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Research Article
Effect of Sage Essential Oil on the Compositional Quality of Anchovy Fish Burger During Freeze Storage

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Abstract

Background and Objective: The quality of fish and fish products changes due to lipid oxidation. The formation of toxic compounds may occur, decreasing the food safety and nutritional quality and causing health damages to the consumer. The purpose of this study was to investigate the compositional quality and sensory evaluation of prepared European anchovy fish burgers supplemented with extracted sage essential oil during frozen storage. **Materials and Methods:** Sage essential oil was isolated by hydrodistillation method and analyzed with GC-MS. The analysis of proximate chemical composition, fatty acid composition and sensory evaluation were carried out on prepared fish burger samples at zero time and after 4 month storage at -18°C, while the analysis of compositional quality (peroxide value PV and free fatty acid FFA) were carried out periodically every month up to 4 month storage period. There was a significant decrease in moisture, protein and lipid of fish burgers after 4 month storage at -18°C. **Results:** The obtained results showed that PV and FFA were less increase in fish burger samples supplemented with sage essential oil than control samples. The investigated sage essential oil caused a significant improve the quality of fish burgers through retarded the spoilage and enhancement the polyunsaturated fatty acids for fish burgers during frozen storage as compared to the control sample. These results could be due to the antioxidative effect of bioactive compounds (α-thujone, camphor, α-pinene and β-thujone) found in sage essential oil. **Conclusion:** In conclusion, the supplementation of anchovy fish burgers with sage essential oil showed a positive effect on the compositional quality and shelf life as antioxidant, anchovy fish burgers were of high quality and high acceptance even after 4 month storage at -18°C.

Key words: Fish burgers, supplementation, sage essential oil, freezing, storage, quality, sensory

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish flesh has unique characteristics as having high protein content with balanced profile of amino acids, polyunsaturated and essential fatty acids with ω-3 series of fatty acids and low level of saturated fat and cholesterol[1-3]. Polyunsaturated fatty acids especially eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA) can reduce the risk of cardiovascular disease, improve mental and visual functions and are involved in inflammatory responses[4]. They may also lead to a decrease in body fat over time and reduce obesity risk[5]. Accordingly, consumption of fish and fishery products are increasing day by day due to increasing awareness of the consumers on health issues[6].

The European anchovy (*Engraulis encrasicolus*) is a small pelagic fish which is widely distributed from the North Sea to Central Africa and throughout the Mediterranean Sea[7]. There is a large quantity of very small fish landed as by-catch which do not find a ready market as anchovy fresh fish in Egypt. European anchovies are small, common saltwater forage fish that are used as human food, fish bait and fish oil[8,9]. The European anchovy is used as many varieties of food from dessert to salty food. The European anchovy contains high-quality of lipid and protein and also highly-balanced distribution in terms of vitamins. It is kind a fish, rich in polyunsaturated fatty acids[10,11]. It is reported that nearly 30% of the total fatty acids in the European anchovy are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)[10,12].

In recent years, the preference of the consumers has significantly directed towards the fast food consumption since there has been a rapid urbanization and an increase in working women population[13]. There have been some studies on the production and quality stability of the fishery fast food products including fish cake, fish crackers, fish balls and fish burgers[14-17].

Fish burgers are very popular and tasty item in fast food industry, which are increasing in popularity and have extensively developed in the world food market. Different studies have been conducted to determine the quality of fish burgers[18-22]. More research works has proved that even fish burgers stored at frozen temperatures can undergo undesirable quality changes due to oxidative rancidity and protein denaturation[20,23-27].

Fish and their products are very perishable foods due to their high water activity, the presence of polyunsaturated fatty acids and neutral pH[28]. The susceptibility of fish oxidation depends not only on fish species, the amount of total lipids and the composition of fatty acids as well as their location in fish muscle tissue[29]. The quality changes due to lipid oxidation results in undesirable changes in taste, odor, color and acceptability. Additionally, the formation of toxic compounds may occur, decreasing the food safety and nutritional quality and causing health damages to the consumer[30,31].

Consequently, employing natural preservatives, antimicrobial, antioxidant substances and stabilisers in the products formulations seems quite logical and necessary. Spices and herbs and their essential extracts have been added to food since ancient times, not only as flavoring agents but also as folk medicine and food preservatives[32-35]. They have been applied in many fish species in showing antimicrobial and antioxidant activities against food-borne pathogens and extending the shelf life of the fish[36-39]. Plants from Lamiaceae family, such as rosemary, thyme, sage, oregano and peppermint, have been recognized for their potent antioxidant activity[40].

Sage (*Salvia officinalis*) is a rich source of phytochemicals including phenolic acids, polyphenols, flavonoid glycosides, anthocyanins, sesquiterpenoids, diterpenoids, sesterterpenes and triterpenes[41-43]. Some researchers have reported that sage or sage extracts, can effectively retard lipid oxidation in different meat and fish products[44-49].

Therefore, the objective of this study was to investigate the effects of different levels of sage extract on physicochemical properties and nutritional value aspects for raw European anchovy fish burgers during frozen storage at -180°C for 4 months.

MATERIALS AND METHODS

Materials: Leaves of sage (*S. officinalis* L.) were collected from Siwa Oasis, Egypt in September, 2018.

Twenty kilogram of European anchovy (*Engraulis encrasicolus*) fish samples were obtained during the autumn season 2018 from artisanal fishermen in El-Maadiya port fishing communities, located in Beheira governorate, Egypt. Fish samples were transported in ice boxes to Food Science Laboratory, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt. Fish samples were weighed, homogenized and kept in a clean well plastic bag and stored at 4°C till analysis.

Fish burger preparation: The European Anchovy fish were weighed and then washed with water, beheaded, gutted and washed in iced condition. Fish were weighed and mixed in order to obtain a fish burger with ratio fish/other ingredients of 55:45, 53:47, 51:49 and 49:51 (w/w). In order to obtain a

Table 1: Recipe of European anchovy fish burgers supplemented with different levels of sage extract

	Treatments			
Ingredients	Control	T1	T2	T3
Minced Fish	55	53	51	49
Potato	15	15	15	15
Soy	10	10	10	10
Flour	10	10	10	10
Spices	3	3	3	3
Salt	2	2	2	2
Sugar	0.5	0.5	0.5	0.5
Garlic	1	1	1	1
Onion	1	1	1	1
Pepper	1	1	1	1
Cumin	1.5	1.5	1.5	1.5
Sage essential oil	0	2	4	6

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively

product with balanced composition of micro and macronutrients different ingredients of vegetable and animal origin such as boiled and crushed potato, Soybean, sage and spices (white pepper and cinnamon, in particular) were added as described in Table 1. The fish burgers were prepared manually for a final weight of 100±5 g, packed in high barrier plastic bags (Nylon/Polyethylene) and divided in control samples and three different concentration of the sage essential oil (2, 4 and 6%) were supplemented to fish burgers, respectively. All samples (control without sage extract and three treatments T1, T2 and T3 with 2, 4 and 6% sage extract, respectively) were stored under freezing temperature (-18°C).

Methods

Extraction of essential oil and Gas chromatography-Mass Spectrometry (GC-MS) analysis: Five hundred grams of sage fresh leaves were submitted to hydrodistillation (with 1000 mL of water) in a Clevenger-type apparatus for 72 min without collecting solvent[50].

The collected essential oil was carried out using gas chromatography-mass spectrometry instrument stands at the Central Laboratories, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m×0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10 using the following temperature program: 40°C for 1 min, rising at 4.0°C min^{-1} to 160°C and held for 6 min, rising at 6 C min^{-1} to 210 C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: relative retention time and mass spectra (authentic chemicals.

Proximate chemical composition analysis: Moisture, Protein, fat and ash of the fish samples were determined according to the standard methods[51].

Extraction of total lipids: Lipid was extracted from prepared anchovy fish burger samples with a mixture of chloroform/ methanol (2: 1 v/v) according to the described method[52].

Peroxide value (PV): The peroxide value as an indicator of primary lipid oxidation was determined in lipids extracted from different fish burgers according to the method presented by Kirk and Sawyer[53]. The results were expressed as mEq active oxygen per kg extracted lipid.

Free fatty acid (FFA): The (FFA) contents of the lipid were determined in lipids extracted from different fish burgers according to Kirk and Sawyer[53] by titration in ethanol against phenolphthalein with 0.02 M sodium hydroxide and expressed as percentage oleic acid.

Fatty acid profile: Preparation of fatty acid methyl esters of total lipids extracted from control and burger samples were performed as follows: Methylene chloride (100 μL) and 1 mL 0.5 M NaOH in methanol were added to oil extracts in a test-tube and heated in a water bath at 90°C for 10 min. The test tubes were removed from the water bath and allowed to cool before addition of 1 mL 14% BF3 in methanol. The test tubes are heated again in a water bath for 90°C for 10 min and cooled at room temperature. One mL distilled water and 200-500 μL hexane was added to the test tubes and then FAME was extracted by vigorous shaking for about 1 min. Following centrifugation, the top layer was transferred into a sample bottle for GC analysis[54]. A sample of 25 mg total lipid was transferred into a screw-cap vial, with 2.5 mL methanolic H_2SO_4 and 1 mL benzene. The vial converted under a steam of nitrogen gas before heating in oven at 90°C for 1.5 h.

Analysis of fatty acid was carried out by Gas Liquid Chromatography (GLC) using Hewlett Packard (HP) 6890 GC with 1 μL injection and flame ionization detector (FID) at 250°C temperature. The fatty acid methyl siloxane capillary column Hp-5 (30 m×0.32 μm ID, 0.25 μm film thickness) was used. Nitrogen was used as the carrier gas (0.8 mL min^{-1} gas flow). The injection temperature was 220°C splitless mode. The temperature program w as 200°C for zero hold min (10°C min^{-1}) and held at this temperature for 9 min. The total run time was 26 min. A standard mixture of methyl esters was analyzed under identical conditions prior to running the samples. The retention times of the unknown samples of methyl esters were compared with those of standard. The relative percentage of the area for each peak was obtained.

Sensory analysis: The sensory evaluation of fish burgers was done by a five-member trained panel from the university. To conduct sensory analyses, fried fish burgers were evaluated with respect to their colour, odour, taste, texture and overall acceptability.

The fish burgers were fried separately by deep-frying in sunflower oil at 160°C for 5 min (frying was carried out immediately during evaluation) were evaluated at zero time immediately after preparation and finally at the end of storage period. After frying, they were cooled and samples were served to the panellists who were asked to evaluate on a 10-point hedonic scale ranging from very poor (8) to very good (10) where: less than 2 is very poor, 2- 4 is poor, 5-6 is normal, 7-8 is good and 9-10 is very good[55].

Statistical analysis: Experimental results were expressed as mean±standard error and subjected to analysis of variance (One way-ANOVA). Means were compared using Tukey's least significant test level of α-0.05, using the R programme.

RESULTS AND DISCUSSION

Chemical composition of sage essential oil: The essential oil of sage growing in Siwa Oasis, Egypt was subjected to detailed GC/MS analysis. Exactly 29 compounds were identified, representing 96.52 % of the total essential oil (Table 2). Results as shown in Table 2 were in agreement with Said-Al Ahl *et al.*[56] and Khedher *et al.*[57].

Table 2: Chemical composition of *S. officinalis* leaves essential oil

Compounds name	RT	Peak area (%)
Camphene	956	0.46
β-Pinene	981	0.67
β-Myrcene	994	0.48
α-Terpinene	1022	0.31
1.8-Cineole	1034	18.06
Limonene	1044	3.52
α-Terpinolene	1090	0.65
Linalool	1098	0.33
α-Thujone	1119	15.14
β-Thujone	1124	4.35
Camphor	1150	26.14
Borneol	1173	3.50
α-Terpineol	1195	1.40
Myrtenol	1204	0.38
Bornyl acetate α	1292	0.71
β-Patchoulene	1377	0.31
α-bourbonene	1393	0.25
β-bourbonene	1396	0.27
α-Gurjunene	1411	0.22
Sinularene	1422	0.21
Calarene	1427	0.16
β-Caryophyllene	1433	4.02
α-Humulene	1465	4.28
α-amorphene	1489	0.31
β-Himachalene	1496	0.60
γ-selinene	1522	3.66
manoyl oxide	1575	4.26
Viridiflorol	1615	1.45
Epimanool	1912	0.42
Total identified compounds		96.52

RT: Retention time (min)

Proximate chemical composition of European Anchovy fish burgers: Chemical analysis was carried in the prepared fish burger samples before and after 4 month storage at -18°C to determine changes occurred in moisture, protein, fat and ash and contents. Nitrogen free extracts (NFE) were determined by differences.

In control fish burger and three treatments supplemented with sage essential oil, the values of moisture content significantly decreased after four month storage at -18°C (Table 3). These results are in agreement with many authors[58-60].

The decrease in moisture content was attributed to the sublimation of ice in frozen storage and the loss of drip during thawing process[3]. Meanwhile, in contrary to these findings, in a study made on crab, Zamir *et al.*[61] found an increasing trend in moisture content during storage and attributed this increase to the loss of water holding capacity of tissue.

The results indicated that protein contents of control and formulated burgers samples significantly decreased after four month storage at -18°C. Some publications reported that the changes in protein content during frozen storage may be due to the loss of some volatile nitrogenous compounds during frozen storage and protein hydrolysis by enzymes which enhanced the loss of water soluble nitrogen with separated drip[3,62]. Gandotra *et al.*[63] attributed protein loss observed during frozen storage of (*Labeo rohita*) to the leaching effect on amino acid and water-soluble protein during thawing, process.

The results indicated that total lipid contents of control and formulated burgers samples significantly decreased after 4 month storage at -18°C.

Table 3: Proximate chemical composition (g/100 g of fresh weight) of European Anchovy fish burgers supplemented with sage extract before (zero time) and after 4 month storage (end time) at -18°C

Storage time and treatments	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Nitrogen free extract (% NFE)
Zero time					
Control	73.26±0.012[a*]	18.34±0.015[a]	3.52±0.010[a]	1.17±0.010[c]	3.71±0.12
T1	73.12±0.018[a]	18.38±0.012[a]	3.50±0.012[a]	1.18±0.012[c]	3.82±0.14
T2	73.24±0.014[a]	18.36±0.018[a]	3.52±0.014[a]	1.19±0.011[c]	3.69±0.11
T3	73.29±0.022[a]	18.84±0.018[a]	3.62±0.014[a]	1.18±0.012[c]	3.07±0.11
End time (After 4 month)					
Control	70.45±0.015[c]	16.44±0.018[c]	2.18±0.021[b]	4.37±0.014[a]	6.56±0.31
T1	70.75±0.012[c]	16.48±0.016[c]	2.48±0.034[b]	4.39±0.012[a]	4.73±0.23
T2	70.65±0.016[c]	16.74±0.020[c]	2.65±0.048[bc]	4.35±0.011[a]	5.61±0.21
T3	71.15±0.020[c]	16.94±0.022[c]	2.98±0.040[c]	4.32±0.014[a]	4.61±0.14

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively. Mean±SE of three replicates, *Means in a column bearing the same letter (a, b, c) are not different (p<0.05)

Table 4: Peroxide value (PV) of European Anchovy fish burgers supplemented with sage extract during 4 month storage at -18°C

	Storage period (month)					
Treatments	0	1	2	3	4	Mean
Control	0.557±0.006	0.960±0.010	5.840±0.010	7.500±0.100	9.090±0.010	4.789[a]
T1	0.480±0.010	0.900±0.010	3.150±0.010	4.500±0.100	4.800±0.100	2.766[d]
T2	0.510±0.010	0.930±0.010	3.050±0.010	5.500±0.100	6.000±0.100	3.198[b]
T3	0.620±0.010	0.950±0.010	4.040±0.010	4.900±0.100	5.300±0.100	3.162[c]
Mean	0.541[e]	0.935[d]	4.020[c]	5.600[b]	6.298[a]	

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively. Mean±SE of three replicates, *Means in a column bearing the same letter (a, b, c) are not different (p<0.05)

However, the burger samples formulated without adding sage extract (control) showed faster rates of lipid decreasing after 4 month storage under the same conditions.

Similar results reported by some authors[3,64]. The decreasing in fat content might be due to oxidation and hydrolysis of lipids which result in the formation of some volatile compounds as aldehydes and ketones. Same finding was mentioned[63].

The results indicated that ash contents of control and formulated burgers samples significantly increased after four month storage at -18°C.

Similar observation was found during frozen storage of some fish products[3,64]. The increase in ash contents of fish products during frozen storage might be attributed to the loss recorded in the concentration of protein and fat content which reflected the increasing found in ash contents.

The increase in nitrogen free extract contents of fish products may be due to the decrease occurred in moisture, protein and fat contents during frozen storage. The variation in the chemical composition of fish is related to nutrition, living area, fish size, catching season, seasonal and sexual variations as well as other environmental conditions[63].

Rancidity of extracted oil from European Anchovy fish burgers

Peroxide Value (PV): The chemical spoilage associated with fish during storage is mainly due to fish lipid degradation (auto-oxidation). In general, fish have high degree of unsaturated lipids than other food commodities. Fish lipids are subjected to two main changes, lipolysis and auto-oxidation. The main reactants in these processes involves atmospheric oxygen and fish unsaturated lipids, leading to the formation of hydroperoxides, associated with tasteless, flavor and accompanied by brown yellow discoloration of the fish tissue. Upon further degradation of hydroperoxides are the formation of strong rancid flavors e.g., aldehydes and ketones, usually associated with spoilt fatty fish species[65].

Several lipid oxidation indices were assessed to follow up the development of oxidation in frozen state. Peroxide value showed primary oxidation products[30,31]. The peroxide test is a measure of the formation of hydroperoxides. An increase in the PV is most useful as an index of the earlier stages of oxidation which on oxidation proceeds and peroxides decrease at final stages and the PV can start to fall[8]. When the peroxide value exceeded 10 meq oxygen kg^{-1} fat of fish or meat, the meat is then considered unfit for human consumption or refused[65].

Peroxide values were measured in extracted oil from European anchovy fish burger samples stored at temperatures -18°C for 4 month (Table 4). Peroxide values ($meqO_2$ kg^{-1}) of control and all the samples supplemented with sage essential oil significantly increased during storage period. Results showed that there was a significant difference in the peroxide value during the storage period. The lowest PV

Table 5: Free fatty acids (FFA) of European Anchovy fish burgers supplemented with sage extract during 4 month storage at -18°C

	Storage period (month)					
Treatments	0	1	2	3	4	Mean
Control	1.140±0.010	2.510±0.010	2.980±0.010	3.157±1.155	4.200±0.010	2.797^{a}
T1	1.320±0.010	2.130±0.010	2.410±0.010	2.960±0.010	4.320±0.010	2.628^{b}
T2	1.250±0.010	2.450±0.010	2.390±0.010	2.810±0.010	4.390±0.010	2.658^{ab}
T3	1.160±0.010	1.970±0.010	2.180±0.010	2.630±0.010	4.220±0.010	2.432^{ab}
Mean	1.218^{e}	2.265^{d}	2.490^{c}	2.889^{b}	4.283^{a}	

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively, Mean±SE of three replicates, *Means in a column bearing the same letter (a, b, c)are not different (p<0.05)

(0.541 meqO_2 kg^{-1}) was detected at zero time (after preparation), while the highest PV (6.298 meqO_2 kg^{-1}) was detected after four month storage. On the other hand results indicated that the treatment 2 samples was the significantly lowest PV (2.766 meqO_2 kg^{-1}), while control samples (Co) was the significantly highest PV (4.789 meqO_2 kg^{-1}). This could be explained that antioxidant sage extract prevented fish burgers from oxidation. Different studies showed that the addition of rosemary extract or sage extract into fish burgers resulted in lower oxidation in treated samples compared to control groups in terms of peroxide values[19,30,31,35,66,67].

Free fatty acid (FFA): Free fatty acids content has been used to establish the grade of deterioration. Lipids (glycerol and fatty acids esters) present in the fish muscle undergo hydrolysis, resulting in the release of free fatty acids. Due to lipid hydrolysis, FFA accumulates in the tissue during frozen storage[31].

Free fatty acids were measured in extracted oil from European anchovy fish burger samples stored at temperatures -18°C for 4 month (Table 5). Free fatty acids values as oleic acid of control fish burger and all the samples supplemented with sage essential oil significantly increased during storage period. Results indicated that the Free Fatty Acids (FFA) was increased significantly through the storage period with minimum number (1.218) at zero time (after preparation), while the maximum number was (4.283) at the fourth month (End time). On the other hand results showed that was significant difference between the four treatments with different sage extract levels. The lowest FFA (2.432) was detected at T3, while the highest FFA (2.797) was detected at Co treatment. Free fatty acids of fish burger samples during storage was found to be in parallel to some studies[31,58]. A similar trend was also observed by the Yerlikaya *et al.*[8] during the refrigerated studies of fish patties. These changes have been attributed to enzymic reactions which take place at a rate governed by the temperature of frozen storage. Tokur *et al.*[18] published that the FFAs were a result of enzymatic decomposition of lipid during chilled and frozen storage of fish products.

Fatty acid composition: Data in Table 6 showed fatty acids composition of oil extracted from control and supplemented European anchovy fish burgers with sage extract (2% as T1, 4% as T2 and 6% as T3) before and after 4 month storage at -18°C. A total of 14 fatty acids were determined by using GC-MS.

In general, results of fatty acid composition showed that saturated fatty acids (SFAs) in fresh fish burgers samples were in the highest levels ranged from 37.48-37.90%, followed by polyunsaturated fatty acids (PUFAs) ranged from 31.31- 33.51 and monounsaturated fatty acids (MUFAs) ranged from 29.01-31.01%.

The obtained results, PUFAs of raw silver carp oil recorded about 35%, SFAs were 34% and MUFAs were 31%.

Regarding to SFAs, palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) and myristic acid ($C_{14:0}$) were the major fatty acids among the SFAs of European anchovy fish burgers and lauric acid ($C_{12:0}$) was in minimum in value (Table 6). Differences were observed between treatments among the total SFAs after 4 month storage at -18°C. Total SFAs increased more in control than supplemented burgers with sage extract (T1, T2 and T3).

The increase in SFAs during the freezing for 180 d was reported in sardine meat (*Sardinella aurita*)[68], which was similar to the results obtained in this study. This performance was due the degradation of PUFAs, which generated low molecular weight compounds and possibly short chain FAs.

The results from this study were in agreement to those reported by Pirestani *et al.*[69] in several species of fishes from South Caspian, when fillets were stored at -8°C during 6 months. Saldanha *et al.*[70] found that Brazilian sardine (*Sardinella brasiliensis*) presented the same FAs performance when was frozen at -8°C for 120 days.

Regarding to MUFAs, oleic acid ($C_{18:1}$) was in the maximum value of oil extracted from fresh anchovy fish burgers (23.24-24.88% of total fatty acids) as compared to other MUFAs. Total MUFAs increased more in supplemented burger samples than control after four month storage at -18°C.

European anchovy fish generally contains a high level of oleic acid and a low level of linoleic acid[71].

Table 6: Fatty Acid profile of extracted oil from European anchovy fish burgers samples at zero time and after 4 month storage (end time) at -18°C

Fatty acids	Treatments			
	Control	T1	T2	T3
Lauric $C_{12:0}$				
Zero time	0.73	0.84	0.81	0.42
End time	1.11	0.95	1.23	0.31
Myristic $C_{14:0}$				
Zero time	6.38	6.66	6.57	6.73
End time	7.38	6.32	6.57	7.03
Palmitic $C_{16:0}$				
Zero time	21.90	21.43	21.62	21.72
End time	24.32	23.22	21.92	21.55
Stearic $C_{18:0}$				
Zero time	8.67	8.97	8.73	8.61
End time	13.34	11.63	10.57	9.12
Total SFA				
Zero time	37.68	37.90	37.73	37.48
End time	46.15	42.12	40.29	38.01
Palmitoleic $C_{16:1}$				
Zero time	4.77	4.91	4.98	4.01
End time	3.63	4.32	4.79	4.89
Oleic $C_{18:1}$				
Zero time	24.88	23.92	23.24	23.51
End time	32.42	33.23	33.76	32.49
9n Eicosaenoic $C_{20:1}$				
Zero time	1.36	1.41	1.42	1.49
End time	ND	0.32	0.63	0.57
Total MUFA				
Zero time	31.01	30.24	29.64	29.01
End time	36.05	37.87	39.18	38.95
Linoleic $C_{18:2}$ n6				
Zero time	3.58	3.02	3.15	3.01
End time	1.53	1.8	3.01	1.13
Linolenic $C_{18:3}$ n3				
Zero time	0.73	0.71	0.73	0.76
End time	0.20	0.33	0.44	0.68
Arachidonic $C_{20:4}$ n6				
Zero time	0.59	0.52	0.55	0.56
End time	ND	ND	ND	ND
EPA $C_{20:5}$ n3				
Zero time	7.43	7.40	7.43	7.38
End time	4.41	5.15	5.10	5.73
DPA $C_{22:5}$ n3				
Zero time	2.36	3.11	2.31	2.09
End time	1.01	1.02	0.90	0.70
DHA $C_{22:6}$ n3				
Zero time	16.62	17.10	18.46	19.71
End time	9.65	11.71	13.08	14.80
Total PUFA				
Zero time	31.31	31.86	32.63	33.51
End time	16.80	20.01	22.53	23.04
PUFA/ SFA				
Zero time	0.831	0.841	0.865	0.894
End time	0.364	0.475	0.559	0.606
PI				
Zero time	1.100	1.143	1.198	1.247
End time	0.578	0.726	0.829	0.940
AI				
Zero time	0.773	0.788	0.782	0.784
End time	1.040	0.854	0.801	0.799
IT				
Zero time	0.350	0.337	0.332	0.324
End time	0.770	0.487	0.453	0.372

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively, EPA: Eicosapentaenoic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, DHA: Docosahexaenoic acid, PUFA: Polyunsaturated fatty acids, PI: Polyene index, AI: Atherogenic, IT: thrombogenic

Regarding to polyunsaturated fatty acids PUFAs, total PUFA decreased more in control than supplemented burgers with sage extract (T1, T2 and T3) after 4 month storage at -18°C. Fresh European Anchovy fish burgers contained high concentrations of n-3, which included docosahexaenoic acid (DHA) presenting the highest concentration (16.62-19.71%) of PUFA followed by eicosapentaenoic acid (EPA) presenting (7.38 -7.43%) of PUFA. When fish burgers were frozen, n-3 concentration decreased. At the end of the trial (4 month of frozen storage), DHA and EPA decreased more in control samples from 16.62% and 7.43% at zero time to 9.65 and 4.41%, respectively after 4 month storage than supplemented sample T3 from 19.71% and 7.38% at zero time to 14.80 and 5.73% after 4 month storage.

During storage, fish are capable of converting PUFA to the shorter chain fatty acids[72-74]. For this reason, saturated fatty acids levels were increased after four month storage at -18°C. These results were in agreement with results of Yildiz *et al.*[75].

Polyunsaturated fatty acids reduction was due to oxidative and hydrolytic reactions that occurred during the storage. Chen *et al.*[76] showed that long hydrocarbon chains and high unsaturation of PUFA made them more susceptible to hydrolytic reactions than the SFA. This susceptibility to these reactions could be influenced by the high content of DHA found in the fresh fish. Because of this PUFAs are the main FAs involved in the processes of oxidation[77].

The effects of storage time on the lipid quality of anchovy fish burgers were examined in this study.

The PUFA/SFA ratio is used to estimate the nutritional quality of lipids and their influence on coronary heart disease[78]. Health guidelines[79] recommend a ratio >0.436 (FAO/WHO, 1994). In the present study, PUFA/SFA ratio was of 0.831 for fresh control burger samples, which was higher than the minimum suggested (0.450) for a human healthy diet[80]. This ratio decreased to 0.364 after 4 month storage at 4°C, which was lower than the minimum suggested for a human healthy diet. On the other hand, this ratio decreased in supplemented burger sample T3 from 0.894-0.606 after 4 month storage at -18°C, which both were higher than the minimum suggested for a human healthy diet.

Similar results in rainbow trout were reported[81]. During the frozen storage, PUFA/SFA ratio decreased significantly ($p \leq 0.05$) due to loss of PUFAs and as expected SFAs increased. Similar to these results, Perez-Mateos *et al.*[82] had observed that this ratio decreased during 90 d storage of surimi fish at -22.3°C.

It has been suggested that EPA+DHA/C16 ratio (polyene index PI) is a good index for a determination of lipid oxidation[83]. PI is an effective parameter for measuring the oxidative rancidity of anchovy fish burgers. During frozen storage, the index declined, while atherogenic (AI) and thrombogenic (IT) indexes increased.

Atherogenic and thrombogenic indexes could be used as a tool in order to compare how healthy was the lipid fraction of different foods[84].

The results of this study could be attributed to DHA being the most reduced PUFA and because it was found in high amounts in the fish burger samples.

Results showed that during the frozen storage, polyene index decreased because the relationship among the PUFA and palmitic acid decreased due to a reduction of DHA and EPA and an increase in palmitic acid concentration. The change in PI value is mainly due to the degradation of DHA and EPA.

In this study, the decrease of PI resulted in an increase in primary and secondary oxidation products (POV and TBARS).

Sensory evaluation: The results of sensory evaluation are one of the most important quality criteria used for determination of shelf life of seafood. The changes of sensory properties (colour, odour, taste, texture and overall acceptability) of fried anchovy fish burgers supplemented individually with 2.0%, 4.0% and 6.0% sage essential oil and untreated (control) during freezing storage at -18±1°C were recorded in Table 7.

The results of the sensory evaluation (colour, odour, test, texture and overall acceptability) of anchovy fish burgers are presented in Table 7. According to the statistical analysis, there were no significant differences ($p>0.05$) in colour, odour, taste and texture between all fish burger treatments at zero time before storage. Significant differences ($p<0.05$) were observed between the control and treated samples after storage at -18±1°C. T2 and T3 were mostly preferred by the panelists. The use of sage extract improved the sensory quality of fish burgers. Similar results have been reported in the other fish products treated with rosemary and sage extracts[34,85,86].

In the present study, it was demonstrated that the addition of essential oils into anchovy fish burgers did not affect certain sensory properties as colour, odour, taste and texture before freezing storage but had an effect on the sensory of the product after 4 month freezing storage. However, the fish burgers used in the present study are a very complex food containing spices such as cumin, white pepper, onions etc. Presumably, the negative effect of essential oils on the sensory attributes of the fish burgers may be masked by

Table 7: Sensory analysis of fish burgers before and after 4 month storage at -18±1°C

	Treatments			
Sensory parameter	Control	T1	T2	T3
Colour				
Zero time	9.40[a]	9.60[a]	9.70[a]	9.70[a]
End time	8.40[b]	7.20[c]	6.60[d]	6.50[d]
Odour				
Zero time	8.60[a]	9.20[a]	9.050[a]	8.60[a]
End time	5.00[b]	6.20[c]	7.00[d]	7.20[d]
Taste				
Zero time	9.60[a]	9.60[a]	9.30[a]	9.40[a]
End time	4.60[b]	7.00[c]	7.80[d]	7.60[d]
Texture				
Zero time	9.20[a]	9.40[a]	9.40[a]	9.70[a]
End time	4.60[b]	7.20[c]	7.40[c]	7.40[c]
Overall acceptability				
Zero time	9.20[a]	9.46[a]	9.34[a]	9.36[a]
End time	4.60[b]	6.90[c]	7.20[d]	7.18[d]

Treatments T1, T2 and T3 are fish burgers supplemented with 2, 4 and 6% sage essential oil, respectively, Mean of ten panelists, *Means in a column bearing the same letter (a, b, c, d) are not different ($p<0.05$)

the ingredients and spices used in the production of fish burgers. The use of higher concentrations than we used in the present study may result in a further increase of the shelf life of fish burgers but high EO concentrations would probably impart unpleasant sensory effects (strong odour and flavour, etc.) on the quality of fish burgers. Natural preservatives such as essential oils can be used as a safe method for storage of fish burgers. According to Orak and Kayisoglu[87], the decrease in the values of sensory analyses was faster than chemical changes during frozen storage.

Oxidation of unsaturated fatty acids could produce ketones, aldehydes, alcohols, hydrocarbons, acids and epoxides that interact with proteins thereby forming off-color during frozen storage[88]. Also the formation of aldehydes and ketones can cause denaturation of myofibrillar proteins and rancid off-flavors that affect the sensory attributes even in a little amount[19].

Even through the added EOs delayed oxidation and extending the shelf-life, their antioxidant activity reduced during storage by increase significantly ($p<0.05$) differences in all individual tested samples during the end of storage periods.

The present study showed that a treatment with 2-4% sage extract kg^{-1} fish burger could effectively delay chemical deterioration, maintain or improve sensory attributes and extend the shelf life of fish burger samples for 4 month during freezing storage. However, further studies are needed with regard to the preservation of fish burgers using natural preservatives, including essential oils, in view of increasing the consumer demand for preservative-free seafood.

CONCLUSION

The obtained results in this study showed the utilization of unaccepted cheap small anchovy fish from consumers in its fresh form in the production of good fish burgers products high safely, having good quality and better acceptability with lowering the costs. The tested essential oils extracted from sage leaves had high effectiveness as an antioxidant and should be utilized for extending the shelf-life and enhancing quality attributes of anchovy fish burger during frozen storage at -18±1°C. The chemical and the sensory analysis revealed that prepared anchovy fish burgers significantly affected by frozen storage for 4 months at -18°C. Anchovy fish burgers supplemented with 4-6% sage extract were of high quality and acceptance even after the storage period is over.

SIGNIFICANCE STATEMENT

This study discovered the significant effect of Egyptian sage essential oil that can be beneficial for keeping quality and sensory evaluation of prepared anchovy fish burger samples during frozen storage at -18°C for 4 months.

This study will help the researchers to uncover the essential oils extracted from medicinal and aromatic plants as sage essential oil as natural antioxidant in meat and fish products.

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