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Quality Changes of Sardine (*Sardina pilchardus* W., 1792) During Frozen Storage

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Özet: ***Donmuş depolama esnasında sardalyalarda (*Sardina pilchardus W., 1792*) meydana gelen kalite değişimleri.*** Bu çalışmada -18°C donmuş depoda bekletilmeden önce -40°C'de şoklanan bütün (Grup A) ve fileto (Grup B) halindeki sardalya balıklarının fiziksel, kimyasal mikrobiyolojik ve duyusal değişimleri belirlenmiştir. İstatistik analiz sonuçlarına gore, iki grubun donmuş depolama esnasındaki TBA, FA (ex) ve FA (dest) değerleri arasında farklar önemli (P<0.05) olarak saptanırken, iki grubun pH ve TVB-N değerleri arasında farklar önemsiz (P>0.05) olarak bulgulanmıştır. Grup A'nın toplam canlı sayısı donmuş depolama sonunda başlangıçtaki değerini korurken, Grup B'nin toplam canlı sayısı depolama esnasında azalma göstermiştir. Grup A ve Grup B'nin koliform bakteri sayısı depolama esnasında aşağı yukarı aynı değerlerde kalmasına karşın, depolama esnasında koagülaz-pozitif *S. aureus* saptanmamıştır. İki grup arasında renk, koku, kas yapısı ve tat özelikleri açısından farklar önemsiz (P>0.05) olarak bulgulanmıştır.

Anahtar Kelimeler: Sardalya, *Sardina pilchardus*; Donmuş depolama; Kalite değişimleri

Abstract: In the present study, physical, chemical, microbiological and sensory changes of sardine as whole (Group A) and fillet (Group B) forms were determined during frozen storage at -18°C, but all samples were shocked at -40°C before. According to the statistical analysis results, there were significant differences (P<0.05) between TBA, FA (ex), FA (dest) values of two groups during the frozen storage while there were no significant differences (P>0.05) between pH and TVB-N values of two groups. It was found that, total viable count of Group A kept its initial value at the end of the frozen storage while total viable count of Group B showed a decrease during the storage period. Coliform bacteria count stayed almost at the same level for Group A and Group B during the storage and coagulase-positive *S. aureus* was not detected. No significant differences (P>0.05) were observed in the colour, smell, chewy texture and flavor properties of two groups.

Key Words: Sardine; *Sardina pilchardus*; Frozen storage; Quality changes.

Introduction

S. pilchardus is an important species caught from the west of Karadeniz, Marmara and Ege seas of Turkey. According to National Statistical Institute data, *S. pilchardus* was caught from these areas as '17 524' tons (Anonymous, 1997). Sardines are consumed most commonly as fresh, and less as frozen and canned in Turkey. In some cases, they are used as a raw material of fish oil and fish meal. Nowadays, the consumption of the foods similar to the fresh form increased the importance of the frozen storage.

Until now on, many studies have been done about the effects of the frozen storage on the fish quality for different fish species (Dawson, Vebersaks & Vebersaks 1978; Fletcher & Statham, 1988; Kundakçı, 1989; LeBlanc, LeBlanc & Blum, 1988; Licciardello, Ravesi, Lundsrom, Wilhelm, Correia & Allsup, 1982; Vareltzis, Zetou & Tsiaras, 1988; Varlık, 1987).

There were also many studies on sardine processing technology (Çaklı, Tokur, Çelik & Taşkaya, 1997; El Marrakchi, Bennour, Bouchriti, Hamama & Togafait, 1990; Ihm, Kim, Joo & Lee, 1992a; Ihm, Kim, Joo & Lee, 1992b; Shimizu, Toyohara & Lanier, 1992; Varlık, Uğur, Gökoğlu & Gün, 1993a).

In some fish processing plants in Turkey, sardine caught as a large amount were frozen without filleting and stored as blocks. Certain blocks were thawed when they were required. It is important to determine the quality changes of the sardine frozen as whole and compare with the quality changes of sardine frozen as fillets.

In the present study, sardine as whole and fillet forms were frozen at -40°C and stored at -18°C. During the storage period, physical (pH value), chemical [TBA, TVB-N, FA(ex), FA(dest)], microbiological (total viable count, coliform bacteria count and coagulase-positive *Staphylococcus aureus* count) and sensory (colour, smell, structure, chewy texture and flavor) changes were evaluated.

Material and Methods

Sardines caught in Urla (a shore district in İzmir) and sold at retail were used as the material in the present study.

The fish bought from fishermen were placed in wooden case with ice and brought to the laboratory immediately. They were washed with tap water. Cleaned fish were divided into two groups.The whole fish (Group A) were shared into the polyethylene bags as 200 g. For the other group (Group B) heads, bones and viscera were removed and then fillets were washed with tap water and shared into the polyethylene bags as 200 g. Both groups were frozen at -40°C for 8 hours and then stored at -18°C.

Group A and Group B were thawed in the refrigerator (+4°C) the night before for the analysis.

Chemical composition of sardine was determined as crude protein (AOAC, 1984), crude fat (Blig & Dyer, 1959), crude ash (AOAC, 1984) and moisture (Ludorff & Meyer, 1973). Analysis was made in 3 parallels.

pH value was measured as described by Lima dos Santos, James and Teutscher (1981) by using digital pH meter (Ebro). Thiobarbyturic acid (TBA, mg malonaldehyde/kg) and total volatile base nitrogen (TVB-N, mg N/100 g) values were determined as described Tarladgis, Watts, Younathan and Dugan (1960) and Antonacopoulos (1971), respectively. The amount of formaldehyde extractable with $HCIO_4$ [free formaldehyde; FA(ex), mg FA/kg] was measured using the Nash test (Nash,1953). Free and bound formaldehyde [FA(dest), mg FA/kg] were measured by steam distillation of acidified samples (Rehbein,1986). Analysis were made in 3 parallels.

The test sample from whole sardine was prepared by aseptically making incision and cutting this plug of tissue from the skeletal frame (Slattery,1998). For all microbiological counts, 10 g of sample were taken and transferred in 90 ml 0.1% peptone water (Difco, 0118-17-0). From the 10^{-1} dilution, other decimal dilutions were prepared. Total viable count was determined by using pour plate method. Plate Count Agar (Difco, 0479-17) was used as medium (Harrigan & McCance, 1976). Plates were incubated at 30ºC for 24-48 h. For coliform bacteria count Most Probable Number method was used. Lauryl Tryptose Broth (Difco, 0241-17-0) was used as medium and confirmation test was made in Brilliant Green Bile 2% (Difco, 0007-17-4). Tubes were incubated at 37ºC for 24-48 h (Harrigan & McCance, 1976). Coagulase-positive *Staphylococcus aureus* count was determined by using spread plate method.

Baird Parker Agar (Difco, 0768-17-3) was used as medium. Plates were incubated at 37ºC for 24-48 h (Harrigan & McCance, 1976).

Analysis were made in two parallels. The results of the total viable counts were given as the mean value of two parallels.The results of the coliform bacteria counts of two parallels were given as separately.

For the determination of sensory quality of sardine scoring test was used. After removing the head and viscera of the whole sardine and washing, they were put into the polyethylene bags and closed tightly. Bags including the samples of Group A (headed and gutted) and Group B were put into boiling water and cooked for 10 minutes. After cooking, they were cooled to 50ºC and samples without salt were served to the panelists to evaluate the sensory attributes (colour, smell, structure, chewy texture, flavor) of the samples using 3-point descriptive scale (Varlık, Uğur, Gökoğlu, & Gün, 1993b). According to the scoring table, scores 2.7 and above indicated the first (very good) quality, scores between 2 and 2.7 indicated the second (good) quality and scores between 1 and 2 indicated the limit of acceptibility (third quality) and scores 0 and 1 indicated the spoiled samples.

Statistical evaluations of the chemical and sensory analysis were made by using 't test' in Microsoft Excel 7.0.

Results and Discussion

According the results of the chemical composition analysis of fresh sardine, crude protein, crude fat, crude ash and moisture rates were found as 18.4±0.12%, 7.1±0.28%, 1.67±0.44%, 72.23±0.43%, respectively.

Table 1 shows the changes of pH, TBA, TVB-N, FA(ex), FA(dest) values of Group A and Group B during the frozen storage at -18°C. pH and TVB-N values were determined for 30 days of the storage while TBA, FA (ex) and FA (dest) values were determined for 60 days (Table 1). pH values of Group A and Group B were measured as 6.57±0.01 on the first day of the storage and pH decreased slightly during the frozen storage at -18°C for both product forms. There were no significant differences between pH values of two groups (P>0.05).

Table 1. Changes in pH, TBA, TVB-N, FA(ex) and FA(dest) values of Group A and Group B during the storage period at -18°C.

Analysis	Group	Days of storage				
		1	7	15	30	60
pH	A	6.57±0.01	6.58±0.01	6.53±0.01	6.45±0.02	- [a]
	B	6.57±0.01	6.54±0.01	6.49±0.01	6.47±0.01	- [a]
TBA	A	0.78±0.01	1.53±0.02	1.48±0.05	1.74 ±0.04	2.93±0.09
	B	1.08±0.04	1.08±0.00	3.41±0.07	3.04±0.03	4.01±0.18
TVB-N	A	13.07±0.47	15.40±0.81	14.47±0.47	16.80±0.81	- [a]
	B	13.53±0.93	16.80±0.81	15.87±0.47	5.40±0.47	- [a]
FA(ex)	A	1.10±0.11	1.33±0.09	1.64±0.12	1.78±0.07	1.94±0.22
	B	0.47±0.11	0.56±0.08	1.18±0.02	1.32±0.11	1.76±0.14
FA(dest)	A	0.56±0.11	0.42±0.08	1.88±0.00	4.31±0.11	4.31±0.11
	B	1.50±0.22	1.59±0.16	3.84±0.16	2.91±0.05	4.38±0.17

Fresh sardine; pH: 6.14±0.01, TVB-N:14.47 ±1.22, FA(ex):2.06±0.59, FA (dest):1.59±0.05; [a]: Not analysed

Waters (1982) showed that pH values of spot *(Leiostomus xanthurus)* stored at -18°C for 12 months as minced and fillet forms decreased slightly during the storage. In the study of Vareltzis *et al*. (1988), it was found that pH values of chub mackerel *(Scomber japonicus collias)* and smooth hound *(Mustelus mustelus)* as fillet forms were almost stable during the storage at -22°C for 8 weeks. An identical behaviour was described for sardine as fillet forms stored at no-frost conditions (Çaklı *et al*.,1997). These results were parallel to the present study.

At the end of 60 days of the storage it was shown that TBA values of Group A and Group B increased to the certain levels (Table 1). TBA value of Group A rose from 0.78±0.01 mg malonaldehyde/kg to 2.93±0.09 mg malonaldehyde/kg while it rose from 1.08±0.04 mg malonaldehyde/kg to 4.01±0.18 mg malonaldehyde/kg for Group B after 60 days of the storage. Statistical analysis results showed that there were significant differences between TBA values of Group A and Group B.

TBA is a good indicator to determine the quality of the fish whether it was frozen, chilled or stored with iced (Tarladgis *et al*., 1960; Vareltzis *et al*., 1988). It suggests that maximum level of TBA value indicating the good quality of the fish frozen, chilled or stored with ice is 5 mg malonaldehyde/kg, while the fish may consume up to the level of 8 mg malonaldehyde/kg TBA value (Schormüller, 1969). The data obtained in the present study suggests that, TBA values of Group A and Group B are in the good quality limits after 60 days of the storage at -18°C.

In the present study, TVB-N value of fresh sardine was measured as 14.47±1.22 mg N/100 g. Irregular changes on TVB-N values of Group A and Group B were revealed during the storage period but TVB-N values were almost close to each other between the two groups (Table 1). According to the statistical analysis results, there were no significant differences between TVB-N values of two groups during the frozen storage at -18°C ($P>0.05$).

TVB-N is used for the determination of the spoilage level and the fish quality during the storage period (Cobb & Venderzont, 1975; Kietzman, Priebe, Rakov & Reichstein, 1969; Oehlenschlager, 1981). In the studies of the storage of frozen different fish species, it is suggested that TVB-N value may be at high level relating with the fish species at the beginning of the storage and may change depending on the spoilage flora and analysis methods (Antonacopoulos, 1971; Kornop, 1976; Rehbein & Oehlenschlager, 1982). Joseph, Surendan and Perigreen (1989) showed that irregular changes on TVB-N values during the frozen storage (-20°C) of Kalawa (*Epinephelus* spp.) were due to the elimination of dissolved volatile constituents through drip. Fluctuations tending to increase on TVB-N values were also reported in the studies of Çaklı *et al*. (1997) working with sardine stored at no-frost conditions and Vareltzis *et al*. (1988) working with chub mackerel and smooth hound stored at -22°C. In the present study, the reason of irregular changes on TVB-N values may be due to the case mentioned above. Generally less than 25 mg N/100 g TVB-N value indicates 'very good quality' for the fishery products (Ludorf & Meyer, 1973; Schormüller, 1968). Considering this value, TVB-N values of Group A and Group B show that even both groups do not exceed 'very good quality' limits in during the storage period.

FA values of Group A and Group B were given as FA(ex) and FA(dest) in Table 1. For fresh sardines FA(ex) value was measured as 2.06±0.59 mg/kg, FA

(dest) value was measured as 1.59±0.05 mg/kg. Depending on the increasing of storage time at -18°C, FA values increased. In Group A, FA (ex) value changed from 1.10±0.11 mg/kg to 1.94±0.22 mg/kg while FA (dest) value changed from 0.56±0.11 mg/kg to 4.31±0.11 mg/kg. In Group B, FA(ex) value changed from 0.47±0.11 mg/kg to 1.76±0.14 mg/kg, FA(dest) value changed from 1.50±0.22 mg/kg to 4.38±0.17 mg/kg. As a result of statistical analysis, significant differences were noticed between FA values of two groups during the storage at -18°C for 60 days ($P<0.05$).

Formaldehyde and dimetylamine (DMA) are produced by the enzymatic (TMOAase activity) degredation of trimethylamine oxide (TMAO), a natural constituent in the muscle of a large number of marine fish and shellfish (Rehbein, 1987). During the freezing of the products, formaldehyde produced is a highyl reactive molecule leading to inter- and intramolecular linkages between protein chains (Aubourg, 1998). As a result, protein denaturation and the loss of quality of the frozen fish have been associated with the formation of formaldehyde (Aubourg, 1998; Orlick, Oehlenschlager & Schreiber, 1991).

There was a slight increase in the amount of free formaldehyde as reported for cod fillet frozen stored at -18°C by Orlick *et al*. (1991). This result is similar to the present study but comparable amounts of free and bound formaldehyde [FA(dest)] of Group A and Group B were formed at the end of 60 days of the storage at -18°C. Since FA (dest) values of Group A and Group B were less than 10 mg/kg, no quality loss was detected on sardine stored at -18°C for 60 days (Rehbein, 1986).

Table 2 and Table 3 show the changes of total viable counts and coliform bacteria counts of Group A Group B during the frozen storage for 90 days. No significant differences occurred on total viable counts and coliform bacteria counts of Group A and Group B during the frozen storage.

Table 2. Total viable counts (cfu/g) of Group A and Group B during the storage period at -18°C.

Group	**Days of storage**				
	1	15	30	60	90
A	$2.2x10^3$	$4.5x10^3$	$1.3x10^4$	$1.1x10^4$	$3.6x10^3$
B	$2.4x10^4$	$7.0x10^3$	$6.9x10^3$	$5.8x10^3$	$4.7x10^2$

Table 3. Coliform bacteria counts (MPN/g) of Group A and Group B during the storage period at -18°C.

Group	**Days of storage**				
	1	15	30	60	90
A	4;4	4;9	4;4	4;4	4;<3
B	4;4	9;25	4;4	4;4	4;<3

Quality assesment by means of total viable count is an important matter. Total viable count of fresh sardine was determined as 6.0×10^6/g. This level does not exceed the maximum level of the acceptibility of fresh or frozen fish, as recommended International Commission of Microbiological Standarts for Foods (ICMSF, 1978). On the first day of the frozen storage, total counts of Group A and Group B dropped to 2.2×10^3/g and 2.4×10^4/g, respectively. In Group A total

viable counts show slightly increase till 60 days and came to approximate its initial value at the end of the storage but Group B showed orderly decrease in the total viable counts and reached to 4.7×10^2 /g. During the storage period , it was shown that total viable counts of Group A and Group B did not reach the maximum level of the acceptability of frozen fish. (ICMSF, 1978).

Coliform organisms and *S. aureus* are good indicators of the standart of hygiene and handling. Coliform bacteria count of fresh sardine was determined as 450/g and 1100/g. Since maximum level of fecal coliform bacteria in fresh or frozen fish was given by ICMSF, it is not possible to put forward an idea for coliform bacteria count in fresh and frozen sardine. According to Harrigan and McCance (1976), since coliform bacteria count should be less than 200/g in salt-water fish, it was found that coliform bacteria count of fresh sardine exceeded the maximum level. On the first day of the storage coliform bacteria counts of Group A and Group B decreased to 4/g because of the frozen effect. These low counts stayed almost same during the storage period of two groups.

Coagulase-positive *S.aureus* was not detected in two groups during the storage period and this result was suitable to the criteria given by Harrigan and McCance (1976) and ICMSF (1978).

Changes in scoring of the sensory attributes of Group A and Group B during the frozen storage for 90 days were shown on Table 4. According to the statistical analysis results, there were no significant differences ($P>0.05$) between colour, smell, chewy texture and flavor properties of two groups but a significant difference ($P<0.05$) was observed on the structure properties of two groups on 55th and 90th days of the storage. On the 7th days of the frozen storage all sensory attributes of the samples were indicated as the 'first quality' by the panelists. On 55th and 90th days of the storage, scores of the sensory attributes dropped to the levels indicating the second (good) quality.

Table 4. Changes in scoring of the sensory attributes of Group A and Group B during the storage period at -18°C.

Quality criteria	Group	Days of storage		
		7	55	90
Colour	A	2.86±0.03	2.63±0.09	2.55±0.09
	B	2.79±0.05	2.45±0.08	2.40±0.10
Smell	A	2.89±0.03	2.55±0.09	2.50±0.08
	B	2.82±0.03	2.47±0.09	2.46±0.09
Structure	A	2.87±0.03	2.40±0.07	2.39±0.07
	B	2.80±0.04	2.12±0.07	2.11±0.08
Texture	A	2.85±0.03	2.41±0.08	2.33±0.08
	B	2.78±0.04	2.19±0.11	2.16±0.11
Flavor	A	2.90±0.01	2.42±0.11	2.39±0.10
	B	2.88±0.02	2.51±0.11	2.31±0.12

Conclusion

In the present study, physical, chemical, microbiological and sensory quality changes of sardine stored at -18°C were studied. During the storage period it was established that all quality criteria mentioned above were not reached to the maximum limits for the acceptibility of the sardine as whole (Group A) and as

fillets (Group B). Even if there were no significant differences (P>0.05) between pH and TVB-N values and sensory attributes exception of the structure properties, in order to make a decision between the two groups longer frozen storage period was advisable.

While the fish were filleted, hygienic rules and mechanical damages manually should be taken into consideration. These cases are more important in small fish like anchovy and sardine. If the fish will be frozen, it is advisable to freeze as single, but if it is not possible sorted fish should be frozen as small blocks since short time for thawing is required.

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