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Combine Effect of Potassium Sorbate and Dry Salting on the Shelf Life Sardine (*Sardina pilchardus*)

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Abstract: Chemical (pH, Total Volatile Basic Nitrogen (TVB-N), Thiobarbutiric Acid Value (TBA), water activity (a_w) and salt amount), microbiological (Mezophile Aerobe Bacteria (MAB), psychrotrophs bacteria, Lactic Acid Bacteria (LAB), enterobacteriaceae, yeast-mould and halophiles bacteria) and sensory changes during storage of dry salted sardine were studied using experimental design with two fish samples (gutted and ungutted) and two salt (dry salted and potassium sorbate with dry salted). Bacterial counts of filet sardine were always lower than those obtained for ungutted sardine samples. Mesophilic counts and enterobacteriaceae for groups A sample exceeded 7 log cfu cm^{-2} after 5 months of storage. Total Volatile Basic Nitrogen (TVB-N) values showed significant increase for groups A and B sardine during storage reaching a value of 44.8 mg N/100 g and 38.2 mg N/100, respectively (5 months) whereas for groups C and D a respective value of 22 mg N/100 g and 16 mg N/100 was recorded, respectively. Thiobarbituric Acid (TBA) values of groups B, C and d increased very slowly whereas for group A samples higher values were obtained reaching a final value of 5.56 mg MA g^{-1} (month 5). Sensory scores decreased with increasing storage time groups C and D (flavor and total assesment).

Key words: Dry salting, sardine, potassium sorbate, microbiological, chemical and sensory quality, Turkey

INTRODUCTION

Sardine (*Sardina pilchardus*) is the most abundant fish species in the Aegean sea of Turkey. Sardine is generally consumed as fresh, canned or salted and the fish is also utilised as fishmeal and fish oil in Turkey (Kilinc, 1998). If sufficient amounts of sardine are available there is the possibility of developing new products with sardines. One possible alternative is the production of marinated sardines. Sardines are very suitable for marination because of their fat contents but usage of marinated sardine is not common in Turkey (Kilinc, 2003). The aim of the present research was to study the physical, chemical and microbiological changes promoted by the dry salting process in sardine fillets. The objectives of salting are to decrease the pH value to soften the texture until it becomes edible and to develop flavour.

Salted fish products are popular in many countries around the world (Basti *et al*., 2006; Lakshmanan *et al*., 2002; Turan *et al*., 2007). Salting is generally aimed at reducing water activity (a_w) to inhibit growth of spoilage microorganisms as well as inactivate autolytic enzymes (Ashie *et al*., 1996; Horner, 1997). The aim of salting is not only to extend the shelf-life of fresh fish but also to provide desirable sensorial changes (Andres *et al*., 2005). Salting can be done by placing fish in a salt solution or by covering with dry salt. The latter method is also called as dry-salting method in which fish is covered with a thin layer of salt (0.65-1.27 cm) between layers (Rahman, 2007). Salting process starts when the surface of fish contacts with salt and is completed when all the fish reach the appropriate salinity, taste, consistency and odor. Salt is a powerful depressor of a_w of the food and it is convenient to use as an inhibitor of microbial growth (Turan *et al*., 2007). Moreover, it is known that chloride ions are toxic for some microorganisms (Leroi *et al*., 2000). Environmental conditions and microbiological quality of the water affect the microbial status of seafood (Basti *et al*., 2006; Feldhusen, 2000).

Organic acids and their salts (acetic, lactic, propionic and sorbic) exert antibacterial activity. They have been traditionally used as food preservatives and are Generally Recognized As Safe substances (GRAS) approved as food additives by E.C., FAO/WHO and FDA (Surekha and Reddy, 2000). Sorbic acid and its salts have several advantages as food preservatives. Initially thought to have only antimycotic activity, they are now known to also inhibit a wide range of bacteria, particularly aerobic catalase-positive organisms (Thomas, 2000). Effective concentrations do not normally alter product taste or odor. These preservatives are also considered harmless (Thomas, 2000). Potassium salt is commonly used because it is more stable. Furthermore, its greater solubility extends the use of sorbate to solutions appropriate for dipping

and spraying (Thomas, 2000). Potassium sorbate has been extensively investigated as an antimicrobial agent for use in meat including fish to extend its shelf life and inhibit the growth of pathogens such as *Salmonella* or *Staphylococcus aureus* (Ahmed *et al*., 2003). Moreover, effective concentrations do not affect sensory characteristics of fish. The ability of sorbic acid and its salts to inhibit *L. monocytogenes* has been studied in laboratory media and in some foods such as cheese, meat products or fish (Dorsa *et al*., 1993; Samelis *et al*., 2001).

MATERIALS AND METHODS

Dry salting process: Sardine (*Sardina pilchardus*) with an average weight of 35-40 g was caught from Aegean sea in Turkey. The fish, off-loaded approximately 12 h after capture were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the laboratuary. Whole fishes were immediately washed. The fishes were divided evenly into 2 groups (filleted and whole fish). Sardine fillets were separated in to four lots: A, dry salting and whole fish; B, dry salting and the fillet; C, dry salting (salt+0.2% potassium sorbate) and whole fish; D, dry salting (salt+0.2% potassium sorbate) and the filleted. The each of the groups were dry salted with ordinary commercial refined salt (Salina salt, Turkey) at a ratio 1 kg fish/0.8 kg salt (A and B groups), 1 kg fish/0.8 kg salt/ 0.2% potassium sorbate (C and D groups) in plastic containers, without drainage.

The first layers of salt were put into plastic containers and then layers of fish were added. The containers were filled with alternate layers of fish and salt. Three replicate experiments were conducted and at regular time intervals (1-5 months) during salting periods, two fish samples from each group were analyzed (chemical, microbiologycal and sensory). Dry salted sardines were taken out and excess of salt eliminated before analysis. The packed fishes were stored at 4°C.

Microbiological analysis: About 25 g of sardine were aseptically weighed and homogenized in a stomacher for 2 min with 225 mL of sterile peptone water (0.1% peptone). Further decimal dilutions were made with the same diluent. The total number of mesophilic micro-organisms was determined on Plate Count Agar (PCA, Oxoid CM 325) following the pour plate method and incubated at 30°C for 72 h (ICMSF, 1986). Psychrotrophs were determined on Plate Count Agar with an incubation temperature of 7°C for 10 days following the pour plate method (ICMSF, 1986). Lactic acid bacteria was determined in MRS (Oxoid) incubated at 30°C for 72 h. Enterobacteriaceae were determined on plates of Violet Red Bile Glucose agar (Difco, Detroit, MI). The plates were overlaid before the incubation at 37°C for 18-24 h (ICMSF, 1986). The halophiles bacteria was plated on to trypticase Soy Agar (TSA) (Hi-Media, India) supplemented with 10% NaCl following spread plate technique (Speck, 1976). The plates were incubated for 24 h at 37°C.

The number of colonies developed on the plates were counted as total halophiles and expressed as cfu g^{-1}. Potato Dextrose Agar (PDA, Oxoid CM 139) was used for moulds-yeast count. Plates were incubated at 30°C for 3-5 days (Harrigan and MacCance, 1976). All the analysis were performed in duplicate.

Chemical analysis: pH values of the samples were determined with pH meter (EDT. GP 353) (AOAC, 1998). The water activity was determined using water activity instrument (AOAC, 1998). The method reported by Varlik was employed in determination of TVB-N amount of the samples. Thiobarbituric acid value (TBA, mg malonaldehyde kg^{-1}) was determined using a spectrophotometric method (Tarladgis *et al*., 1960).

While determining the salt content (%), 5 g of the sample was homogenized and diluted to 250 mL using distilled water. This mixture was filtered after heating in water bath for 1 h. Then, 25 mL of filtrate was mixed with the indicator (5% K_2CrO_4) and titrated with $AgNO_3$ (0.1 N). The content of salt was calculated using the following formula:

$$\text{Salt (\%)} = \frac{[0.00585 \times \text{AgNO}_3\ \text{(mL)}]}{[\text{Amount of sample (g)}]} \times 1000$$

Sensory analysis: For the sensory analysis, samples were washed with water. The groups A and B were performed fillets. Sensory analysis was performed then fried. The samples were then presented to the eight panellists in small aluminium trays. The panellists were selected and trained according to ISO standards (ISO 8586-11993). The quality of each sample was classified using characteristics to describe the texture, taste, color, smell, appearance and total assessment. Each characteristic was scored using a point scale ranging from 1-5. The samples were scored from 1-5 where 1 means very bad, 2 bad, 3 normal, 4 good and 5 very good. Sensory analysis were performed until 3 months.

Statistical analysis: Analysis of the data was conducted using Statistical Analysis System (SAS) package programme. Values between groups and within group between days were compared. Data were subjected to

variance analysis in accordance with 3×11×3×1 factorial design and in terms of fix effects and inter-variable interactions so that repetition number×sampling time×test groups×number of samples examined at one instance from each test group. According to General Linear Models (GLM) procedure, Fisher's smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (Anonymous, 1996). Alpha value was determined as 0.05.

RESULTS AND DISCUSSION

The changes in the microflora (mezophile aerobe bacteria, psychrotrophs bacteria, halophiles bacteria, yeast- mould, LAB and Enterobacteriaceae) of A, B, C and D filleted sardine samples are shown in Table 1. The changes chemical result are shown in Table 2. Sensory scores of dry salted sardine significantly decreased ($p<0.05$) throughout the storage (Table 3).

The initial (raw) MAB (Table 1) of sardine was 4.84 log cfu g^{-1} which is a relatively high bacterial load in agreement with the results (Frangos *et al*., 2010) for whole ungutted rainbow trout. A (salted, ungutted sardine) samples exceeded the value of 8 log cfu g^{-1} for MAB, considered as the upper acceptability limit for fresh salted species (ICMSF, 1986) on month 5 of storage while B (salted, filet sardine), C (salted with potassium sorbate, ungutted sardine) and D (salted with potassium sorbate, fillet sardine) samples did not reach this value throughout the 5 months storage period. B, C and D sardine samples had significantly ($p<0.05$) lower MBA count compared to A samples between 2 and 5 months of storage. There are no reports in the literature on the effect of combination with dry salting, potassium sorbate in freshwater fish species. Frangos *et al*. (2010) found that salting rainbow trout fillets both reduced the initial MBA and extended the shelf-life from 4 days to at least 12 days at 5°C according to microbiological results.

Kilinc found that raw sardine fillets MBA counts 4.5×104 cfu g^{-1}, after the sardine fillets were put into barrels, all these microorganisms were inhibited. After marination, the same negative results for microbiological counts were also found in other studies (Aksu *et al*., 1997; Fuselli *et al*., 2003).

The ICMSF (1986) has established an aerobic mesophilic count limit of 7 log cfu g^{-1} for fish that is fit for human consumption. This value (7.58) was reached on months 4 of storage (groups A). The MAB counts, cod and perch fillets applied potassium sorbate were reduce at the beginning while increased at the end of during storage time (Khuntia *et al*., 1993). The results were different from present study. Lynch and Potter (1982) reported that cod mince increased both control and sample with potassium sorbate during storage time. Initial counts of

Table 1: Result of microbiolgical analysis of sardine dry salted storaged 4°C

		Storage (months)					
Microorganisms (log cfu g^{-1})	Example	Raw	1	2	3	4	5
Mezophile aerobe bacteria	A	$4.84^{c,z}$	$5.30^{bc,z}$	$6.43^{b,z}$	$6.11^{b,z}$	$7.58^{a,z}$	$8.12^{a,z}$
	B	$4.84^{a,z}$	$4.61^{a,z}$	$5.29^{a,z}$	$5.49^{a,z}$	$5.41^{a,y}$	$5.54^{a,y}$
	C	$4.84^{a,z}$	$4.72^{a,z}$	$4.89^{a,y}$	$5.01^{a,z}$	$5.33^{a,y}$	$5.19^{a,y}$
	D	$4.84^{a,z}$	$4.20^{a,z}$	$4.80^{a,y}$	$4.02^{a,y}$	$4.67^{a,y}$	$4.10^{a,y}$
Psychrotrophs bacteria	A	$3.64^{b,z}$	$4.87^{b,z}$	$4.84^{b,z}$	$5.67^{a,z}$	$5.74^{a,z}$	$5.89^{a,z}$
	B	$3.64^{b,z}$	$4.63^{a,z}$	$4.85^{a,z}$	$4.77^{a,z}$	$5.46^{a,z}$	$5.73^{a,z}$
	C	$3.64^{a,z}$	$4.07^{a,z}$	$3.91^{a,z}$	$4.92^{a,z}$	$4.43^{a,z}$	$4.75^{a,z}$
	D	$3.64^{b,z}$	$3.84^{b,z}$	$4.61^{b,z}$	$4.92^{a,z}$	$4.74^{b,z}$	$5.93^{a,z}$
Lactic acid bacteria	A	$3.89^{b,z}$	$4.04^{b,z}$	$5.30^{a,z}$	$5.79^{a,z}$	$6.22^{a,z}$	$6.53^{a,z}$
	B	$3.89^{a,z}$	$4.46^{a,z}$	$4.92^{a,z}$	$4.47^{a,z}$	$4.28^{a,z}$	$4.85^{a,z}$
	C	$3.89^{a,z}$	$3.91^{a,z}$	$3.89^{a,y}$	$3.73^{a,y}$	$3.76^{a,y}$	$3.97^{a,y}$
	D	$3.89^{a,z}$	$3.78^{a,z}$	$3.20^{a,y}$	$3.94^{a,y}$	$3.43^{a,y}$	$3.49^{a,y}$
Enterobacteriaceae	A	$4.48^{b,z}$	$4.46^{b,z}$	$5.98^{a,z}$	$6.00^{a,z}$	$6.18^{a,z}$	$7.01^{a,z}$
	B	$4.48^{a,z}$	$4.84^{a,z}$	$4.94^{a,y}$	$4.39^{a,z}$	$4.79^{a,y}$	$4.34^{a,y}$
	C	$4.48^{a,z}$	$4.12^{a,z}$	$4.90^{a,z}$	$4.74^{a,z}$	$4.79^{a,z}$	$4.72^{a,y}$
	D	$4.88^{a,z}$	$4.09^{a,z}$	$4.70^{a,y}$	$4.72^{a,z}$	$5.04^{a,z}$	$4.90^{a,y}$
Yeast-Mould	A	$4.43^{b,z}$	$4.78^{b,z}$	$5.80^{a,z}$	$6.12^{a,z}$	$6.37^{a,z}$	$6.59^{a,z}$
	B	$4.43^{b,z}$	$4.90^{b,z}$	$5.62^{a,z}$	$5.99^{a,z}$	$5.15^{a,z}$	$6.86^{a,z}$
	C	$4.43^{a,z}$	$4.91^{a,z}$	$4.83^{a,z}$	$4.97^{a,z}$	$4.85^{a,z}$	$4.89^{a,y}$
	D	$4.43^{a,z}$	$4.07^{a,z}$	$3.73^{a,y}$	$4.46^{a,y}$	$4.61^{a,y}$	$4.81^{a,y}$
Halophiles bacteria	A	$3.59^{b,z}$	$3.72^{b,z}$	$4.89^{b,z}$	$5.13^{b,z}$	$6.27^{a,z}$	$7.12^{a,z}$
	B	$3.59^{b,z}$	$3.66^{b,z}$	$4.43^{b,z}$	$4.89^{b,z}$	$5.26^{a,z}$	$6.11^{a,z}$
	C	$3.59^{b,z}$	$3.72^{b,z}$	$4.56^{a,z}$	$4.28^{b,z}$	$5.15^{a,z}$	$5.72^{a,y}$
	D	$3.59^{b,z}$	$3.87^{b,z}$	$3.89^{b,y}$	$4.89^{a,z}$	$5.45^{a,z}$	$5.27^{a,y}$

A: ungutted; B: gutted; C: 0.2% potassium sorbate, ungutted; D: 0.2% potassium sorbate,gutted. a-c: Means within a column lacking a common superscript letter are different ($p<0.05$). z, y: Means within a row lacking a common superscript letter are different ($p<0.05$). Values are means for three trials at each groups ($n = 4×3$)

Table 2: Result of chemical analysis of sardine dry salted storaged 4°C

		Storage (months)					
Chemical analysis	Example	Raw	1	2	3	4	5
pH	A	6.720^{a,z}	6.450^{a,z}	6.320^{a,z}	6.410^{a,z}	6.400^{a,z}	6.450^{a,z}
	B	6.720^{a,z}	6.540^{a,z}	6.280^{a,z}	6.120^{a,z}	6.910^{a,z}	6.180^{a,z}
	C	6.720^{a,z}	6.260^{a,z}	6.680^{a,z}	6.030^{a,z}	6.650^{a,z}	6.150^{a,z}
	D	6.720^{a,z}	6.330^{a,z}	6.940^{a,z}	6.220^{a,z}	6.820^{a,z}	6.870^{a,z}
TVB-N	A	10.240^{c,z}	19.600^{b,z}	25.200^{b,z}	28.300^{b,z}	36.400^{ab,z}	44.800^{a,z}
	B	10.240^{b,z}	15.400^{b,z}	17.800^{b,z}	21.600^{a,z}	33.400^{a,z}	38.200^{a,z}
	C	10.240^{b,z}	13.200^{b,z}	19.200^{a,z}	15.000^{b,z}	21.300^{a,y}	22.000^{a,y}
	D	10.240^{b,z}	16.000^{b,z}	18.000^{a,z}	23.000^{a,z}	18.000^{a,y}	16.000^{b,y}
TBA	A	1.030^{c,z}	1.560^{c,z}	2.030^{c,z}	3.040^{ab,z}	4.070^{a,z}	5.560^{a,z}
	B	1.030^{b,z}	1.130^{b,z}	2.170^{a,z}	2.210^{a,y}	2.590^{a,y}	3.010^{a,y}
	C	1.030^{c,z}	1.760^{b,z}	2.070^{b,z}	3.650^{a,z}	4.420^{a,z}	3.890^{a,y}
	D	1.030^{c,z}	2.130^{b,z}	2.780^{b,z}	3.560^{a,z}	3.890^{a,z}	3.970^{a,y}
a_w	A	0.979^{a,z}	0.748^{a,z}	0.743^{a,z}	0.745^{a,z}	0.745^{a,z}	0.741^{a,z}
	B	0.979^{a,z}	0.740^{a,z}	0.743^{a,z}	0.745^{a,z}	0.741^{a,z}	0.740^{a,z}
	C	0.979^{a,z}	0.743^{a,z}	0.745^{a,z}	0.746^{a,z}	0.741^{a,z}	0.743^{a,z}
	D	0.979^{a,z}	0.721^{a,z}	0.739^{a,z}	0.745^{a,z}	0.746^{a,z}	0.743^{a,z}
Salt	A	*	20.890^{a,z}	20.920^{a,z}	21.920^{a,z}	21.330^{a,z}	21.730^{a,z}
	B	*	22.770^{a,z}	22.930^{a,z}	20.190^{a,z}	21.450^{a,z}	21.320^{a,z}
	C	*	21.560^{a,z}	20.980^{a,z}	21.130^{a,z}	22.050^{a,z}	21.560^{a,z}
	D	*	20.570^{a,z}	21.870^{a,z}	21.450^{a,z}	22.530^{a,z}	21.770^{a,z}

A: ungutted; B: gutted; C: 0.2% potassium sorbate, ungutted; D: 0.2% potassium sorbate,gutted. a, b: Means within a column lacking a common superscript letter are different ($p<0.05$). z, y: Means within a row lacking a common superscript letter are different ($p<0.05$). *: Not analyzed. Values are means for three trials at each groups (n = 4×3)

Table 3: Result of sensory analysis of sardine dry salted storaged 4°C

		Storage (months)		
Features	Example	1	2	3
Colour	A	4.62^{a,z}	4.81^{a,z}	4.81^{a,z}
	B	4.62^{a,z}	4.68^{a,z}	4.50^{a,z}
	C	4.37^{a,z}	4.43^{a,z}	4.24^{a,z}
	D	4.62^{a,z}	4.56^{a,z}	4.68^{a,z}
Odor	A	4.68^{a,z}	4.68^{a,z}	4.56^{a,z}
	B	4.18^{a,z}	4.21^{a,z}	4.36^{a,z}
	C	4.21^{a,z}	4.14^{a,z}	4.11^{a,z}
	D	4.12^{a,z}	4.43^{a,z}	4.01^{a,z}
Texture	A	4.81^{a,z}	4.81^{a,z}	4.49^{a,z}
	B	4.81^{a,z}	4.60^{a,z}	4.50^{a,z}
	C	4.75^{a,z}	4.56^{a,z}	4.93^{a,z}
	D	4.60^{a,z}	4.50^{a,z}	4.81^{a,z}
Flavor	A	4.60^{a,z}	4.60^{a,z}	4.56^{a,z}
	B	4.43^{a,z}	4.31^{a,z}	4.39^{a,z}
	C	2.56^{a,y}	2.75^{ab,y}	1.93^{ab,y}
	D	2.12^{a,y}	1.93^{b,y}	1.81^{ab,y}
View	A	4.75^{a,z}	4.60^{a,z}	4.61^{a,z}
	B	4.42^{a,z}	4.56^{a,z}	4.59^{a,z}
	C	4.49^{a,z}	4.31^{a,z}	4.31^{a,z}
	D	4.33^{a,z}	4.39^{a,z}	4.42^{a,z}
Total assesment	A	4.67^{a,z}	4.67^{a,z}	4.55^{a,z}
	B	4.53^{a,z}	4.69^{a,z}	4.61^{a,z}
	C	3.72^{a,y}	3.99^{a,y}	3.98^{a,y}
	D	3.13^{a,y}	3.01^{a,y}	3.70^{a,y}

A: ungutted; B: gutted; C: 0.2% potassium sorbate, ungutted; D: 0.2% potassium sorbate, gutted. a, b: Means within a column lacking a common superscript letter are different ($p<0.05$); z, y: Means within a row lacking a common superscript letter are different ($p<0.05$). Values are means for three trials at each groups (n = 4×3)

Psychrotrophs bacteria (raw) for sardine samples was 3.64 log cfu g^{-1} (Table 1). Psychrotrophs reached final (5 month) counts of 5.89, 5.73, 4.75 and 5.93 log cfu g^{-1}, respectively for A, B, C and D sardine samples. According to variance analysis, no significant differences were found between psychrotrophs counts of sardine fillets during the salted process at 4°C ($p>0.05$) (Table 1). It was reported by Kilinc (2003) 7.6×104 cfu g^{-1} raw metarials while psychrotrophs were inhibited. This results are different than the findings. Figueroa *et al.* (1990) found that the mesophilic and psychrotrophic bacterial populations increased with storage time and reached counts of 106-107 CFU cm^{-2} after 2 weeks of storage in flake ice. In contrast, mesophilic and psychrotrophic microorganisms reached end of month 4. By Shaw *et al.* (1983) found that there was no significant difference between samples immersed potassium sorbate solutions 3% concentrations during storage. This case, the findings similar to.

Growth of LAB is presented as a function of storage time at 4°C in Table 1. The initial LAB numbers 3.89 log cfu g^{-1} while this value increased during storage time all groups (Table 1). Shalini *et al.* (2001) reported that potassium sorbate was performed *Lethrinus lentjan* fillets and increased the end of storage LAB. This situation compatible present study. There were significant differences ($p<0.05$) in LAB counts between samples in dry salted (A and B groups) and dry salted with potassium sorbate (Table 1). LAB usually do not dominate in the microflora of the raw material and only certain species have been found in fish from temperate and cold marine waters and their surrounding environment (Gonzalez *et al.*, 2000). It could be speculated that lactic acid bacterial strains thriving in the processing

environment contaminate the maatjes herring after thawing during further processing. The exposure of fish products to in-house flora during several processing steps has been suggested earlier (Bagge-Ravn *et al.* 2003). The halophiles bacteria and yeast and mould counts of raw material were 4.43 and 3.59 log cfu g^{-1}, respectively. After the sardine fillets were put into barrels, all these microorganisms were increased (Table 1). Enterobacteriaceae were also found to be part of the spoilage microflora of both whole gutted and filleted sardine storage time.

Enterobacteriaceae counts (Table 1) were higher ($p<0.05$) for groups A than for whole groups sardine samples by approximately 1-2 log cfu cm^{-2} throughout the entire storage period of 5 month, reaching final levels of 7.01 log cfu cm^{-2}. The population of this group was lower than that obtained for other bacteria in this study which is in agreement with results reported for different fish at the end of the product's shelf-life (Tejada and Huidobro, 2002). During the storage TVB-N values significantly ($p<0.05$) increased (Table 2). There were significant differences ($p<0.05$) in TVB-N values between samples in dry salted (A and B groups) and dry salted with potassium sorbate (Table 2). While initial TVB-N contents of salted sardines were 10.24 mg/100 g, this value increased to 44.8 mg/100 g and 38.2 mg/100 g in sardine A and B groups, respectively.

El-Marrackchi *et al.* (1990) stated that TVB value was more useful in assessing the degree of sardine deterioration than in evaluating the changes occuring during the first stages of storage. It is suggested that TVB-N value is affected by species, catching season and region, age and sex of fish. A level of 30 mg/100 g has been considered the upper limit above which fishery products are considered unfit for human consumption (Sikorski *et al.* 1989). TVB-N value upper limits which end of months 4, groups A and B (Table 2).

Gokoglu *et al.* (1998) found that TVB-N value in fresh sardine increased from 13.2-64.8 mg/100 g during refrigerated storage. It was reported that TVB-N value of 8.3 mg/100 g in anchovy marinated using acetic acid of 2% increased to 15.1 mg/100 g at the storage of 150 days (Aksu *et al.*, 1997). In another report TVB-N value in anchovy marinated with acetic acid of 4% and stored at 4°C increased from 9.8-14 mg/100 g during the storage of 8 months (Dokuzlu, 2000).

While the initial TVB-N values in the samples were similar to findings of other researchers, the increase in TVB-N values during the storage was higher than others. The probable reason of these differences is differences in fish species and different methods (like marinations). The pH in fresh fish flesh is almost neutral. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh (Shenderyuk and Bykowski, 1989). The increase in pH indicates the loss of quality. pH value in raw fish flesh was found 6.72. Nunes *et al.* (1992) found a pH value of 6.1 in sardine. The pH value in sardine found by El Marrackchi and Gokoglu were 5.83 and 6.2, respectively. There was not a significant difference ($p>0.05$) in pH between samples dry during storage (Table 2).

The highly unsaturated lipids in fat-rich fish are easily susceptible to oxidation that results in a rancid smell and taste as well as alterations in texture, colour and nutritional value (Olafsdottir *et al.*, 1997). TBA (thiobarbituric acid) value is a widely used indicator for the assessment of degree of lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish is 5 mg malonaldehyde kg^{-1} while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MA)/kg (Schormuller, 1969).

In the present study, the TBA value of fresh raw sardine was 1.03 mg MA kg^{-1}. After the treatment processes, sharp increases ($p<0.01$) in the initial TBA values to high levels of 5.56, 3.01, 3.89 and 3.97 were measured for groups A, B, C and D, respectively. During storage, there was a tendency towards an increase in TBA values up to a maximal point (at 5 months of storage, groups A).

The decrease in TBA content after the peak point has been attributed to the interaction between MA and decomposition products of protein to give tertiary degradation products (Fernandez *et al.*, 1997; Reddy and Setty, 1996). The present result indicated that oxidative rancidity in marinated fillet samples increased throughout the entire period of storage at 4°C and its level was within the acceptability limits for fish consumption for B, C and D groups. However, groups A values (end of storage time) than higer acceptability limits (5.56 mg MA kg^{-1}). TBA levels were reported in salted anchovies during 9 weeks of ripening in cans at 20°C (Hernandez-Herrero *et al.*, 2002) and also in non-pasteurized sardine marinade in tomato sauce during 6 months of storage at 4°C (Kilinc and Cakli, 2005) than higer this study who also concluded that TBA may not be a reliable criterion for anchovies salted under the condition used. The salt and water activity values of sardine fillets insignificant ($p>0.05$) during the storage periods (Table 2). Sensory scores of dry salted sardine significantly decreased ($p<0.05$) throughout the storage (Table 3). Sensory scores of sardine salted with potassium sorbate were significantly higher ($p<0.05$) than those found in sardine dry salted (A group). The samples had good quality up to 5 months.

CONCLUSION

In this study, the shelf life of gutted and ungutted sardine samples in salted and potassium sorbate with salt were determined as 5 months at 4°C. At the end of this study while the differences between salt, a_w and pH value of salted and potassium sorbate with salted samples were not statistically significant ($p>0.05$), the difference between TVB-N, TBA, MAB, LAB, Enterobacteriaceae, yeast and moulds count of salted and potassium sorbate with salted sardine samples were significant ($p<0.05$). The dry salted and fillets process affect the shelf life of the sardine. At the end of the storage of 5 months A group were found unconsumable according to the results of chemical and microbiological values and sensory analysis.

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