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Effect of Different Salting Methods on Physicochemical Properties, Quality Characteristics and Microbiological Analysis of Sardine (Sardina Pilchardus) during Ripening at Ambient Temperature

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Abstract

In recent years, sardines (*Sardina pilchardus*) are pelagic fishes of notable gastronomic and economic importance around the world, and their consumption is very large. Therefore, the effect of different salting methods on physicochemical properties, quality characteristics and microbiological analysis of sardine during ripening at ambient storage conditions for 90 days were investigated. The proximate chemical composition of fresh sardine was: 69.46 ± 0.77% moisture, 18.41 ± 0.12% crude protein, 10.77 ± 0.33% crude fat, 1.28 ± 0.21% ash, 0.08% carbohydrate on ww. It was found that the protein, ash, and salt contents of the salted sardine samples increased, while the contents of moisture, and pH decreased during ripening at storage period. Lipids content did not show a typical trend of changes within salting duration at ambient temperature. TBARS and TVB-N values of sardine were found to be 0.17 ± 0.48 mg MA/kg and 27.52 mg/100g at the beginning, 4.74 ± 0.9 and 5.28 ± 0.135 mg malonaldehyde/kg, 83.16 and 85.68 mg/100g at the end of the ripening period for scientific and commercial samples, respectively. The microbiological quality parameters analyzed in this study indicates that the sardine samples were of high quality and safe for utilization either as fresh sardine or for salting processing and use. Generally, characterization of salted sardines in terms of their safety and chemical composition will greatly help in designing the optimum conditions in developing the method of salting process, leading to higher consumer acceptability of the salted sardines. In conclusion, the present data suggest the superiority of scientific salting method over commercial salting method applied.

Keywords: Sardina pilchardus; Salting; Quality characteristics; TBARS; TVB-N; Microbiological analysis; Ripening

Introduction

Fish is one of the most important sources for the provision of animal protein and has been widely accepted as a good source of protein for the maintenance of healthy human's body [1]. In addition, it provides several other essential nutrients such as vitamins A and B particularly in liver, K and E vitamins, and it is a good source of some minerals like phosphorus, iron, zinc, iodine and calcium [2]. In general, the proximate composition of fresh fish is given as 66–81% water, 16–21% protein, 0.2–15% fat, 1.2–1.5% ash and 0–0.5% carbohydrate. The global contribution of fish and fish products as sources of protein is high, ranging from 10 % to 15% of the human food across the world [3]. Small pelagic fishes such as sardine (Sardina pilchardus), anchovy, herring, mackerel and sardinella (Sadinella aurita) have a strong worldwide economic, livelihood, nutrition and ecological importance, as (i) they represent about 25% of the global landings of capture fisheries and (ii) they function a key role in maintaining environmental processes in marine systems, occupying a fundamental intermediate trophic level in pelagic

ecosystems [5-6]. The nutritional assessment of small pelagic fishes and more specifically sardines exhibited their richness in essential fatty and amino acids, minerals, vitamins and are characterized by their high digestibility. Sardines are an excellent source of polyunsaturated fatty acids such as docosahexaenoic acid (C22:6n-3, DHA), docosapentaenoic (C22:5n-3, DPA) and eicosapentaenoic acid (C20:5n-3, EPA), which have various health enhancing properties [7,8]. Regular consumption of sardines allows the prevention of considerable many diseases such as diabetes, cardiovascular and cancer inflammatory diseases [9]. Thus, sardines consumption for human being is advisable due to its high nutritional quality given by the appropriate balance of amino acids and healthy unsaturated fatty acids [10,11]. The annual average price registered for sardines ranged between 1.02 and 1.58 Euros per kilogram. Fish quality is spontaneous in nature and is very complicated concept, which includes physiochemical, microbiological, nutritional and biochemical attributes. The freshness of fish degrades after death due to the changes in lipid and protein fractions, the formation of biogenic amines and the microbiological spoilage. This results in the deterioration of nutritional value and sensory quality of fish and therefore led to a very short of shelf life and hence needs at-most attention to maintain the quality of fish for longer duration [12]. Preservation of fish considers greater importance to prevent the loss of this nutritionally rich natural resource. Drying, salting and canning are the most common methods used for preserving fish and seafood products primarily small pelagic from spoilage and decay [13]. Of the various preservation techniques, food salting process represents an effective obstacle to the microbial growth in fishery products, especially in anchovies and sardines. However, when salt fails to penetrate fish tissues and remove water (water available for the encouragement of microbial growth which causes the spoilage) potential pathogenic and spoilage microorganisms can easily grow. Concentration of salt from 6 to 10% in the tissues will prevent the activity of most spoilage bacteria. Salting is performed either by brine, dry, or injection salting or a combination of these methods. Dry salting has been the most widely used methods by the industry. It is the traditional salt-curing method used during processing of salted fish in many countries. The salting method leads to obtain a tender fishery product particularly pleasant aroma and taste, due to the diffusion of salt into fish tissues and subsequently to the enzymatic reactions that decompose proteins and fats during marinating [14]. In Egypt, sardines fish consumption is very large and generally consumed as fresh or salted and also used as fish meal. Furthermore, the implementation of innovative technique for the preservation of sardines might represents an important economic way for several manufacturing companies located around the world. Therefore, the main objective of the present research was to characterize the chemical composition of fresh sardine (*Sardina pilchardus*) obtained from Fayoum governorate, Egypt and to evaluate the effect of different salting methods on physicochemical properties (proximate composition, lipid oxidation indices, pH, and salt content), quality characteristics and microbiological analysis of sardine during ripening at ambient storage conditions for 90 days.

Material and Methods

Fish samples

About 15 kg of fresh sardine fish (*S. pilchardus*) were purchased from local fish market (Fayoum governorate). After that, they were transferred to the laboratory of food science and technology department, faculty of agriculture, Fayoum University in polyethylene bags with crushed ice at within 1 hour. Up on arrival to the laboratory, the sardine was quickly washed with tap water. The average length and weight of fish sardine samples were 22 cm and 88 g, respectively.

Edible salt

Commercial Sodium chloride salt was used. It composed of 98.5% sodium Chloride, 30- 70 ppm, potassium iodate, and 0.3% humidity.

Plastic containers and polyethylene bags

Queen containers (28×20×14cm) produced by Khorshed Plastic company and polyethylene bags were used.

Salting treatment

Raw sardine fish was divided into two equal batches: (a) Traditional batch: whole fish samples were washed with tap water, left on ambient temperature (22 ± 2) for 24 hours before salting technique. (b) Scientific batch: Fish samples were washed carefully by tap water and salted directly. Sardine fish batches (traditional and scientific) were salted by commercial salt at 20 % salt concentration (w\w). Each batch was dry salted as follows: Different layers of fish with salt were well mixed as well as abdomen cavity and gills of fish were filled with salt and finally packed in polyethylene bags. In addition, the bottom and surface layers of salt were added. The polyethylene bags were put into plastic containers and tightly closed. All containers were covered by black polyethylene bags and stored at room temperature (22 ± 2ºC) for 90 days.

Preparation of samples for analysis

Raw sardine fish and salted samples picked in salt were picked up from the container, and the salt above the fish surface was completely removed. Then fresh and salted samples.

Analytical Methods

Chemical composition analysis of sardine fish

Moisture, crude protein, fat, ash and salt contents were determined according to method described by [15].

pH value

5 g of raw and salted sardine fish samples were homogenized with 50 ml of distilled water and filtered using filter paper. The pH value of filtrate was measured using digital pH meter according to the method of [16].

Total volatile bases nitrogen (TVB-n) content

TVB-N content was determined by the method described by Pearson using Macro-Kjeldehl distillation apparatus as follow: 10g of minced fish sample were mixed with 100 ml distilled water, 200 ml distilled water, 2g MgO and antifoaming agent were added [17]. 25 ml of 2% boric acid solution were added into 500 ml receiving flask and a few drops of mixed indicator (0.1g of methyl red and 0.1g of methylene blue to 100 ml of ethanol) where the condenser terminal must be dipped in boric acid solution. After boiling by heating, the condenser was washed with distilled water and titrate the distillate with sulfuric acid (0.1 N). Multiply the titration (minus blank) by 14 to obtain the TVB-N as mg N per 100g sample

Thiobarbituric acid (TBA) value

TBA was determined calorimetrically in minced fish flesh samples as described by [18]. Ten grams of minced fish flesh were macerated with 50 ml of distilled water for 2 min, washed into a distillation flask with 47.5 ml distilled water, and 2.5 ml of hydrochloric acid (4 N) were added. A volume of 50 ml distillate was collected, and from which 5 ml were pipette into glass coppered tube and mixed with 5 ml of TBA reagent. The mixture was heated in boiling water bath for 35 min. after cooling; the optical density was measured against the blank at 538 nm. The method based on the spectrometric quotation of the pink complex formed after reaction of one molecule of malonaldhyde (MDA) with two molecules of TBA. The TBA value was expressed as mg malonaldehyde per kg sample.

Microbiological analysis

10 gm. of fish sample were aseptically weight and homogenized with 90 ml of sterile saline water for 1 min from each treatment. The homogenized samples were serially diluted using 9 ml sterile saline for bacteriological analysis. Total viable count (TVC), yeasts and molds count were examined during ripening periods. Total viable count was determined by using nutrient agar medium [19]. Yeasts and molds count were enumerated on malt agar as mentioned by [20].

Statistical analysis

The statistical analysis of the results obtained was carried out according to SPSS version 16 software program 2007.

Result and Discussion

Proximate composition of fresh sardine

Over the last decades Arabian countries have witnessed a rapid growth and development in different industrial and economic sectors, with increase in population Chemical and biochemical procedures for the evaluation of food quality are more credible and accurate, since they eliminate personal opinions on the product quality. Moreover, the knowledge of proximate analysis of seafood is fundamentally important for the application of different processing techniques and for storage stability. The proximate composition of raw sardine fish obtained from local market, Fayoum governorate, Egypt conducted at day 0 is shown in (Table 1).

Table 1: *Proximate composition biochemical characteristics (pH, TVB-N and TBARS) and salt content of fresh sardine samples.*

Quality attributes	Sardine (*Sardina pilchardus*)
Moisture %	69.46 ± 0.77
Ash %	1.28 ± 0.21
Crude fat %	10.77 ± 0.33
Crude protein %	18.41 ± 0.12
Carbohydrate % **	0.08
Salt content %	1.08 ± 0.58
pH value	5.75
TBA(mg MA\ kg)	0.17 ± 0.48
TVB-N (mg\100 g)	27.52

The results indicated that the proximate chemical composition of raw sardine fish used in the experiments on wet weight basis was: 69.46 ± 0.77% moisture, 18.41 ± 0.12% crude protein, 10.77 ± 0.33% crude fat, 1.28 ± 0.21% ash, 0.08% carbohydrate. These results are comparable to previous findings for similar sardine pelagic fish samples that protein being the main constituents, followed by lipids, ash and carbohydrate content is low, around 0.5 % reported that the chemical composition of raw sardine was 69.20% moisture, 24.1% protein, 3.6% fat and 2.22% ash, while found the proximate composition of sardine (*Sardinella Brasiliensis*) was 70.41%, 22.73%, 2.75% , 4.41, 1.08 and 0.08% moisture , protein , fat , ash , NaCl and carbohydrate contents, respectively. Another author reported that proximate chemical composition of sardine (*Sardina pilchardus*) was 66.0%; 13.5%; 16.0 and 2.7% for moisture; lipid; protein; and ash, respectively [20-21]. Data are mean ± SD of three replicates; TVB-N is the total volatile base nitrogen; TBARS is the thiobarbituric acid reactive substances value. Based on wet weight % by difference reported that the proximate chemical composition of sardine varies greatly from one species to another and from one individual to another depending on nutrition, habitat, age, size of fish, gender, and months of capture, as well as variations in environmental season conditions. Also suggested that harvesting season and species specific physiological characteristics due to physiological reasons and changes in environmental conditions, such as spawning, migration, and starvation or well-feeding, can bring variability in the proximate composition of small- sized fish species. Of notes, the total fat content of sardine (10.77 %) was higher than those reported by who found that the small pelagic fish include sardine had a crude fat content between 3.26% and 3.85%. Besides the species, Fish lipid content variation depends on the season, geographic regions, type of production, typical maturity, and nutrition as well as whether the species is being cultured or living in the wild [22-24]. According to the classification of fish by whereby fish can be classified as lean fish (< 2% fat); low fat (2–4% fat); medium fat (4–8% fat); and high fat (> 8% fat), the fresh sardine in our study can be classified as high-fat fish. These findings were supported by previous reports of**,** who reported that the flesh of fast-moving, migratory species (such as tuna, mackerel, herring, anchovy, and sardine) contains more dark muscle tissue and more fat. In contrast reported that sardine species and Indian mackerel are low and medium-fat fish. The results showed also that the sardine small pelagic fish possessed considerably higher protein content (18.41%) and therefore can be considered as a good source of protein [25-28].

Effect of salting method on chemical composition of salted sardine during ripening at ambient temperature

Moisture content: Shows the moisture content of sardine samples salted by scientific and commercial methods and stored for 90 days at ambient temperature [29]. Salting process reduces the moisture content in foods, due to osmotic dehydration, whereby in our research salting led to a reduction of up to 10% in moisture content of the fresh sardine (Table 2). Moisture content of raw sardine samples was 69.46%, this values decreased to 48.04 and 49.44% after 15 days of salting by scientific and commercial methods, respectively. There was a further decrease in the moisture content after 30 days of ripening, while after 45 days the moisture content was increased in both scientific and commercial samples. After 60 days moisture content was decreased again to 45.68 and 49.38% for scientific and commercial samples, respectively. While after 90 days moisture content was increased to 50.57 and 51.03% for scientific and commercial treatments, respectively. Same trend found by who found that after salting process of anchovy, the moisture content decreased from 75.5% in fresh fish to 54.16% in salted fish and the loss in moisture content was accompanied by increase in the salt and ash contents. Also, reported that moisture content of fresh sardine fillets (Sardina aurita) (73.62%) decreased continuously from the surface to the bottom of the fillets. However, found no significant change in moisture content values for salted-pressed spotted sardine samples stored under ambient conditions during the ripening period of 6 weeks. Another study by who reported that moisture and lipid contents in raw European eels varied during ripening and storage The lower moisture content of the food helps prolong its shelf-life, quality and prevents propagation of pathogenic and spoilage microorganisms, due to a decrease in water activity. From the it could be concluded that the decreasing in moisture content of salted sardine was slightly higher in scientific treatment samples than commercial salting samples.

Protein content: Data presented in showed the protein content of scientific and commercial salted sardine samples during ripening at ambient storage (22 ± 2ºC) for 90 days. Protein content of sardine samples increased during the ripening period whereby the protein content of scientific and commercially salted sardine samples were found to be 24.34 ± 1.15 and 20.60 ± 0.325 %after 90 days, respectively [30]. This increase in protein content may due to the reduction in moisture contents during storage of salted sardine samples. These data are not in accordance with that obtained by Michael who found that the crude protein did not significantly change throughout the six weeks of salting. Also, reported that during the salting method, the changes in protein structure like protein denaturation of cod occurred when brine concentration raised from 20 to 25% due to the protein salting-out and the amount got lower than that obtained when using 20% brine. Therefore, the present data suggest the superiority of scientific salted sardine in protein content during ripening over commercial salted sardine.

Table 2: *Effect of salting methods on proximate chemical composition of salted sardine during ripening at ambient temperature.*

Ripening period in (day/ambient)	Moisture content % (ww)		Protein content % (ww)		Lipid content % (ww)		Ash content % (ww)	
	Scientific	Commercial	Scientific	Commercial	Scientific	Commercial	Scientific	Commercial
0	69.46 ± 0.77	69.46 ± 0.94	18.41 ± 0.12	18.41 ± 0.12	10.77 ± 0.33	10.77 ± 0.33	1.28 ± 0.21	1.28 ± 0.21
15	48.04 ± 0.62	49.44 ± 0.25	23.69 ± 2.66	21.38 ± 2.33	13.61 ± 0.29	15.29 ± 0.59	14.04 ± 0.63	13.37 ± 0.03
30	46.83 ± 0.64	48.48 ± 0.24	25.07 ± 8.48	24.52 ± 2.01	13.90 ± 0.42	13.77 ± 0.03	13.75 ± 0.72	13.33 ± 0.93
45	48.11 ± 0.21	50.39 ± 0.74	22.93 ± 1.15	21.73 ± 0.90	12.33 ± 1.28	11.69 ± 0.34	16.07 ± 1.45	15.40 ± 0.33
60	45.68 ± 0.13	49.38 ± 0.89	26.33 ± 1.13	21.15 ± 0.78	11.28 ± 0.41	12.76 ± 0.29	16.11 ± 0.41	15.79 ± 0.25
75	46.64 ± 0.21	50.26 ± 0.26	25.06 ± 1.76	21.95 ± 2.04	13.68 ± 0.45	13.13 ± 0.92	15.72 ± 0.08	15.25 ± 0.98
90	50.57 ± 0.32	51.03 ± 0.39	24.34 ± 1.15	20.60 ± 0.33	7.04 ± 0.48	11.63 ± 0.6	17.05 ± 1.04	16.57 ± 0.96

Table 3: *Hanges in salt content, pH values, total volatile base nitrogen (TVB-N), and thiobarbiuturic acid (TBARS) values of salted sardine during ripening at ambient temperature.*

Ripening period in (day/ambient)[b]	Salt content % (ww)		pH values		TVB-N values (mg/100gm)		TBARS values (mg/kg)	
	Scientific	Commercial	Scientific	Commercial	Scientific	Commercial	Scientific	Commercial
0	1.08	1.08	5.75	5.75	27.52	27.52	0.17 ± 0.48	0.17 ± 0.48
15	18.91	22.68	5.48 ± 0.005	5.54 ± 0.005	39.56	42.58	6.06 ± 0.09	5.17 ± 0.03
30	16.45	26.32	5.57 ± 0.005	5.60 ± 0.005	103.32	57.96	5.07 ± 0.06	4.95 ± 0.92
45	20.01	30.45	5.60 ± 0.005	5.43 ± 0.005	60.48	40.44	4.21 ± 0.74	5.86 ± 0.12
60	23.55	18.42	5.54 ± 0.095	5.61 ± 0.005	52.92	80.64	4.02 ± 0.1	4.96 ± 0.78
75	20.41	15.843	5.35 ± 0.005	5.24 ± 0.005	83.16	85.68	4.42 ± 0.39	5.58 ± 0.25
90	29.54	24.00	5.34 ± 0.005	5.32 ± 0.005	66.15	95.50	4.74 ± 0.9	5.28 ± 0.14

Lipid content: The rancidity of salted fish, which negatively affect the quality and the technological characteristics of the finished product, was found to increase related to many factors such as fish species, lipid content, time of salting, and the degree of unsaturation of fatty acids shows the changes of lipid content of scientific and commercial salted sardine samples during ripening at ambient storage (22 ± 2ºC) for 90 days. After 15 days lipid content increased in both scientific and commercial salting treatments to 13.61 and 15.29%, respectively. This increase may be due to the loss of water as a result of salting process and breakdown of lipoprotein during salting process and release of lipids to tissue muscles [31]. Also, lipid content increased after 30 days in scientific salted samples to 13.9%, while decreased in commercial treatment to 13.77% at the same period. Same trend reported by Cha who found little differences in total lipids due to salt concentration and the time of salting within the same fish species. At 45 days lipid content decreased to 12.33% in scientific treatment samples and 11.69% in commercial treatment samples. The decreasing in lipid content, may be due to their insolubility in water, which diffusion over the cell walls to the brine solution and/or the hydrolysis of phospholipids and triglycerides, which is catalyzed by phospholipases and lipases and liberation of free fatty acids (FFA) that is soluble in water [32-34]**.** At the end of ripening the lipid content in scientific and commercial samples reached a value of 7.04 and 11.63%, respectively from an initial value of 10.77, indicating a stable and good final product quality from scientific methods**.** The obtained data agreed with who found that lipid content of fresh Bolti, Labes , Karmout and Kannome decreased during salting and storage and observed relatively increased in lipid content in salted fish after salting. In contrast found that the lipid and moisture contents of eels remained stable after salting process

and exhibited no significant differences relative to the fresh raw sample.

Ash content: Ash content is a good index of the mineral content in fish product. The given data in show the contents of ash in scientific and commercial salting treatments of sardine samples during ripening at ambient storage (22 ± 2°C) for 90 days. There was a general increase in ash content during ripening at ambient conditions. At the end of storage the ash content of scientific and commercial salted sardine samples were found to be 17.05 ± 1.04 and 16.57 ± 0.96%, respectively [35-37]. The increasing in ash content during salting process duration probably due to the effect of extracted lipid which helps to make a crusted surface on every dried fish and the effect of crashed scales and bones in dried fish and meat. Moreover, the presence of remains of salt during preparation of samples for determination and this consequently causes an increase in the ash content. The obtained results agreed with who found that the ash content of salted sardine ranged between 15.15-17.04 %. Another study by Michael who observed that the ash content of salted-pressed sardine throughout the six weeks of storage ranged from 11.8 to 13.0%. Variations in chemical composition are not unusual as who observed variations in chemical composition of salted fish, mainly in protein and moisture during salting process.

Effect of salting method on biochemical quality of salted sardine during ripening at ambient temperature

Salt content: It is important to determine the salt content of fresh seafood so as to establish a baseline for future preservation procedures include salting. NaCl is added to foods for its effects on functional, preservation and sensory characteristics (Table 3). Shows the NaCl contents of salted sardine fish during ripening at ambient storage (22 ± 2ºC) for 90 days. There was a variation of salt content during salting process period, and this may be due to changes in the muscle structure of the fish, which has an effect on water-holding capacity and concomitant salt retention ability of fish [38-40]. For commercial and scientific treatment samples, NaCl contents markedly increased from an initial value of 1.08% to 24.00 and 29.54% at the end of ripening at ambient storage, respectively. The obtained data are agreed with who found that the salt content of salted mullet at 20% salt was 18.88% after 15 days and 29.3% after 90 days of ripening at ambient storage. Also found that the weight gain of salted products depends not only on the brine concentration, but also on the brining temperature and time. However, other findings indicated lower salt content (5.14–6.12%) throughout salting for Eritrean sardines. The salt content of the fresh sardine fish samples were comparable to the findings of Negasi. pH values: pH is the most critical factor affecting microbial growth, quality and spoilage of foods. pH is widely used to measure fish deterioration; it has been common to measure the pH of the muscle tissue. Mean pH values of the salted sardine samples in our study was acidic (5.75), which is in agreement with who found the pH of fish ranged from 5.7 to 6.6. These results also agreed with who found that pH values ranged between 5.47 and 6.31 in traditional salted mullet fish samples obtained from some Egyptian markets. Found that pH value of salted sardine and salted mackerel ranged from 4.89- 5.60 and 4.68-5.32, respectively [41-43]. The pH of newly killed fish is neutral, but after death there is a speedy fall as glycogen breaks down and consequently the formation of lactic acid. On contrast found that the mean pH values of salted small pelagic fish ranged from 7.08–7.30 involve spotted sardine 7.08 ± 0.01. A post-mortem pH of 7 or above is a guide of starvation with depleted carbohydrate stores. The difference can be due to life history before and after harvest, the species chemical composition, fishing ground, and the pH of the aquatic environment. The change in pH of fish could owe to stress during harvesting leading to gathering of lactic acid and/or production of volatile bases by the autolytic action on protein and other compounds. As shown in pH values decreased during ripening at ambient storage in both treatments samples. Same trend found by Hernandez-herrero who found that pH of anchovy muscle appreciably decreased from 6.13-5.72 during the first week of ripening. But in a study by Ana who observed a slight pH increment for sardine samples during the period of ripening. The decrease in pH value from 5.75 to 5.34 and 5.32 of 7.1% and 7.5% of scientific and commercial salted sardine fish, respectively may be due to the ionic strength of the solution inside of the cells. Indeed, salting methods can have an effect on the dissociation of amino acids and small peptides leading to a decrease in pH.

Thiobarbituric acid-reactive substance (TBARS) value

The highly unsaturated fatty acids of fish are easily susceptible to oxidation, resulting in alterations in colour, texture and nutritional value as well as a rancid smell and taste. TBARS is a good indicator of the quality of the fish and is commonly used to measure the secondary oxidative products produced by lipid hydroperoxides decomposition. Shows the effects of the two different salting methods and the ripening period on the formation of thiobarbituric acid reactive substances in sardine [44-45]. The initial value of TBARS (0.17±0.48 mg malonaldehyde /kg) was in agreement with those mentioned by some authors for the same species indicating that the sardine sample in the present study has a good quality. Results showed that the TBARS levels in both salting methods increased sharply to 6.06 and 5.17 mg malonaldehyde /kg after 15 days in scientific and commercial samples, respectively. This is may be due to higher release of free iron and other prooxidants from the muscle and home proteins might undergo degradation, thus lipid oxidation become more pronounced. Consequently the produced

secondary oxidation compounds should arise especially from peroxides breakdown. Additionally, the marked increase in TBARS of sardine may be related with the laboratorial analysis conditions, which promoted some lipid oxidation. In agreement with the current results, an increased formation of secondary oxidation products was observed in canned sardine (Sardina pilchardus) during storage [46]. Reported a marked increase in protein hydrolysis in lizardfish during extended storage. Contrary, a marked decrease of the TBARS value has already been found for canned fish. Thereafter, TBARS values were decreased after 30 days of ripening to 5.07 and 4.95 mg malonaldehyde /kg, in both scientific and commercial treatments samples, respectively. This is may be due to the autooxidation of secondary oxidative products releasing aldehydes or carboxylic acids. TBARS are reported to be interacted with nucleophilic compounds present in the muscle and facilitate the fluorescent compounds formation. Moreover, TBARS probably lost partly into the brine packaging medium and not be determined when analysing the sardine. After the 45th day, for scientific method samples the TBARS values were lower compared to commercial method samples and remained quite low throughout the entire period of ripening. After this time, TBARS values did not provide a general trend about the effect of the ripening period. At the end of the ripening period, the samples reached the maximum values of 4.74 ± 0.9 and 5.28 ± 0.14 mg malonaldehyde /kg for scientific and commercial samples respectively, which is below the reported critical values. In general, maximum acceptable levels of TBARS in small pelagic fish are regarded to be consumed up to 8 mg malonaldehyde /kg.

Total volatile base nitrogen (TVB-N) value

Total volatile base nitrogen (TVB-N) is the most reliable measurement for evaluation of freshness indices of seafood and indicates sign of spoilage and decay during ripening and storage. Among the more general chemical parameters of fish spoilage are total volatile bases, indicative of protein breakdown, and trimethylamine (TMA) resulting from bacterial proteolysis [47-49]. High TVB-N values is regarded as spoiled and unacceptable for consumption as trimethylamineoxide (TMAO) is reduced to formaldehyde, dimethylamine and TMA by spoilage bacteria or by the action of endogenous enzymes. Data presented in show the effect of ripening periods at ambient storage condition (22 ± 2ºC) for 90 days and the method of salting on TVB-N content of salted sardine fish products. At start, the initial TVB-N content of salted sardine was 27.52 mg/100g, which increased with the ripening period up to 30 days to 103.32 and 57.96 mg/100gm in scientific and commercial samples, respectively. Same trend found by Aimen who found that the TVB-N content increased during the ripening and storage time. They concluded that the enzymatic reaction and higher microbial counts, particularly the growth of halophilic bacteria, which breakdown compounds like amino acids, trimethylamine oxide (TMAO), peptides, etc., resulted in an increase in the basic nitrogen fraction for sardine fish. Reported that the initial TVB-N values for sardine and anchovy patties were 13.66 and 17.37 mg/100g, respectively and these values increased progressively throughout salting. In another research, initial TVB-N levels of fishballs produced from tench and pike perch were reported as 11.2 and 11.4 mg/100 g, respectively then these values increased to 36.2 and 39.6 mg/100 g in 14 days The TVB-N value in the salted sardine in this study was higher than reported by Mohan for the sardine stored for 12 days. At 45 days TVB-N contents decreased to 60.48 and 40.44 mg/100gm in scientific and commercial samples, respectively. On other hand, after 60 days of ripening TVB-N contents increased until the end of ripening of 90 days. These results agreed with who found that the amount of TVB-N decreased in eviscerated and non- eviscerated Bori tissues during salting which was due to leaching. Reported the decrease in anchovy TVB-N content may be due to a part of TVB-N content diffused into the brine with other nitrogen fractions. This fluctuation in TVB-N content in salted fish samples was accordance relatively with that obtained by many authors such as **[5**. This wide range of TVB-N for the finished product might be due to the effect of the different salting treatments on the quality such products. The legal limits of acceptability set for this index at 35 mg/100 g for TVB-N were exceeded throughout ambient storage period, which indicates that the salted sardine samples is unacceptable, in contrast to fresh sample. Generally, due to the changes in climate conditions, season, processing, and industrial growth, there could be wide differences in the biochemical constituents of the fish and fish products.

Microbiological characteristics changes of salted sardine fish treatments

The microbiological safety of the fresh and salted sardines was ascertained through the assessment of microbiological parameters, including total viable count (TVC), yeasts and molds. Total viable count is an important criterion for quality evaluation of fish and fishery products. Since the International Commission Regulation 2073/2005 and subsequent modifications recommended the shelf-life threshold for fresh fish and their products at 10^7 cfu/g for total viable count, thus TVC was considered in this study to measure the microbiological quality of the considered samples. It could be recommended the standard levels of TVC of fish freshness are as following $<10^4$ cfu /g, 10^4 - 10^6 cfu /g and $>10^6$ cfu /g number of microbe colonies for fresh fish, sub fresh fish and deteriorated fish respectively [50-53]**.** The changes in total viable count (TVC) with the ripening duration for the scientific and commercial

salted sardine samples are given in The initial total viable bacteria in the sardine samples at zero time was 4.5 $\times 10^3$ cfu/g confirming the data found by Mohan but was higher than the values reported by Stamatis and Arkoudelos for fresh sardines, which may be due to the handling in preparation, processing, distribution and storage of sardine. As evidenced by the (Table 4). The sardines used in this study were characterized by a good microbiological quality, as indicated by a low total viable count under the critical limits of microbe colonies for fresh fish. The limits of bacterial counts were 10^5 cfu/g in Egyptian standards specification (288, 2005).

Table 4: *Changes in microbiological characteristics of salted sardine (Sardina pilchardus) during ripening.*

Ripening period in (day/ ambient)	TVC ($\times 10^3$cfu/g)		Yeasts and molds ($\times 10^2$cfu/g)	
	Scientific	Commercial	Scientific	Commercial
0	4.5	4.5	1	1
5	1	4	1.5	2
0	3	10	3	3

At the end of ripening period of 90 days, TVC reached 3 $\times 10^3$ and 1 $\times 10^4$ cfu/g for scientific and commercial samples, respectively. The increase of TVC may be due to the multiplication of microbial counts that can able to growing under commercial salting method conditions. The obtained results were lower than found by Gennari who found the aerobic bacterial count of fresh Mediterranean sardines (*clupea pilchardus*) was 3.5×10^4 cfu/g. Furthermore, found that during 28 days of salting, TVC increased significantly and the sensory analysis correlated well with microbiological analysis of fish. Yeasts and molds are normally distributed in nature and generally contaminate fish during processing, handling, storage and exposure to other unhygienic environmental factors, and thus considered a causative agent for rapidly spoilage of such foods. The yeasts and molds counts of the microbial investigated during the storage of salted sardines are reported in Table 4. At a zero time yeasts and molds counts were 1×10^2cfu/g, with the ripening period their counts showed an increasing trend in both treatments. These results agreed with who found that the mean values of total yeast count /g of vacuumed packed fesiekh and salted sardine fillet were 3.9×10^2 and 3×10^2, respectively. Also, Reported that the yeast and mold counts of sardine were 20 cfu/g and 10 cfu/g, respectively. It could be concluded that the commercial salted sardine contained more bacteria, yeasts and molds than scientific one probably due to the type of method used. Therefore, the present data suggest the superiority of scientific salting method over commercial salting method during ambient storage [54-57]. In this study, the microbiological assessment analyzed of sardines is indicative of superior sardine flesh quality, considering the proposed lower limits for TVC, yeast and mold.

Conclusion

Salting is a simple, low-cost and appropriate technology for pretreatment of sardine, giving a product with a good sensory properties, even at ambient conditions. The present research provides valuable information on moisture, fat, protein, ash, pH, and salt contents of fresh Egyptian sardine. Current study clearly shows the effectiveness of salting methods on chemical, microbial, and quality changes of sardine at ambient storage condition. Regarding the quality of the sardine, TVN and TBARS analysis were efficient indicators for the extent of oxidation during ripening. The extent of lipid oxidation were very low for scientific method samples, indicating its effectiveness in restraining secondary oxidation as well. Based on the oxidation and microbiological quality indicators, the sardines from both salting treatments were below maximum allowable levels and therefore, acceptable to the consumer. Thus, certain processes such as salting could be used to obtain a product which maintains almost all its nutritional characteristics with a longer shelf life.

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