

DETERMINATION OF SHELF LIFE OF MARINATED CARP FILLETS

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Abstract: In the present study, sensory, microbiological and chemical changes during manufacturing and storage of marinated and baked fillets were investigated. For this purpose, the fillets were divided in to three groups. One group was control and the other two groups were marinated with two different marination mixes (group A and group B). The control fillets were cooked at 150 °C for 55 minutes. The marinated groups were cooked after holding at +4 °C for 6 hours. Central temperatures of cooked fillets were chilled to +4 °C and vacuum packaged. Vacuumed fillets stored at +4 °C. Then fillets were analysed for sensory, microbiological (mesophilic aerobic bacteria, psychophilic aerobic bacteria, mesophilic anaerobic bacteria, yeast and mould counts) and chemical (pH, moisture, total volatile – nitrogen, tiobarbituric acid and salt) on 0, 7 th,14 th,28 th,42 nd,56 th,70 th,84 th and 98 th days of storage. In the microbiological analysis, counts of total mesophilic aerobic bacteria, psychophilic aerobic bacteria, mesophilic anaerobic bacteria, yeast and mould counts were determined as < 10 cfu/g in all groups during the storage. There was no significant difference among the groups in pH, moisture, TVB-N and salt level ($p>0.05$). The TBA value increased during the storage period in control group. As for the TBA value, the difference between control group and group A and group B was significant ($p<0.05$). When fillets have been investigated from sensory characteristics, A and B products found beter than control group throughout the storage period.

Key words: carp fillet, marination, baking, sensory, microbiological and chemical quality

Introduction

Fresh seafood is a highly perishable product and spoilage developing in aerobically stored fish typically consists of Gram-negative psychophilic non-fermenting rods. Thus, under aerobic ice storage, the flora is composed almost

exclusively of *Pseudomonas* spp. and *Shewanella putrefaciens* (Davies *et al.*, 2001). The *Enterobacteriaceae* count is considered as another index of fish quality because it is related to storage in ice, washing and evisceration (Emblem *et al.*, 2001). A monitoring of these microorganisms has been suggested as a measure of fish quality. Also, risk management decisions should take into account the whole food chain from primary production to consumption, and should be implemented in the context of appropriate food safety infrastructures, for instance regulatory enforcement, food product tracing and traceability systems. In the fish processing chain managing risks should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during food preparation, storage and transport, retail and catering (Davies *et al.*, 2001).

Protein represents the major component of fish feeds. It is a source of energy for the fish, but also a medium for micro-organisms, especially proteolytic bacteria and ammonifiers. Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.

Fresh fish is consumed in different patterns (fried, baked, steamed etc.); it can also be consumed by transformation into different fish products (Cardinall *et al.*, 2004). Several technological processes can be employed in order to preserve the freshness of fish flesh.

Sauce compositions prepared with natural additives improve the flavor, aroma, appearance, color and texture features of food; they also preserve the food against microbial, chemical and physical effects (Altuğ *et al.*, 2001). In addition, they facilitate and improve technological process (Damarlı *et al.*, 1992).

Fish fat becomes easily oxidized as it includes high level of unsaturated fats, natural catalysts in its structure (i.e. hem pigment), and abundance of vitamins A and D. Some spices in the sauce improve the aroma and flavor of food; they also have antimicrobial and antioxidant characteristics (Damarlı *et al.*, 1992).

In our country, the sales figures of food made of sea products which are readily consumable are not high. It is extremely important that fish is transformed into ready or semi-ready products so that the animal-based protein gap can be closed and consumption of fish harvested during hunting season can be ensured in periods other than their seasons. In our region *Cyprinus carpio* is produced abundantly but not preferred by the public as its organoleptic features are low. This study was conducted so as to improve organoleptic features of *Cyprinus carpio* in our region and lengthen its shelf life, so that a contribution can be made to the economy.

Material and Methods

Raw materials and process. Material of the study was *Cyprinus carpio* (*Cyprinus carpio* Linnaeus, 1758) hunted in Keban dam lake. The study consisted of three trials and in every trial fish with approximately 15 kgs live weight was used. Fish hunted from Keban dam lake were transported to the laboratory in cold chain. Their heads were chopped, skins were stripped and internal organs were taken out. Then fillets cleaned out from muscles, fishbones and bones were divided into 64 parts with similar thickness, each of which weighed 70-80 grams. Two different types of sauces were used in the study (A and B). Prepared fillets were separated in to three groups. The first group was assigned as the control group. Three percent salting process was applied to control group samples. Second group was left in the sauce with formula A (Table 1) for 6 hours at + 4°C, whereas third group was left in the sauce with formula B (Table1) for 6 hours at + 4°C. The weight of the sauce used was 20% of the fish weight. Fillets placed on a tray were baked for 55 minutes in the oven (Arçelik, MF 2009) at 150°C. Central temperature of the fillets was measured with K probed thermocouple (HI 9057 KJT thermocouple, Hanna instruments, Portugal). Organoleptic appearance of the product was also taken into account during baking. After the baking process was completed, the tray was covered with aluminum foil and fillets were rapidly cooled in deep freezer until their central temperature fell to + 4°C. The cold fillets were packaged with vacuum. Vacuumed fillets were preserved at + 4°C and examined on days 0, 7, 14, 28, 42, 56, 70, 84 and 98 in terms of organoleptic, microbiological and chemical features.

Table 1. Sauce combinations

	A Group		B Group	
	Amount(g)	Rate(%)	Amount(g)	Rate(%)
Tomato Paste	200	20	300	30
Lemon juice	200	20	300	30
Oil	200	20	100	10
Garlic	100	10	100	10
Onion	-	-	50	5
Thyme	10	1	20	2
Red Pepper	10	1	10	1
Black Pepper	10	1	10	1
Cumin	-	-	10	1
Salt	30	3	20	2
Water	240	24	80	8

Physical and chemical analysis. pH values of the samples were determined with pH meter (EDT. GP 353) (AOAC, 1990). The method reported by Varlık (1993); was employed in determination of TVB-N amount of the samples. The amount of malonaldehit formed with fat oxidation in samples was calculated in mgs (Tarladgis, 1960). Salt level of the samples was determined according to Mohr method (55). Moisture was determined according to TSE (1974).

Microbiological analysis. For all microbiological counts, 10 g of sample were taken and transferred into 90 ml 0.1% peptone water (Difco, 0118-17-0) and homogenized. From the 10^{-1} dilution, other decimal dilutions were prepared. Plate Count Agar (Oxoid CM 325) was used in order to count mezophilic aerobe bacteria of the samples. Implanted plates were incubated at 35°C for 48 hours and colonies formed were counted. Wort Agar (Merck 1.10130) was employed for counting yeasts in the samples. Plates were incubated at 30°C for 5 days and the colonies formed were counted. Sabouraud Dextrose Agar (Acumedia 7150 A) was used for counting mould in the samples. Plates were incubated at 30°C for 5 days and the colonies formed were counted. Brewer Agar (Oxoid) was used to count the total mesophilic anaerobe microorganisms. Plates were incubated at $30 \pm 1^\circ\text{C}$ under anaerobe conditions for 3 days. Colonies formed at the end of incubation were counted. Plate Count Agar (Oxoid CM 325) was used in order to count the total psychophilic aerobe bacteria. Plates were incubated at 5°C for 10 days and colonies were counted (Harrigan *et al.*, 1976).

Sensory analysis. Samples were examined in terms of crispiness, flavor, saltiness, appearance and total assessment. For this purpose samples were analyzed by a group of 8 panelists against preset criteria. The samples were scored from 1 to 5, where 1 very bad, 2 bad, 3 normal, 4 good and 5 very good (Kurtcan and Gönül, 1987). The samples were evaluated color, smell, flavor, appearance, crispiness, saltiness and total assessment.

Statistical analysis. Analysis of the data was conducted using Statistical Analysis System (SAS) package programmed. Values between groups and within group-between days were compared. Data were subjected to variance analysis in accordance with $3 \times 11 \times 3 \times 1$ factorial design and in terms of fix effects and inter-variable interactions so that “repetition number x sampling time x test groups x 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 (Anonim, 1996). Alpha value was determined as 0.05.

Results and Discussion

Raw material of microbiological analysis are given in Table 2. Total mesophilic aerobic bacteria, total mesophilic anaerobe bacteria, total psychophilic aerobic bacteria, yeast and mould were determined to be <10 kob/g on all sampling days in all groups.

Table 2. Raw and sauced fillets of number microorganisms (\log_{10} kob/g) (n=3)

Microorganisms	T.F. ^a	Sauced Groups	
		A ^b	B ^c
Mezophilic aerobic	4.16	3.62	3.3
Psicrofilic aerobic	4.25	3.79	4.02
Mezophilic anaerobic	1.74	<10	<10
Yeast	3.71	2.56	2.8
Mould	3.77	2.77	2.8

a : Raw Fillet, *b* : A Groub, *c* : B Groub

Changes detected during the production and preservation of fish fillets are given in figure 1. The pH value of the prepared sauce was determined as 5.63 in group A and 4.8 in group B. 6.51 pH value found in fresh fillet decreases after saucing process and becomes 5.89 in samples from group A and 5.35 in samples from group B. irregular pH changes were observed in all three groups during preservation (Figure 1.a.). It was found out that the difference between groups and in the same group-between days was not significant ($p>0.05$).

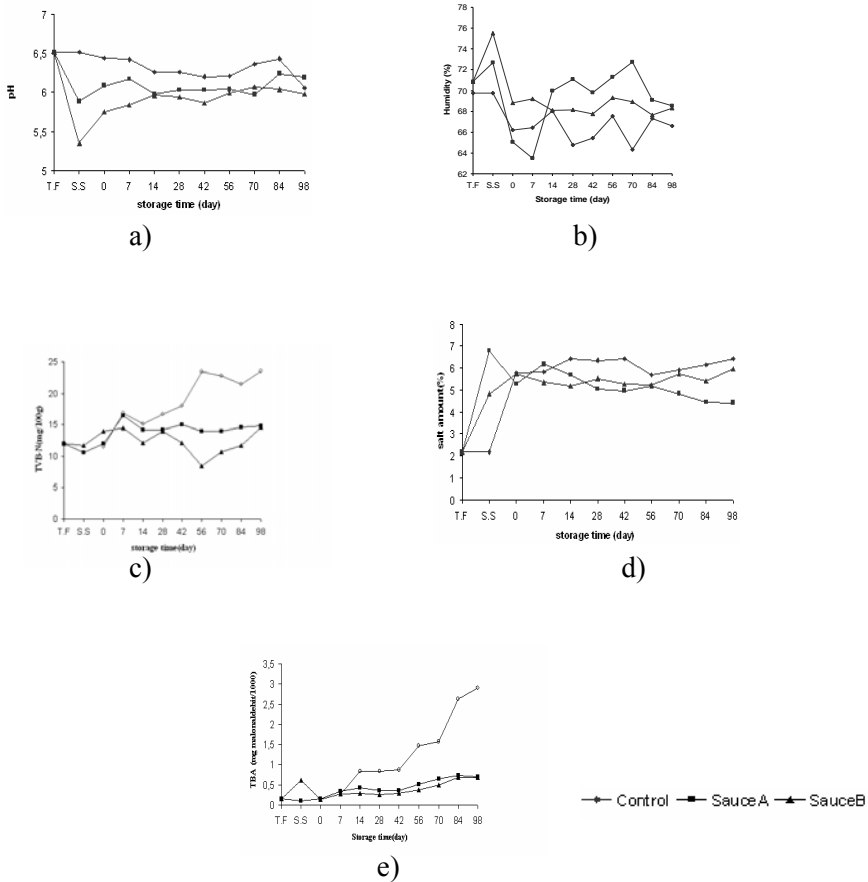


Figure 1. a: Changes in pH of during storage and production. b: Changes in humidity of during storage and production. c: Changes in TVB-N of during storage and production. d: Changes in salt of during storage and production. e: Changes in TBA of during storage and production. T.F.: Raw Filet. S.S: Marination.

The humidity changes of samples are given in the figure 1.b. It was determined that changes between groups and within all three groups during production and preservation periods was insignificant ($p>0.05$).

TVB-N is used for determination of the spoilage level of fish during the storage period TVB-N values detected in control group samples were higher compared to the other two groups; this value was 11.56 mg/100 g on day 0, and increased to 23.53 mg/100 g on day 98. During production and preservation of fillets, TVB-N values of samples form groups A and B increased slower compared to the control group (Figure 1.c). In statistical analysis, it was determined that the difference between days within the same group was not significant ($p>0.05$) in terms of TVB-N values. When a comparison was made between groups, it was found out that the difference between control group and group B on day 56 was statistically

significant, but difference on other days was insignificant ($p > 0.05$). When samples are statistically evaluated, it was found out that difference between groups was not significant ($p > 0.05$), but when days within a group are compared, it was found out that the difference between raw fillet and sauced fillets was significant ($p < 0.05$), whereas difference between other days was insignificant ($p > 0.05$).

The TBA value of raw material was found to be 0.153 mg/1000 g. This value decreased to 0.104 mg/1000 g in group A after saucing process, whereas it increased to 0.612 mg/1000 g in group B. On the day 0 of preservation, TBA values was found as 0.143 mg/1000 g in control group and group B, whereas it was 0.161 mg/1000 g in group A. TBA value in control group increased regularly during preservation. TBA number in sauced groups was found to be close to each other; it was found to be 0.703 mg/1000 g on day 98 of preservation in group A and 0.688 mg/1000 g in group B (Figure 1. d.). Statistically speaking, it was determined that the difference between days within group in groups A and B were not significant ($p > 0.05$), whereas the difference between days 0, 7, 14, 28 and 42 and other groups was significant ($p < 0.05$). It was also found out that the difference between control group and groups A and B on days 84 and 98 was significant ($p < 0.05$). TBA numbers detected in samples of group B were rather low in all preservation days compared to the samples in group A, whereas the results were statistically examined the difference turned out to be insignificant ($p > 0.05$).

Changes detected during the production and preservation of fish fillets in determined sensory quality values are given in figure 2.

As a result of sensory evaluation of samples, when the scores they received in terms of total assessment, it can be seen that the lowest scores belonged to control group, whereas the highest scores were represented by group A. Samples were evaluated in terms of colour, smell, flavour, appearance and total assessment; as a result, it was detected that the difference between control group and groups A and B was statistically significant ($p < 0.05$), whereas in terms of crispiness and saltiness, the difference between groups remained insignificant ($p > 0.05$).

Total number of mesophilic aerobic bacteria in fresh fillet was found as $4.16 \log_{10}$ kob/g on average. *Patır et al.*, (2001) determined 6.70×10^4 kob/g total number of mesophilic aerobic bacteria in carp fillet in the beginning, which is higher than the value obtained in this study. Values can differ depending on contaminations during preparation of the fillets used in experiments.

As regards preservation of fish flesh using different methods (using potassium sorbate, curing, marination etc.) there is lots of information in the literature; however, there are few resources on such preservation of same carp fillets (using thermal process and saucing).

Rosnes et al., (1999), applied thermal process at 70 °C for 15 minutes after they vacuumed sauced trout fillets and preserved them at 4 and 10 °C (sous-vide method). At the end of day 42, researchers found the total number of mesophilic aerobic bacteria to be $< 1 \log_{10}$ kob/g of fillets kept at 4 °C. These values are in agreement with the values that we found. The same study determined this value to

be $6 \log_{10}$ kob/g on day 17 and above $8 \log_{10}$ kob/g on day 42 in fish stored at 10 °C. Applied temperature level was lower and preservation period was shorter compared to those employed in our study.

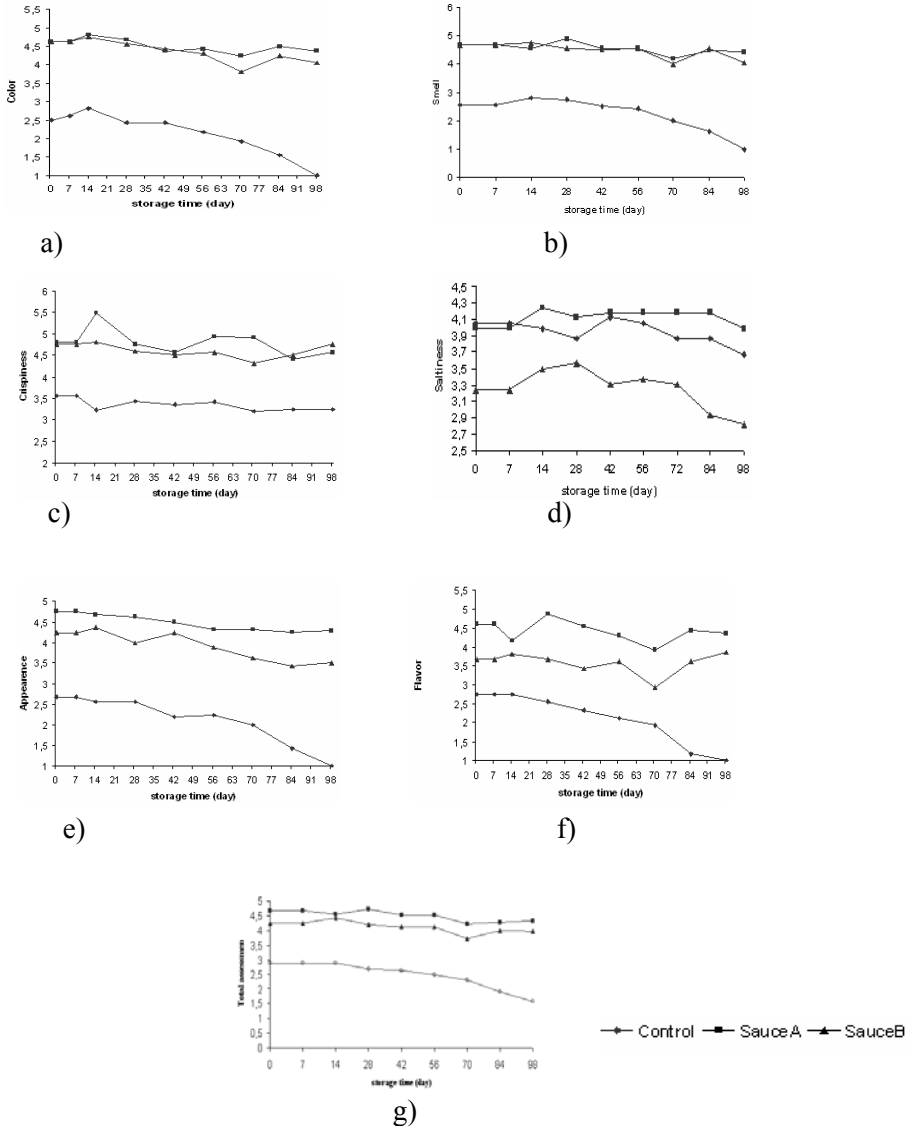


Figure 2. a: Changes in color of during storage and production. **b:** Changes in smell of during storage and production. **c:** Changes in flavor of during storage and production. **d:** Changes in appearance of during storage and production. **e:** Changes in crispiness of during storage and production. **f:** Changes in saltiness of during storage and production. **g:** Changes in total assessment of during storage and production. T.F.: Raw Filet. S.S: Marination.

Bergslien (1996); preserved trout fillets at 2 °C to which 10 minutes of thermal process was applied at 65 °C. On day 7 of preservation, the total number of mesophilic aerobic bacteria was found to be above 5 log₁₀ kob/g. However, even the value determined in fresh fillet (4.16 log₁₀ kob/g) in the study is lower than this value, which was found as <10 kob/g on day 7 of the study. In their study, *Simpson et al., (1994)* applied 65 °C (71 and 105 minutes) and 75 °C (37 and 40 minutes) thermal process to sauced spaghetti and sauced meat, preserved it at 5 and 15 °C, and checked the total number of mesophilic aerobic, mesophilic anaerobic and lactic acid bacteria. In the study, they determined that samples preserved at 5 °C maintained their quality for more than 35 days, whereas samples maintained at 15 °C lost their organoleptic features turned sour on day 14. This finding can be attributed to the fact that microorganisms injured by the effect of heat but which became active again at preservation temperature proliferated.

Sauced trout fillets were preserved at 2 °C for 45 days by applying 3,3 minutes of thermal process at 90 °C (*Gonzalez, 2004*). In the study, psychophilic aerobic bacteria number found as 5 log₁₀ kob/g in fresh fillet decreased below 1 log₁₀ kob/g. When the product obtained with the same method is preserved at 10 °C, psychophilic aerobic bacteria number was again found to be below 1 log₁₀ kob/g. Although the temperature and heating period was low, findings obtained coincide with the findings of our study. In the same research (*Gonzalez, 2004*), number of mesophilic aerobic in fresh fillet were found as 4.4 log₁₀ kob/g, whereas number of mesophilic anaerobes was found as 1.5 log₁₀ kob/g. However, this value is higher than the findings obtained in this study. Sauced trout fillets were exposed to 5 minutes of thermal process at 70 °C and preserved at 10 °C; at the end of day 45, number of mesophilic aerobic bacteria was found as above 7.5 log₁₀ kob/g. applied temperature, heating period and preservation temperature cause difference in terms of microbial quality of the product.

Gonzalez et al., (2005) subjected the salmon fillets to which olive oil and salt was added to 5 minutes of thermal process at 65°C and 10 and 15 minutes of thermal processes at 90 °C, and preserved them at 2 and 10°C. In fresh fillet, number of mesophilic aerobic bacteria determined as 4.77 log₁₀ kob/g decreased to 2 log₁₀ kob/g at the end of day 45 in fillets which were treated with 90°C for 10 minutes. This finding differs from ours. It is believed that this difference can be explained by the difference in method, applied temperature and additives included in the fillets.

Sardine fish were filled in jars after they were marinated with different sauces; when the central temperature reached 70 °C, they were subjected to thermal process for 20 minutes. In the study, samples were preserved at 4 °C and examined for 6 months in terms of total number of mesophilic aerobic, psychophilic aerobic, lactic acid bacteria, yeasts and fungi. In the end, it was found out that the number of mentioned microorganisms was below <10 kob/g (*Kılınç and Çaklı, 2005*). This

study is in agreement with our findings although it yielded partially different results.

Generally, preservation period of sauced fish fillets changes according to the microbial load in the beginning, applied temperature, packaging type and preservation temperature.

Determination of total volatile basic nitrogen (TVB-N) used in finding the freshness of fish flesh and fish products is an important parameter. The value that we found in fillet (12.03 mg/100 g) is close to the value found by *Patır et al.*, (2001) for fresh carp, which was 11.67 mg/100 g. In addition, if we compare with the values obtained by researchers using different types of fish, the findings reached by *Turan and Erkoyuncu (1997)*, 4.20 mg/100 g, *Yapar and Yetim (2000)* for anchovy fillets 7.10 mg/100g, are much lower than our findings. However, they are lower than the findings of *Tunç (1994)*, *Metin (1995)* and *İzgi (1996)* for fresh trout (15.33 mg/100 g and 17.97 mg/100 g). Throughout the study, TVB-N amounts obtained during preservation from all three groups did not transcend the suggested criteria and, it was concluded that, taking these criteria into account, they were in good quality. Thermal process, vacuum packaging and preservation at +4 °C did not cause much increase in TVB-N amount. *Kaya (1994)* found TVB-N amount at cured trout and bonito preserved at room temperature to be 48.6 mg/100 g on day 5; the findings for salmon was 45.2 mg/100 g. the same researcher (*Kaya, 1994*) found out that if preserved in refrigerator for 50 days, trout produced 58.4 mg/100 g, bonito produced 57.6 mg/100 g and salmon produced 55.8 mg/100 g TVB-N. *Cardinall et al.*, (2004) reported that TVB-N amount of cured and vacuum-packaged Atlantic salmon which were preserved at +4°C for 2-3 weeks was found as 22.4 mg/100 g.

TBA number was found as 0.153 mg malonaldehit/1000 g in the carp fillet which was used in this study; this number showed a relative increase during production and preservation. However, the increase in both groups was lower than the increase observed in control group. Antioxidant lycopene in tomato paste and citric acid in lemon juice may have prevented oxidation in sauced groups. It is believed that the increase in TBA number of control group can be attributed to the fact that it did not include any sauces. Vacuumed packaging prevents oxidation of products (*Ariyani 2000*); however, there can be a small amount of oxygen in the vacuum package which can trigger oxygen oxidation. The reason for which TBA number remained high in control group was that there was no such barriers which could prevent oxidation at the initial phase. During the study, TBA numbers found in all three groups were found to lower than suggested levels (7-8 mg malonaldehit/1000 g).

Taking into all organoleptic evaluation criteria, samples of group A got higher scores than group B, and control group samples received the lowest scores. This result is attributed to the effect and composition of sauces.

Conclusion

The reason for using *Cyprinus carpio* in the research is that it is a less costly, abundant, and easily treatable type of fish. Turkey has favorable climatic condition for carp growing. However, hunting season of carp matches with the season when sea fish is most abundant, which is an important disadvantage for carp growing. In this case, carp loses its competitive power and marketed at prices far below its value. For this reason, *Cyprinus carpio* which cannot be consumed fresh can be turned into various products and its consumption outside the production season can be popularized. Three basic criteria must be observed so as to ensure microbial safety in sauced products; namely, thermal process at proper temperatures, rapid cooling of the product to a proper temperature and its preservation at low temperature. Thermal process applied to fillets is economic and free from chemical residues; it also terminates the microorganisms which cause spoilage and improves organoleptic characteristics of the product, which are regarded as its advantages.

It has been determined that carp fillets, which were preserved at 4°C after being sauced, baked and vacuum packaged, maintained their organoleptic, microbiological and chemical quality for at least 98 days.

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Određivanje roka trajanja mariniranih fileta šarana

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Rezime

U ovom istraživanju, ispitivane su senzorne, mikrobiološke i hemijske promene tokom procesa proizvodnje i skladištenja mariniranih i pečenih fileta. U tu svrhu, fileti su podeljeni u tri grupe. Jedna grupa je bila kontrola, a druge dve su bile marinirane korišćenjem dve različite smeše za mariniranje (grupa A i grupa B). Kontrolni fileti su kuvani na 150°C u trajanju od 55 minuta. Marinirane grupe su kuvane nakon čuvanja na 4°C u trajanju od 6 sati. Centralna temperatura skuvanih fileta je smanjena, ohlađena na 4 °C i fileti su vakumirani. Vakumirani fileti su

skladišteni na 4 °C. Nakon toga fileti su analizirani, i to: senzorne, mikrobiološke (mezofilne aerobne bakterije, psihofilne aerobne bakterije, mezofilne anaerobne bakterije, broj plesni) i hemijske osobine (pH, vlaga, ukupni isparljivi – azot, tiobarbiturna kiselina i so) 0, 7.,14.,28.,42.,56.,70., 84. i 98. dana skladištenja. U mikrobiološkoj analizi, brojevi mezofilnih aerobnih bakterija, psihofilnih aerobnih bakterija, mezofilnih anaerobnih bakterija, plesni, kvasaca su određivani kao <10 cfu/g u svim grupama tokom skladištenja. Nije bilo signifikantne razlike među grupama u pH, sadržaju vlage, TVB-N i nivou soli ($p>0.05$). TBA vrednost se povećala tokom skladištenja u kontrolnoj grupi. U vezi sa TBA vrednošću, razlika između kontrolne grupe i grupa A i B je bila signifikantna ($p<0.05$). U ispitivanju senzornih karakteristika fileta, A i B proizvodi su imali bolje osobine tokom čitavog perioda skladištenja.

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