Kapreeta fish fillet were 2.1 meq peroxide/kg fish fat and 1.1 mg malonaldehyde/kg, increased after smoking to be 2.6 meq peroxide/kg fish fat and 2.9 mg malonaldehyde/kg in untreated smoked Kapreeta samples at zero time storage. On the other hand, PV and TBA of treated fish fillets were less than untreated samples (1.9 meq peroxide/kg fish fat and 1.8 mg malonaldehyde/kg treated with 5% thyme oil and to 1.7 meq peroxide/kg fish fat and 1.7 mg malonaldehyde/kg for treated with 5% sage oil). According to Augbourg and Ugliano (2002) and Yanar *et al.* (2007) lipid oxidation was enhanced by method of salting, salting time, smoking and drying method. In the present study lipid, oxidation values were lower than the general PV and TBA limit for smoked fish as mentioned by Egyptian Organization for Standardization (2005). which reported that PV values should not be above 10-20 meq/kg fish fat and TBA values not exceed 4.5 mg malonaldehyde/kg (Frangos *et al.*, 2010 and Emir and Ozpolat, 2013).

During storage period at 5 ± 1 °C, PV and TBA increased more than recorded by Egyptian Organization for Standardization (2005)., untreated smoked fish fillets increased to 15.4 meq peroxide/kg fish fat and 4.6 mg malonaldehyde/kg after 30 days. PV and TBA of treated fish fillets were less than untreated samples (12.5 meq peroxide/kg fish fat and 4.5 mg malonaldehyde/kg after 60 days for 5% thyme oil and 11.4 meq peroxide/kg fish fat and 4.5 mg malonaldehyde/kg for 5% sage oil after 75 days storage).

In food suitable for consumption, the TBA values might reach the upper limit of 7 to 8 mg of MDA kg^{-1} (Emir and Ozpolat 2013); in "perfect material," the TBA value should be less than 3 mg of MDA/kg, and in "good material," the TBA value should be no more than 5 mg of MDA kg^{-1} . The TBA values indicate the degree of rancidity of products, and values greater than 3-4 mg of MDA kg^{-1}

indicate a loss of product quality (Papadopoulos *et al.*, 2003 and Frangos *et al.*, 2010).

The use of thyme essential oil to protect muscle foods against oxidation has been reported in the literature. Mariutti *et al.* (2008) and Erkan *et al.* (2011) observed that sage oil and thyme oil was an effective means of controlling lipid oxidation in chicken and fish meat, as reflected in thiobarbituric acid reactive substance values. There are two possible reasons for this phenomenon in the effectiveness of this product: 1, reduction in TBARS using thyme and sage is related to peroxide-scavenging enzyme activity, which could reduce unsaturated fatty acid and total unsaturated fatty acid oxidation and 2, some active components in the sage and thyme essential oils may involve desaturase and elongase activities (Mariutti *et al.*, 2008).

The initial free fatty acids (FFA) value for fresh kapreeta fish was 1.7% increased after smoking (zero time storage) in untreated smoked fish to 2.9 and to 2.6 and 2.2 oleic acid percentage in treated samples with 5% thyme oil and 5% sage oil, respectively. FFA values increased with storage time (Table 3); however the values in the control samples were higher than other samples during storage. FFA values found to be 4.6% after 30 days in untreated smoked fish and to 4.1 and 4.2 oleic acid percentage in treated samples with thyme or sage essential oils after 60 and 75 days. FFA is said to contribute to off flavor of the product and cause textural alterations by complexing with protein (Al-Reza *et al.*, 2015). The results established the effectiveness of sage and thyme essential oils as antioxidants which were greater in activities to inhibit the synthesis of free fatty acid in the treated samples than control samples during refrigerated storage.

6. Fatty acids composition

Fatty acids composition of fresh and smoked kapreeta fish fillets during refrigerated storage are presented in Tables (4). In fresh kapreeta fish, polyunsaturated fatty acids (PUFAs) constitute the majority of the fatty acids composition (36.23% of total fatty acids), followed by saturated (33.07% SFAs) and monounsaturated fatty acids (30.70% MUFAs). The total polyenes content included eicosapentaenoic acid 20:5 (n-3) (EPA) and docosahexaenoic acid 22:6 (n-3) (DHA) were being the prominent polyunsaturated fatty acids. In smoked kapreeta fish, saturated (SFAs) constitute the majority of the fatty acids composition (37.26% of total fatty acids), followed by polyunsaturated fatty acids (35.14% PUFAs) and monosaturated fatty acids (27.60% MUFAs). Fatty acid profiles were followed and observed after storage. SFAs and MUFAs levels increased more while PUFAs levels decreased more in untreated samples during refrigerated storage.

The decrease of PUFAs percentage may indicate the oxidation of these unsaturated fatty acids during storage. Such changes were not observed in sage or thyme treated lot. The uses of natural antioxidant in fish fillets have induced the stability of fatty acids profiles without altering their composition and minimize oxidation. These results are in accordance with those reported by Serdaroglu and Felekoglu (2005). Due to their high degree of unsaturation, EPA and DHA are readily oxidized. Such characteristic has suggested the use of the

polyene index [(EPA + DHA)/16:0] and PUFAs/SFAs to evaluate oxidative deterioration of polyunsaturated fatty acid in fish lipids (Wada and Fang, 1992 and Rahimabadi *et al.*, 2016). In this study, the polyene index and PUFAs/SFAs values in control samples decreased from 1.70 % to 1.52 % and from 1.35 % to 1.20 % following 15 days of storage, respectively. No changes were observed in polyene index and PUFAs/SFAs values for thyme treated lot. It has been reported that sardines treated with rosemary extract and onion juice, retained ratio of [EPA + DHA/16:0] statically constant during storage (Serdaroglu and Felekoglu 2005 and Colakoglu *et al.*, 2011).

4. Microbial quality

The activity of microorganisms is the most important factor limiting the shelf life of fish and fish products. Total viable count (TVC) is the most common microbiological method aimed to detect and enumerate high proportion of the microbial population as possible. In practice, this usually means mesophilic, aerobic or facultatively anaerobic bacteria, which account for the major part of the microflora in fish. A TVC method can only provide an estimate of the microbial population based on those cells that are recoverable under the test conditions.

TVC of fresh kapreeta fish was 4.2 log₁₀ cfu/g (Table 3) indicating good fish quality, but after the samples were subjected to hot smoking, the TVC was reduced to 2.8 log₁₀ cfu/g in untreated samples and to 1.8 and 1.2 log₁₀ cfu/g in treated samples with 5% thyme and 5% sage essential oils at day 0 of storage. Karra (1978) reported that smoking caused a decrease in total microbial count by an average of 94.7% of the original number in dogfish fillets. This occurrence could be attributed to the effects of dehydration and antimicrobial activity of the smoke constituents besides the high temperature during hot smoking (Rorvik, 2000).

TVC increased exponentially with storage time. At day 15 of storage, TVC in untreated samples was 4.1 log₁₀ cfu/g and increased to 5.6 log₁₀ cfu/g at day 30. On the other hand, TVC in smoked kapreeta fish fillets treated with 5% thyme or 5% sage oils was 2.9 and 2.1 log₁₀ cfu/g at day 15 and increased to 4.5 and 3.3 log₁₀ cfu/g at day 45. Microbial load sharply increased on the 75th day of storage and reached 7.3 and 6.2 log₁₀ cfu/g in both treated smoked fish fillets. TVC is an important criterion for quality evaluation; the maximum recommended bacterial count for good quality products is 5.7 log₁₀ cfu/g, and the maximum recommended bacterial count for marginally acceptable quality products is 7 log₁₀ cfu/g (ICMSF, 1986). Considering these values, it is possible to say that TVCs of smoked kapreeta fish exceeded the microbiological limits of acceptability after 15 days of storage for untreated samples and for 60 days storage for treated samples with 5% thyme oil and 75 days for treated samples with 5% sage oil. Kolodziejska *et al.* (2002) reported that the initial TVCs of hot smoked mackerel were 1.6 log₁₀ cfu/g prior to storage and 4.7 log₁₀ cfu/g after 21 days of storage at 8°C. These results showed that thyme and sage essential oils having the greatest antimicrobial activity. A number of essential oils and some of their components have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Lambert *et al.* 2001; Burt 2004). Thyme contains high concentrations of

phenolic compounds including carvacrol, thymol, p-cymene and γ -terpinene (Komaki *et al.*, 2015). The thyme and sage essential oils can be considered effectively inhibitory on the total aerobic flora. Similar results were observed by several researchers (Cadun *et al.*, 2008, Duman *et al.*, 2012; Can and Ersan, 2013). According to the results of fish quality and lipid stability, smoked Kapreeta fish fillets treated with 5% sage oil prior smoking recorded highest quality and high shelf life (up to 75 days), followed by fish fillets treated with 5% thyme extract (up to 60 days), while untreated fish fillet recorded the lowest quality and shelf life (from 15 to 30 days).

5. Sensory quality

Sensory evaluation together, with chemical and microbial characteristics have been used extensively to assess the quality of smoked kapreeta fish fillets. Therefore, the effect of storage at refrigerated temperature ($5 \pm 1^\circ\text{C}$) on the organoleptic attributes of appearance, flavour, texture and overall acceptability of smoked fillets was studied. Sensory evaluation in this study was conducted by panelists. A nine point hedonic scale was used, a score of nine being the best, one being the worst and four being the borderline of acceptability. The obtained results are shown in Table (5). The appearance of smoked fish is one of the most important organoleptic properties mainly due to its effect on the acceptability of these products by consumers. Smoked fish fillets showed a good appearance score 8 after smoking at 0 time storage in both untreated and treated smoked fish fillets with 5% thyme or sage essential oils prior smoking. During storage the appearance scores tended to decrease until the smoked samples were completely rejected organoleptically after 30 days for untreated smoked fish samples (5) and after 60 days for smoked fish samples treated with 5% thyme oil (5) prior smoking and after 75 days for smoked fish samples treated with 5% sage oil (5) prior smoking. These data indicated the effect of thyme and sage extracts on the appearance scores and acceptability of treated smoked kapreeta fish during storage.

Regarding to the effect of storage on the texture scores and acceptability of smoked kapreeta fish fillets, the obtained results illustrated that the untreated texture score and treated with 5% thyme or 5% sage of smoked fish at 0 time storage was 7. The texture scores of smoked kapreeta fish fillets during storage showed the same trend as for appearance scores and acceptability. Flavour is a major sensory attribute for smoked fish quality. The specific aroma and taste of smoked fish were formed due to the effect of volatile compounds from smoking and essential oils as sage and thyme extracts. Fresh smoked kapreeta fish fillets (0 time storage) recorded very good flavour (taste and odor). The flavour scores of smoked kapreeta fish fillets during storage showed the same trend as for appearance and texture scores and acceptability. It could be observed that the overall acceptability scores of smoked fillets did not alter within 15 days of untreated fish and within 45 and 60 days of treated fish fillets with 5% thyme or 5% sage essential oils prior smoking, respectively. These findings for sensory evaluation were in a good agreement with the chemical and microbial characteristics of smoked kapreeta fish fillets. Similar results have been reported in other recent studies (Ozpolat *et al.*, 2010, Duman *et al.*, 2012, Egbal *et al.*, 2013 and Yildiz, 2016).

CONCLUSION

From the above results, it can be concluded that liquid smoking can significantly influence the physicochemical properties of smoked kapreeta fish fillets by imparting antioxidant and antimicrobial properties and by influencing the sensory attributes. The present work has demonstrated that sage and thyme essential oils have antioxidative and antimicrobial properties that can retard oxidative rancidity and inhibit microbial growth, thus, extending the shelf life of the smoked fish. This is justified by the low TBA and peroxide values, as well as microbial count of the sage or thyme treated samples, compared to the untreated samples. Organoleptically, the general pattern of consumer preference to the products indicates that smoked kapreeta fish fillets treated with sage oil followed by thyme oil samples were most acceptable in relation to storage stability compared with control samples. According to the present results, the shelf life of kapreeta fish fillets was estimated as 15 days for untreated smoked fish and 60 days for smoked fish treated with 5% thyme oil and 75 days for smoked fish treated with 5% sage oil under refrigerator storage. Further research is required to focus on understanding the mechanisms of action, in particular concentrations of active ingredients of both sage and thyme essential oils which applied to liquid smoked fish products.

Table (1). Sensory evaluation of purchased fresh kapreeta (like tuna) fish

Panelist	Specification				
	Eye	Gill	Belly	Texture	Average
1	8	7	8	7	7.5
2	8	8	9	9	8.5
3	8	7	7	8	7.5
4	9	8	9	8	8.5
5	8	8	9	9	8.5
Average	8.2	7.6	8.2	8.2	8.1

Table (2). Proximate composition of fresh and liquid smoked Kapreeta (like tuna) fish filets (mean \pm SE).

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Fresh fish	71.75 \pm 2.45	24.54 \pm 1.2	2.11 \pm 0.40	1.60 \pm 0.34
Smoked fish (no treatment)	53.28 \pm 1.80	36.15 \pm 1.50	4.13 \pm 0.66	5.84 \pm 0.86
Smoked fish (pretreated with 5% thyme oil)	54.50 \pm 1.68	36.90 \pm 1.22	3.40 \pm 0.46	5.60 \pm 0.76
Smoked fish (pretreated with 5% Sage oil)	54.74 \pm 1.74	37.03 \pm 1.20	3.02 \pm 0.36	5.21 \pm 0.46

Table (3). Quality and lipid stability parameters of smoked Kaprreta (like tuna) fish filets during refrigerated storage at 5 ± 1 °C for 90 days (mean ± SE)

Storage Period (days) / Fish treatments	Salt (g)	pH	TVB (mgN/100g)	TMA (mgN/100g)	Peroxide value (meq peroxide/kgfat)	TBA (mg malonaldehyde/kg fat)	FFA (oleic acid %)	TVC log ₁₀ cfu/g
Fresh fish	1.7 ± 0.02	6.4 ± 0.2	14.1 ± 1.1	5.5 ± 0.2	2.1 ± 0.1	1.1 ± 0.2	1.7 ± 0.1	4.2 ± 0.2
Smoked fish								
No Treatment								
0 days	1.75 ± 0.02	6.1 ± 0.1	11.2 ± 1.2	4.2 ± 0.3	2.6 ± 0.1	2.9 ± 0.2	2.9 ± 0.1	2.8 ± 0.2
15 days	1.78 ± 0.03	6.3 ± 0.1	23.3 ± 1.5	6.6 ± 0.4	8.3 ± 0.1	3.3 ± 0.3	3.3 ± 0.1	4.1 ± 0.2
30 days	1.80 ± 0.03	6.6 ± 0.2	32.2 ± 1.8	9.8 ± 0.4	15.4 ± 0.2	4.6 ± 0.3	4.6 ± 0.1	5.6 ± 0.2
45 days	1.85 ± 0.03	6.7 ± 0.1	48.1 ± 2.2	11.4 ± 0.5	19.8 ± 0.2	5.8 ± 0.4	5.4 ± 0.1	6.8 ± 0.2
60 days	1.85 ± 0.03	6.7 ± 0.1	53.2 ± 2.4	12.6 ± 0.4	24.1 ± 0.3	9.5 ± 0.4	5.9 ± 0.1	8.9 ± 0.2
75 days	1.90 ± 0.03	6.8 ± 0.1	55.8 ± 2.5	14.2 ± 0.4	27.7 ± 0.3	11.7 ± 0.5	6.6 ± 0.1	12.1 ± 0.3
90 days	1.90 ± 0.03	6.9 ± 0.2	58.9 ± 2.5	15.8 ± 0.5	32.8 ± 0.5	13.7 ± 0.5	6.9 ± 0.1	15.8 ± 0.3
5% Thyme oil								
0 days	1.75 ± 0.02	6.1 ± 0.2	9.4 ± 0.8	4.1 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.6 ± 0.1	1.8 ± 0.1
15 days	1.75 ± 0.02	6.2 ± 0.1	12.5 ± 1.1	5.6 ± 0.2	3.1 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.9 ± 0.1
30 days	1.75 ± 0.02	6.3 ± 0.1	18.4 ± 1.3	7.5 ± 0.3	7.8 ± 0.2	2.2 ± 0.2	2.8 ± 0.1	3.3 ± 0.1
45 days	1.77 ± 0.02	6.4 ± 0.1	25.3 ± 1.5	8.5 ± 0.3	10.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.1	4.5 ± 0.1
60 days	1.78 ± 0.02	6.4 ± 0.1	32.1 ± 1.1	9.5 ± 0.4	12.4 ± 0.3	4.5 ± 0.2	4.1 ± 0.1	4.5 ± 0.2
75 days	1.80 ± 0.02	6.5 ± 0.1	36.2 ± 1.3	11.4 ± 0.4	16.8 ± 0.2	5.3 ± 0.3	5.3 ± 0.1	7.3 ± 0.2
90 days	1.85 ± 0.02	6.6 ± 0.2	43.7 ± 1.4	13.6 ± 0.4	28.8 ± 0.2	6.5 ± 0.2	5.8 ± 0.1	10.3 ± 0.2
5% Sage oil								
0 days	1.75 ± 0.02	6.1 ± 0.2	8.1 ± 0.5	3.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	2.2 ± 0.1	1.2 ± 0.1
15 days	1.75 ± 0.02	6.1 ± 0.1	11.1 ± 0.5	4.5 ± 0.1	2.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.1 ± 0.1
30 days	1.77 ± 0.02	6.2 ± 0.1	15.3 ± 0.7	5.5 ± 0.2	4.7 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	2.6 ± 0.1
45 days	1.79 ± 0.02	6.2 ± 0.1	18.2 ± 1.1	7.1 ± 0.2	7.9 ± 0.1	3.1 ± 0.1	2.8 ± 0.1	3.3 ± 0.1
60 days	1.79 ± 0.02	6.3 ± 0.1	27.3 ± 1.2	8.5 ± 0.3	9.5 ± 0.2	3.4 ± 0.2	3.3 ± 0.1	4.3 ± 0.2
75 days	1.80 ± 0.02	6.4 ± 0.1	33.2 ± 1.4	9.4 ± 0.3	11.4 ± 0.2	4.5 ± 0.2	4.2 ± 0.1	6.2 ± 0.2
90 days	1.82 ± 0.02	6.5 ± 0.2	38.1 ± 2.1	10.8 ± 0.3	15.5 ± 0.2	5.3 ± 0.2	5.2 ± 0.1	7.1 ± 0.2

Table (4). Fatty acid composition of fresh and smoked Kaprrreta (like tuna) fish filets during refrigerated storage at 5 ± 1 °C for 90 days Values are % of total fatty acid expressed as mean of three replicates

Fatty acids	Fresh fish	Smoked fish (no treatment)				5% Thyme extract				5% Sage extract			
		0 day	30 days	60 days	90 days	0 day	30 days	60 days	90 days	0 day	30 days	60 days	90 days
12:0	0.80	0.43	0.53	0.85	0.80	0.45	0.48	0.52	0.55	0.43	0.40	0.40	0.45
14:0	5.40	6.30	7.40	7.20	7.25	5.80	6.67	6.65	6.90	5.30	5.90	5.90	6.20
16:0	21.10	23.52	24.58	25.52	25.60	23.50	23.40	24.50	25.40	23.52	23.20	23.75	23.90
18:0	5.22	6.26	7.20	7.50	8.20	6.10	6.30	6.70	6.90	6.26	6.00	6.00	6.65
22:0	0.55	0.75	0.70	0.75	0.70	0.80	0.75	0.75	0.75	0.75	0.75	0.75	0.75
SFAs	33.07	37.26	40.41	41.82	42.55	36.65	37.60	39.12	40.50	36.26	36.25	36.80	37.95
16:1 n-7	8.60	8.30	8.00	8.10	8.10	8.10	8.10	8.05	8.00	8.30	8.40	8.40	8.30
18:1 n-9	19.60	17.20	17.00	17.05	17.10	17.15	17.10	17.05	17.00	17.20	17.90	17.80	17.30
20:1 n-9	2.50	2.10	2.05	2.05	2.10	2.45	2.10	2.10	2.05	2.10	2.20	2.20	2.10
MUFAs	30.70	27.60	27.05	27.20	27.30	27.55	27.30	27.20	27.05	27.60	28.50	28.40	27.70
18:2 n-6	9.40	9.24	8.74	8.24	8.10	9.20	9.10	8.80	8.50	9.24	9.20	9.10	9.05
18:3 n-3	4.50	3.10	2.80	2.39	2.25	2.90	2.80	2.70	2.65	3.10	3.40	3.30	3.20
20:4 n-3	1.76	1.60	1.50	1.50	1.40	1.50	1.50	1.45	1.40	1.60	1.60	1.60	1.50
20:5 n-3 (EPA)	8.40	5.50	5.10	5.05	5.10	5.80	5.50	5.40	5.10	5.60	5.50	5.30	5.20
22:5 n-3	ND	1.50	1.30	1.40	1.20	1.30	1.50	1.40	1.30	1.40	1.45	1.40	1.30
22:6 n-3 (DHA)	12.17	14.20	13.10	12.40	12.10	15.10	14.70	13.93	13.50	15.20	14.10	14.10	14.10
PUFAs	36.23	35.14	32.54	30.98	30.15	35.80	35.10	33.68	32.45	36.14	35.25	34.80	34.35
PUFAs/SFAs	1.1	0.94	0.81	0.74	0.71	0.97	0.93	0.86	0.80	1.00	0.97	0.95	0.91

Table (5). Organoleptic quality of smoked kaprreta (like tuna) fish during storage at 5 ± 1 °C for 90 days (mean ± SE)

Storage Period (days) / Smoked Fish treatments	Appearance	Texture	Flavour	Overall acceptability
No Treatment				
0 days	8	7	9	8
15 days	6	5	7	6
30 days	4	4	4	4
45 days	3	3	3	3
60 days	2	2	2	2
75 days	1	1	1	1
90 days	1	1	1	1
5% Thyme extract				
0 days	8	7	9	8
15 days	8	8	8	8
30 days	5	6	6	6
45 days	5	5	5	5
60 days	5	5	5	5
75 days	4	4	4	4
90 days	2	2	2	2
5% Sage extract				
0 days	8	7	9	8
15 days	8	8	8	8
30 days	7	7	7	7
45 days	6	6	6	6
60 days	6	6	6	6
75 days	5	5	5	5
90 days	3	3	3	3

REFERENCES

- Abdel-Hamied, A.A., Nassar, A.G. and El-Badry, N. (2009).** Investigations of antioxidant and antibacterial activities of some natural extracts. *World Journal of Dairy and Food Sciences*, 4.. 1-7.
- Abou-Tor, E. M. (2002a).** Production of hot smoked fillets/steaks and fish fingers from tuna-like fish. 4th International Conference for food industries quality control. Alex. University.
- Abou-Tor, E. M. (2002b).** Quality changes and shelf-life of hot smoked filliets. *J. Agric. Sci. Mansoura Univ.*, 27.. 6847-6861.
- Akhtar, P., Gray, J. I., Booren, A. M. and Garling, D. L. (1998).** Effect of dietary components and surface application of oleoresin rosemary in lipid stability of rainbow trout (*Oncorhynchus mykiss*) muscle during refrigerated and frozen storage. *Journal of Food Lipids*, 5.. 43-45.
- Al-Flailih, A.M. (2009).** A study of some aromatic plant extracts at Algabal Al Akhdar in Libya as natural antioxidants. Ph.D. Thesis. Food Science Dept., Faculty of Agric., Alex. University.

- Al-Reza, S., Karmaker, S., Hasan, M. and and Nannur, R. (2015).** Effect of Traditional Fish Processing Methods on the Proximate and Microbiological Characteristics of Laubuka dadiburjori During Storage at Room Temperature. *Journal of Fisheries and Aquatic Science*, 10.. 232-243.
- AMC (1979).** Analytical Method Committee. Recommended method for the examination of fish and fish products. *Analyst*, 104: 434-439.
- AOAC (1990).** Salt 938.08 Official Methods of Analysis (15th Edn). Association of Official Analytical Chemists, Washington DC.
- AOAC (2007).** Official methods of analysis. (20th edn), Association of Analytical Chemists International, Gathersburg, MD, USA.
- APHA (1976).** American Public Health Association. In:Speck, M.L. (Ed.), Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. Pages 701.
- Aruoma, O. I., Halliwell, B., Aeschbach, R., and Looliger, J. (1992).** Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica*, 22.. 257–268.
- Asiedu, M.S., Julsham, K. and Lie, O. (1991).** Effect of local processing methods on three fish species from Ghana: Part I, Proximate composition, fatty acids, minerals, trace elements, and vitamins. *Food Chemistry*, 40: 309321.
- Augbourg, S. and Ugliano, M. (2002).** Effect of brine pretreatment on lipid stability of frozen horse mackerel (*Trachurus trachurus*). *European Food Research and Technology*, 215.. 91-95.
- Balbaa, S.I., Hilal, S.H. and Zaki, A.Y. (1981).** Medicinal plant constituents. Edited by General Organization for University and School Books.
- Basaga, H., Tekkaya, C., and Acitel, F. (1997).** Antioxidative and free radical scavenging properties of rosemary extract. *Lebensmittel – Wissenschaft und Technologie*, 30, 105–108.
- Bilgini, F., Unlusayin, M. and Gunlu, A. (2008).** The Determination of the Shelf Life and Some Nutritional Components of Gilthead Seabream (*Sparus aurata* L., 1758) after Cold and Hot Smoking. *Turk. J. Vet. Anim. Sci.*, 32.. 49-56
- Birkeland, S. and Bjerkeng, B. (2005).** The quality of coldsmoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. *International Journal of Food Science and Technology*, 40.. 963-976.
- Boran, G., Karacam, H. and Boran, M. (2006).** Changes in the quality of fish oils due to storage temperature and time. *Food Chemistry*, 98.. 693-698.
- Burt, S. (2004).** Essential oils: their antibacterial properties and potential applications in foods:a review. *Int. J. Food Microbiol.*, 94.. 223-253.
- Cadun, A., Kisla, D. and Cakli, S. (2008).** Marination of deep-water pink shrimp with rosemary extract and the determination of its shelf-life. *Food Chem.*, 109.. 81-87.
- Can, O.P. (2011).** Combine effect of potassium sorbate and dry salting on the shelf life sardine (*Sardina pilchardus*). *J. Food Technol.*, 9.. 43-49.
- Can, O.P. and Ersan, M. (2013).** Response surface methodology for optimizing the marination conditions during the processing of rainbow trout fillets. *J. Anim. Plant Sci.*, 23.. 1595-1602.
- Carbonell, I., Izquierdo, L. and Costell, E. (2002).** Sensory profiling of cooked gilthead sea bream (*Sparus aurata*): Sensory evaluation procedures and panel training. *Food Sci Technol Int.*, 8.. 169-177.

- Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Morkore, T. and Vallet, J.L. (2001).** Relation of smoking parameters to the yield colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Research International, 34.. 537-550.
- Connell, J.J. (1995).** Control of Fish Quality. 4th Edn., Wiley-Blackwell Publishers, London, England, ISBN-13: 9780852382264, Pages: 256.
- Colakoglu, F., Basri, H. and Ormanc, C. (2011).** Effect of marination and smoking on lipid and fatty acid composition of thornback ray (*Raja clavata*) and spiny dog fish (*Squalis acanthias*). Eur. Food Res. Technol., 232.. 1069–1075
- Daramola, J.A., Fasakin, E.A. and Adeparusi, E.O. (2007).** Changes in physicochemical and sensory characteristics of smoke-dried fish species stored at ambient temperature. African Journal of Food, Agriculture, Nutrition and Development, 7.. 1684-5358.
- Darvishi, H., Azadbakht, M. Rezaeiasl, A. and Farhang, A. (2013).** Drying characteristics of sardine fish dried with microwave heating. J. Saudi Soc. Agric. Sci. 12.. 121–127.
- Dessoiki, T. M. (1971).** Studies on smoking of fish. M.Sc. Thesis. Faculty of agriculture, Ain Shams University.
- Dillon, R., Pattel, T. and Martin, A.M. (1994).** Microbiological control of smoking operations. In: Martin A.M. (ed.), Fisheries processing, Memorial University of Newfoundland. St. Johns' Canada, Chapman and Hall, pp. 51–81.
- Donelli, J. K. and Robinson, D. S. (1995).** Free radicals in foods. Free Radical Research, 22.. 147-176.
- Duman, M., Coban, O. and Ozpolat, E. (2012).** The determination of the effect to shelf life of marinated crayfish (*Astacus leptodactylus* Esch., 1823) with rosemary and thyme essential oils additive. Kafkas Üniv. Vet. Fak. Derg., 18.. 745-751.
- Egbal, O., Hawa, A. and Kalthom, E. (2013).** Investigating the Quality Changes of Hot Smoked *Clarias lazera* at Refrigerated Temperature ($5\pm 1^\circ\text{C}$). Journal of Agriculture and Food Sciences, 1.. 27-32.
- Egyptian Organization for Standardization (2005).** Ministry of Industry. Cairo, Arab Republic of Egypt.
- El-Akeel, A.T. (1988).** Chemical, microbial and sensory evaluation of smoked catfish. Ph.D. thesis. Faculty of agriculture, Cairo University.
- Emir, O. and Ozpolat, E. (2013).** The effects of different concentrations of rosemary (*Rosmarinus officinalis*) extract on the shelf life of hot-smoked and vacuum-packed *Luciobarbus esonicus* fillets. J. Food Process. Pres. 37: 269-274.
- Erkan, N., Ulusoy, S. and Tosun, S. (2011).** Effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout. J. Fur. Verbrauch. Lebensm., 6.. 419–426.
- Ercan, N. (2012).** The Effect of Thyme and Garlic Oil on the Preservation of Vacuum-Packaged Hot Smoked Rainbow Trout (*Oncorhynchus mykiss*) Food Bioprocess Technol, 5:1246–1254
- Fawole, M.O. and Oso, B.O. (1995).** Laboratory manual on microbiology. (2nd Edition), Spectrum books Ltd, Ibadan, Nigeria 77-78.
- Fawole, O., Ogundian, M., Anyandiran, T. and Olagunji, O. (2007).** Proximate and mineral composition in some selected fresh water fishes in Nigeria. Internet Journal of Food Safety, 9.. 52-55.

- Frangos, L., Pyrgotou, N. Giatrakou, V., Ntzimani, N. and Savvaidis, I. (2010).** Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. *Food Microbiol.*, 27.. 115-121.
- Frankel, E. N. (1993).** In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends in Food Science and Technology*, 4.. 220-225.
- Folch J., Lees, M. and Bloune, S.G. (1957).** A simple method for their isolation and purification of total lipids from animal tissues. *Biological Chemistry*, 266.. 497-509.
- Ghaly, A.E., Dave, D., Budge, S. and Brooks, M.S. (2010).** Fish spoilage mechanisms and preservation techniques: Review. *Am. J. Applied Sci.*, 7.. 859-877.
- Gokoglu, N., Cengiz, E. and Yerlikaya, P. (2004).** Determination of the shelf life of marinated sardine (*Sardina pilchardus*) stored at 4 °C. *Food Control.*, 15.. 1-4.
- Goulas, A. E., and Kontominas, M.G. (2005).** Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chem.*, 93.. 511–520.
- Huss, H.H. (1988).** Fresh fish quality and quality changes Danish International Development Agency, FAO, Rome: 43-45.
- ICMSF (1986).** International commission on microbiological specifications for foods. Sampling plans for fish and shellfish. In ICMSF (Ed.), ICMSF, Microorganisms in foods. Sampling for microbiological analysis: Principles and scientific applications (Vol. 2, 2nd ed.). Toronto, Canada: University of Toronto Press.
- Iheagwara, M.C. (2013).** Effect of Ginger Extract on Stability and Sensorial Quality of Smoked Mackerel (*Scomber scombrus*) Fish. *J. Nutr. Food Sci.*, 3.. 3 – 13.
- Jenkins, R. (2010).** Natural Wood Smoke Technologies. Ohio State University.
- Jittinandana, S., Kenney, P.B., Slider, S.D. and Kiser, R.A. (2002).** Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. *Journal of Food Science*, 67.. 2095-2099.
- Karra, H.A. (1978).** Chemical and technological studies on the utilization of dogfish. M. Sc. Thesis, Fd. Yech. Dept. Faculty of Agriculture, University of Alexandria, Egypt.
- Karpinska, M., Borowski, J. and Danowska-Oziewicz, M. (2001).** The use of natural antioxidants in ready-to-serve food. *Food Chemistry*, 72.. 5-9.
- Karlsdottir, G. M., Sveinsdottir, G. H., Kristinsson, V., Dominique, B. and Arason, S. (2014).** Effects of temperature during frozen storage on lipid deterioration of saithe (*Pollachius virens*) and hoki (*Macruronus novaezelandiae*). *Food Chem.*, 156.. 234–242.
- Kahkonen, M.P., Hopia, A.I., Heikki, J.V., Raucha, J.P. and Pihlaga, K. (1999)** Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem.*, 47.. 3954-3962.
- Kilinc, B. and Cakli, S. (2005).** Determination of the shelf life of sardine (*Sardine pilchardus*) marinades in tomato sauce stored at 4 °C. *Food Control*, 16.. 639-644.
- Kirk, R.S. and Sawyer, R. (1991).** Pearson's Composition and Analysis of Foods (9th Edn). Longman Scientific and Technical, London, Pages: 510 -511.
- Kolodziejska, I., Niecikowska, C., Januszewska, E. and Sikorski, Z.E. (2002).** The microbial and sensory quality of Mackerel hot smoked in mild conditions. *LebensmittelWissenschaft und-Technologie*, 35.. 87-92.

- Komaki, A., Hoseini, F. Shahidi, S. and Baharlouei, N. (2015).** Study of the effect of extract of *Thymus vulgaris* on anxiety in male rats. J. Tradit. Complement. Med., 22.. 42-49.
- Korish, M., Badiaa, A., Samir, Y. and Ammar, A. (2008).** Fish cake from tuna-like fish flesh improve its consumption acceptability. J.Agric. Res. Kafer El-Sheikh Univ., 34.. 114 – 129.
- Lacroix, M., Smoragiewicz, W., Pazdernik, L., Koné, M. I. & Krzystyniak, K. (1997).** Prevention of lipid radiolysis by natural antioxidants from rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.). Food Research International, 30.. 457-462.
- Lambert, R.J., Skandamis, P.N., Coote, P.P. and Nychas, G.I. (2001).** A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol., 91.. 453-462.
- Maksimovic, Z., Stojanovic, D., Sostaric, I., Dajic, Z. and Ristic, M. (2008).** Composition and radical-scavenging activity of *Thymus glabrescens* Willd. (*Lamiaceae*) essential oil. J. Sci. Food Agric., 88.. 2036–2041.
- Marc, C., Kaaker, R. and Mboofung, C.M. (1997).** Effect of salting and smoking method on the stability of lipid and microbiological quality. J. Food Qual., 22.. 517–528.
- Martin, E. M., O'Bryan, C. A., Lary, R. Y., Griffis, C. L., Vaughn, K. S. and Marcy, J. A. (2010).** Spray application of liquid smoke to reduce or eliminate *Listeria monocytogenes* surface inoculated on frankfurters. Meat Sci., 85.. 640–644.
- Mariutti, L. R., Orlien, V., Bragagnolo, N., and Skibsted, L. H. (2008).** Effect of sage and garlic on lipid oxidation in high pressure processed chicken meat. European Food Research and Technology, 227(2).. 337–344.
- Mejlholm, O. and Dalgaard, P. (2002).** Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products. Letters in Applied Microbiology, 34.. 27-31
- Metcalfe, L. D., Schimitz, A. A. and Pelka J. R. (1966).** Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. Annexe Chemistry, 38: 524-535.
- Nyarko, H.D., Obodai, E.A., Boamponsem, L.K., Coomson, S.S. and Aniwe, Y. (2011).** Microbial profile of smoked sardine (*Sardillella aurita*) at smoking sites and market centres of Tema, Ghana. Applied Science Research, 3.. 443-453.
- Oduor-Odote, P., Shitanda, D., Obiero, M. and Ituu, G. (2010 a).** Drying characteristics and some quality attributes of *Rastrineobola argentea* (Omena) and *Stolephorus delicatulus* (kimarawali). African Journal of Food Agriculture Nutrition and Development, 10.. 2998–3014.
- Oduor-Odote, P.M., Obiero, M. and Odoli, C. (2010 b).** Organoleptic effect of using different plant materials on smoking of marine and freshwater catfish. African Journal of Food Agriculture Nutrition and Development, 10.. 2658-2677.
- Olayemi, F.F., Raji, A.O. and Adedayo, M.R. (2012).** Microbiological quality of catfish (*Clarias gariepinus*) smoked with Nigerian Stored Products Research Institute (NSPRI) developed smoking kiln. International Research Journal of Microbiology, 3.. 426-430.
- Osman, H., Suriah, A. R. and Law, E. C. (2001).** Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. Food Chemistry, 73.. 55-60.
- Ozogul, Y., Ozogul, F. and Kuley, E. (2006).** Effect of modified atmosphere packaging on fish and fish products. J. Fisheries & Aquat. Sci., 23.. 193-200.

- Ozpolat, E., Coban, Z. and Patir, B. (2010).** Farklı oranlarda asetik asit ve eugenol ile hazırlanan gokkuşacağı alabalığı (*Oncorhynchus mykiss*) marinatlarının duyuusal özellikleri. J. Fisheries Sciences.com., 4.. 329-336.
- Patrick-Iwuanyanwu, K.C., Wegwu, M.O. and Ayalogu, E.O. (2007).** The protective nature of garlic, ginger and vitamin E on CCl₄-induced hepatotoxicity in rats. Asian Journal of Biochemistry, 2.. 409-414.
- Papadopoulos, V., Chouliara, I., Badeka, A., Savaidis, I.N. and Kontominas, M.G. (2003).** Effect of gutting on microbiological, chemical and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. Food Microbiology, 20.. 411-420.
- Pearson, D. (1982).** Pearson's Chemical Analysis of Foods . 8th (ed). Esan HE, Kiriltris , Sawyer R, Churchill Livingstone London. pp. 113 - 507.
- Red Arrow, (2014).** Environmental Benefits of Using Condensed Natural Smokes. Red Arrow, Products Company LLC.
- Rezaei, M., Hosseini, S. F., Langrudi, H. E., Safari, R., and Hosseini, S. V. (2008).** Effect of delayed icing on quality changes of iced rainbow trout (*Onchorynchus mykiss*). Food Chemistry, 106.. 1161–1165.
- Rodriguez, C.J., Besteiro, I. and Pascual, C. (1999).** Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. Journal of Science of Food and Agriculture, 79.. 1473-1480.
- Rorvik, L. M. (2000).** *Listeria monocytogenes* in the smoked salmon industry. Int. J. Food Micro. 62..183–190.
- Ruiz-Capillas, C. and Moral, A. (2005).** Sensory and biochemical aspects of quality of whole bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. Food Chemistry, 89.. 347-354.
- Salindeho, S. and Mamuja, J. (2015).** Physico-Chemical Characteristics and Fatty Acid Profiles of Smoked Skipjack Tuna (*Katsuwonus pelamis*) from Several Producers in Bitung Municipality, North Sulawesi, Indonesia. Int.J. Chem. Tech. Res., 8.. 356-361.
- Selmi, S. and Sadok, S. (2008).** The effect of natural antioxidant (*Thymus vulgaris* Linnaeus) on flesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage. Pan-American Journal of Aquatic Sciences, 3.. 36-45
- Serdaroglu, M. and Felekoglu, E. (2005).** Effects of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. Journal of Food Quality, 28.. 109-120.
- Shakila, R.J., Vijayalakshmi, K. and Jeyasekaran, G. (2003).** Changes in histamine and volatile amines of six commercially important species of fish of the Thoothukkudi coast, India stored at ambient. Food Chemistry, 82.. 347-352.
- Siripongvutikorn, S., Patawatchai, C. and Usawakesmanee, W. (2009).** Effect of herb and spice pastes on the quality changes in minced salmon flesh waste during chilled storage. Asian Journal of Food and Agro-Industry, 2.. 481-492.
- Stolyhwo, A., Kołodziejaska, I. and Sikorski, Z. E. (2006).** Long chain polyunsaturated fatty acids in smoked Atlantic mackerel and Baltic sprats. Food Chem., 94.. 589–595.
- Stoick, S. M., Gray, J. L., Booren, A. M., and Buckley, D. J. (1991).** Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone and sodium tripolyphosphate. Journal of Food Science, 56.. 597–600.

- Thompson, D. C. and Trush, M. A. (1986).** The toxicological implications of the interaction of butylated hydroxytoluene with other antioxidants and phenolic chemicals. *Food Chemistry and Toxicology*, 24.. 1189-1195.
- Topuz, O.K., Yerlikaya, P., Ucak, I. and Buyukbenli, H.A. (2014).** Effects of olive oil and olive oil-pomegranate juice sauces on chemical, oxidative and sensorial quality of marinated anchovy. *Food Chem.*, 154.. 63-70.
- Ucak, I., Ozogul, Y. and Durmus, M. (2011).** The effects of rosemary extract combination with vacuum packing on the quality changes of Atlantic mackerel fish burgers. *Inter. J. Food Sci. Technol.*, 46.. 1157-1163.
- Varlet, V., Serot, T. and Prost, C. (2010).** Smoke flavoring technology in seafood. Pp. 233–254 in L. M. L. Nollet and F. Toldra, eds. *Handbook of seafood and seafood products analysis*. CRC Press, Boca Raton, FL.
- Viuda-Martos, M., Mohamdy, M.A., Fernandes-Lopez, J., Abd Elrazek, K.A., Omer, E.A., and Sendra, E. (2011).** In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. *Food Control.*, 22.. 1715–1722.
- Wada, S. and Fang, X. (1992).** The synergistic antioxidant effect of rosemary extract and atocopherol in sardine oil model system and frozen crushed fish meat. *Journal of Food Processing and Preservation*, 16.. 263-274.
- Yanar, Y. (2007).** Quality changes of hot smoked catfish (*Clarias gariepinus*) during refrigerated storage. *Journal of Muscle Foods*, 18.. 391–400
- Yildiz, P.O. (2016).** Effect of thyme and rosemary essential oils on the shelf life of marinated rainbow trout. *The Journal of Animal and Plant Sciences*, 26.. 665-673.
- Zaki, M. M. (2010).** Effect of sage on the quality and oxidative stability of prepared beef burgers during frozen storage. M.Sc. Home Economics Department, Faculty of Specific Education, Alexandria University.

المخلص العربي

جودة وثبات سمك الكبريئة المدخن بسائل التدخين خلال التخزين المبرد

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يتواجد سمك الكبريئة في مصر ضمن الأسماك البحرية ولكن لا يلقي قبولا لدى المستهلك المصري لإغمقاق لون اللحم المشوب بالدم ووجود الدم ضمن خلايا اللحم بالداخل لذا فإن هذا النوع من الأسماك يجب تصنيعه وحفظه بطرق مختلفة منها التعليب والتدخين. الهدف من هذا البحث هو دراسة تأثير التدخين بالسائل والزيوت العطرية الطيارة وإضافتها للسمك قبل التدخين علي جودة لحم سمك الكبريئة خلال التخزين المبرد لمدة ٩٠ يوم. تم تقدير التحاليل الأولية في السمك الطازج والمدخن وكذا التحاليل الكيماوية والميكروبيولوجية والحسية لجميع العينات المدخنة كم تم التعرف على تركيب الأحماض الدهنية خلال مراحل التخزين وذلك لتقدير الجودة وثبات اللييدات لسمك الكبريئة المدخن.

أوضحت النتائج انخفاض في نسبة الرطوبة وزيادة كل من نسب البروتين واللييدات والرماد بعد التدخين. وقد ارتفع قليلا قيم pH بالتخزين. كما أوضحت النتائج أن كل من (TVB-N) النتروجين الكلي المتطاير ومركب النتراي مثل أمين (TMA) ورقم البيروكسيد وتقدير حمض الثيوبوترك (TBA) وكذا الأحماض الدهنية الحرة (FFA) كلها ارتفعت خلال التخزين بشكل أعلى في السمك غير المعامل عن السمك المعامل بالزيوت الطيارة لكل من الزعتر والمريمية وذلك التخزين. بينما انخفضت معدلات التقديرات الحسية خلال التخزين. وقد أوضح تركيب الأحماض الدهنية أن نسبة ٣٥.١٤% أحماض دهنية عالية درجة عدم التشبع متواجد في السمك غير المعامل وازداد إلى ٣٥.٨٠% في السمك المعامل بواسطة ٥% زيت الزعتر وإلى ٣٦.١٤% في السمك المعامل بواسطة ٥% زيت المريمية وكانت أحماض (EPA) إيكوزا بنتا إينويك، (DHA) ديكوزا هيكسا إينويك هي أعلى نسبة للأحماض الدهنية عالية عدم التشبع. وانخفاض مستوي الأحماض غير المشبعة يوضح مدي حدوث الأكسدة خلال مراحل التخزين وكان هذا الانخفاض والتغير في الحد الأدنى في عينات السمك المعاملة بالزيوت الطيارة لكل من الزعتر والمريمية قبل التدخين. ووفقا لنتائج التحاليل الكيماوية والميكروبيولوجية والحسية فقد وجد أن سمك الكبريئة المدخن كان له درجة جودة عالية وثبات للبييدات لمدة صلاحية ٣٠ يوم في السمك غير المعامل وازدادت فترة الصلاحية إلى ٦٠ يوم في السمك المعامل بزيت الزعتر وازدادت أكثر إلى ٧٥ يوم في السمك المعامل بزيت المريمية تحت ظروف التخزين المبرد.

ويمكن تلخيص أن فلية سمك الكبريئة المعامل بالزيوت الطيارة للزعتر والمريمية قبل التدخين كان له تأثير إيجابي فعال علي الجودة وفترة الصلاحية للسمك المدخن وكذا الاحتفاظ بثبات الأحماض الدهنية دون التأثير عليها وبالتالي تقليل الأكسدة.

