Determination of Biogenic Amine Formation and Microbiological Changes in Carp (Cyprinus carpio L., 1758) During Frozen Storage

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Abstract: It has been identified that total number of aerobic mesophilic microorganisms (TAM), total number of aerobic psychrophilic microorganisms (TAP), number of Pseudomonas spp. (PS), number of yeast and molds (Y/M), number of microorganisms in coliform group (CG), number of fecal streptococci (FS), number of microorganisms in Enterobacteriaceae group (EB) and number of microorganisms Lactobacillus-Leuconostoc-Pediococcus group (LB) and values of pH, quantities of total volatile basic nitrogen (TVB-N) have been determined, data of sensory analysis has been evaluated by hedonic scale of whole and gutted specimens of carp (Cyprinus carpio L., 1758) which were whole and gutted frozen kept under -18 ºC at 1st, 15th, 30th, 60th, 90th, 120th days and biogenic amines tryptamine (TRM), β-phenylethylamine (PEA), putrescine (PUT), cadaverine (CAD), histamine (HIM), tyramine (TYM), spermidine (SPD) and spermine (SPM) were assayed by High Performance Liquid Chromatography (HPLC) method. According to the results of the research: In Frozen Whole Fish (FWF) and Frozen Gutted Fish (FGF) samples, psychrophilic microorganisms constitute the dominant microorganism group, the mean pH values of FWF and FGF samples were 6.53±0.12 and 6.63±0.11, and TVB-N values were 13.38±1.39 and 12.87±0.81 respectively, the samples were found to be in “good” quality class in terms of sensory evaluations. It was determined that all biogenic amines remained below the recommended limit values during the whole storage period, according to the changes in chemical and sensory properties, that these fish preserved their consumable properties.

Keywords: Biogenic amines, frozen carp fish, microbiological and chemical properties, sensory properties.

Dondurulmuş Halde Muhafaza Edilen Sazan Balığında (Cyprinus carpio L., 1758) Biyojen Amin Oluşumu ve Mikrobiyolojik Değişimlerin Belirlenmesi

Öz: Bu çalışmada; -18 ºC’de bütün (Dondurulmuş Bütün Balık/DBB) ve temizlenmiş (Dondurulmuş Temiz Balık/DTB) olarak muhafaza edilen sazan balıklarında (Cyprinus carpio L., 1758), muhafazanın 1., 15., 30., 60., 90. ve 120. günlerinde toplam aerobik mezofilik mikroorganizma (TAM), toplam aerobik psikrofilik mikroorganizma (TAP), Pseudomonas spp. (PS), maya/küf (MK), koliform grubu mikroorganizma (KG), fekal streptokok (FS), Enterobacteriaceae grubu mikroorganizma (EB) ve Lactobacillus-Leuconostoc-Pediococcus grubu mikroorganizma (LB) sayıları, pH değerleri, Toluaduç Bazık Azot (TVB-N) miktarları, duyusal analiz verileri ve Yüksek Performanslı Sıvı Kromatografisi (HPLC) ile de biyojen amin (triptamin (TRM), β-fenilettilamin (PEA), putresin (PUT), kadaverin (CAD), histamin (HIM), tyramin (TYM), spermidin (SPD) ve spermin (SPM)) konsantrasyonları belirlenmiştir. Araştırma sonuçlarına göre; DBB ve DTB örneklerinde psikrofilik mikroorganizmaların dominant mikroorganizma grubunu oluşturdukları, DBB ve DTB örneklerinde muhafaza süresi sonunda pH değerlerinin ortalaması sırasıyla 6.53±0.12 ve 6.63±0.11 ve TVB-N değerlerinin de 13.38±1.39 ve 12.87±0.81 arasında değiştiği, örneklerin muhafaza sonunda duyusal değerlendirmeler yönünden “iyi” kalite sınıfında yer aldığı tespit edilmiştir. Biyojen aminlerin tavanının tüm muhafaza süresince önerilen limit değerlerin altında kaldığı, mikrobiyolojik, kimyasal ve duyusal özelliklerdeki değişimlere göre muhafaza süresi sonunda bu balıkların tüketilebilir özellikleri koruduğu tespit edilmiştir.

Anahtar Kelimeler: Biyojen aminler, dondurulmuş sazan balığı, duyusal özellikler, mikrobiyolojik ve kimyasal özellikler.

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1. Introduction

Fish and other seafood, which have a special place among animal foods as they are low-calorie foods besides their contents of high levels of protein, unsaturated fatty acids (eicosapentaenoic acid-EPA, docosahexaenoic acid-DHA, etc.), vitamin (niacin, folic acid, Vitamins A, D, E, K, and B, etc.), and mineral matter (phosphorus, calcium, iodine, selenium, etc.), are invaluable food sources. [1-3]. All over the world, the family “Cyprinidae” has an important place among the fish species produced through hunting or aquaculture; and silver and bighead carp, Indian major carp, grass carp, common carp, goldfish carp, and crucian carp are produced 7 million tons on average and the other Cyprinid species are produced more than 2 millions annually [4].

If fish are not stored under proper conditions after hunting/harvesting, they rapidly spoil because of various hydrolytic enzymes in their structures, natural microflora, and subsequent microbial contaminations [5, 6]. Time and severity of spoilage in fish vary depending on the density and interaction of microbial contamination, fish species and size, fishing and harvesting methods, hunting area, hygiene of the boat and other transport vehicles, processing techniques and storing conditions [7, 8], physical and chemical properties of the water in which fish live, transport and storing temperature and time, water activity of fish meat (aw), high level of pH after death, presence of large amounts of NPN and TMAO, and oxidation/reduction potential [9-11]. Physical methods, chemical parameters, microbiological and sensory criteria are used in determining the quality of fish and other seafood. Biogenic amines, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and trimethylamine (TMA) are the most widely used chemical criteria [12, 13].

Bioactive amines are organic bases with low molecular weight, which form as a result of metabolic activities of plants, animals, and microorganisms [14,15]. These amines occur by decarboxylation of free amino acids, thermal decomposition, hydrolysis of components with nitrogen, and transamination of aldehydes or ketones in fresh and processed foods [16, 17]. Histamine, β-phenylethylamine, tyramine, cadaverine, putrescine, tryptamine, spermidine, spermine, and agmatine are the biogenic amines that are most commonly found in foods and drinks [18, 19]. Biogenic amines, which are used as an indicator for spoilage, have great importance in terms of public health as both they are evaluated as a quality indicator and index of microbial spoilage, and their toxic effects [20, 21].

Frozen fish is described as a product that is deep-frozen at temperatures below -18 °C. The temperatures of these fish are between -18.0 °C and -30.0 °C throughout the storage period in general [22]. The freezing method is one of the preferred methods for extending the shelf life of fish and other seafood, as it prevents the loss of nutritious items and is an easy-to-use method without adding any additives [23]. Determining the maximum time that seafood can be stored in a healthy and quality way and revealing their hygienic quality parameters are very important in terms of ensuring continuity of supply of products that are obtained during hunting and/or harvest seasons for consumption during the periods when hunting is forbidden and/or outside the harvest seasons [24].

This study aimed to determine the changes that occurred in the microbiological, chemical, and sensory quality parameters of frozen carps throughout the storage period and reveal the effects of changes in these parameters on public health and shelf life.

2. Materials and Method

2.1. Supply and preparation of the samples for the analyses

In the study, 100 carps, which were hunted in the Sarımehmet reservoir in Van province of Turkey during a night on a February, were used. The fish were put into polyethylene Styrofoam boxes (2-4 °C) and brought to the laboratory in about 1 hour. The average length and weight of the fish were found to be 32.77±2.49 cm and 416.77±37.70 g, respectively. The fish were divided into two lots. The fish in the first lot (Frozen Whole Fish/FWF) were manually packed in Styrofoam plates with at least 3 pieces on each plate (1.250 kg on average) in sterile conditions and wrapped with stretch film. As for the fish in the second lot (Frozen Gutted Fish/FGF), heads, internal organs, and gills were removed in aseptic conditions and then packed in the same way. The FWF/FGF samples were shocked at -40±1 °C in a deep-freezer (UCF, 10 SF, TR) for 4 hours and then stored in a deep-freezer (UCF, 310 SSL, TR) at -18±1 °C. The FWF/FGF samples were kept in a refrigerator at 4±1 °C for 6-10 hours before the analysis.
was carried out and in this way they thawed. On the 1st, 15th, 30th, 60th, 90th, 120th days of the storage, muscle samples collected from the samples in aseptic conditions were mixed and homogenized, and two-recurrence microbiological and chemical analyses were performed.

2.2. Microbiological analyses

10 g of homogenates were put into sterile stomacher bags, 90 ml of physiological saline water with sterile peptone were added into them, and then they were homogenized in the stomacher (Interscience Bag Mixer 400 P, Germany) for five minutes. The decimal dilutions of the samples up to 10^8 cfu/ml were prepared and microbiological inoculations were performed. The petri plates inoculated on the same medium were left for incubation in aerobic conditions at 37 °C on the Plate Count Agar (Oxoid® CM463) for 24-48 hours for total aerobic mesophile (TAM) microorganisms count, and at 7 °C for 7-10 days for total aerobic psychrophile microorganisms (TAP) count. The petri plates that were inoculated on the Pseudomonas Agar (Oxoid® CM559+SR103) for the count of Pseudomonas spp. (PS) were left for incubation at 25 °C for 48-72 hours and the colonies that yielded a positive result in the oxidase (Oxidase Identification Sticks, Oxoid® BR64) test were counted [25]. In the yeast/mold (Y/M) count, the petri plates that were inoculated on the Potato Dextrose Agar (Oxoid® CM 139B) medium were left for incubation at 20-25 °C for 5-7 days [26]. Violet Red Bile Agar (Oxoid® CM107) was used for the coliform group microorganisms (CG) count and the petri plates were left for incubation at 37±1 °C for 24-48 hours [25, 27]. The inoculation was carried out on the Slanetz&Bartley Medium (Oxoid® CM377) for the count of fecal streptococci (FS) and the petri plates were left for incubation firstly at 35 °C for 4 hours, and then at 44-45 °C for 44 hours. The inoculation was performed on the Violet Red Bile Glucose Agar (Oxoid® CM485) for the count of Enterobacteriaceae (EB) and the petri plates were left for incubation at 30±1 °C for 24 hours. For the count of lactobacilli (LB), the petri plates that were inoculated on Rogosa Agar (Oxoid® CM0627) in double layers at 35±1 °C for 72 hours [25]. The colonies occurred after the incubation was counted.

2.3. Chemical analyses

2.3.1. Determination of the pH

10 g of homogenate prepared for the pH analysis were diluted with 1:1 distilled water and the probe of the pH-meter (Hanna® PH890) was submerged into this mixture and pH value was determined 45 sec later [28].

2.3.2. Determination of the amount of TVB-N

It was carried out according to the method modified by Antonacpoulos [13]. In this method, 50 g of fish meat was homogenized, 10 g of it were put in a balloon, 2-3 drops of silicone antifoam, 1 g of magnesium oxide (MgO), and 100 ml of distilled water were added on it. The tubes were placed into the heater caps of a water vapour distillation device (Kjeldahl), which had been run and brought to the appropriate temperature setting before. 100 ml of distilled water, 10 ml of 3% boric acid (H₃BO₃), and 7-8 drops of Tashiro indicator (0.5 g of methylene red (Merck 1.06076, Germany)+250 ml 95% ethyl alcohol) were added to the distillation output in a 500 ml Erlenmeyer flask. The distillation was terminated when a 200 ml of distillate was formed. The distillate collected in an Erlenmeyer flask was titrated with a 0.1 N hydrochloric acid (HCl) to the point where the current color turned to a pinkish-dull light color. The amount of TVB-N occurred as a result of the distillation was calculated as follows: The amount of TVB-N (mg) = the amount of spent 0.1 N HCl (ml) ×1.4×100/The amount of sample (g).

2.3.3. Determination of the number of biogenic amines

2.3.3.1. Extraction of the samples

2g of the homogenate prepared for the analyses were put into a plastic Falcon tube, 125 µl internal standard (1.7 Diamino heptane, Merck, Germany) and 10 ml of perchloric acid (70% HClO₄, Merck,
Germany) solutions were added, and then the mixture was homogenized in a cooled alcohol-ice bath in an ultra turrax homogenizer (IKA® Yellowline DI Basic, USA) for 3 minutes. This homogenate was centrifuged at 3000 rpm, 4 °C for 10 minutes (Sigma® 3-30K, Germany), and the clear part on the top was filtered into a 25 ml volumetric flask with a Whatman (Filter lab® 1240 90 mm) filter paper. 10 ml of perchloric acid were again added into the plastic Falcon tube, mixed in a vortex (Heidolph Reax Top D-91126, USA) for 1 min., centrifuged in the same way before, and its clear part was filtered into the same volumetric flask in the same way. The filtrate was completed to 25 ml with the perchloric acid solution [29].

2.3.3.2. Derivatization of amine standards and samples

The amine standards that were used, namely tryptamine hydrochloride (93650), β-phenylethylamine hydrochloride (77905), putrescine dihydrochloride (P7505), cadaverine dihydrochloride (33220), histamine dihydrochloride (53300), tyramine hydrochloride (93820), spermidine trihydrochloride (85580), and spermine tetrahydrochloride (85607), were obtained from the Sigma-Aldrich (Germany) company. Each biogenic amine standard was weighed in a 25 ml volumetric flask with a precision scale (ANDGR 200, Japan), and completed to 25 ml by dissolving with ultra-pure water. 100 ppm and 10 ppm dilutions were prepared from this main stock. 1 ml samples were taken from the amine standard dilutions and each one of the extracted samples into 5 ml amber volumetric flasks, then 200 µl 2 N NaOH (Merck, Germany), 300 µl saturated NaHCO₃ (Merck, Germany) and 2 ml dansyl chloride (5-dimethylamino naphthalene-1-sulfonyl chloride, D2625-5G, Sigma-Aldrich, UK) (10 mg dansyl chloride/1 ml acetone (Merck, Germany)) were added, and after that the mixture was stirred in a vortex for 20 sec. The mixtures were incubated in an oven (Nüve® EN 120, TR) at 40 °C for 45 minutes, the incubation temperatures of the tubes were lowered to room temperature by keeping them in a dark medium for 15 minutes, and 100 µl of ammonium (25% NH₃, Merck, Germany) were added on it at the end of this period, and kept in a dark medium at room temperature for 30 minutes after being stirred in a vortex for 20 seconds. The total volume was completed to 5 ml by adding the mixture of ammonium acetate: acetonitrile (Merck, Germany) (v:v, 1:1), the samples were centrifuged at 2500 rpm at 4 °C for 5 minutes after they were stirred in a vortex, filtered through a 0.45 µm syringe filter, placed into 2 ml amber vials (LC/MS Certified Amber Glass 12 x 32mm Screw Neck Vial, 2 ml, Waters), and made ready for HPLC analysis [29].

<table>
<thead>
<tr>
<th>Amin Standards</th>
<th>RSD%</th>
<th>CV%</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptamine</td>
<td>6.52</td>
<td>1.24</td>
<td>0.025</td>
<td>0.311</td>
</tr>
<tr>
<td>β-phenylethylamine</td>
<td>9.02</td>
<td>2.40</td>
<td>0.038</td>
<td>0.014</td>
</tr>
<tr>
<td>Putrescine</td>
<td>1.35</td>
<td>1.97</td>
<td>0.028</td>
<td>0.086</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>2.73</td>
<td>0.96</td>
<td>0.039</td>
<td>0.117</td>
</tr>
<tr>
<td>Histamine</td>
<td>2.72</td>
<td>1.56</td>
<td>0.033</td>
<td>0.101</td>
</tr>
<tr>
<td>Tyramine</td>
<td>6.41</td>
<td>1.27</td>
<td>0.309</td>
<td>0.937</td>
</tr>
<tr>
<td>Spermidine</td>
<td>5.13</td>
<td>0.79</td>
<td>0.114</td>
<td>0.436</td>
</tr>
<tr>
<td>Spermine</td>
<td>3.24</td>
<td>1.80</td>
<td>0.109</td>
<td>0.331</td>
</tr>
</tbody>
</table>

RSD%: Relative Standard Deviation, CV%: Coefficient of Variation, LOD: Limit of Detection, LOQ: Limit of Quantification.
2.3.3.3. Configuration of the HPLC Device

Pump: Shimadzu LC-20 AT series, 2 pieces, Degasser Unit: Shimadzu DGU-20A 5, Auto Sampler: Shimadzu SIL20AC (cooled, 4 °C), Detector: Shimadzu SPD-M20A, DAD detector, Column Oven: Shimadzu CTO-10AS VP (heated), System Control: Shimadzu CBM-20 ALITE, Japan. Device Software: LC solution 1.12 SP1. The amount of injection: 20 μl, Flow rate: 0.8 ml/min, Column type: C 18 (Inertsil® ODS-2.5 μm, 4.6 x 150 mm, Japan), Detector type: DAD, Column oven temperature: 40 °C, Carrier phase A: 0.1 mole ammonium acetate, Carrier phase B: Acetonitrile (50%-90% 16th min, 16-22nd min 90%, 22-30th min 50% post-run), Wavelength: 254 nm.

2.4. Sensory analyses

They were conducted by a group of 6 expert panelists according to the scoring system that was developed by Paulus et al. [30]. Throughout the study, much attention was paid to the selection of these panelists from the same people. The “color, smell, taste, and overall acceptability” criteria of the samples were evaluated according to the hedonic scale (9.00-7.00 points “very good”, 6.90-4.10 points “good”, 4.00 “can be consumed” and 3.90-1.00 points “spoiled”).

2.5. Statistical analyses

Descriptive statistics for the continuous variables out of the characteristics discussed were stated as mean and standard deviation. Kruskal-Wallis test was used for the comparisons of the groups in terms of continuous variables. Tukey multiple comparison tests were used to determine different groups. Pearson correlation coefficients were calculated separately in each group for determining the relationship between the characteristics. The statistical significance level was set at 5% in the calculations and SPSS (version 23, Windows; Inc., Chicago, IL) software was used for the calculations [31].

3. Results

The results of the microbiological counts (log cfu/g), chemical parameters, sensory analyses, and biogenic amine levels (mg/kg–ppm/ wet weight) of the FWF and FGF samples taken in the stated days were given in Table 2.
Table 2. Changes in parameters analyzed during storage whole and gutted fish stored at -18 °C.

<table>
<thead>
<tr>
<th>Parameters examined</th>
<th>Storage Time (day)</th>
<th>Application form</th>
<th>Number of microorganisms (Logia Chlg)</th>
<th>Statistical Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>2nd day</td>
<td>3rd day</td>
<td>4th day</td>
</tr>
<tr>
<td></td>
<td>WF</td>
<td>GF</td>
<td>WF</td>
<td>GF</td>
</tr>
<tr>
<td>Total Aerobic Mesophile (TAM)</td>
<td>5.19±0.02 A</td>
<td>5.06±0.09 A</td>
<td>4.99±0.02 A</td>
<td>5.53±0.18 A</td>
</tr>
<tr>
<td>Total Aerobic Psychrophile (TAP)</td>
<td>2.55±0.04 A</td>
<td>2.75±0.01 A</td>
<td>2.94±0.07 A</td>
<td>3.25±0.14 A</td>
</tr>
<tr>
<td>Pseudomonas (PS)</td>
<td>1.84±0.01 A</td>
<td>2.26±0.14 A</td>
<td>2.80±0.12 A</td>
<td>3.60±0.18 A</td>
</tr>
<tr>
<td>Yeast/Mold (YM)</td>
<td>2.42±0.01 B</td>
<td>2.33±0.01 B</td>
<td>2.35±0.01 B</td>
<td>2.44±0.01 B</td>
</tr>
<tr>
<td>Coliform group (CG)</td>
<td>2.48±0.01 A</td>
<td>2.48±0.01 A</td>
<td>2.48±0.01 A</td>
<td>2.48±0.01 A</td>
</tr>
<tr>
<td>Pece Staphylococci (PS)</td>
<td>3.36±0.18 A</td>
<td>3.36±0.18 A</td>
<td>3.36±0.18 A</td>
<td>3.36±0.18 A</td>
</tr>
<tr>
<td>Enterobacteriaceae (EB)</td>
<td>1.50±0.01 B</td>
<td>1.50±0.01 B</td>
<td>1.50±0.01 B</td>
<td>1.50±0.01 B</td>
</tr>
<tr>
<td>Lactobacilli (LB)</td>
<td>1.86±0.13 A</td>
<td>1.86±0.13 A</td>
<td>1.86±0.13 A</td>
<td>1.86±0.13 A</td>
</tr>
<tr>
<td>PH</td>
<td>6.67±0.05 A</td>
<td>6.67±0.05 A</td>
<td>6.67±0.05 A</td>
<td>6.67±0.05 A</td>
</tr>
<tr>
<td>TVB-N (mg/100 g)</td>
<td>11.48±0.29 A</td>
<td>11.48±0.29 A</td>
<td>11.48±0.29 A</td>
<td>11.48±0.29 A</td>
</tr>
<tr>
<td>Parameters examined</td>
<td>Biogenic Amines (mg/kg ppm) (wet weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Application form</td>
<td>1st day</td>
<td>2nd day</td>
<td>3rd day</td>
</tr>
<tr>
<td></td>
<td>WF</td>
<td>1.04±0.04b</td>
<td>1.13±0.04b</td>
<td>1.22±0.08b</td>
</tr>
<tr>
<td></td>
<td>GF</td>
<td>1.04±0.04b</td>
<td>1.13±0.04b</td>
<td>1.22±0.08b</td>
</tr>
</tbody>
</table>

**Table 2. (Cont.)**

<table>
<thead>
<tr>
<th>Statistical Values</th>
<th>Mean±SD</th>
<th>Max</th>
<th>Min</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Putrescine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
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<td>Histamine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
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<tr>
<td>Tyramine</td>
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<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Spermidine</td>
<td>1.13±0.04</td>
<td>1.22±0.08</td>
<td>1.04±0.04</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*SD: Standard Deviation, ND: Not Detected, (C.O.D): Whole Fish, GF: Gutted Fish.

*Different from the GF group, statistically significant (P<0.05).

A, B, C, D, E: — Different upper cases in the same row represent statistically significant differences between storage days (P<0.05).
4. Discussion and Conclusion

The freezing process, to be applied for protecting the appearance and texture, smell and taste, and extending the storage period of fish that are hunted/harvested freshly and are not subjected to any technological process provides advantages to consumers [32]. In the food safety of seafood, it is known that aquatic environment and relevant processes during hunting/harvesting and various production phases have a very close relationship with the hygienic quality of products [12].

The total numbers of aerobic mesophile microorganisms (TAM) in the FWF and FGF samples were found to decrease in the following days of the storage and increase again in the later days, and finally, on the last day of the storage they were found to be 2.12±0.31 and 2.51±0.04 log10 cfu/g, respectively (Table 2). Similar findings were reached in the studies conducted with ungutted silver carp (Hypophthalmichthys molitrix) [33], Nile tilapia fish (Oreochromis niloticus) [34], and tilapia fish (Oreochromis mossambicus) [35]. These researchers reported that the TAM numbers in the samples they examined decreased during the early days of the storage, and this might be because of the cold shock effect, and an increase has been observed in the following days of storage. The cold shock effect, which is caused by the freezing process, may bring about decreases in TAM number during the early periods of storage [36]. It was observed that the TAM numbers in the FWF and FGF samples have not exceeded 7 log10 cfu/g, which is the limit according to the Regulation on Seafood [37]. A negative correlation (0.845) was detected between the amount of TAM and putrescine in the FGF samples (p<0.01). The TAM numbers of the FGF samples detected in this study were found to be higher than those of the FWF samples. When a fish is gutted, its physical integrity is lost; and microorganism load may increase in the edible muscular parts because of the contaminations that occur in the gills and that are especially caused by internal organs during the gutting processes [38].

The total numbers of aerobic psychrophile microorganisms (TAP) in the FWF and FGF samples fluctuated throughout the storage period, decreased at the end of the storage and were found to be 5.20±0.23 and 5.11±0.24 log10 cfu/g, respectively (Table 2). Similar to the findings of this research, the studies on whole pike-perch (Sander Lucioperca) [39] and fish burgers from deep flounder (Pseudorhabdus livingstonii) and brushtooth lizardfish (Saurida undosquama) [40] reported that psychrotrophic microorganisms constituted the dominant group of microorganisms and decreased towards the end of the storage. Different findings were detected in different carp fish samples (Cyprinus carpio) stored at -18 ºC for 90 days, and it was reported that the number of psychrotroph microorganisms increased during the storage period and this increase might be because of the contamination, which occurred during all of the preparation processes beginning from hunting, and storage temperature and time [41].

The numbers of Pseudomonas spp. (PS) increased in the FWF and FGF samples throughout the storage, and they were found to be 3.33±0.02 and 3.26±0.10 log10 cfu/g, respectively, at the end of the storage (Table 2). Popelka et al. [32] found that the number of PS increased throughout the storage and was 2.85±0.57 log10 cfu/g on average at the end of the storage in the whole trout and trout fillets (Oncorhynchus mykiss) samples, which were stored at -18 ºC for 180 days. They also reported that the number of PS remained at a lower level in the freezing method. Hasan et al. [42] detected the number of PS in silver carp (Hypophthalmichthys molitrix) fillets to be 5.90 log10 cfu/g. Ehsani and Jasour [39] reported that they could not detect any PS at the outset of the storage in the whole pike-perch (Sander Lucioperca) samples, but they reported that it was 1.89 log10 cfu/g at the end of the storage. The increase in the pseudomonas species towards the end of the storage can be attributed to the better competing of waterborne pseudomonas species, which include obligate psychrophilic species, with other species and their ability to reproduce in cold environments [43]. It is considered that the different results on the number of PS in this study and other studies maybe because of the microbial load of the fish in the water that they hunt or differences in the temperature and duration of the storage.

It was observed that the numbers of yeast/mold (Y/M) in the FWF and FGF samples at the beginning of the storage were 1.84±0.08 and 2.16±0.06 log10 cfu/g, respectively, they increased throughout the storage and there occurred a decrease in the FGF samples on the 120th day of the storage (Table 2). Unlike the results of this research, Khanipour et al. [44] reported that Y/M was not detected in the breaded kilka (Clupeonella cultriventris) samples stored at -18 ºC for 120 days throughout the whole storage period. Javadian et al. [45] reported that they detected the number of Y/M in the whole rainbow trout (Oncorhynchus mykiss) samples, which were stored at -18 ºC for 90 days, to be 4.62 log10
cfu/g at the beginning of the storage, but there occurred a decrease after a 30-days storage period and this decrease might be because of the cold shock effect. While a positive correlation (0.671) was found between the number of Y/M and putrescine in the FGF samples (p<0.05), a negative correlation (0.583) was detected between the number of Y/M and spermidine (p<0.05).

The numbers of coliform group (CG) microorganisms in the FWF and FGF samples were found to be <1 log_{10} cfu/g on the 1st day of the storage and no changes were detected in the number of CG until the 120th day of the storage (Table 2). According to the Regulation on Seafood [37], the legal limits for the number of CG microorganisms in frozen fish were set to be 160/g minimum and 210/g maximum. It was observed that the numbers of CG microorganisms in the examined samples were below these limits.

Enterococci are commonly found in nature and have a high potential to contaminate food. Therefore, they should be evaluated together with total aerobic mesophilic microorganisms and coliform group microorganisms rather than alone as an indicator of fecal contamination in foods [46]. The numbers of fecal streptococci (FS) in the FWF and FGF samples were found to be <1 log_{10} cfu/g on the 1st day of the storage and no changes could be detected in the number of FS until the 120th day of the storage (Table 2). Bhosale and Patange [47] similarly reported that they could not detect any FS in the Catla catla (Bloch) samples throughout the storage period.

The number of Enterobacteriaceae (EB) can often be evaluated as a quality index during the processes of the gutting of internal organs, washing, and cooling of fish with the help of ice [48, 49]. While any significant difference was not observed between the numbers of EB in the FWF samples at the beginning and end of the storage, a decrease of about 0.60 log_{10} cfu/g was detected in the FGF samples (Table 2). Unlike the results of this research, Cai et al. [50] reported that the number of EB in the Japanese sea bass (Lateolabrax japonicas) fillets, which were stored at -18 ºC for 90 days, was found to be 1.33 log_{10} cfu/g at the beginning of the storage and the numbers of EB were low throughout the storage period. The researchers stated that this was an expected situation, which could be explained by the fact that these microorganisms tended to develop slowly at low temperatures and were not of competitive flora. Significant fluctuations (p<0.05) were observed in the number of EB in the FWF and FGF samples. In the FWF samples, a significant positive correlation (0.849) between the number of EB and putrescine (p<0.01), a negative correlation (0.657) between the number of EB and tryptamine (p<0.05), and a significant negative correlation (0.733) between the number of EB and β-phenylethylamine were detected (p<0.01). In the FGF samples, significant negative correlations (p<0.05) were detected between the number of EB and β-phenylethylamine (0.665), tyramine (0.677), and spermine (0.635).

It was observed that the numbers of Lactobacillus-Leuconostoc-Pediococcus group microorganisms (LB) fluctuated in both two samples throughout the 120 days of storage, and they were found to be <1 log_{10} cfu/g in the FWF samples and 1.25±0.07 log_{10} cfu/g in the FGF samples at the end of the storage period (Table 2). It was observed that the fluctuations in the number of LB in the samples in both two groups were statistically significant (p<0.05), and it is thought that these fluctuations may be because the LB species in the samples are different or each LB species behaves differently against the cold shock effect. Some lactobacillus species are reported to be able to maintain their lives in freezing processes with their cold shock proteins [51].

In the studies conducted to determine the shelf life in different carp species, the low pH values detected in the samples at the beginning of the storage might be due to the amount of inorganic phosphate, occurred by degradation of ATP in muscle tissue or accumulation of lactic acid, occurred as a result of anaerobic glycolysis [52, 53]. While the pH value varies between 6.0-6.5 in fresh fish [54], the changes in pH that are observed throughout the storage are reported to depend on the characteristics of fish (such as species, size, muscle type, way of nutrition, hunger/satiety, pre-death stress) and environmental conditions (such as hunting/harvesting season, geographical location of the water where hunting is performed, composition of the water, storage conditions) [50, 55, 56]. In this study, it was observed that at the end of the storage period the pH values in the FWF and FGF samples were on average 6.53±0.12 and 6.63±0.11, respectively, and remained below 6.80-7.00, reported as the limit for consumption [13].

Increases in the amount of TVB-N, which is one of the chemical methods used in determining the freshness of fish and fishery products, occur mainly as a result of enzymatic and microbial activities such as deamination of free amino acids, degradation of nucleotides, and oxidation of amino acids [57,
In the FWF and FGF samples, it was detected that the TVB-N values increased throughout the storage period and were 13.38±1.39 and 12.87±0.81 on average at the end of the storage. The TVB-N values detected this study were found to be similar to the findings detected in the silver carp (Hypophthalmichthys molitrix) mince [59], while they were found to be higher than the values found in the whole trout and trout fillets (Oncorhynchus mykiss) samples [32].

Organoleptic properties are seen as an important parameter in assessing and a better understanding of shelf life and freshness in fish by consumers [60]. The samples were included in the "good" quality class in terms of sensory evaluations throughout the 120 days of storage. Tokur et al. [61] detected decreases in the sensory analysis (color, smell, taste, and overall acceptability) scores of the mirror carp (Cyprinus carpio) fillets, which were stored at -18 °C for 150 days, throughout the storage period, and reported that acceptability in fish and fishery products that are stored by freezing should be based on sensory evaluations. In the FWF and FGF samples, which were examined in parallel with the findings of these researchers, slow but statistically significant (p<0.05) decreases were observed in the sensory analysis scores between 9.00±0.00 and 5.16±0.36 throughout the storage period.

The harmful effects of biogenic amines in terms of health are observed when they are taken with foods in great quantities or when deficiencies occur in the detoxification system of people who receive excess biogenic amines.

In the samples of both two groups examined in the study, it was found that tryptamine, β-phenylethylamine, cadaverine, tyramine, spermidine, and spermine levels except for putrescine level fluctuated throughout the storage process, and the difference between the analysis periods was statistically significant (p<0.05). The amounts of putrescine and cadaverine were found to be higher in the FWF samples than those in the FGF samples. It was found that the amount of histamine in the FGF samples examined throughout the storage was below the limit of detection, while it could be detected only on the 30th and 60th days of the storage in the FWF samples, and its highest level was 0.87 ppm throughout the whole storage period (Table 2). Ehsani and Jasour [33] reported that the amount of histamine in the ungutted silver carp (Hypophthalmichthys molitrix) was 27 ppm at the end of the storage, and Ekici et al. [51] reported that no histamine could be detected in the pearl mullet (Chalcalburnus tarichi) samples throughout the storage period. Spermidine and spermine were the amines detected at the highest amount in both two ways of application (Table 2). Bardocz [62] states that spermidine and spermine are the amines that are present in the natural structures of live cells. Yu et al. [63] reported that spermidine and spermine are polyamines that are naturally found in fish muscle and their amounts fluctuated mainly towards decreasing throughout the storage. Mohan et al. [64] reported that biogenic amine levels in fish might vary depending on the species, amount of free amino acid in muscles, contents of the digestive system at the time of death, and harvest season. General condition of a fish at the time of capture, hygienic conditions throughout the processes during hunting/harvesting and the storage, hygienic conditions of vehicles such as boat-vessel and level of their cooling systems, capacity of cooling units and hygienic conditions of transport systems may affect the occurrence of different biogenic amines in various fish species at different levels [33, 65]. The biogenic amines that were analyzed throughout the whole storage period were found to be much below the limit values permitted in terms of consumption. The biogenic amine concentrations determined in the samples in the study are lower than the findings in the bighead carp (Aristichthys nobilis) fish obtained by Hong et al. [66] and whole pike-perch (Sander Lucioperca) fish obtained by Ehsani and Jasour [39]. It is considered that these different results maybe because of the differences in the fish species, preparation of the samples, and storage times.

Regarding the findings on the samples that were stored by freezing, it was found that the TAP microorganisms constituted the dominant flora, the limit values on the microbiological quality permitted in terms of TAM microorganism numbers were not exceeded throughout the whole storage and the samples preserved their good quality properties. It was detected that fluctuations in the analyzed biogenic amine concentrations were observed throughout the storage period; however, the values detected in the samples were below the limit values suggested by the researchers. Although there is no significant difference between keeping the fish samples whole or gutted based on the average microbiological values, it was found that keeping carps whole is safer than keeping them gutted. Eating habits of people change in time and fish and other seafood consumption is increasing and they are taking place in diets more especially in developed countries. Fish and other seafood, a delicious and healthy food source, are among the foods that can easily spoil due to their nutritious compositions. Therefore,
to prevent the adverse effects of fish and other seafood on human health, risk factors arising from the products in this food group, their quality parameters, and legal limits should be determined; control activities, and protective and preventive measures to have complied with these parameters should be taken. In fish and seafood industry, protective and preventive measures to be taken from the time of harvest and/or hunting, control activities, implementing hygienic rules strictly, and revealing deviations from the detected quality parameters and limit values will make very significant contributions in terms of public health.

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References


