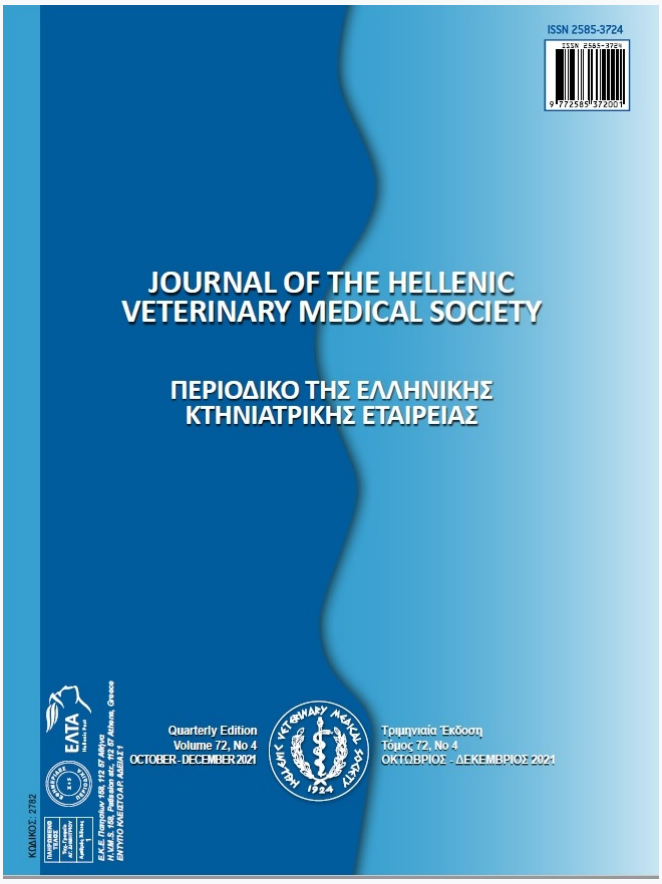
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Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



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doi: 10.12681/jhvms.29368

To cite this article:

AKSOY, A., SEZER, Ç, ATASEVER, M., ÇETINKAYA, A., & GÜLBAZ, G. (2022). Determination of microbiological quality and histamine levels in rainbow trouts (Oncorhynchus mykiss). *Journal of the Hellenic Veterinary Medical Society*, *72*(4), 3321−3328. https://doi.org/10.12681/jhvms.29368

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J HELLENIC VET MED SOC 2021, 72(4): 3321-3328
ΠΕΚΕ 2021, 72(4): 3321-3328

Research article
Ερευνητικό άρθρο

Determination of microbiological quality and histamine levels in rainbow trouts *(Oncorhynchus mykiss)*

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ABSTRACT: This study aims to determine the microbiological quality and histamine levels of fresh-marketed rainbow trouts (*Oncorhynchus mykiss*). The total mesophilic aerobic bacteria (TMAB), total psychrophilic aerobic bacteria (TPAB), coliform, fecal coliform, *Enterobacteriaceae, Pseudomonas*, *Staphylococcus-Micrococcus* and mold-yeast numbers were detected as 4.24, 4.00, 1.10, 0.17, 1.50, 3.05, 0.28 and 3.82 $\log_{10}$ cfu/g, respectively. *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and *Vibrio* spp bacteria were found in 18 (37.5%), 14 (29.1%), 8 (16.6%) and 14 (29.1%) respectively. Twenty-eight out of 51 *Listeria* isolates (54.9%) were verified through PCR analysis as *L. monocytogenes*. It was determined that the histamine level in the samples was above the detectable level (>2.5 mg/kg) in 8 of the samples (16.6%)determined using an ELISA method. It was concluded that the detection of foodborne pathogens in fresh-marketed fish samples potentially constitutes a public health hazard.

Keywords: Rainbow trout, Microbial quality, Histamine, ELISA, PCR

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Date of initial submission: 07-11-2020
Date of acceptance: 31-05-2021

INTRODUCTION

Fish and fishery products are among the main sources of proteins and functional components that are very important for human health (Han et al., 2017). In addition, fish meat contains water-soluble B and C and fat-soluble A, D, E and K vitamins in adequate and balanced amounts (Kara et al., 2020). Despite the difficulties the fish and fishery products industry faces, fish constitutes a significant part of the diet in many countries. It is appreciated throughout the world due to its high nutritional value and delicious taste. Therefore, it is very important in the international fishing industry to ensure the safety of edible fish (Han et al.,2017).Although fish is a rich nutritional source of animal origin and healthy in many aspects, it is an extremely perishable food (Junior et al.,2014). The microbial condition of the seafood after it is caught is closely related to environmental conditions, the microbiological quality, temperature and salt content of water, the distance between the areas of fecal contamination and fishing, natural bacterial growth in water, hunting methods, and cooling conditions (Feldhusen, 2000).

There are different types of bacteria that cause spoilage of fish and fishery products and such spoilage is largely connected to the processing and preserving methods. Yet, the composition of the microflora on newly caught fish may be due to the microbial content of the water the fish live in. The microflora that causes spoilage of fish consists of bacteria such as *Salmonella* spp., *Enterohemorrhagic Escherichia coli*, *Campylobacter jejuni*, *Y. enterocolitica*, *L. monocytogenes*, *Bacillus anthracis*, *Bacillus cereus, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Vibrio vulnificus*and *Vibrio parahaemolyticus* (Han et al., 2017).In unpreserved fish, microbial spoilage is mainly a result of Gram-negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas spp*. and *Shewanella spp*.) tend to spoil chilled fish (Ghaly et al., 2010). Kuley et al. (2017), identified *Enterobacter cloacae, Serratia liquefaciens, Proteus mirabilis, Photobacterium damseale, Pseudomonas luteola, Pantoea spp., V. vulnificus, Stenotrophomonas maltophila, Acinetobacter lwoffii,Pasteurella spp*. and *Citrobacter spp.* from spoiled fish.Some psychrotrophic pathogens can multiply in chilled food without causing a significant change in its sensory properties. *Pseudomonas* species cause deterioration in refrigerated food products by forming the dominant microflora during cold storage. Therefore, the presence of these microorganisms in foods poses a great risk as they cause both food poisoning and food spoilage (Popelka et al., 2016).

Pathogenic bacteria associated with seafood may be examined under three groups, i.e. bacteria constituting the normal components of the marine or port environment: *Vibrio cholerae, V. parahaemolyticus, V. vulnificus, L. monocytogenes, C. botulinum* and *Aeromonas hydrophila*; enteric bacteria growing due to fecal contamination: *Salmonella* spp., pathogenic *E. coli, Shigella* spp., *Campylobacter* spp. and *Y. enterocolitica*; and bacteria growing due to bacterial contamination during processing: *B. cereus*, *L. monocytogenes*, *Staphylococcus aureus* and *C. perfringens* (Feldhusen, 2000).

Histamine, which is the most important cause of food poisoning, is a biogenic amine (Cavanah and Casale, 1993; Ehsanet al., 2015). It is mainly formed by decarboxylation of amino acids due to enzyme activities of microorganisms (Shalaby, 1996; Chen et al., 2010;Akyol et al.,2015). Biogenic amine poisoning is often caused by histamine. Histamine poisoning is also named as “scombroid fish poisoning” as it is often associated with the consumption of scombroid fish such as tunas, mackerels and sardines (Halasz et al., 1994). In fish and fishery products, biogenic amines can be considered indicators of both quality and safety. Because of its toxicity, histamine is a biogenic amine with regulatory limits for fishery products (Visciano et al., 2020). Consumption of food that is high in histamine content causes poisoning with clinical symptoms such as nausea, vomiting, diarrhea, abdominal cramps, headache, rash, asthma and hypotension (Cavanah and Casale, 1993; Maintz and Novak,2007).A highlevel of biogenic amine in food causes food poisoning. Particularly, a high level of histamine in fishery products is a significant indicator of bacterial spoilage. In this regard, the measurement of histamine level is very important in the determination of the hygienic quality of fish.

This study aims to determine the microbiological quality and histamine levels of fresh-marketed rainbow trouts.

MATERIAL AND METHODS

Samples

The samples were obtained from three different sales points (fresh fish retailers) between February and April 2016 in Kars, Turkey. A total of 48 fish fillets consisting of 16 samples from each sales point

were analyzed. The fillets were prepared by the fisherman (internal organs were removed, beheaded, filleted and washed). The fillets were collected in sterile plastic bags. The samples were brought to the laboratory under the cold chain and microbial analysis was made immediately. Samples were stored at -20 °C until used in histamine analysis.

Microbiological analysis

Twenty-five g of each fish fillet sample was measured and homogenized in 225 ml sterile physiological saline solution. After homogenization, decimal dilutions of the samples were prepared and inoculated into suitable mediums by pour plate and streak plate methods, i.e. into Plate Count Agar (PCA, Oxoid CM 325) at 30°Cfor 48 h for Total Mesophilic Aerobic Bacteria (TMAB), Plate Count Agar (PCA, Oxoid CM 325) at 7°C for 10 days for Total Psychrophilic Aerobic Bacteria (TPAB), Violet Red Bile Lactose Agar (VRBA, Oxoid CM 0107) at 37°C for 24 h for Coliform group bacteria, Violet Red Bile Lactose Agar (VRBA, Oxoid, CM 0107) at 44.5°C for 24-48 h for Fecal coliform group bacteria, VRB Glucose Agar (VRBG, Oxoid CM 485) at 35°C for 48 h for *Enterobacteriaceae*, Baird Parker Agar (Oxoid CM 275) at 37°C for 24-48 h for *Staphylococcus- Micrococcus*, Pseudomonas Agar (Oxoid CM 559) and C-F-C Supplement (Oxoid SR 103) at 30°C for 48 h for *Pseudomonas*, and Potato Dextrose Agar (Oxoid CM 139) at 22°Cfor 5-10 days for Yeast-Mold; and the incubation was conducted under the abovementioned conditions (Harrigan, 1998).

***Y. enterocolitica* isolation and identification**

Y. enterocolitica isolation was performed by the conventional inoculation method. 25 gr the fish fillet sample was homogenized in a 225 ml Yersinia selective enrichment broth (Merck 1.16701) medium and incubated at 30 °C for 24 h. From this pre-enrichment culture, inoculation was made to the Yersinia Selective Agar (Oxoid CM0653) medium involving Yersinia selective supplement (Oxoid SR0109) by streak plate method and incubated at 30°C for 24 h. Colonies with crimson red centers and transparent zones at the periphery were considered suspicious and stored in the agar slant. For the identification of the isolated suspicious colonies, inoculation into the Gram staining, simmons citrate, motility, Voges Proskauer (VP), urea, Kligler Iron Agar reaction and Triple Sugar Iron media was made and lactose, sucrose and glucose use, gas and hydrogen sulphide (H_2S) formation, lysine decarboxylase, ornithine decarboxylase, nitrate reduction, catalase, oxidase and indole tests were conducted (FDA, 2007).

***L. monocytogenes* isolation and identification**

L. monocytogenes isolation was performed by the FDA method. 25 gr the fish fillet sample was homogenized in 225 ml Buffered Listeria Enrichment Broth (Oxoid, CM 897) medium and incubated at 30 °C for 4 h. Then, a selective supplement **(**Listeria Selective Enrichment Supplement, Oxoid SR141) was added and incubated at the same temperature for 44 h. From this pre-enrichment culture, inoculation into the Listeria Selective Agar medium (LSA, Oxoid CM 856) involving Listeria Selective Supplement (Oxoid SR140) was made by streak plate method. The LSA plates were incubated at 37°C for 48-72 h. After incubation, blackish green-brown colonies 2-3 mm in diameter surrounded by a black zone and having a sunken center that grew in the medium were evaluated as *Listeria* suspected colonies and stocked at the agar slant. For the identification of the isolates, Gram staining, catalase, oxidase, indole, Methyl Red (MR), VP, nitrate reduction, motility, CAMP and carbohydrate fermentation tests were performed (Seeliger andJones,1986; Hitchins, 1992;Harrigan, 998).

***Vibrio* spp. isolation and identification**

Twenty-five g the fish fillet sample was added into225 ml alkaline peptone (Merck, 101800. 0500) medium and homogenized on the Stomacher. It was left to incubation at 35-37ºC for 8 h. Then, it was inoculated into Thiosulphate Citrate Bile Salt Sucrose Agar medium (TCBS, Merck, 110263.0500) by loop and the Petri dishes were left to incubation at 35-37 °C for 18- 24 h. After the incubation, round, yellow, green or blue-green colonies2-3 mm in diameter that grew in the TCBS agar were evaluated as suspicious colonies and stocked in the agar slant. For the identification of the isolated strains, inoculation was made into the Gram staining, oxidase (Bactident Oxidase, Merck), catalase, Triple Sugar Ironmedia, the lactose, sucrose and glucose use, gas and hydrogen sulfide (H_2S) formation, indole, VP and lycine decarboxylase tests were conducted, and the results were evaluated (FDA, 1992).

***Salmonella* spp isolation and identification**

Twenty-five g of each fish fillet sample was measured and homogenized with 225 ml buffered peptone water and incubated at 37 °C for 24 h. Following incubation, 0.1 ml was taken without enrichment and

inoculated into Rappaport Vassiliadis Broth (Oxoid, CM 669) and incubated at 42 °C for 18-24h. After incubation, inoculation was made on the selective solid media of Brilliant Green Agar (Oxoid, CM 263), Hektoen Enteric Agar (Oxoid, CM 419) and Xylose Lysine Deoxycholate Agar (Oxoid, CM 469) by streak plate method and incubated at 37 °C for 18-24 h. Ten of each typical colony that grew in the selective agars were taken and stored in the agar slant. For the identification of the isolates, urea test, acid and gas production from glucose in Triple Sugar Iron Agar, lactose and sucrose use, H_2S formation, lysine decarboxylase, VP and indole tests were conducted and the results were evaluated. For serological analysis, the Salmonella latex test (Oxoid FT 203) was performed on the isolates (Andrews and Hammack, 1995;ISO, 2002).

E. coli O157:H7 isolation and identification

Twenty-five g of each fish fillet sample was measured and homogenized with 225 ml Modified Tryptic Soy Broth (mTS, Merck 1.09205), then incubated at 35-37°C for 18 h. Following enrichment, inoculation was made into cefixime-tellurite (Merck 1.109202) added SMAC agar (Merck 109207) by streak plate method and incubated at 41-42°C for 18-24 h. Sorbitol-negative colorless colonies that grew in the medium were taken and inoculated into 4-methylumbelliferyl-D-glucuronide (Oxoid, BR0071) added MacConkey Agar (Oxoid, CM007) and incubated at 41-42°C for 18 h, then MUG-negative colonies were selected and stocked in the agar slant. Gram staining, indole, MR, VP, citrate, hydrogen sulfide formation, lactose, gas production from glucose, motility and lysine decarboxylase tests were performed on the isolates (Feng and Weagant, 2011; Harrigan,1998).

Detection of histamine

For determining the histamine levels of the trout samples, the Ridascreen Histamine ELISA test kit (R-Biopharm AG, R1601, Darmstadt, Germany) was used. The analysis was made according to the test procedure. The absorbance value was measured in the ELISA reader (Microplate reader, BioTekELx800) at 450 nm in 10 min (Ridascreen R1601, 2014). In the evaluation of the results obtained, Ridasoft Win PC Software was used.

Identification of isolates through the molecular method

Genomic DNA isolation

Genomic DNA extraction in bacteria isolates was conducted by DNA extraction kit (Qiagen-Mericon DNA Bacteria Kit and Mericon DNA Bacteria Plus Kit, Germany) for Gram-negative and Gram-positive bacteria in accordance with the recommendations of the manufacturer.

L. monocytogenes genetic identification

For genomic DNA amplification, the method recommended by Aznar and Alarcon(2003) was used in modified form. The target gene in the identification was selected as hylA. LM1: CCTAAGACGCCAATCGAA and LM2: AAGCGCTTGCAACTGCTC primers were used (Border et al.,1990). For PCR, 25 µl master mix was prepared. PCR buffer (10 mmol/L, Tris-HCI, pH 8.8; 1.5 mmol/L $MgCI_2$, 50 mmol/L KCI, 0.1%Triton X-100), 1 µmol/Lof each primer (IDT, USA), 100 µmol/L of each dNTP (Thermo Scientific, R0181), 1 U Taq DNA Polymerase (Thermo Scientific, EP0402) and 5 µl genomic DNA were used. The thermal conditions applied for the PCR were initial denaturation at 94°C for5 min, 30 cycles of denaturation at 94°Cfor 30 sec, annealing at 50°Cfor 1 min, synthesis (extension) at 72°Cfor 1 minandfinal extension at 72 °Cfor 5 min in the TProfessional Basic Thermocycler (Biometra, Model TProfessional Basic Gradient) device. In order to observe the PCR products, 0.5 X TBE (Tris-Borate-EDTA) was conducted in 1% agarose gel. *L. monocytogenes* (ATCC 7644) was used as positive control and the *hlyA* gene was visualized at 702 bp under UV illumination.

Y. enterocolitica genetic identification

For genomic DNA amplification, the method recommended by Lucero Estrada et al. (2011) was used in modified form. The target genes in the identification were selected as virF (591 bp) and ail (170 bp),and multiplex PCR was applied. virF-F: TCATGGCAGAACAGCAGTCAG virR: ACTCATCTTACCATTAAGAAG and ailF: ACTCGATGATAACTGGGGAG ailR: CCCCCAGTAATCCATAAAGG primers were used (Hussein et al.,2001). For PCR, 25 µl master mix was prepared. PCR buffer x1 (10 mmol/L, Tris-HCI, pH 8.8; 50 mmol/L KCI, 0.1% Triton X-100), 1.5 mM $MgCI_2$, 10 pmol of each primer (IDT, USA), 200 µmol/L of each dNTP (Thermo Scientific, R0181), 1 U Taq DNA Polymerase (Thermo Scientific, EP0402) and 5 µl genomic DNA were used. Thermal conditions were initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94°Cfor 30 sec, annealing at 60 °Cfor 30 sec, synthesis (exten-

sion) at 72 °Cfor 1 minand final extension at 72 °Cfor 4 minas applied in the TProfessional Basic Thermocycler (Biometra, Model TProfessional Basic Gradient) device. In order to observe the PCR products, 0.5 X TBE (Tris-Borate-EDTA) was conducted in 2% agarose gel. As a positivecontrol,*Y. enterocolitica* (ATCC 9610) was used.

*V. cholerae*genetic identification

For genomic DNA amplification, the method suggested by Sheikh et al. (2012) was used in modified form. In the identification, ompW-F: CACCAAGAAGGTGACTTTATTGTG ompW-R: GAACTTATAACCACCCGCG primers were used, and a target gene, ompW (588 bp) was selected (Nandi et al., 2000). For PCR, 25 µl master mix was prepared. PCR buffer x1 (10 mmol/L, Tris-HCI, pH 8.8; 50 mmol/L KCI, 0.1% Triton X-100), 2.5 mM $MgCI_2$, 10 pmolof each primer (IDT, USA), 2,5 mM dNTP (Thermo Scientific, R0181), 1 U Taq DNA Polymerase (Thermo Scientific, EP0402) and 5 µl genomic DNA were used. The thermal conditions were initial denaturation at 94 °Cfor 5 min, 28 cycles of denaturation at 94 °Cfor 30 sec, annealing at 64 °Cfor 30 sec, synthesis (extension) at 72°Cfor 30 secand final extension at 72 °Cfor 6 minas applied in the TProfessional Basic Thermocycler (Biometra, Model TProfessional Basic Gradient) device. In order to observe the PCR products, 0.5 X TBE (Tris-Borate-EDTA) was conducted in 1.5% agarose gel. *V. cholerae* (RSKK 751) was used as a positive control.

E. coli O157:H7 genetic identification

For amplification, the method suggested by Kim et al. (2005) was used in modified form. In the identification, the target genes were selected as stx1 (614 bp) and stx2 (779 bp). stx1-F: ACACTGGATGATCTCAGTGG, stx1-R: CTGAATCCCCCTCCATTATG and stx2-F: CCATGACAACGGACAGCAGTT, stx2-R: CCTGTCAACTGAGCAGCACTTTG primers were used (Fagan et al., 1999).For multiplex PCR,a 25 µl master mix was prepared. PCR buffer (10 mmol/L, Tris-HCI, pH 8.8; 50 mmol/L KCI, 0.1% Triton X-100), 3 mM $MgCI_2$, 1 µM of each primer (IDT, USA), 0.2 mM of each dNTP (Thermo Scientific, R0181), 1 U Taq DNA Polymerase (Thermo Scientific, EP0402) and 4 µl genomic DNA were used. The thermal conditions applied for the PCR were initial denaturation at 94 °Cfor 3 min, 35 cycles of denaturation at 94 °Cfor 20 sec, annealing at 58 °Cfor 40 sec, synthesis (extension) at 72°Cfor 90 secand final extension at 72 °C for 5 min as applied in the TProfessional Basic Thermocycler (Biometra, Model TProfessional Basic Gradient) device. In order to observe the PCR products, 0.5 X TBE (Tris-Borate-EDTA) was conducted in 1.5% agarose gel. *E. coli* (ATCC 43894) was used as a positive control.

Statistical analysis

The statistical analysis of the findings of the study was performed in the SPSS 20 software package by chi-square test.

RESULTS

Microbiological analysis and PCR results of the isolates

The microbiological analysis results of the analyzed fish samples are shown in Table 1 and Table 2. The identification of the *Y. enterocolitica* and *V. cholerae* isolates was performed by biochemical tests, and they were not genetically verified by the PCR analysis. The identification of the *E. coli* O157:H7 isolates was performed by biochemical verification tests, and none were shown by the PCR to produce Shiga toxin. Out of 51 *Listeria* isolates, 28 (54.9%) were verified as *L. monocytogenes. L. monocytogenes* was identified in 9 out of 48 (18.75%) fish samples according to the PCR result (Figure 1).

Table 1. Microbiological quality parameters (in $\log_{10}$ cfu/g) of rainbow trout samples (n: 48)

Microorganism	Mean ± SD	Min.	Max.
TMAB	4.24±0.94	2.60	7.92
TPAB	4.00±0.70	<1	4.70
Total coliform	1.10±1.29	<1	3.08
Fecal coliform	0.17±0.70	<1	3.49
Enterobacteriaceae	1.50±1.44	<1	3.68
*Pseudomonas*spp.	3.05±1.26	<1	4.60
Staphylococcus-Micrococcus	0.28±0.74	<1	2.60
Mold-Yeast	3.82±0.37	3.08	4.82

Table 2. Presence of pathogenic microorganisms in rainbow trout samples

Microorganism	Number of samples (%)	Number of positive samples (%)
Y. enterocolitica	48	18 (37.5%)
L. monocytogenes	48	14 (29.1%)
E. coli O157:H7	48	8 (16.6%)
Vibrio spp.	48	14 (29.1%)
Salmonella spp.	48	Not detected
S. aureus	48	Not detected

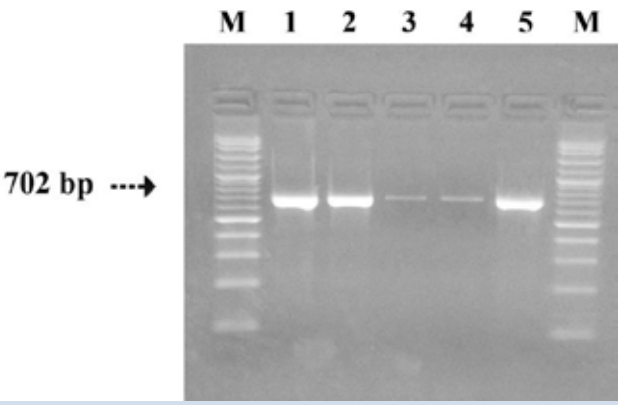


Figure 1. Amplification products obtained from the PCR. Band M :100-bp DNA ladder, Band 1-4: *L. monocytogenes* PCR amplification products, Band 5: Positive control for *L. monocytogenes*

Histamine analysis results

In the histamine analysis performed by the ELISA method, it was detected that the majority of the samples showed a histamine level below 2.5 mg/kg. Eight samples yielded numerical results. The highest histamine level was measured as 10.05 mg/kg. A statistical difference existed between the histamine levels of the samples ($P<0.05$).

DISCUSSION

In this study, microbial quality and histamine levels of fresh rainbow trout were evaluated. The numbers of TMAB, TPAB, Coliform, Fecal coliform, *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus-Micrococcus* and Mold-Yeast were found to be 4.24, 4.00, 1.10, 0.17, 1.50, 3.05, 0.28, and 3.82 $\log_{10}$ cfu/g, respectively. In terms of foodborne pathogens, *Y. enterocolitica, L. monocytogenes, E. coli* O157:H7 and *Vibrio* spp were found to be positive in 18 (37.5%), 14 (29.1%), 8 (16.6%) and 14 (29.1%) samples respectively, whereas none of the samples were positive for *Salmonella* spp and *S. aureus*. Popelka et al. (2016) reported in fillets of rainbow trout the numbers of total viable, psychrotrophic bacteria and *Pseudomonas spp*. as 4.55, 4.55 and 1.28 log cfu.g^{-1} respectively. In our study, the number of psychrotrophic bacteria and *Pseudomonas spp*. is higher than the findings of the researchers. Ucak et al. (2020) reported the numbers of initial total viable, psychrotrophic bacteria, yeast-mold and *Enterobacteriaceae* as 1.543, 1.932, 1.194 and3.390$\log_{10}$ cfu/g respectively. The findings of the researchers are quite low compared to our findings in terms of total viable, total psychrotrophic bacteria and yeast and mold numbers. The number of *Enterobacteriaceae* is higher than our findings. Arslan (2019) reported in fillets of rainbow trout the numbers of initial TMAB, TPAB, *Enterobacteriaceae* and Yeast-Mould as 4.07, 4.36, 2.00 and 2.00 $\log_{10}$ cfu/g respectively. These results are in parallel to our study findings in terms of the number of TMAB and TPAB. The number of *Enterobacteriaceae* was found to be higher than our results. The number of yeast- mold is lower than our findings. Eltholth et al. (2018) reported that the number of positive samples for *E. coli*, *L. monocytogenes*, *Salmonella* spp*, S. aureus* and *V. parahaemolyticus* were 8.0%, 7.7%, 3.3%, 13% and 12.3%, respectively. In a study analyzing different fish species, Eizenberga et al. (2015) reported that the total number of bacteria was 5.58 to 7.84 $\log_{10}$cfu cm^{-2}, 11 out of 20 fish samples were positive for *L. monocytogenes* and all samples were negative for *Salmonella*. In our study, the total number of aerobic bacteria was 4.24 $\log_{10}$ cfu/g, *L. monocytogenes*positive samples were 14 (29.1%), and all samples were negative for *Salmonella*. In a study examining the effect of the traditional marination process on certain features of rainbow trout fillets throughout cold preservation, the initial total number of mesophilic bacteria was determined as 3.9 log and psychrophilic bacteria as 3.5 log in the control group samples (Maktabi et al., 2016). In our study, the total number of mesophilic bacteria and psychrophilic bacteria were determined as 4.24 and 4.00 $\log_{10}$ cfu/g, respectively. The results of our study are inconsistent with the findings of the researches. In a study analyzing different types of fish, Onmaz et al. (2015) reported 5 (5%) *Salmonella* spp and 9 (9%) *S. aureus* infected samples. Another study reported in two of the tilapia fillet samples, coagulase-positive *Staphylococcus* and fecal contamination in the muscles (Junior et al., 2014). Again, consistently with this study, our study detected an average of 0.17 $\log_{10}$ cfu/g fecal coliform but no *S. aureus.* The differences between similar studies may be attributed to seasonal and environmental differences, microbial quality of the water in which the fish were caught, distance between the fishing area and the sales area, contamination that may occur during the transportation and marketing of fish, unsuitable cold preservation conditions and contamination that may occur due to the personnel working in manual processing.

The acceptable level of histamine is specified as 100 ppm in the Turkish Food Codex (TFC) whereas the limit value is identified as 200 ppm (TFC, 2011). According to EU Regulation (EC) No 2073/2005, the recommended histamine level in fish is 100-200 mg/kg (EC, 2005). The presence of biogenic amines above the recommended limit in foodstuff causes

serious health problems and high dosages may even cause fatalities (Akyol et al., 2015). This study was conducted to determine the presence of such compounds that are extremely significant for food quality and constitute a threat to human health, as a result of which it was determined that in all the samples, the histamine levels were below the limit considered as toxic. Emir Çoban and Patır (2008) reported that the average histamine levels in fresh-marketed anchovies, horse mackerels, mackerels, rainbow trouts, goatfish and cyprinus carpio were 24.24, 26.49, 25.81, 12.21, 11.27 and 10.33 ppm, respectively, concluding that the histamine levels did not exceed the recommended limit. Sadeghi et al. (2019)reported that the lowest and highest histamine concentration in canned tuna fish were 2.14±0.17 and 21.69±0.11 mg/100 g of fish respectively.The authorsstated that the amount of histamine in the tuna was below the standard limit (<50 mg histamine/100 g).In another study on canned tuna fish, in a total of 80 fish samples, the average histamine level was found as 10.97±9.86 mg kg^{-1} which complied with the limits permitted in the TFC (Er et al., 2014). Hosseini et al. (2014), in a study where they observed the change in the histamine concentration level during storage in ice for 18 days, reported that they did not detect histamine on days 1 and 3 of storage. As a result of a study analyzing the histamine levels in fresh and canned fish, Evangelista et al.(2016) reported no histamine in fresh tuna, tilapia and rainbow trout samples, but 44.6% of the canned tuna samples involved histamine below the allowed limits. In our study, a total of 48 samples revealed that in 8 samples (16.6%), the histamine levels were above 2.5 mg/kg whereas in 40 samples (83.3%), they were below 2.5 mg/kg. The highest rate was measured as 10.05 ppm. It was determined that the fish samples involved histamine at statistically different rates ($P<0.05$).Our results are highly consistent with the studies conducted by various researchers.

CONCLUSION

In this study, it was determined that fresh fish were contaminated with important foodborne pathogens such as *Y. enterocolitica*, *L. monocytogenes*, *E. coli* O157: H7 and *Vibrio* spp. It was concluded that this situation may pose an important public health hazard, especially due to the consumption of undercooked or raw fish. On the other hand, it has been pleasantly found that the histamine levels were below the specified limits and therefore much lower than the level that may harm human health. It is essential that the manufacturers and consumers are informed about foodborne pathogens, the manufacturers satisfy suitable cold storage conditions at sales points and the consumers are informed about the health risks associated with inadequately cooked products. It is crucial for consumer health to organize training programs for fishing industry personnel about the issues to take into consideration during the fish cleaning process and about cross contamination. As a result, it is suggested that the necessary hygienic precautions are taken at every step of the process from the hunting of the fish until putting it on the market, for the sake of protecting public health against foodborne pathogens and enabling safe food consumption.

ACKNOWLEDGEMENTS

This study was supported by the Coordination Office for Scientific Research Projects, Kafkas University, under project no. 2014-MMF-45.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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