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Determination of Biogenic Amine Formation, Microbiological and Sensory Changes in Carp (*Cyprinus carpio* L., 1758) Stored at Cold (4 °C)

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ABSTRACT In this research; it was aimed to determine the shelf life of whole (Cold Whole Fish/CWF) and gutted (Cold Gutted Fish/CGF) carp samples (*Cyprinus carpio* L., 1758) stored at 4 °C for 14 days by analyzing microbial load, chemical parameters, and sensory analysis scores and concentrations of biogenic amines. 0, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th on days of storage microbiological analyzes, chemical, sensory analysis evaluation, and biogenic amines levels were detected by using High Performance Liquid Chromatography (HPLC). According to the research results; it was determined that Total Aerobic Psychrophilic Microorganism (TAP) and *Pseudomonas* spp. formed the dominant microflora during storage in CWF and CGF samples the acceptable limits were not exceeded until the 10th day in terms of TVB-N values, and according to the sensory analysis evaluations, carp samples were not consumable after the 6th day. In both application forms of the carp samples stored in the cold, putrescine and cadaverine were dominant biogenic amines. It has been determined that keeping the carp whole is safer than keeping it after gutted, based on the microbiological values of the 8th day when the samples were rejected sensory. In addition, it is thought that putrescine and cadaverine can be evaluated as indicator biogenic amines in determining the freshness of carp fish.

Keywords: *Biogenic amines, Carp, Chromatography, Microbiology, Storage.*

ÖZ

Soğukta (4 °C) Muhafaza Edilen Sazan Balıklarında (*Cyprinus carpio* L., 1758) Biyojenik Amin Oluşumu, Mikrobiyolojik ve Duyusal Değişikliklerin Belirlenmesi

Bu araştırmada; 4 °C'de 14 gün süreyle muhafaza edilen bütün (Soğukta Bütün Balık/SBB) ve temizlenmiş (Soğukta Temiz Balık/STB) sazan balığı örneklerinin (*Cyprinus carpio* L., 1758) mikrobiyal yükü, kimyasal parametreleri, duyusal analiz puanları ve biyojen amin konsantrasyonları saptanarak raf ömürlerinin belirlenmesi hedeflenmiştir. Depolamanın 0., 2., 4., 6., 8., 10., 12., ve 14. günlerinde sazan örneklerinin mikrobiyolojik, kimyasal ve duyusal analiz değerlendirmeleri ile örneklerin yüksek basınçlı sıvı kromatografisiyle (HPLC) biyojen amin düzeyleri saptanmıştır. Araştırmanın sonuçlarına göre; SBB ve STB örneklerinde muhafaza süresince Toplam Aerobik Psikrofilik Mikroorganizma (TAP) ve *Pseudomonas* spp.'lerin hakim mikroflorayı oluşturdukları, TVB-N yönünden 10. güne kadar kabul edilebilir değerlerin aşılmadığı ve duyusal analiz değerlendirmelerine göre ise 6. günden sonra tüketilebilir nitelikte olmadıkları tespit edilmiştir. Soğukta muhafaza edilen örneklerin her iki uygulama şeklinde de putresin ve kadaverinin dominant biyojen aminler olduğu belirlenmiştir. Örneklerin duyusal olarak reddedildiği 8. gündeki mikrobiyolojik değerler baz alındığında sazan balıklarının bütün halde muhafaza edilmesinin temizlenerek muhafaza edilmesinden daha güvenli olduğu saptanmıştır. Ayrıca, sazan balıklarında tazeliğin belirlenmesinde putresin ve kadaverinin indikatör biyojen aminler olarak değerlendirilebileceği düşünülmektedir.

Anahtar Kelimeler: *Biyojenik aminler, Kromatografi, Mikrobiyoloji, Muhafaza, Sazan.*

INTRODUCTION

Decarboxylation of amino acids in foods is carried out by naturally occurring enzymes (endogenous decarboxylase enzymes) or enzymes produced by microorganisms (exogenous decarboxylase enzymes) (Müller et al. 2022). Formation of biogenic amines (BAs) with decarboxylation of amino acids is achieved by the removal of the α-carboxyl group in the structure of the related amino acid. When this process is carried out by microbial decarboxylases, they are called as biogenic amines (Ozogul and Ozogul 2019). In addition, BAs can also be formed as a result of reductive amination and transamination of aldehydes and ketones, or as a result of body tissue activity (Ozogul and Ozogul

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2019; Martuscelli et al. 2021). In addition to the presence and amount of free amino acids and the presence of microorganisms with decarboxylase activity factors such as presence of fermentable carbohydrates and co-enzymes, presence of vitamins, water activity (a_w), presence of carbon sources, oxygen density, pH and temperature play a role in the production of BAs in foods (Buňka et al. 2012; Sivamaruthi et al. 2021; Vasconcelos et al. 2021; Wójcik et al. 2021). BAs most commonly found in foods and beverages include histamine (HIS), β-phenylethylamine (PAE), tyramine (TYM), cadaverine (CAD), putrescine (PUT), tryptamine (TRM), spermidine (SPD), spermine (SPM) and agmatine (AGM) (Vasconcelos et al. 2021). In addition to these, it has been reported that octopamine and dopamine can be found in fish in addition to meat and some meat products (Abuhlega and Ali 2022). Since BAs are thermostable, they are difficult to be removed once formed (Müller et al. 2022). BAs, which are enzymatic degradation products, have special importance in terms of public health due to their toxic effects as well as being a quality indicator and having microbial deterioration index, especially in fish and fish products (Ruiz-Capillas ve Herrero 2019; Ucar et al. 2021; Wójcik et al. 2021). In fish, spoilage begins in a short time due to reasons such as loose connective tissue, intense enzymatic activity, high pH, water content and storage conditions (Ruiz-Capillas and Herrero 2019; Abuhlega and Ali 2022). The most important factors in degradation are postmortem enzymatic autolysis, natural microflora, microbial contaminations and oxidation of lipids (Speranza et al. 2021; Müller et al. 2022). The microflora of the skin, gills, and intestinal contents, as well as the water itself, are responsible for primary contamination. Physical contaminants such as hunting/harvesting, processing, transport and sales/marketing can cause secondary contamination. Both situations can cause deterioration and food poisoning occurs as a result of consuming these products (Sheng and Wang 2021; Speranza et al. 2021). Basically, four criteria are used to evaluate and monitor the quality of fish and these are physical, sensory, chemical and microbial qualities. Sensory features mainly include the fish's appearance (eyes, skin and gills), texture, smell and color (Wu et al. 2021). It is quite common to use parameters such as pH, Trimethylamine/Trimethylaminoxide (TMA/TMAO), Total Volatile Basic Nitrogen (TVB-N), Thiobarbituric acid (TBA), BAs, sensory analyzes and microbiological analyzes (*Enterobacteriaceae*, *Pseudomonas* spp., lactic acid bacteria, yeast etc.) (Ozogul 2009; Tavares et al. 2021). Also adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inosine monophosphate (IMP), inosine, hypoxanthine formation and K value, free fatty acids, peroxide value and total proteolytic activity, BAs are used in the evaluation of spoilage in fish and fish products (Ocano-Higuera et al. 2011; Tavares et al. 2021; Wu et al. 2021). Toxic substances such as BAs are formed with deterioration which can be harmful to human health. Determination of the presence and concentration of BAs provides important benefits in determining the toxicological risks for public health as well as demonstrating the hygiene quality of fish and processed fish products (Wójcik et al. 2021). For maintaining the freshness of the fish and extending the shelf life, many methods such as smoking, marinating, cold and freezing are applied to inhibit autolytic enzyme activity and microorganism growth (Ochrem et al. 2014).

In this study, it was aimed to determine the shelf life of carp fish storaged in the cold as whole and cleaned. For this purpose, changes in microbiological, chemical, sensory parameters and BAs levels were determined in carp stored in the cold.

MATERIAL AND METHODS

Van Yuzuncu Yil University Animal Researches Local Ethic Committee decided that the study "does not need Animal Researches Local Ethic Committee approval" in the approval certificate dated 28/09/2017 and numbered 2017/09.

Supply of Samples and Preparation for Analysis

The samples (N:165) used in the study were selected according to their lengths (32.77±2.49 cm) and weights (416.77±37.70 g) from a total of 300 fish (Sarımehmet Dam Lake/VAN) caught at one time by Van Lake Fisheries Enterprise in February/2011.

The samples were brought to the laboratory in a short time (~1 h) in frosted polyethylene styrofoam boxes and the preparation stage was started without waiting. While fish in the first lot are packaged as a whole (Cold Whole Fish/CWF); fish in the second lot were washed with cold water in previously sterilized buckets, which are used as drinking water, after removing the heads, internal organs and gills under aseptic conditions using sterile gloves, scalpel and scissors (Cold Gutted Fish/CGF). Then they were manually packed in sterile styrofoam plates with at least 3 fish (~1.250 kg) on each plate and wrapped with cling film after being filtered for 15 minutes in sterile strainers. Except for the initial analysis (day 0), samples were kept in a refrigerator (Arcelik, 8825 SBS NY, TR) at 4±1 °C until the day of analysis. On the 0, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, and 14^{th} days of storage, muscle tissues (dorsal and lateral) were taken from the samples under aseptic conditions, and microbiological and chemical analyzes were performed with two replications from the prepared homogenates.

Microbiological Analyses

Decimal dilutions (10^{1-8} cfu/ml) of the samples homogenized (5 min) in the stomacher (Interscience Bag Mixer 400 P, Germany) as 10 g homogenate + 90 ml sterile PTS (physiological saline with peptones) in sterile stomacher bags were prepared and microbiological analyzes were carried out after inoculation of relevant medium (Table 1).

Chemical Analyses

For pH analysis, homogenate (10 g) of each sample was first diluted with distilled water (*1:1*) and then measured with the help of a pH-meter (Hanna® PH890, USA) (Manthey et al. 1988). In order to determine the amount of Total Volatile Basic Nitrogen (TVB-N), 10 g were taken from the homogenized samples, transferred to Kjeldahl tubes, and silicon defoamer (2-3 drops) +1 g magnesium oxide (MgO, Merck, Germany) +100 ml distilled water was added. Distillation was carried out by placing the tubes in the Kjeldahl distillation apparatus (Elektromag-MX 425, Turkey). A flask containing 100 ml of distilled water and 10 ml of 3% boric acid (H_3BO_3, Merck, Germany) and a Tashiro's indicator (7-8 drops, 0.5 g Methyl Red (Merck 1.06076, Germany) +250 ml ethanol (95%, Merck, Germany)) is placed at the distillation outlet. Approximately 200 ml of distillate was collected. The collected distillate was titrated with 0.1 N hydrochloric acid (37% HCl, Merck, Germany) until an opaque color was obtained. TVB-N level in the samples (TVB-N (mg 100 g^{-1})=0.1 N HCl used in the titration (ml)x1.4x100/Sample amount (g)) was calculated (Varlık et al. 1993).

Biogenic Amines Analyses

BAs analysis was performed according to the method of Eerola et al. (1993), and consists of the stages of sample extraction, derivatization and injection into High Performance Liquid Chromatography (HPLC) device.

a- Derivatization of amine standards:

The amine standards (tryptamine hydrochloride-93650, β-phenylethylamine-77905, putrescine dihydrochloride-P7505, cadaverine dihydrochloride-33220, histamine dihydrochloride-53300, tyramine hydrochloride-93820, spermidine trihydrochloride-85580 and spermine tetrahydrochloride-85607, Sigma, UK), which are going to be used in this study, were weighed in a volumetric flask with a precision balance (ANDGR 200, Japan) and master stock solutions (1000 ppm) were prepared by making up to 25 ml with ultrapure water.

Table 1. Media and incubation conditions for microbiological analysis.

Microorganism	Medium	Supplement	Incubation			References
			Temperature	*Time (h/day)*	*Condition*	
Total Aorobic Mesophilic Microorganisms (TAM)	Plate Count Agar (PCA) (Oxoid® CM463) (Pour plate)	-	37 °C	24-48 h	Aerobic	Pichhardt, 1993
Total Aerobic Psychrophilic Microorganisms (TAP)	Plate Count Agar (PCA) (Oxoid® CM463) (Pour plate)	-	7 °C	7-10 day	Aerobic	Pichhardt, 1993
***Pseudomonas* spp. (PS)**	*Pseudomonas* Agar (PSA) (Oxoid® CM559) (Spread plate)	Glycerol (5 mL) + (Oxoid® SR103)	25 °C	48-72 h	Aerobic Oxidase (+) (Oxidase Identification Sticks, Oxoid® BR64)	Pichhardt, 1993
Yeast and Molds (Y/M)	Potato Dextrose Agar (PDA) (Oxoid® CM139B) (Pour plate)	Tartaric Acid (10%) (pH=3.5±0.1)	20-25 °C	5-7 day	Aerobic	Koburger ve Marth, 1984
Coliform Group Microorganisms (CG)	Violet Red Bile Agar (VRBA) (Oxoid® CM107) (Pour plate)	-	37±1 °C	24-48 h	Aerobic	Harrigan ve Mc Cance, 1976; Pichhardt, 1993
Fecal Streprococci (FS)	Slanetz & Bartley Agar (S&B) (Oxoid® CM377) (Pour plate)	-	35+44-45 °C	4 h+44 h	Aerobic	Pichhardt, 1993
***Enterobactericeae* (EB)**	Violet Red Bile Glucose Agar (VRBGA) (Oxoid® CM485) (Pour plate)	-	30±1 °C	24 h	Aerobic	Pichhardt, 1993
***Lactobacillus-Leuconostoc-Pediococcus* group Microorganisms (LB)**	Rogosa Agar (RA) (Oxoid® CM0627) (Pour plate)	Glacial Acetic Acid (pH=5.7)	35±1 °C	72 h	Anaerobic	Pichhardt, 1993

Dilutions of 100 and 10 ppm were prepared from the main stock solution. Amine stock solution mixtures in the form of 12.5 µl, 25 µl, 50 µl and 100 µl amine stock solutionx8 (mix, 100 ppm), 20 µlx8 (mix, 1000 ppm) and 50 µlx8 (mix, 10 ppm) were prepared in different 5 ml volumetric flasks, 50 µl of internal standard (1.7 Diamino heptane, Merck, Germany) was added to the stock solution and then made up to 1 ml with 0.4 M perchloric acid (70% HCIO4,

Merck, Germany) in order to determine the curves for amine standards. 200 µl of 2 N sodium hydroxide (NaOH, Merck, Germany), 300 µl of saturated bicarbonate (NaHCO3, Merck, Germany) and 2 ml of dansyl chloride (5-dimethylaminonaphtalene-l-sulfonyl chloride, D2625-5G, Sigma-Aldrich, UK) were added to this mixture, respectively and vortexed for 20 seconds (Heidolph Reax Top D-91126, USA). The mixture was left incubation in an incubator (45 min at 40 °C). Afterwards, the incubation temperature was reduced to room temperature by keeping it in the dark for 15 min. 100 µl of 25% ammonia (25% NH3, Merck, Germany) was added and mixed in a vortex for 20 seconds and kept in a dark environment at room temperature for 30 min. It was completed up to 5 ml with a mixture of ammonium acetate (CH3COONH4, Merck, Germany): Acetonitrile (CH3CN, Merck, Germany) (1:1, v:v), was vortexed and was centrifuged at 2500 rpm for 5 minutes at 4 °C (Sigma® 3-30K, Germany). Centrifugates were filtered through an injector-tipped filter (Millex®-LCR Syringe type filter, 0.45µm, Ireland) and taken into 2 ml amber vials (LC/MS Certified Amber Glass 12 x 32mm Screw Neck Vial, 2 ml, Waters, USA) and calibration was completed by making analyzes with HPLC (Table 2).

b- Extraction and derivatization of the samples:

During the extraction process of the samples, 125 µl of internal standard and 10 ml of 0.4 M perchloric acid (70% $HClO_4$, Merck, Germany) were added onto the homogenate (2 g) in the centrifuge tube and passed through a homogenizer (IKA® Yellowline DI Basic, USA) for 3 minutes in a cooled alcohol-ice bath. Then it was centrifuged at 3000 rpm/10 min at 4 °C and the upper clear part was collected by filtration through filter paper (Filter lab® 1240 90 mm, Spain). 10 ml of perchloric acid was added to the centrifuge tube again and mixed in the vortex, and filtered after centrifugation and taken on the first filtrate. The collected filtrate was collected up to 25 ml with perchloric acid. Extraction of the samples was performed in triplicate. For derivatization of the samples, 1 ml was taken from the collected filtration into 5 ml amber volumetric flasks. After this stage, the procedures applied for derivatization of BA standards were followed and three repetitive analyzes were performed for each sample in HPLC.

c- Configuration of the HPLC Device:

C 18 (Inertsil® ODS-2, 5 µm, 4.6 x 150 mm, Japan) column was used for BAs analysis. HPLC (Prominence LC-20A Modular HPLC System, Japan) configuration was determined as follows: injection amount: 20 µl, flow rate: 0.8 ml/min, Diode-Array Detection (DAD), column oven temperature 40 °C, mobile phase A (Ammonium acetate), mobile phase B (Acetonitrile 50%-90% 16th min, 16-22nd min 90%, 22-30th min 50%, post-run), 254 nm wavelength (Eerola et al. 1993).

Sensory Analyses

Sensory analyzes were carried out by a group of 6 panelists. Panelists are between the ages of 33 and 40 working at Van YYU Faculty of Veterinary Medicine, it was made by 6 panelists 3 women and 3 men who are associates. During the study, attention was paid to the presence of the same panelists. The samples were scored and evaluated according to the hedonic scale (0-9 points) in terms of color, odor, taste and general acceptability criteria as "very good" (9.00-7.00), "good" (6.90-4.10), "can be consumed" (4.00) and "spoiled" (3.90-1.00) (Ekici et al. 2011).

Statistical Analysis

Descriptive statistics for the continuous variables were presented as Mean and Standard deviation, while count and percentages for categorical variables. Kruskal-Wallis test was performed to compare groups. Following the Kruskal-Wallis test, Dunn multiple comparison test was used to determine different storage days. Pearson correlation coefficients were calculated separately in groups to determine the relationship between traits. Statistical significance level was considered as 5% and SPSS (ver: 23) statistical program was used for all statistical computations (Özdamar 2010).

RESULTS

The results of the microbiological counts (log_{10} cfu/g), chemical parameters, sensory analyses, and BAs levels (mg kg^{-1}–ppm/wet weight) of the CWF and CGF samples taken in the stated days were given in Table 3. The correlation data between the examined parameters of the fish samples stored at 4 °C as whole (CWF) and gutted (CGF) are given in Table 4-5.

Table 2. HPLC values of biogenic amines standard solutions *RSD%*, *CV%*, *LOD* and *LOQ*.

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

RSD%: Relative Standard Deviation*, CV%:* Coefficient of Variation, *LOD:* Limit of Detection, *LOQ:* Limit of Quantification.

Table 3. Changes in the parameters analyzed during storage in fish stored at 4 °C as whole and gutted.

Parameters examined		Storage Time (day)								Statistical Values		
Number of microorganisms (log_{10} cfu/g)	App. form	0 day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	Min.	Max.	Mean
TAM	WF	3.10±0.08^{D}	#4.42±.0.04^{C}	#4.23±0.35^{C}	4.99±0.14^{B}	5.07±0.12^{B}	5.28±0.33^{B}	5.47±0.08^{B}	6.10±0.20^{A}	3.04	6.24	4.83±0.90
	GF	3.14±0.10^{D}	3.08±.0.17^{D}	3.14±0.08^{D}	4.56±0.06^{C}	5.15±0.16^{B}	5.55±0.05^{A}	5.20±0.13^{B}	5.53±0.22^{A}	2.96	5.69	4.42±1.09
TAP	WF	5.85±0.25^{E}	#6.90±0.13^{D}	7.35±0.25^{CD}	7.39±0.13^{CD}	7.75±0.21^{BC}	#7.95±0.05^{AB}	8.28±0.16^{A}	8.24±0.33^{AB}	5.68	8.47	7.46±0.79
	GF	5.63±0.04^{E}	5.32±0.09^{E}	6.34±0.22^{D}	7.36±0.17^{C}	7.77±0.02^{B}	8.31±0.10^{A}	8.25±0.27^{A}	8.58±0.10^{A}	5.26	8.65	7.20±1.23
Pseudomonas spp.	WF	3.09±0.13^{E}	#3.72±0.10^{CD}	3.63±0.06^{D}	#3.98±0.08^{C}	3.79±0.13^{CD}	4.57±0.12^{B}	4.44±0.19^{B}	6.15±0.11^{A}	3.00	6.22	4.16±0.90
	GF	2.98±0.09^{E}	2.80±0.08^{E}	3.31±0.11^{D}	3.07±0.23^{DE}	4.17±0.12^{C}	4.25±0.07^{C}	4.78±0.06^{B}	6.46±0.06^{A}	2.75	6.50	3.98±1.19
Yeast/Mold (Y/M)	WF	1.85±0.21^{D}	#3.05±0.13^{B}	3.07±0.10^{B}	3.55±0.09^{A}	#2.44±0.04^{C}	#2.27±0.05^{C}	3.57±0.11^{A}	3.28±0.32^{A}	1.70	3.65	2.88±0.62
	GF	1.47±0.10^{D}	2.24±0.05^{C}	3.26±0.12^{AB}	3.14±0.15^{B}	3.47±0.18^{AB}	3.51±0.03^{B}	3.17±0.21^{B}	3.57±0.13^{A}	1.40	3.66	2.98±0.72
Coliform group (CG)	WF	1.82±0.16^{D}	#3.48±0.09^{B}	#3.78±0.11^{A}	#3.90±0.09^{A}	3.20±0.13^{BC}	3.31±0.10^{B}	2.95±0.18^{C}	4.01±0.11^{A}	1.70	4.09	3.30±0.69
	GF	1.64±0.14^{E}	2.60±0.08^{C}	2.56±0.06^{D}	2.44±0.03^{D}	2.99±0.12^{D}	3.48±0.04^{C}	3.33±0.07^{B}	3.81±0.11^{A}	1.54	3.86	2.86±0.67
Fecal streptococci (FS)	WF	<1	1.17±0.13^{C}	#2.58±0.07^{A}	#2.38±0.10^{AB}	#2.33±0.14^{AB}	#2.30±0.04^{AB}	2.15±0.21^{B}	2.52±0.10^{A}	<1	2.63	2.05±0.60
	GF	<1	<1	1.15±0.21^{D}	1.45±0.21^{C}	1.61±0.19^{C}	2.61±0.05^{AB}	2.50±0.02^{B}	2.82±0.01^{A}	<1	2.82	1.77±0.74
Enterobacteriaceae (EB)	WF	2.10±0.11^{E}	#4.04±0.11^{D}	#4.77±0.04^{C}	4.78±0.23^{C}	4.82±0.16^{C}	#4.97±0.02^{BC}	#5.27±0.09^{B}	#5.79±0.24^{A}	2.02	5.96	4.56±1.08
	GF	2.34±0.06^{G}	3.28±0.06^{F}	4.15±0.07^{E}	4.43±0.14^{D}	5.31±0.14^{C}	5.51±0.02^{C}	6.05±0.13^{B}	6.63±0.06^{A}	2.30	6.67	4.71±1.39
Lactobacilli (LB)	WF	1.59±0.16^{E}	#3.20±0.09^{D}	#3.13±0.05^{D}	#4.43±0.11^{C}	#5.61±0.04^{A}	#5.54±0.05^{A}	#4.92±0.01^{B}	4.86±0.06^{B}	1.48	5.64	4.16±1.36
	GF	2.13±0.10^{F}	2.28±0.04^{F}	2.57±0.09^{E}	3.56±0.06^{D}	4.07±0.10^{C}	5.26±0.07^{A}	5.30±0.10^{A}	4.68±0.16^{B}	2.06	5.37	3.72±1.26
pH	WF	6.54±0.23^{C}	6.64±0.04^{BC}	6.49±0.12^{C}	#6.58±0.04^{BC}	#6.62±0.06^{BC}	#6.81±0.01^{AB}	#6.60±0.01^{BC}	6.89±0.01^{A}	6.38	6.90	6.64±0.15
	GF	6.52±0.06^{D}	6.65±0.00^{C}	6.78±0.01^{B}	6.75±0.04^{B}	6.89±0.04^{A}	6.67±0.02^{C}	6.78±0.04^{B}	6.95±0.02^{A}	6.48	6.96	6.75±0.13
TVB-N (mg 100 g^{-1})	WF	11.34±0.20^{E}	#11.41±0.10^{E}	#12.67±0.10^{DE}	14.35±0.50^{CD}	#16.45±0.50^{C}	26.95±0.50^{B}	28.00±1.98^{B}	30.80±1.98^{A}	11.20	32.20	18.99±7.93
	GF	11.76±0.20^{CD}	12.32±0.20^{C}	10.50±0.20^{D}	11.97±0.89^{CD}	13.51±0.30^{C}	25.69±0.69^{B}	30.45±0.49^{A}	26.61±1.57^{B}	10.36	30.80	17.85±7.95
Sensory analysis	WF	9.00±0.00^{A}	7.92±0.65^{B}	6.62±0.16^{C}	4.04±0.35^{D}	1.87±0.42^{E}	1.00±0.00^{F}	1.00±0.00^{F}	1.00±0.00^{F}	1.00	9.00	4.08±3.20
	GF	9.00±0.00^{A}	8.50±0.31^{B}	6.54±0.32^{C}	4.08±0.29^{D}	2.08±0.29^{E}	1.00±0.00^{F}	1.00±0.00^{F}	1.00±0.00^{F}	1.00	9.00	4.15±3.26

#: ↓ For a variable, the difference between whole and gutted applications within the same storage days is statistically significant ($p<0.05$). A, B, ...H: → Shows the difference between storage days within the same application method ($p<0.05$). App.: Application, TAM: Total Aerobic Mesophilic Microorganisms, TAP: Total Aerobic Psycrophilic Microorganisms; TVB-N: Total Volatile Basic Nitrogen, BAs: Biogenic Amines, ND: Not Detected (<LOD), WF: Whole Fish, GF: Gutted Fish.

Table 3. Following.

BAs (*n=3*) (mg kg^{-1}-ppm) (wet weight)		0 day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	Min.	Max.	Mean
Tryptamine (TRM)	WF	#1.25±0.06^{F}	1.10±0.08^{G}	#1.54±0.15^{CD}	#1.61±0.07^{C}	1.47±0.09^{DE}	#2.72±0.04^{B}	#2.90±0.05^{A}	#1.36±0.17^{EF}	0.98	2.94	1.75±0.65
	GF	0.92±0.07^{F}	1.04±0.14^{F}	1.26±0.15^{E}	1.51±0.04^{D}	1.53±0.04^{D}	3.41±0.20^{C}	4.92±0.07^{B}	5.67±0.20^{A}	0.83	5.99	2.53±1.79
β-phenylethylamine (PEA)	WF	#1.36±0.09^{C}	#0.43±0.02^{F}	#1.02±0.09^{D}	#1.80±0.11^{B}	#0.71±0.08^{E}	#2.40±0.30^{A}	#0.76±0.05^{E}	#0.12±0.04^{G}	0.08	2.90	1.07±0.71
	GF	1.05±0.11^{B}	1.05±0.12^{B}	2.06±0.10^{A}	1.12±0.06^{B}	1.06±0.10^{B}	0.59±0.06^{C}	0.62±0.08^{C}	0.45±0.04^{D}	0.41	2.26	1.00±0.48
Putrescine (PUT)	WF	1.65±0.04^{H}	#3.44±0.26^{F}	#2.20±0.32^{G}	#5.66±0.26^{E}	#7.34±0.29^{D}	#14.29±0.34^{B}	#16.24±0.11^{A}	#10.85±0.41^{C}	1.58	16.37	7.71±5.25
	GF	1.68±0.10^{E}	0.94±0.07^{F}	1.60±0.18^{E}	1.15±0.16^{EF}	2.57±0.16^{D}	15.59±0.11^{C}	21.07±0.32^{B}	24.61±0.09^{A}	0.85	25.46	8.65±9.52
Cadaverine (CAD)	WF	#1.64±0.04^{H}	#3.45±0.08^{G}	#4.51±0.42^{F}	#17.29±0.61^{G}	#41.81±1.22^{A}	#37.87±0.75^{C}	#36.05±0.45^{D}	#38.67±0.33^{B}	1.57	42.96	22.66±16.8
	GF	0.40±0.01^{F}	0.52±0.20^{F}	0.30±0.02^{F}	1.51±0.08^{E}	5.39±0.10^{D}	17.85±0.51^{C}	32.55±0.47^{B}	38.99±0.76^{A}	0.27	39.86	12.19±14.9
Histamine (HIS)	WF	#1.30±0.04^{D}	#0.32±0.04^{F}	#ND	#0.74±0.03^{E}	#2.49±0.21^{C}	#4.89±0.08^{A}	#3.14±0.08^{B}	#1.25±0.03^{D}	ND	5.03	1.77±1.56
	GF	ND	ND	0.15±0.03^{D}	0.16±0.01^{D}	0.23±0.01^{D}	0.75±0.04^{C}	2.43±0.07^{A}	2.25±0.28^{B}	ND	2.57	0.75±0.96
Tyramine (TYM)	WF	#1.25±0.01^{F}	#1.41±0.04^{E}	#1.37±0.03^{E}	#2.00±0.03^{D}	#2.41±0.07^{C}	3.32±0.19^{A}	#2.52±0.06^{B}	#2.43±0.05^{BC}	1.23	3.56	2.09±0.68
	GF	1.07±0.03^{F}	0.96±0.03^{G}	1.53±0.04^{D}	1.29±0.05^{E}	1.36±0.05^{E}	3.37±0.06^{B}	3.52±0.02^{A}	2.84±0.06^{C}	0.93	3.76	1.99±1.01
Spermidine (SPD)	WF	#1.24±0.00^{E}	#1.13±0.01^{F}	1.27±0.02^{E}	#1.45±0.02^{D}	#1.42±0.03^{D}	#1.76±0.02^{D}	#1.57±0.01^{C}	#1.66±0.02^{B}	1.11	1.79	1.44±0.21
	GF	1.18±0.01^{E}	1.05±0.04^{F}	1.23±0.03^{D}	1.35±0.01^{C}	1.22±0.03^{D}	1.33±0.01^{D}	1.96±0.01^{A}	1.85±0.03^{B}	1.00	1.98	1.40±0.31
Spermine (SPM)	WF	#1.28±0.02^{E}	#1.25±0.06^{F}	#1.15±0.01^{G}	#1.54±0.02^{B}	#1.11±0.01^{H}	#1.67±0.01^{A}	#1.31±0.01^{D}	#1.48±0.01^{C}	1.10	1.69	1.35±0.19
	GF	1.19±0.02^{D}	1.05±0.02^{F}	1.34±0.01^{C}	1.33±0.04^{C}	1.52±0.02^{B}	1.14±0.00^{E}	1.55±0.04^{B}	1.77±0.02^{A}	1.02	1.79	1.36±0.23

#: ↓ For a variable, the difference between whole and gutted applications within the same storage days is statistically significant ($p<0.05$). A, B, ...H: → Shows the difference between storage days within the same application method ($p<0.05$). App.: Application, TAM: Total Aerobic Mesophilic Microorganisms, TAP: Total Aerobic Psycrophilic Microorganisms; TVB-N: Total Volatile Basic Nitrogen, BAs: Biogenic Amines, ND: Not Detected (<LOD), WF: Whole Fish, GF: Gutted Fish.

Table 4. Correlations between parameters examined in fish stored at 4 °C as a whole (*: p<0.05, **: p<0.01).

Parameters examined	pH	TVB-N	SA	TAM	TAP	PS	Y/M	CG	FS	EB	LB	TRM	PEA	PUT	CAD	HIS	TYM	SPD	SPM
pH	1																		
TVB-N	,667**	1																	
Sensory analysis	-,558*	-,871**	1																
TAM	,645**	,808**	-,896**	1															
TAP	,514*	,783**	-,901**	,934**	1														
Pseudomonas spp.	,738**	,855**	-,730**	,848**	,740**	1													
Yeast/Mold (Y/M)	-,041	,264	-,300	,529*	,506*	,411	1												
Coliform group (CG)	,335	,244	-,381	,650**	,595*	,529*	,668**	1											
Fecal streptococci (FS)	,251	,488	-,694**	,692**	,783**	,535*	,428	,681**	1										
Enterobacteriaceae (EB)	,423	,679**	-,815**	,917**	,949**	,750**	,646**	,785**	,832**	1									
Lactobacilli (LB)	,475	,676**	-,932**	,863**	,876**	,582*	,285	,486	,703**	,823**	1								
Tryptamine (TRM)	,187	,675**	-,649**	,431	,592*	,277	,167	-,038	,378	,427	,513*	1							
β-phenylethylamine (PEA)	-,019	-,054	-,030	-,183	-,139	-,265	-,330	-,153	,071	-,197	,071	,433**	1						
Putrescine (PUT)	,523*	,920**	-,881**	,772**	,808**	,657**	,269	,146	,447	,656**	,763**	,838**	,100	1					
Cadaverine (CAD)	,561*	,822**	-,967**	,833**	,827**	,672**	,137	,250	,606*	,711**	,919**	,541**	-,029	,838**	1				
Histamine (HIS)	,404	,642**	-,673**	,383	,452	,251	-,298	-,233	,194	,239	,616*	,799**	,470**	,801**	,718**	1			
Tyramine (TYM)	,587*	,775**	-,895**	,715**	,733**	,558*	-,023	,208	,541*	,606*	,873**	,715**	,319*	,882**	,893**	,867**	1		
Spermidine (SPD)	,607*	,891**	-,896**	,756**	,742**	,711**	,110	,235	,624**	,633**	,773**	,671**	,292*	,862**	,847**	,748**	,917**	1	
Spermine (SPM)	,560*	,516*	-,421*	,437	,314	,507*	,117	,278	,241	,298	,371	,420**	,575**	,497**	,322*	,464**	,605**	,696**	1

TVB-N: Total Volatile Basic Nitrogen, SA: Sensory analysis, TAM: Total Aerobic Mesophilic Microorganisms, TAP: Total Aerobic Psycrophilic Microorganisms.

Table 5. Correlations between parameters examined in fish stored at 4 °C as a gutted (*: p<0.05, **: p<0.01).

Parameters examined	pH	TVB-N	SA	TAM	TAP	PS	Y/M	CG	FS	EB	LB	TRM	PEA	PUT	CAD	HIS	TYM	SPD	SPM
pH	1																		
TVB-N	,334	1																	
Sensory analysis	-,659**	-,790**	1																
TAM	,596*	,756**	-,967**	1															
TAP	,669**	,773**	-,984**	,966**	1														
Pseudomonas spp.	,714**	,791**	-,786**	,767**	,808**	1													
Yeast/Mold (Y/M)	,776**	,460	-,825**	,740**	,801**	,597*	1												
Coliform group (CG)	,683**	,804**	-,869**	,815**	,826**	,842**	,806**	1											
Fecal streptococci (FS)	,498*	,929**	-,910**	,883**	,905**	,869**	,653**	,890**	1										
Enterobacteriaceae (EB)	,794**	,788**	-,954**	,894**	,940**	,874**	,863**	,937**	,895**	1									
Lactobacilli (LB)	,510*	,880**	-,971**	,948**	,951**	,745**	,719**	,849**	,933**	,906**	1								
Tryptamine (TRM)	,538*	,936**	-,780**	,752**	,800**	,923**	,549*	,853**	,939**	,859**	,820**	1							
β-phenylethylamine (PEA)	-,177	-,722**	,587**	-,704**	-,568*	-,599*	-,179	-,583*	-,703**	-,525*	-,659**	-,696**	1						
Putrescine (PUT)	,447	,965**	-,754**	,730**	,769**	,895**	,474	,817**	,934**	,813**	,819**	,985**	-,717**	1					
Cadaverine (CAD)	,529*	,941**	-,758**	,740**	,779**	,928**	,495	,828**	,921**	,839**	,805**	,993**	-,714**	,987**	1				
Histamine (HIS)	,540*	,903**	-,703**	,661**	,722**	,885**	,450	,757**	,850**	,803**	,745**	,965**	-,623**	,940**	,970**	1			
Tyramine (TYM)	,329	,961**	-,805**	,765**	,809**	,750**	,575*	,817**	,937**	,803**	,901**	,882**	-,609**	,911**	,862**	,829**	1		
Spermidine (SPD)	,520*	,847**	-,704**	,648**	,723**	,813**	,424	,655**	,795**	,766**	,724**	,920**	-,555**	,883**	,920**	,968**	,781**	1	
Spermine (SPM)	,843**	,483	-,656**	,618*	,701**	,815**	,564*	,567*	,581*	,758**	,544*	,682**	-,287*	,604**	,697**	,718**	,404**	,769**	1

TVB-N: Total Volatile Basic Nitrogen, SA: Sensory analysis, TAM: Total Aerobic Mesophilic Microorganisms, TAP: Total Aerobic Psycrophilic Microorganisms.

DISCUSSION AND CONCLUSION

The determination of the total microbial load is one of the important parameters for the determination of spoilage due to the lack or insufficiency of thermal measures in all transport, processing and preservation stages of the food, starting from the raw material supply, with the full compliance of the hygiene and sanitation rules of the food enterprises. Besides, it also provides valuable data in terms of determining the onset of spoilage and the possible shelf life according to the food product, and providing information about the contamination level during the production processes (Temiz 2003). TAM counts at the beginning of storage in CWF and CGF samples were 3.10±0.08 and 3.14±0.10 $\log_{10}$ cfu/g, respectively. According to our results and low initial TAM counts indicate the good quality of samples (Table 3). According to International Commission on Microbiological Specifications for Foods (ICMSF) (1986), it has been reported that the microbial load during harvest or fishing for most aquatic products can vary between 2-5 $\log_{10}$ cfu/g (<5.69 $\log_{10}$ cfu/g good quality). Although there are studies which have similar results to our research for TAM count at the beginning of storage (Fan et al. 2009; Zhang et al. 2011) reports with different results are also available (Krizek et al. 2004; Hasani and Hasani 2014). It is thought that microbial flora and load in the waters from which the samples were obtained, hunting/harvest period, preparation processes, transport conditions and durations, and possible contaminations might be effective on the differences between the research results. Microflora of the water and temperature are effective on the initial microbial load in freshwater fish (Ehsani and Jasour 2012). It was evaluated that TAM counts were 5.07±0.12 and 5.15±0.16 $\log_{10}$ cfu/g, respectively, on the 8th day when spoilage was observed at both application forms (CWF and CGF) in terms of sensory analysis, however it was determined that the limit (7 $\log_{10}$ cfu/g) determined for TAM count in marine and freshwater fish according to ICMSF (1986) and Erdem et al. (2022) were not exceeded during the 14-day storage period. Correlation was found between TAM counts and TAP, *Pseudomonas* spp. (PS), Coliform group (CG), Fecal Streptococci (FS), *Enterobacteriaceae* (EB), Lactobacilli (LB), pH, TVB-N, sensory analysis, putrescine, cadaverine, tyramine and spermidine ($p<0.01$) and Yeast/Mold (Y/M) ($p<0.05$) (Table 4). On the other hand, in CGF samples, a correlation at $p<0.01$ was determined between TAM count and TAP, PS, Y/M, CG, FS, EB, LB, TVB-N, sensory analysis, tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, tyramine and spermidine; and a correlation at $p<0.05$ was determined between TAM count, and pH and spermine (Table 5). It is expected that the values of other microorganism groups will be high if TAM count is high. There is a positive correlation between the TAM counts of the samples in both groups and the other microorganism groups at different levels in terms of statistical analysis. At the same time, other microorganism groups can grow in the medium and incubation conditions at which the TAM count is determined. At the end of the storage period, the TAP count of the samples was found to be 2 $\log_{10}$ cfu/g more than the TAM count on average (Table 3). This can be related to the microflora differences of the samples at the beginning of storage, and the fact that the samples were composed of fish caught in the winter season and psychrophilic microorganisms formed the dominant microflora. It was determined that a correlation of TAP count was present with sensory analysis results and chemical parameters at different levels ($p<0.01$, $p<0.05$) (Table 4-5). In this study, it was thought that psychrophilic microorganisms are more effective on the formation of BAs in both applications. The level of increase in the number of PS is similar to the increase in TAM count and PSs were found to constitute the majority of the microbial load in the samples. Microflora, which was initially heterogeneous in fresh fish kept in cold aerobic conditions, became dominant in the later stages due to competition with psychrotrophic *Pseudomonas* and *Shewanella* species, and as a result, spoilage has occurred (Sterniša et al. 2020). Increases and decreases in the PS count during the storage process can be due to inhibition as a result of competition between microorganisms. It was determined that PS counts were statistically significantly increased ($p<0.05$) in CWF and CGF samples, and reached 6.15±0.11 and 6.46±0.06 $\log_{10}$ cfu/g on the 14th day, respectively (Table 3). It was determined that the PS count in all fish was generally higher than the cleaned ones, but the PS count increased more in the cleaned fish starting from the 8th day of storage (Table 3). In this study, it was seen that *Pseudomonas* spp. are dominant microorganisms in CWF and CGF samples. Significant ($p<0.05$) increases and decreases were observed in CG and Y/M counts during storage and these values were found to be 4.01±0.11 and 3.81±0.11 $\log_{10}$ cfu/g, and 3.28±0.32 and 3.57±0.13 $\log_{10}$ cfu/g, respectively, in CWF and CGF samples on the 14th day (Table 3). It has been determined that the Y/M counts were generally similar in both applications. At the beginning of storage (0 day), the number of CGs in the CWF and CGF samples were determined 1.82±0.16 $\log_{10}$ cfu/g and 1.64±0.14 $\log_{10}$ cfu/g, respectively. It may be related to possible cross-contaminations of the water in which the samples are caught, the tools-equipment used in the fishing and the boat and/or transport process or during the preparation of the samples. Coliform microorganism contamination can occur both from the waters where fish are caught/harvested, and from transportation and all other processes following the fishing/harvest process. For this reason, two processes should be evaluated together in the detection of these microorganisms, which are considered as indicators (Patir and Inanli 2005).

While fecal streptococcus (*Enterococcus* spp.) counts in CWF and CGF samples was found to be <1 $\log_{10}$ cfu/g at the beginning of storage; it was found to be 2.52±0.10 $\log_{10}$ cfu/g in CWF samples and 2.82±0.01 $\log_{10}$ cfu/g in CGF samples at the end of storage (Table 3). In both applications, it was determined that the *Enterobacteriaceae* count has increased during storage, and the highest values of 5.96 and 6.67 $\log_{10}$ cfu/g were determined in CWF and CGF samples, respectively (Table 3). While some of the species in the *Enterobacteriaceae* family have fecal origin and pathogenic characteristics, some of them are saprophytic. This family is accepted as a hygiene and sanitation indicator rather than an indicator of fecal contamination (Temiz 2003). *Enterobacteriaceae* count is evaluated as a quality index from a different aspect in the processes of cleaning, washing and cooling the internal organs of fish with the help of ice. It is recommended to monitor these microorganisms in the evaluation of fish quality, and *Enterobacteriaceae* in the entire food chain from production to consumption is taken into account. *Pseudomonas* microorganism count is evaluated in the establishment of new practices and regulations for food safety, food tracking and traceability systems. For the risk management process determined in fish, it is very important to obtain clear information by means of such microorganisms in order to better

understand and determine the danger at all stages, including technological product processing, transportation and storage, sales and supply (Popovic et al. 2010). It was determined that there were increases and partial decreases in lactobacilli count (*Lactobacillus-Leuconostoc-Pediococcus*) during storage (Table 3). Reproduction of microorganisms can be affected by different environmental factors. Lactobacilli can produce lactic acid and bacteriocins, and this may contribute to the selective growth of these microorganisms in the environment during the spoilage processes (Li et al. 2011). It is considered that the initial high lactobacillus load may be due to differences in fish species and/or sample preparation. In this study, it was determined that some microorganism counts in CGF samples were partially higher than that in CWF samples on different days of storage. Although repeated washing was performed during the preparation of CGF samples, blood clots and other residues formed during the cleaning of internal organs are thought to be effective. Microbial quality in fish meat may vary depending on the characteristics of the water in which catch/harvest is performed and the sanitation conditions between the fish markets and sales points. When the fish is cleaned, the edible muscle parts can be contaminated because of microorganisms originating from gills and the internal organs during the cleaning processes (Krizek et al. 2004; Abuhlega and Ali 2022).

The pH value of fish and fish products is accepted as one of the important parameters for determining the freshness. It is stated that the pH of fish can vary between 5.5 and 7.1 depending on the season and species, but a value between 6.8-7.0 is generally recommended (Varlık et al. 1993; Ozogul 2009). It was determined that there were fluctuations in pH values in both applications during the storage period, the pH values increased in the following periods, and reached the level of 6.89±0.01 and 6.95±0.02 in CWF and CGF samples, respectively, at the end of storage (Table 3). It has been reported that the decrease in the pH level at the beginning of storage is due to the inorganic phosphates released as a result of the accumulation of lactic acid, the increase in the amount of dissolved CO_2 or the destruction of ATP during the energy production by anaerobic glycolysis. On the other hand, it has also been reported that the increase in the subsequent periods is caused by volatile bases such as NH_3, TMA and alkaline products such as BAs due to endogenous enzymes and microbial activities (Fan et al. 2009; Ochrem et al. 2014; Yu et al. 2016). TVB-N, which is another indicator of spoilage in fish, is mainly formed by the degradation of primary, secondary and tertiary amines, proteins and non-protein nitrogen (NPN) components (such as ammonia), and is more related to microbial activities (Shi et al. 2012). According to our study results, the increases in TVB-N and BAs are compatible with each other. It is seen that the samples have a shelf life of 10 days in terms of TVB-N values according to Regulation on Seafood (2008) (<20 mg 100 g^{-1}) in both applications (Table 3). According to the sensory analysis results of CWF and CGF samples, it was determined that the samples were spoiled on the 8th day of storage. Hasani and Hasani (2014) and Ocherm et al. (2014), results on sensory analyzes in carp fillets are similar to our research results.

The general condition of the fish during catching, the operations applied during the hunting/harvest processes and the hygienic conditions throughout the storage, the hygienic condition of vehicles such as boats, the level of their cooling systems, the capacity and hygienic characteristics of the cooling units may cause different levels of BAs to occur in various fish species (Ehsani and Jasour 2012). BAs, an enzymatic degradation product used as an indicator of spoilage; have a special importance especially in fish and fish products in terms of public health due to both quality indicator and microbial deterioration index and their toxic effects (Krizek et al. 2002; Ruiz-Capillas and Herrero 2019; Ucar et al. 2021; Wójcik et al. 2021). In our study, fluctuations in the amount of tryptamine and β-phenylethylamine were determined during the storage period (Table 3). It was determined that the amount of β-phenylethylamine varied between 0.08 and 2.90 ppm as the lowest and highest values, respectively. On the other hand, the amount of tryptamine was found to be 1.47±0.09 and 1.53±0.04 ppm in whole and cleaned samples, respectively on the 8th day when the samples were evaluated as "spoiled" in terms of sensory. Bakar et al. (2010) and Yu et al. (2016) stated in their research that there were fluctuations in tryptamine and β-phenylethylamine concentrations during storage, but these fluctuations were insignificant and could not be taken into account. Tryptamine and β-phenylethylamine can cause migraine and various cardiovascular disorders such as narrowing of the vessels, increase in blood pressure and hypertension. While no toxic dose is reported for tryptamine in foods, the toxic dose for β-phenylethylamine is reported to be 30 ppm (Mah et al. 2019; Wójcik et al. 2021). It is observed that the concentration of β-phenylethylamine in the samples during the entire storage period is less than 3 ppm and remains within the safe limits for human consumption. Especially the *Enterobacteriaceae* family and *Pseudomonas* species are accepted as sources of cadaverine and putrescine (Buňka et al. 2012). Zhang et al. (2015) reported that putrescine and cadaverine were dominant BAs during storage in common carp fillets stored for 18 days and reported that these BAs increased during storage and would be appropriate for measuring quality changes in common carp fillets. Kordiovská et al. (2006), stated that the amines which have best correlation with microorganism counts in the samples are putrescine and cadaverine, and reported that these two amines can be considered as the most objective indicator in the evaluation of quality in carp fish. In general, putrescine and cadaverine suppress histamine oxidation and increase histamine toxicity in humans (Buňka et al. 2012; Abuhlega and Ali 2022). It has been reported that two amines, histamine and tyramine, cannot be used as quality indicators of carp fish since the increase in the concentration of these two amines is revealed during the matrix decomposition phase. On the other hand, it is reported that the amount of putrescine or putrescine+cadaverine can be evaluated together in the evaluation of quality (<20 mg kg^{-1} good quality, 20-45 mg kg^{-1} acceptable and >45 mg kg^{-1} bad quality for putrescine+cadaverine) since putrescine and cadaverine have similarly flat kinetics and cause slow pre-sensory manifestations (Krizek et al. 2002; Krizek et al. 2004).

According to our research results within the framework of this evaluation, the amount of putrescine and cadaverine in CWF and CGF samples was 7.34 and 2.57 ppm and 41.81 and 5.39 ppm, respectively on the 8th day of sensory rejection of samples. On the 6th day of storage, the amount of putrescine+cadaverine in CWF and CGF samples was found to be 22.95 and 2.66 ppm, respectively (Table 3). It was observed that the amount of cadaverine detected in the CGF samples in the first 12 days of the storage was lower than in the CWF samples. Low cadaverine level may be associated with lower proteolytic enzyme activity in

CGF samples compared to CWF samples. BAs are formed by decarboxylation of free amino acids as a result of the activity of bacteria containing decarboxylase enzymes (Shi et al. 2012). It was determined that the amount of putrescine in CWF samples decreased on the 14th day of the storage. Microbial deamination and decarboxylation activities of many kinds of amino acids decreased in the late period of storage, which might be due to the inhibition effects of microbial metabolites like ammonia and amines (Zhuang et al. 2023). In both applications, the amount of putrescine+cadaverine was found to be of poor quality with 49.15 ppm in CWF samples and good quality with 7.96 ppm in CGF samples on the 8th day of sensory rejection of the samples (Table 3). A correlation of ($p<0.05$-$p<0.01$) was determined between the amount of putrescine and the amount of cadaverine, tryptamine, β-phenylethylamine, histamine, tyramine, spermidine and spermine in CWF and CGF samples (Table 4-5). According to the results of that study; an increase in the amount of putrescine and cadaverine was determined in accordance with the increase of microorganisms in both applications during the whole storage period, similar to the literature and it was observed that the changes in the amount of TVB-N showed parallelism. It is considered that these two BAs can be considered as suitable quality indicators (index) in determining the quality of mirror carp stored in the cold.

According to our research results, it was determined that the increase in histamine and tyramine concentrations in the samples was limited during the storage period and the amount of histamine and tyramine in CWF and CGF samples was 2.49-0.23 ppm and 2.41-1.36 ppm, respectively, on the 8th day when sensory spoilage was observed (Table 3). While histamine is considered to be one of the important problems in marine fish, it is ignored in freshwater fish, where the amount is low compared to marine fish (Abuhlega and Ali 2022). For tyramine, less than 800 ppm is defined as a safe dose, while even 6 ppm can cause poisoning reactions in people taking MAO inhibitors. The first signs of poisoning appear 1 to 2 hours after consumption and may cause migraine, gastrointestinal complaints, tachycardia, increase in blood sugar, noradrenaline release and hypertension. It is reported that the daily intake of tyramine should not exceed 800 ppm, and the level of 1080 ppm is accepted as a toxic dose according to the recommendations of the European Food Safety Authority (EFSA). Tyramine can react with nitrite in the presence of acidic environments and turn into mutagenic components such as 3-diazotyramine (Mah et al. 2019; Wójcik et al. 2021). In both applications, it was determined that the amount of tyramine during the entire storage period was well below the limit values specified in the literature. Histamine poisoning causes allergic reactions such as tingling in the tongue, rash, vomiting, diarrhea, burning sensation, headache and dizziness, nausea, drop in blood pressure, vasodilation, intracranial bleeding, palpitations or difficulty breathing, itching and redness of the skin, fever and hypertension (Mah et al. 2019; Wójcik et al. 2021). According to Turkish Food Codex (2011) and Regulation on Seafood (2008), legal limits for histamine are regulated as minimum 100 ppm and maximum 200 ppm (100-200 ppm) (n:9/c:2). It is seen that the histamine level in both applications during storage is below the limits allowed by Turkish Food Codex (2011), Regulation on Seafood (2008), FDA (Food and Drug Administration) and EC (European Community). While the amount of spermidine and spermine in CWF samples were between 1.11 and 1.79 ppm and 1.10 and 1.69 ppm, respectively; a fluctuation was observed for the amount of spermidine and spermine between 1.00 and 1.98 ppm and 1.02 and 1.79 ppm in CGF samples (Table 3). It is stated that the amount of spermidine and sperm is clearly in low concentration and fluctuates, there can be a tendency for the amount of spermidine to decrease slowly over time, the low and fluctuating concentrations of spermidine and sperm make it difficult to establish a relationship with these amines in defining the decomposition process, these two amines are toxicologically insignificant in carp fillets stored in the cold which have a low correlation with other parameters and are not important as a quality indicator (Krizek et al. 2002; Krizek et al. 2004; Zhang et al. 2015).

In the light of the results of the samples stored in the cold; it was determined that TAM, TAP and *Pseudomonas* spp. increased during the whole storage period and constituted the dominant flora. It was also determined that there were high correlations between BAs and other parameters and these microorganisms. It was determined that putrescine and cadaverine were the highest increasing BAs during storage, and they were positively correlated with other parameters and negatively correlated with sensory analysis scores. According to the results, it was concluded that the evaluation of putrescine and cadaverine as indicator BAs in determining the freshness of carp fish can be appropriate. Although there is not much difference microbiologically between keeping fish samples as a whole or cleaned, it is considered that the whole preservation is safer than the preservation after cleaning based on the average microbiological values on the 8th day when the samples were rejected in a sensory manner.

CONFLICTS OF INTEREST

The authors declare that there has no conflict of interests.

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