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Effect of potassium metabisulphite, glaze and vacuum on shelf life of frozen blacksea anchovy (E*ngraulis encrasicolus*, Linnaeus 1758)

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ABSTRACT

The effects of glaze, potassium metabisulphite and vacuum treatments were investigated on physical (thaw loss, cook loss, pH), chemical (TVB-N, TBA), microbiological (total mesophilic, physcrophilic bacteria; total *Enterobacteriacea)* and sensory properties of anchovy stored at -30°C for 180 days.

The number of total mesophilic and physicrophilic bacteria showed alterations (2.95-3.75Log CFU/g and 2.50-3.68 Log CFU/g, respectively) and *Enterobactericea* were not observed in any samples during frozen storage period for all groups. Treatments with vacuum, glaze and metabisulphite showed positive effect on the protection of sensory properties of anchovy stored at -30°C for 180 days. All the sensory and chemical results show that the shelf life of anchovy stored at -30°C after packed (control), glazed+packed (B), dipped in metabisulphite solution+glazed+packed (C) and vacuumed (D) were 90, 150, 90 and 180 days, respectively.

KEYWORDS

Anchovy;
Glaze;
Metabisulphite;
Vacuum;
Frozen storage;
Packaging.

INTRODUCTION

The aim of cold storage in seafood is to extend its shelf life and to limit microbiological and chemical deterioration. Freezing is both widely used to prevent microbial spoilage and to slow chemical and enzymatic reactions in fish muscle. However, deteriorates in fish muscle during frozen storage, largely because of the myofibriller proteins undergo denaturations and aggregation[1]. Freezing and cold storage is an efficient method on seafood preservation but it must be emphasized that it does not improve product quality. The final quality depends on the quality of the seafood at the time of freezing as well as other factors during freezing, cold storage and distribution[2]. Various factors, such as quality of the initial raw material, storage conditions before freezing, freezing temperature, the rate of freezing, vacuum packaging or packaging materials and prevention against oxidation (light and oxygen) can affect frozen fish quality[3]. Lipid oxidation of fish can be prevented or reduced by low storage temperature[4], glazing and vacuum packaging in order to remove oxygen and treatment of antioxidants[4,5].

Numerous studies have focused on quality related with processes occuring in the temperature interval between 0 and -30°C, and it is well known that storage at

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-20°C or higher is insufficient in keeping a high quality[6]. Burgaar and Jørgensen[6] reported that after 18 months of frozen storage at -30°C, the values of thiobarbituric acid reactive substances (TBARS) in rainbow trout were still below what had been reported as the sensory detection limit. And they recommended that based on the overall behavior of the quality-related parameters, temperature of frozen storage for rainbow trout is -30°C or below. In addition, frozen fish quality depends on amount of weight loss by dehydration, oxidation degree, chemical and microbiological spoilage of fish muscle. Especially weight loss by dehydration and lipid oxidation are connected with the surface area of fish where contact with cold airflow. These negative effects can be prevented by covering the surface with glaze and packaging materials or using food additives like metabisulphite, citric acid, ascorbic acid and phosphates.

About 75% of the world's total seafood is used for human consumption, 25% is converted into fish meal and other nonfood products, 40% is consumed as wet fish without any further technological processing or preservation, about 20% is converted into deep frozen products, 8% is transformed into cured products, and another 8% into canned products[7]. Anchovy (*Engraulis encrasicolus*) is commercially an important fish in Turkey. Total seafood production in Turkey was reported as 644852 tons in 2012. A significant portion (about 25, 43%) of this harvest is anchovy[8]. The amount of total export and import of seafood were 74000 tons and 65384 tons in 2012, respectively. In 2012, while the amount of fresh and chilled anchovy exported was 204 tons, frozen anchovy amount was 356 tons[9]. As seen as above, it is commonly consumed as fresh besides it is exported to other countries as frozen. In several researches have been investigated the effect of frozen storage conditions and some factors on frozen sea foods quality (2, 6, 10-17). In addition, there is little studies related to frozen anchovy at different temperatures[18-20].

The objectives of this research were to observe the effects of (i) glazing, (ii) potassium metabisulphite and (iii) vacuum packaging on physical, chemical and microbiological changes of frozen anchovy during frozen storage (180 days).

MATERIALS AND METHODS

Raw material and sampling

Fresh Black Sea anchovy (*Engraulis encrasicolus*) were purchased total 37kg from a local market and transported to the laboratory on ice within 5 hour after the harvest. All the fish were washed with tap water several times. After, fish were divided into four groups (A, B, C, and D). The experiment was performed with two replicate. Fish in A and B groups were put into polystyrene dish. Fish in C group were dipped into potassium metabisulphite solution (50ppm for 1kg) for 2 minutes. These three groups were packed with shrink film. Fish in D groups were vacuum packed into oriented polyamide-polyethylene 30cm x 18cm bags (thickness 98µm, oxygen permeability of 47.6 ml/m^2/24h at 23°C and water vapor permeability of 3.48 g/m^2/24h at 23°C). Bags were sealed using Abant packaging machine (Abant MG 42, Adapazarı, Turkey).

In experiment, 48 polystyrene dishes were used that had 751±0.23g anchovy. All the fish (A, B, C and D groups) were frozen in freezer at -35°C. For fish in groups of B and C, the glazing was carried out by dipping of fish into a container of cold water (0°C) for 6-8 sec. Then all the fish were packed and stored at -30°C for 180 days.

Proximate analysis in fresh anchovy

For proximate analysis, about 1 kg anchovy were used. Analyses were carried out on the homogenized muscle of the raw material. The moisture (925.10) and crude ash (923.03) contents were determined as described by AOAC[21] and crude protein content (960.52) was calculated by converting the nitrogen content determined by the Kjeldahl method (Nx6.25)[22]. Crude lipid content (991.36) was determined by acid digestion prior to continuous extraction using petroleum ether (b.p. 40-60°C) in a Soxtec system[21]. Energy value was calculated by Atwater method[23]. The analyses were run in triplicate.

Preparation of fish samples for analysis

Two packed frozen fish were selected from each group for each sampling day and thawed in microwave (160W, 7min).

Quality analysis

Thaw drip, the cook loss

After thawing process, each package were weighted and percent drip loss calculated according to the following formula[5]:

%drip loss= (initial weight raw material-weight at sampling)/

(initial weight raw material) x 100

After drip loss calculated, the percent water loss in cooking was determined using the method described by Varlık et al.[24]. For this analysis, 200g fish were divided to glass jar. After cooking for 30 min in boiling water, the glass jar were kept in cool until decrease to 50°C; the fish tipped on strainer and kept for 20min. Filtrate was weighted and the water loss (%) in cooking was calculated with proportion.

After, the rest of thawed fish were homogenized for other analysis.

pH determination

pH was determined using the method described by Manthey et al.[25].

Determination of total volatile basic nitrogen

Analysis was carried out according to the Lücke-Geidel method modified by Antonacopoulus[26]. Results were expressed as mg TVB-N/100g.

Determination of lipid oxidation

The distillation method of Tarladgis et al.[27] was used to determine the degree of lipid oxidation in fish. TBA values were expressed as mg of malondialdehyde (MDA)/kg of sample.

Microbiological analysis

For microbiological analysis, sampling was carried out aseptically. Ten g of sample diluted with 90 ml of peptone salt solution for each microbiological analysis. Then, samples were homogenized for 2 min and prepared serial dilutes needed for plating. The following media and incubation condition were used. Microbial counts were expressed as LogCFU/g. Total mesophilic aerobic bacteria (TMAB) were determined using Plate count agar (PCA, Merck code: 105463.0500) after incubation for 2 day at 37°C[28]. Total psychophilic bacteria (TPB) were enumerated on PCA and incubated at 7°C for 10 days[28]. Total *Enterobacteriacea* were enumerated on Violet Red Bile Dextrose (VRBD-Merck code:1.10275) and incubated for 24h at 37°C[29].

Sensory analysis of cooked fish

Sensory analysis of cooked fish was determined by the method of Varlık et al.[24]. The attribute of cooked fish (odor, texture, taste) (3-2.7=very good, 2.7-2=good, 2-1= medium, <1= spoilage) were evaluated by eight experienced judges.

Statistical analysis

Differences between the samples were evaluated by MINITAB 13 by one-way and two-way analysis of variance (ANOVA) using a significance level of $p<0.05$. Tukey's test was used for significant differences.

RESULTS AND DISCUSSION

The results of proximate analysis of anchovy are shown in TABLE 1. Proximate composition values of anchovy have been reported by Ayas[30] were as 19.56% crude protein, 4.72% crude fat, 73.80% moisture and 1.39% crude ash. He found different results from our results except for crude ash value. In November, proximate composition of anchovy in Black sea-Turkey was stated as 66.34% moisture, 15.68% crude fat and 1.48% crude ash[31]. Özden[32] reported that protein, lipid, ash and water contents of anchovy were 18.02%, 10.32%, 1.62% and 69.76%, respectively. Contents of crude protein and crude lipid of anchovy in Sinop region-(Black Sea) in November, December and January was declined 17.24%, 17.36%, %16.94% and 18.57%, 15.91%, 15.57% respectively[33]. Proximate composition is influenced by season, water temperature and spawning cycle which also determined by species[34].

Changes in physical and chemical parameters of anchovy stored at -30°C were given in Figure 1.

The results concerning with thaw drips losses are in Figure 1(a). There were no differences ($p>0.05$) between the metabisulphite+glaze group and vacuum group during the storage period. The thaw drips losses of both groups were higher than glaze group after the day of 30th. During the storage, the thaw drip losses regularly increased in the control and metabisulphite groups. In vacuum group, drip losses firstly increased to 1.86% at 60th day and then decreased to 1.80% but the differences between them were insignificant ($p>0.05$) at that days. The minimum drip loss in the thawing was found in glaze group (0.60%) at the day of 60th. This

TABLE 1 : Proximate composition of anchovy

Moisture (%)	Crude Fat (%)	Crude Protein (%)	Crude Ash (%)	Carbohydrate (%)	Calory (kcal)
62.86±0.03	10.64±0.04	22.71±0.04	1.48±0.01	2.31±0.08	195.88±0.25

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decrease may be from effect of glaze layer covering the fish. At the end of the storage, maximum drip loss (3.47%) was found in metabisulphite group and this result were similar with control and vacuum groups ($p>0.05$). These results indicated that metabisulphite and vacuum treatments on thaw drip loss were not af-

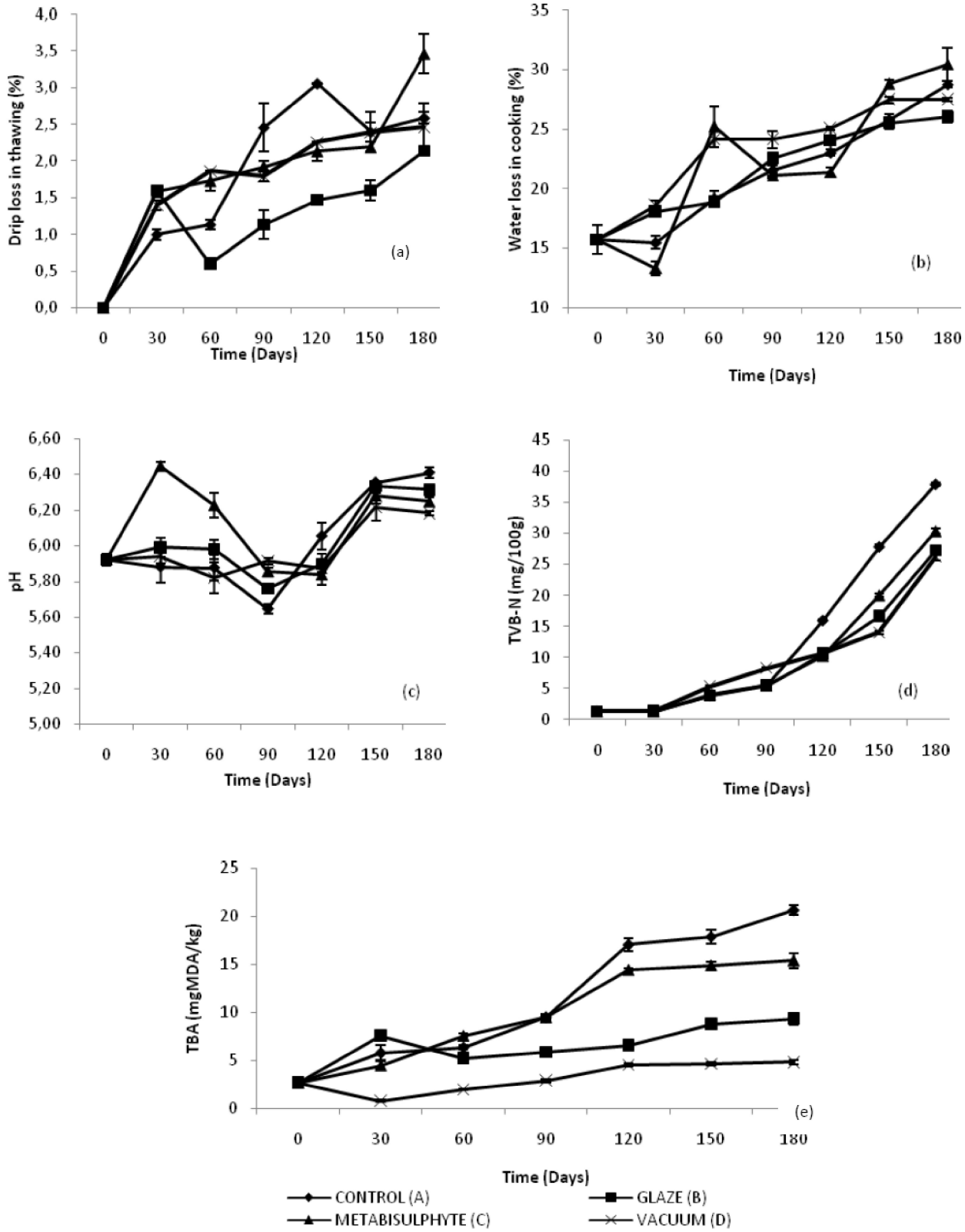

Figure 1 : Changes in physical and chemical parameters; drip loss in thawing (%) (a), water loss in cooking (%) (b), pH (c), TVB-N (mg/100g) (d), TBA (mgMDA/kg) (e)

fect as well as glazing process. Santos and Regenstein[5] reported that white hake and mackerel packed with vacuum, both treated and untreated antioxidant (erythorbic acid), gave significantly higher values than other groups (with or without erythorbic acid and packed, with or without erythorbic acid and water glazed). Turan et al.[13] also reported that using of food additive (phosphate) may not be more important than glazing+packing treatments of rainbow trout in terms of drip loss.

The water losses in cooking are shown in Figure 1(b). At the beginning of the storage (0th day), the cooking loss value of fresh anchovy was 15.72% in all groups. This value regularly increased during the storage. At the day of 30th, cooking loss value of metabisulphite+glaze groups was lower according to the 0th day ($p<0.05$). The water loss in cooking of glaze and vacuum groups were not important ($p>0.05$) after the day of 60th and 90th days. At the end of the storage, cooking loss values of groups (A, B, C, and D) were 28.75%, 26.03%, 30.47% and 27.44%, respectively. Only glaze process was more effective than the other applications.

At the beginning of the storage, the pH value was determined as 5.92 in all groups. Orak and Kayışoğlu[20] reported that the initial pH value of whole, gutted and filleted anchovy (*Engraulis encrasicolus*) were 6.50, 6.51 and 6.51, respectively. These differences may be from the freshness degree of fish. The initial low pH is owing to the high amount of lactic acid, while the increase in the pH at the end of the storage period is due to the production of basic components caused by the enzymatic spoilage of the fish muscle. In generally, the pH values of all the groups except for metabisulphite+glaze group regularly increased ($p<0.05$) during the storage. The pH value of fish packed with only stretch film (control) increased to 6.41 at the end of the storage periods. pH values of fish in glaze group, metabisulphite+glaze group and vacuum group were 6.31, 6.25, and 6.18, respectively. During storage, minimum pH value was measured at vacuum group (5.82) at 60th day, also at the end of the storage minimum pH value was measured at vacuum packed anchovy. In fish stored in aerobic conditions, the increase in pH may be owing to the production of volatile bases by aerobic spoilage bacteria. This spoilage and production of volatile bases can be prevented by vacuum packaging. As shown in Figure 1(c), pH values of control and glaze groups were statistically similar ($p>0.05$) during 180 days. pH values of glaze and metabisulphite+glaze groups (B and C) were significantly different ($p<0.05$) at 60th day but they were similar in other days ($p>0.05$). pH values of C group dipped potassium metabisulphite solution were generally higher than control and glaze group between the 30th and 90th days. Similar results were reported by Omar[17] that the pH was slightly higher in frozen shrimp treated ice/dipped sodium metabisulphite. The pH changes in vacuum, control and glaze groups group were statistically similar during 60th days ($p>0.05$). During the storage, although the pH values in all the groups increased and none of them did not exceed the consumability limit values reported as 6.8-7.0[35]. The pH of live fish muscle is close to the value 7.0[36]. It has to be supported by other chemical and sensory analysis[26].

At the beginning of the storage, as expected, the TVB-N level was low (1.35 mg/100g) in fresh anchovy. Köse et al.[37]; Orak and Kayışoğlu[20] reported that; TVB-N values of fresh anchovy were 4.24 and 16.1 mg/100g, respectively. TVB-N value of anchovy used in our study was approximately 3 and 12 times lower than thoses in these literatures. TVB-N is documented as a quality index of fish freshness. TVB-N value of some fish species is related to non-protein nitrogen content of fish, which is changes depend on type of fish feeding, season of catching, fish size as well as other environmental factors. Also it is directly related to microbial activity in the fish tissue[38]. All groups showed an increase in TVB-N values after 30th day ($p<0.05$) Figure 1d. TVB-N values of all groups were similar on 30th and 60th days ($p>0.05$). TVB-N values of vacuum group were significantly higher ($p<0.05$) than the other groups at the day of 60th and 90th. TVB-N value of control group was 15.98 mg/100g ($p<0.05$) while the TVB-N value of other groups did not exceed 10.67 mg/100g in 120th day. In the 150th day, TVB-N value of control group reached to 27.86 mg/100g, the same day TVB-N values of B, C and D groups were 16.67, 20.04 and 14.05 mg/100g, respectively. At the day of 150th, the TVB-N values of all groups were different statistically ($p<0.05$), moreover this differences went ahead after 150th day. At the end of the storage, the lowest TVB-N value was determined in glaze (27.19mg/100g) and vacuum (26.16 mg/100g) groups ($p>0.05$). The level of 35 mg/100g has been considered as the maximum limit, which above fishery prod-

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uct are considered spoiled[26]. Only control group exceed this limit value at the end of the storage (180 days). Similar results for anchovy[19,20], for other fish species[39,40]; and for mussel (Gökoğlu et al., 2000), stored frozen have been previously reported by the researches.

TBA number can be used in the lipid oxidation determination of the products having extractable or not extractable oils such as meat products[41]. TBA values of anchovy are shown in Figure 1(e). Initial value of TBA was 2.68 mgMDA/kg for fresh anchovy whereas final values of TBA were 20.62 (control), 9.35 (B), 15.38 (C) and 4.80 mgMDA/kg (D). According to the Varlýk et al.[35], TBA value of 5 mg MDA/1000g of fish flesh are usually regarded as a good value. At 30th day, TBA value of all groups were significantly different from each other ($p<0.05$). The lowest TBA value (0.78 mgMDA/kg) was found in vacuum group. TBA value of glaze group (B) firstly increased to 7.57 mgMDA/kg (30th day) and then decreased to 5.27 mgMDA/kg (60th day). Changes in these days can be affected by the amount of glaze covering the fish surface. Because after the 30th day, TBA values of glaze group were found fewer and slowly increased when compared control and metabisulphite+glaze groups ($p<0.05$). TBA values of anchovy vacuumed were slowly increased and had the minimum TBA values during storage period. Potassium metabisulphite solution did not affect fat oxidation. It has been proposed that maximum TBA value is 5 mgMDA/kg, while the fish may be consumed up to a level of 8 mgMDA/kg in TBA value[42]. Control, glaze and metabisulphite+glaze groups exceed this limit value at 90th, 150th, and 90th, respectively. However, vacuum group did not exceed good value (5 mgMDA/kg) during storage period. It can be said that optimum methods for frozen anchovy were vacuum and glaze in terms of lipid oxidation. Similar to our result, Santos and Regenstein[5] was reported that the use of vacuum packaging or glazing resulted in less oxidative deterioration than the use of the antioxidant and packing the sample in the bags with no vacuum.

Figure 2 presents the total mesophilic bacteria (TMB), psyrophilic bacteria (TPB) and sensory scores of the fish. In our study, the initial TMB count (2.95 LogCFU/g) of anchovy was found very low and good level according to the initial bacteria loads (7.2 LogCFU/g, 4.2 LogCFU/g, 3.17 LogCFU/g) found by other researches, respectively (18, 19). TMB counts of all groups did not exceed to 3.76 LogCFU/g during storage period. During storage period, average TMB counts of control, glaze, metabisulphite+glaze and vacuum groups were 3.23, 3.06, 3.04 and 3.01 LogCFU/g, respectively ($p>0.05$). At the end of the storage period, the TMB counts in the all groups (A, B, C and D) were, 2.54, 2.68, 2.63 and 2.72 LogCFU/g, respectively. It has been recomended that the upper limit of aerobic bacteria for frozen fish should be $1x10^7$/g[43]. During the storage, all groups did not exceed this limit value for TAB. Karaçam and Boran (18) reported that total mesophilic bacteria counts of whole anchovy stored at -18^{o}C was $6.3x10^3$ g^{-1} (3.79 LogCFU/g) at the end of the 180th days. In our study TMB counts were lower than in this study. This may be higher from initial TMB count and storing temperature.

At the beginning of the storage, initial TPB count of anchovy was 2.50 LogCFU/g. In all groups, firstly TPB counts were increased until 60th day and then decreased until the end of the storage time. During storage, maximum TPB count (3.68 LogCFU/g) was found in control group at 60th day. At 180th day, TPB counts of groups (A, B, C and D) were 2.21, 2.33, 2.36 and 2.35 LogCFU/g, respectively. At the beginning and end of the storage (180 days), TPB counts of anchovy were reported as 6.07 LogCFU/g and 3.65 LogCFU/g, respectively by Karaçam and Boran[18].

The initial total *Enterobactericea* count of anchovy was 0.98 LogCFU/g. During storage period, *Enterobactericea* were not observed in any group.

The results of sensory evaluation of anchovy are presented in Figure 2 (c). During storage, sensory scores of cooked anchovy in all groups regularly decreased. At the 30th day, the sensory scores of groups were found similar ($p>0.05$). The group with metabisulphite+glaze was statistically similar to control group during storage period ($p>0.05$). Despite exceed the limit value (8 mgMDA/kg) the TBA values after 60th day of control and metabisulphite+glaze groups, both sensory scores were in acceptable limits and rancid odor/taste were not sensed. At the day of 180th, sensory scores of groups (A, B, C and D) were 0.95, 1.32, 1.26 and 1.64, respectively. The sensory score of only control group was decreased to under the one puan and this group was found unconsumable. Especially sensory score of vacuum group was higher than the other groups ($p>0.05$) at the end of the 180day. Treatments of vacuum, glaze and

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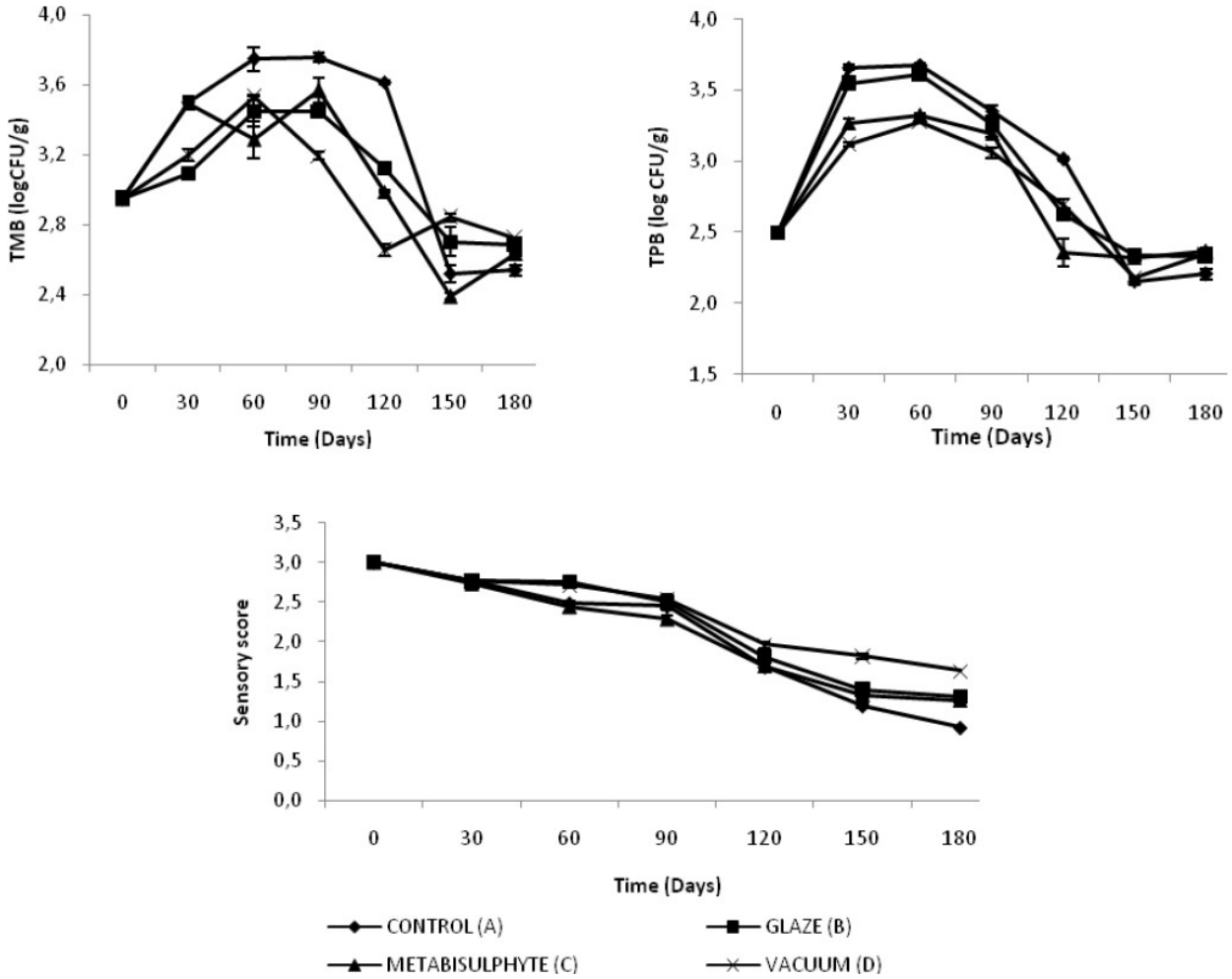

Figure 2 : Changes in microbiological and sensorial parameters; total mesophylic bacteria (TMB) (LogCFU/g) (a), total psycrophilic bacteria (TPB) (LogCFU/g) (b), sensory scores (c)

metabisulphite showed positive effect on the protection of sensory properties of anchovy.

CONCLUSION

All the results of the research shows that the shelf life of anchovy stored at –30°C and frozen at -35°C after packed (control), glazed+packed (B), dipped in metabisulphite solution+glazed+packed (C) and vacuumed (D) are 90, 150, 90 and 180 days, respectively. Metabisulphite+glaze treatment on anchovy stored at -30°C is not effective while only glaze treatment is effective. In our study, glaze treatment (B) has increased 1.5 times the shelf life of fish, also vacuum increased approximately 2 times.

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