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A Characterization Study of Hot Smoked Rainbow Trout for Each Production Stages

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Abstract **– Chromatographic, chemical, microbiological, physical methods were used to evaluate the effects of processing steps on the quality changes of hot smoked rainbow trout manufactured in a commercial plant. Hot smoked rainbow trout (R1) and cooked rainbow trout (R2) were produced, vacuum-packed, and kept in a refrigerated storage for 21 days. Characterization techniques include proximate analysis, TBA, TVB-N, pH, color, microbiological and biogenic amine analysis by HPLC. TBA, and TVB-N levels increased during storage. Significant differences ($p<0.05$) were observed between R1 and R2 groups. Microbiological counts increased during refrigerated storage. Biogenic amine amount was determined and histamine was the main amine, which ranged between 0.83-15.60 mg/kg for R1 and 1.15-53.65 mg/kg for R2. There were fluctuations of the biogenic amine amounts in hot smoked rainbow trout. Evisceration had no impact on accumulation of biogenic amines. Marination process decreased the amount of histamine in rainbow trout while the histamine concentration in the marination solution increased. Smoking had a reducing effect on biogenic amines accumulation. Total biogenic amine amount of samples were below EU-permitted levels. As a result, the microbiological parameters such as total aerobic bacteria, *Enterobacteriaceae*, *Lactobacillus,* psychotropic bacteria count are the most important parameters for longer shelf life in hot smoked rainbow trout.**

Keywords **– Biogenic Amine, Fish Quality, Smoking, Shelf Life, Rainbow Trout, Histamine.**

I. INTRODUCTION

Fish is an extremely perishable food product. About 27% of the fish caught are consumed fresh, whereas the remaining 73% is processed with food preservation methods such as, salting, freezing, canning, marinating, and smoking [1].

As a conventional method, smoking is used to conserve fish products all over the world. The smoking process has effects on sensory parameters such as flavor, texture, aroma, and color. Smoking procedure is usually performed to lower water activity levels (drying), extend shelf life by the antimicrobial, and antioxidant effects of the phenolic compounds released in the wood smoke. Smoking process is subdivided into two sections considering the temperature of the smoke applied. Cold smoking is performed below 30°C throughout the process to allow some drying as well as preservation by the deposition of smoke components. With hot smoking, this process can be accomplished in various phases, during the process, smoke temperature varies within 40–100°C and core temperature of the fish might rise up to 85°C.

Hot smoked fish is produced from various fish species such as; salmon, eel, herring, anchovy, sardine and particularly rainbow trout with having a substantial economic importance throughout the world, specifically by European customers. Rainbow trout is a high quality product with significant economic importance and nutritional value with being exported to various European countries. In 2015, the total production of fish from aquaculture in Turkey was 240, 334 tons, rainbow trout (*Oncorhynchus mykiss*) being the most widely cultivated fish species (108, 038 t) (Data from administrative register of Minister of Food Agriculture and Livestock of Turkey).

Rainbow trout (*Oncorhynchus mykiss*) is retailed as whole fresh fish stored on ice, vacuum packaged fillets, or processed with hot or cold smoking. Usually, hot smoked fish stored under vacuum packaging (anaerobic conditions) at refrigerated temperature is quite susceptible to deterioration and, based on microbial quality and sensory properties, has a restricted shelf life between 21 days to 28 days at refrigerated temperature [2]. Fish deterioration occurs primarily as a result of microbial, chemical and enzymatic activities, that leads to quality loss and spoilage [3]. The fish micro flora under vacuum packaging is dominated by several gram-positive bacteria, primary lactic acid bacteria with less numbers of other bacteria such as, *Pseudomonas spp., Enterobacteriaceae, Micrococci*, *Enterococci* etc.

Biogenic amines are non-volatile heterocyclic, aliphatic, and alicyclic organic bases with low molecular weight formed in fish by free amino acids decarboxylation, deamination, and transamination of ketones and aldehydes [4]. Biogenic amines such as histamine (HIM), putrescine (PUT), cadaverine (CAD), tyramine [5], tryptamine (TRM) are widely distributed in fish and fish products. Histamine is one of the major risk factor in fish and fish products, which is produced by microbial decarboxylation of histidine in consequence of inappropriate handling of fish during storage or processing. Histamine poisoning is commonly linked to scombroid fish poisoning due to the relevant connection of the illness with spoiled scombroid fish consumption such as mackerel, tuna, skipjack, saury, and bonito. However, non-scombroid fish such as sardines, pilchards, anchovies, herring, marlin, mahi-mahii and bluefish have also been involved in incidents of this illness [6].

Amines accumulation is related to bacterial spoilage, which is determined at low levels in fresh fish flesh. The quality of the raw material, time, storage, and processing conditions are crucial to the biogenic amine accumulation. There are many studies conducted about marine fish; however, there are only few numbers of studies about fresh water fish. Restricted data is available in the literature on effects of processing stages, and processing conditions in a fish processing plant therefore, the objective of the current

study was to investigate the effect of processing steps on shelf life of vacuum-packaged, hot smoked rainbow trout fillets stored at 4 ± 0.5°C using chemical, microbiological, and biogenic amine analysis.

II. Materials and Methods

Raw Material

Rainbow trout (*Onchorynchous mykiss*), of average weight 300 g and average length 350 mm, were obtained from a Turkish aquaculture farm located in Sivas Province. Following the harvest, rainbow trout were transferred to the fish processing plant at North Point Black sea Fish Co., Samsun, Turkey, in a cooled truck in order to keep cold chain. Fresh rainbow trout were processed into hot smoked rainbow trout at the day of arrival. The flow diagram of the fish production is given in figure A. The fish were instantly eviscerated and washed with tap water. Rainbow trout samples were immersed in containers with a brine at a ratio of 1:1 (w/w) for 24 h at 4°C. The brine had 8% (w/v) NaCl.

After brining, fish were drained and smoked at 72° C by using oak sawdust for 2 hours and 16 minutes (Kerres Fishsmoker JS H-2850). Control group was cooked at 72° C for 2 hours 16 minutes. Hot smoked fish and cooked fish were cooled in cold room, filleted, and vacuum packaged. Final products were blast frozen at -35°C and brought to laboratory in polystyrene foam Fish are blast frozen in order to prevent quality loss and deterioration during the transportation to markets. After 0, 7, 14 and 21 days at 4°C, three randomly chosen fish were analyzed in triplicate.

Proximate Analysis

Protein, ash and moisture content was determined triplicate for hot smoked rainbow trout. Protein content was analyzed according to the Kjeldahl Method [7]. Crude protein was calculated by multiplying total Kjeldahl nitrogen by 6.25. Samples were burned at 550°C for 4 h till the color of samples turned ash grey and ash content was determined from the weight difference of the sample. Moisture content was calculated gravimetrically after being oven-dried at 105°C for 8 h until constant weight was gained.

Raw material arrival (max 4°C)*
↓
Calibration/Grading
↓
Cleaning/Gutting
↓
Brining
↓
1st brine solution*
2nd brine solution*
↓
Draining
↓
Smoking (Interior temperature max 72°C)
↓
Resting in cold room
↓
Filleting, beheading, skinning
↓
Vacuum packaging*

Fig. A Flow Chart of Production
*is the processing phases where the samples are taken

Chemical Analysis

pH value was measured in homogenized slurry of 10 g of fish sample in 100 mL of distilled water using a pH meter (Eutech, Cyberscan pc-510). Total volatile basic nitrogen (TVB-N) was estimated with the distillation equipment (Buchi Distillation Unit K-350) according to the method [8]. Thiobarbituric acid (TBA) was determined as reported by [5] and lipid oxidation products were expressed as malondialdehyde (MA, mg/kg) equivalents. Salt content was calculated using Mohr Method [9].

Microbiological Analysis

Hot smoked rainbow trout samples (25g) were mashed and transferred aseptically to a Stomacher bag containing 225 ml of 0.85% normal sterile saline and homogenized for 180 seconds using a Stomacher (Aes Smasher Stomacher, France). Total viable bacteria counts, *Enterobacteriaceae*, psychrotropic and lactic acid bacteria were determined. Plate count agar (PCA) was used for the enumeration of total viable count and psychrotropic bacteria. Plates were incubated 2 days at 30°C and 7 days at 4°C respectively. For total *Enterobacteriaceae* and lactic acid bacteria, violet red bile agar (VRBA) and MRS agar were prepared and pour plate method was used. Plates were incubated for 5 days at 30°C for lactic acid bacteria count (MRS agar) and 24 h (VRBA) at 37°C for *Enterobacteriaceae* count. Microbiological data were expressed as a logarithm of the number of colony forming units (log cfu) per gram of sample.

Determination of Biogenic Amines

Biogenic amine content of the samples was determined according to the method [10]. Two grams of samples were taken and homogenized with 10 ml of 0.4 M perchloric acid via Ultra-turrax blender (IKA, T25, Germany). Centrifugation was performed at 3000 rpm for 10 min. Supernatant was collected and the volume was brought to 25 ml with 0.4 M perchloric acid. The solution was filtered through Whatman number 1 filter paper. Derivatization was done with 2mL of dansyl chloride. Following the derivatization, samples and standarts were filtered through 0.45µm pvdf filters (Isolab). 20-µL of filtered samples were injected into a Shimadzu HPLC system equipped with a pump (Shimadzu LC-20AT), photodiode array detector (Shimadzu SPD–M20A), column oven (Shimadzu CTO-10AS VP) set at 40°C, auto sampler (Shimadzu SIL-10A), and data station (Shimadzu CBM-20A). Chromatographic separation was performed using a gradient elution of Ammonium acetate (0.1 M, solvent A) and acetonitrile (100%, solvent B) the gradient-elution program began at 50% solvent B and ended at 90% solvent B in 25 min. Equilibration of the system was done for 10 minutes before the following analysis. The analyses were carried out at a flow rate 1 mL/min with an ODS-2 column (15 cm × 4.6 mm, 4 µm, Spherisorb, Sigma Aldrich). The quantitative determinations were carried out with internal standard (1.7-diaminoheptane) method, by using peak areas. Biogenic

amine contents were stated as mg/kg. The limit of detection (LOD) was between 0.005-0.050 mg/kg and limits of quantification (LOQ) were between 0.010 - 0.100 mg/kg for different biogenic amines.

Color Measurements

Instrumental color analyses were performed using a Hunterlab ColorFlex EZ-45/0 spectrophotometer (Reston, VA, USA). The measurements were done directly on the flesh of hot smoked rainbow trout fillets after opening the vacuum packages. Four measurements were performed on each fillet for three times. Hunter L (lightness; 100 = white, 0 = black), a (redness; +, red; −, green), b (yellowness; +, yellow; −, blue) values were measured.

Statistical Analysis

SPSS Version 21 for Macintosh (SPSS Inc., 119 Chicago, IL, USA, 2012) one-way analysis of variance [11] and Duncan was used for all of the statistical analyses. $P<0.05$ value was used to identify significant differences for the rainbow trout samples.

III. Results and Discussion

Proximate Composition

The mean (± SD) compositional contents of moisture, protein, salt and ash (g/100 g fish muscle) in the hot smoked rainbow trout and fresh rainbow fillet analyzed were 68.82 ± 1.92, 23.86 ± 0.60, 1.8 ± 0.34, 2.55 ± 0.59, and 72.62 ± 2.54, 19.78 ± 0.64, 0.4 ± 0.28, 1.58 ± 0.15 respectively.

pH value and moisture content of rainbow trout flesh decreased, while ash, protein, and salt amount increased at the end of the marination, and smoking process. Protein content increment in hot smoked rainbow trout could be linked with the salting and smoking process. Previously, reference [12] reported the proximate composition of hot smoked rainbow fish, which was smoked at 2 hours at 80°C contained 66.70% moisture, 22.06% protein, 3.50% ash. It was stated that moisture, protein, and ash contents of raw and smoked rainbow trout were 67.00, 21.23, 1.48, and 61.14, 26.53, 1.71, respectively [13]. The variations of the proximate composition of smoked fish were caused by different factors, such as smoking methods (hot or cold), smoking time, the state of nutrition, fish size, fish reproduction cycle, salting method (dry or wet) and salt concentration.

Thiobarbituric Acid Reactive Substances (TVB-N value)

TVB-N values of fresh, cooked, and hot smoked rainbow trout fillets with vacuum packaging can be seen in Table I.

The TVB-N in fish species is mainly formed by trimethylamine, dimethylamine, and ammonia and is linked generally to deterioration of fish quality since both trimethylamine and ammonia are produced by spoilage microorganisms [14]. The levels of 30–35 mg/100 g muscle have been recommended as limit of acceptability for various fish products including smoked fish [15]. TVB-N value of fresh rainbow trout samples was found to be 14,45 mg/100 g which was higher than the value reported by reference [16]. Following the smoking procedure, TVB-N level increased up to 16.56 ± 0.34 mg/100g. This result may indicate that autolytic process may form volatile amine compounds.

At the end of the 21 days of storage period, TVB-N values increased to 19.74 ± 0.38 21.89 ± 0.77 mg/100 g, for hot smoked rainbow trout and cooked rainbow trout, respectively.

The TVB-N value in fish varies according to fish species, age, region, and sex of fish [17]. Freshwater fish muscle has 10-20 mg of TVB-N/100g after harvesting and the initial TVB-N value in hot smoked rainbow trout was 16.56 mg/100g and reached level of 19.74 mg/100g after 21 days of storage at 4 °C. Smoking process slightly influenced the TVB-N level of smoked rainbow trout. TVB-N value of cooked rainbow trout was higher than smoked products and the level of TVB-N increased gradually. From the start of the storage till the 7th day of storage, there were no significant differences ($p > 0.05$) among all of the samples. No sample reached the limit level of 35 mg/100 g flesh. The value of 25 mg/100g flesh was recommended as the highest admissible TVB-N level for trout [3]. Our findings were below this value. The initial TVB-N value of hot smoked rainbow trout is slightly higher than those reported by references [18], [19] and approximately similar to the values reported by references [20], [21].

Table I. The results of chemical analysis of hot smoked rainbow trout fillets with vacuum packaging and cooked samples (Mean ± SD)

Storage duration(d)	R1	R2
TBA value		
0	1.65 ± 0.11^{a}	4.39 ± 0.21^{a}
7	2.68 ± 0.30^{b}	8.38 ± 0.52^{b}
14	3.38 ± 0.31^{c}	8.55 ± 0.19^{b}
21	3.65 ± 0.24^{d}	9.41 ± 0.15^{c}
TVB-N value		
0	16.56 ± 0.34^{a}	21.89 ± 1.64^{a}
7	16.42 ± 1.69^{a}	22.41 ± 1.41^{a}
14	18.97 ± 0.75^{b}	22.48 ± 0.09^{a}
21	19.74 ± 0.38^{b}	21.89 ± 0.77^{a}
pH value		
0	6.54 ± 0.06^{a}	6.57 ± 0.02^{a}
7	6.66 ± 0.01^{b}	6.58 ± 0.05^{a}
14	6.61 ± 0.02^{b}	6.70 ± 0.04^{b}
21	6.61 ± 0.01^{b}	6.76 ± 0.05^{b}

Values are means ± SD of three replicates.

TBA Value

TBA is a second breakdown product of lipid oxidation and commonly used as an indicator of lipid oxidation. The lipid oxidation is evidenced by measuring malondialdehydes [22], which are the initial reaction products of polyunsaturated fatty acids with oxygen [23]. Malondialdehyde [22] is formed by multiple scissions of cyclic internal hydroperoxides originating from fatty acids with three or more double bonds during lipid oxidation. MDA reacts with TBA at low pH and high temperature, resulting in formation of pink color complex with an absorption maximum at 532nm. This fast and cheap analysis is suitable for the analysis of lipid oxidation in products containing fish oil without prior extraction of lipids.

The acceptability limit of TBA value of 5 mg MA/Kg of

tissue was proposed to indicate good quality for Pacific salmon (*Onchorhynchus nerka*). In the present study, the TBA value of fresh rainbow trout was 1.25 mg MDA/kg (Table I). Following the brining and smoking processes, sharp increases in the TBA value of final product reached to level of 1.65 mg MDA/kg. The initial TBA value in the cooked rainbow trout was 4.39 mg MDA/kg which increased to 9.41 MDA/kg at the end of 21 days of storage at 4°C. It can be evaluated that smoking had a positive effect on the TBA formation compared to fresh rainbow trout. TBA values were higher in cooked rainbow trout than smoked rainbow trout and showed increment during storage period. Smoking delays microbiological and oxidative changes. Consequently, TBA value was inadequate for evaluating the quality of hot smoked rainbow trout, which is in agreement with the results of [24], [25], [18]. The initial TBA value in hot smoked rainbow trout was 2.43 mg MDA/kg which was higher than the values reported by [21, 26] and similar to [27]. In a previous study done by [28], initial TBA values of cultured rainbow trout were 7.65 mg MDA/kg for ungutted trout and 10.43 mg MDA/kg for filleted trout. These values reached to 16.21 and 19.41 respectively.

Higher TBA value can be related to the storage conditions before processing the fish. TBA value increased to 3.38 mg MDA/kg at the 14th day of storage and decreased to 1.65 mg MDA/kg at the 21th day of storage at 4°C. This fluctuation can be a result of several interactions between MDA and proteins, glucose, amino acids, and other fish constituents during storage [23].

pH Value

Table I shows changes in pH values of hot smoked rainbow trout fillets. Initially, the pH of the fresh rainbow fillets was 6.56. It was similar to the value with that reported by reference [29]. Significant differences were seen during the first 2 weeks of storage. ($P<0.05$) Initial pH value of hot smoked rainbow trout fillets was 6.54 and increased to 6.61 at the end of storage. The decrease of pH value at 14 days of storage might be related to the decomposition of glycogen, creatine phosphate, and ATP, dissolution of CO_2 in fish muscle, while the following increase is because of the production of volatile basic materials as a result of protein degradation by either microbial or endogenous enzymes [30]. Higher pH values as well as TVB-N values were observed in cooked rainbow trout samples. In a study done by reference [31] pH values were slightly reduced in fish flesh with the carbonic acid dissociation. Reference [18] reported the pH value of trout in the range of 6.26-6.74 during refrigerated storage. Similar findings were stated by reference [19].

Microbiological Analysis

The initial microbial load of fresh rainbow trout was 3.45 log cfu/g while this value in freshwater fish might show difference as a result of various conditions such as transportation and water temperature. In compliance with the current literature data, total viable counts of various freshwater fish species ranges between 2 and 6 log cfu/g [28]. The initial total count of hot smoked rainbow trout was 3.13 log cfu/g which is slightly higher than reference [18]. The initial total count showed a load ranging between 2.88-4.2 log cfu/g in hot smoked vacuum packaged rainbow trout [27], [32]. Total viable bacteria counts (TVC) increased with the time of storage (Figure-B and C). On weeks 1 and 3, total viable count of vacuum packaged hot smoked rainbow trout was 3.25 log/cfu and 6.00 log/cfu, respectively. Reference [33] reported 7.04 log cfu/g microorganisms in iced trout fillets stored for 20 days. Total viable bacteria number exceeded 6 log cfu/g at the end of storage time, which is considered near to the maximum level of acceptability (7 log cfu/g) for smoked fish products. As seen in figure C, for cooked rainbow trout, 7.60 log cfu/g was reached at the end of 21 days of storage and the tendency to rise had begun at 15 days of storage. Bacterial growth in our study was inhibited by smoking process which might be due to bacteriostatic or bactericidal effect resulted from oak saw dust smoke.

Lactic acid bacteria count increased along with the storage period (Figure B and C). LAB was reported as part of natural micro flora of fresh rainbow trout fillets [21], [34]. In our study, the initial counts of LAB for hot smoked rainbow trout was 2.5 log cfu/g and increased to 4.26 log cfu/g at the end of 21 days of storage at 4°C. No significant differences were observed in LAB values of cooked and smoked samples. From the presence of LAB in the finished products, it can be concluded that these species were capable of surviving cooking and smoking. At the end of storage at various cooled temperatures, LAB were found in numbers ranging from 6 to 8 log cfu/g [35]. Similar result was reported by reference [21].

LAB seem to form the main micro flora of the vacuum-packed smoked fish at the end of the storage period generally, since they are well adapted to the conditions prevalent in these products: low pH, vacuum packaging, higher salt content, refrigerated storage [36], [37].

Enterobacteriaceae were also found to be part of the spoilage micro flora of vacuum packaged smoked fish. In hot smoked rainbow fillets, the initial *Enterobacteriaceae* count was low as 0.3 log cfu/g and reached to 5.27 log cfu/g at the end of storage time. Reference [18] reported higher (0.93 log cfu/g) initial *Enterobacteriaceae* count for rainbow trout stored at 2 ± 1°C. Reference [38] reported 2.0 log cfu/g *Enterobacteriaceae* for fresh rainbow trout. 6.6 log cfu/g was observed in vacuum packaged rainbow trout after 15 days of storage at refrigeration temperature [27]. The initial total number of *Enterobacteriaceae* on the hot smoked rainbow trout and cooked rainbow trout was lower than 1 log cfu/g and raised to 5.27 log cfu/g and 5.68 log cfu/g. Smoking reduced the *Enterobacteriaceae* count throughout the storage duration.

At refrigerated temperatures, gram-negative psychotropic bacteria (PSI) are the main group of microorganisms, causing spoilage of aerobically stored fresh and processed fish. Initial psychrotrophic bacteria of hot smoked rainbow trout and cooked rainbow trout was below 1 log cfu/g and increased with a storage period until the end of storage time. Smoking had an antimicrobial effect on PSI bacteria, as it can be seen from the figure B.

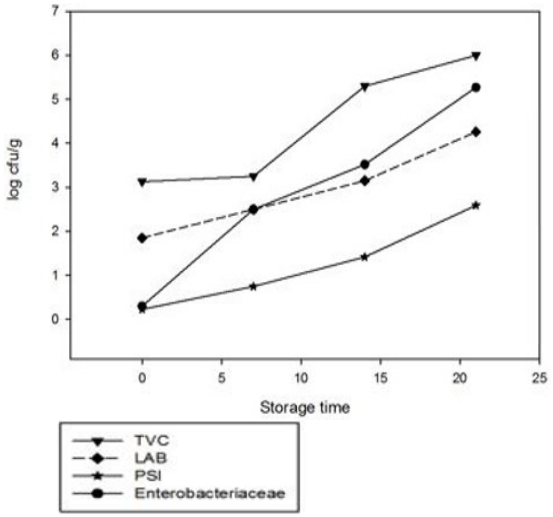

Fig. B. Microbiological results of R1 during 21 days of storage at 4°C

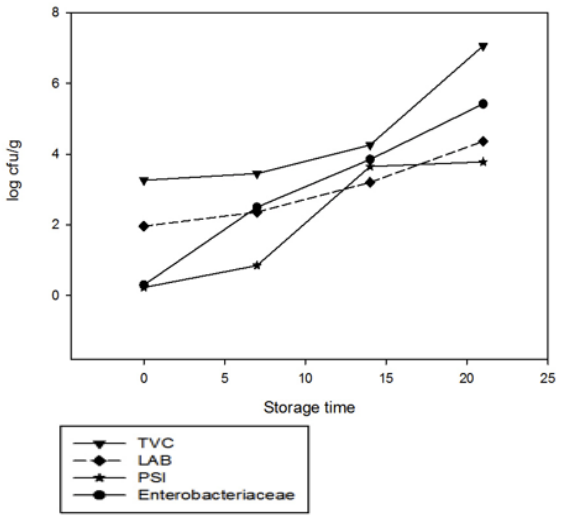

Fig. C. Microbiological results of R2 during 21 days of storage at 4°C

Biogenic Amines

The contents of biogenic amines in fresh, brined, smoked rainbow trout, cooked rainbow trout and brine solutions are summarized in Table II and III. Histamine, putrescine**,** cadaverine, tyramine, tryptamine and were main amines found trout fillets.

The first and second brine solutions used in the processing phase had higher histamine levels than the samples. Special recipe with brine used in factory could contribute this. As shown in Table III, among the tested brine solutions histamine represented the highest value in the range of 6.72–38.09 mg/kg followed by cadaverine (0.00–32.17 mg/kg). Initial putrescine amount of trout was found as 1.76 mg/kg, while cadaverine was 5.62 mg/kg at raw rainbow trout. After brining step, putrescine level increased to 3.26 mg/kg. The initial value of tyramine was 4.57 mg/kg while tryptamine was not detected in raw rainbow trout samples. Tyramine showed fluctuations between 0-8.81 mg/kg. These results are in agreement with reference [18].

Initial concentrations of five BAs were very low in all samples indicates the high quality of raw rainbow trout used in this study. Biogenic amines exist either as a natural component of fresh fish or as a result of spoilage and bacterial growth [39]. 5 mg/kg putrescine is considered a level of as an early notice of autolytic degradation in ice-stored trout muscle [40]. In our samples this level was not obtained during the 21 day storage at 4°C.

When fish is captured, rapid chilling below 10°C is recommended in order to inhibit biogenic amine forming bacteria, for longer storage temperatures below 0°C is needed. Decarboxylase enzymes continue producing histamine and other biogenic amines even though the storage temperature is decreased below refrigerated temperature, as lower temperature disrupt bacterial growth not the enzyme activity. It was verified that histamine production was 31 times higher at 10°C, and 4 times higher at 4°C compared to 0°C in tuna. They also showed that icing temperature retarded the histamine formation [41]. Histamine, a toxic amine and precursor of scombroid fish poisoning, was detected in range between 0.83-15.60 mg/kg in hot smoked rainbow trout fillets. According to the EU Regulation No 2073/2005, only histamine levels are limited for fishery products at 200 mg/kg for fresh fish and up to 400 mg/kg for fish products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine. FDA considers histamine a danger to health if it is higher than 50mg/kg. During 21 days of storage at 4°C biogenic amines amount in our samples was lower than this level. Reference [39] reported similar results. Reference [18] stated that histamine content of chilled rainbow trout was 2.9 mg/kg at 0 day and reached to maximum level of 8.1 mg/kg at 20 day. Reference [11] reported 0.4 mg/kg histamine in rainbow trout stored at 3°C. Histamine formation in another study was detected at very low concentration (1.61 mg/kg) in rainbow trout during 18 days of ice storage [42]. High levels of histamine were reported in various smoked fish products, which exceed either the EU-permitted or FDA levels [6], [43]. However, reference [6] reported that sterilization of the product and denaturation of the enzymes can be provided by means of smoking process. This treatment also helps preservation of the product to a certain degree without destroying the histamine already formed within the product. Moreover, misuse of time and temperature parameters in preparation of raw material may result in formation of histamine during hot smoking procedure. It can be concluded that minimal loads of *Enterobacteriaceae* (<6logCFU/g) and decreased histidine content in live rainbow trout may lead to a lower level formation of histamine within the filleted rainbow trout samples. Quality of the fresh fish may be identified by histamine content in accordance with HACCP application [44]. On the other hand, in a previous study by [39] showed that histamine is formed only in the final stage of storage in rainbow trout. This suggests that using histamine as a freshness indicator is not meaningful for trout samples. Initial putrescine amount of hot smoked trout was found as 3.26 mg/kg, while cadaverine value was 4.19 mg/kg at the beginning of storage. A consistent increase in putrescine and cadaverine amount of vacuum-packaged trout fillets were reported during cold storage.

Table II. The results of biogenic amines of hot smoked rainbow trout with vacuum packaging

Storage days	Sample code	Histamine (HIS)	Putrescine (PUT)	Cadaverine (CAD)	Tyramine (TYR)	Tryptamine (TRP)
0	R1	11.00 ± 4.13^{b}	3.26 ± 5.64^{a}	4.19 ± 3.76^{a}	5.31 ± 3.50^{b}	14.47 ± 17.70^{a}
	R2	18.26 ± 30.65^{a}	22.76 ± 39.43^{b}	ND	6.65 ± 3.54^{a}	ND
7	R1	1.80 ± 0.23^{a}	ND	ND	1.33 ± 0.07^{a}	1.84 ± 2.25^{a}
	R2	11.71 ± 20.28^{a}	ND	16.78 ± 4.63^{ab}	13.87 ± 3.82^{ab}	18.66 ± 32.33^{a}
14	R1	1.77 ± 1.45^{a}	1.74 ± 2.83^{a}	6.12 ± 9.36^{a}	1.55 ± 1.81^{ab}	2.99 ± 2.11^{a}
	R2	ND	54.93 ± 57.74^{b}	28.42 ± 27.12^{b}	12.92 ± 4.97^{ab}	ND
21	R1	1.33 ± 0.76^{a}	ND	1.03 ± 0.99^{a}	5.17 ± 1.64^{b}	ND
	R2	13.66 ± 23.65^{a}	9.34 ± 16.18^{b}	17.03 ± 3.28^{ab}	17.40 ± 2.04^{b}	18.44 ± 31.94^{a}

Table III. The results of biogenic amines in processing phases

Samples	HIS	PUT	CAD	TYR	TRP
Fresh rainbow trout	1.57 ± 2.73^{a}	1.76 ± 3.05^{ab}	5.62 ± 3.73^{a}	4.57 ± 1.54^{a}	ND
1st brine solution	3.36 ± 5.32^{b}	ND	11.66 ±17.82^{a}	ND	1.84 ± 2.26^{a}
2nd brine solution	13.54 ± 7.09^{c}	4.39 ± 1.94^{b}	0.65 ± 1.13^{a}	3.07 ± 5.31^{a}	3.02 ± 2.77^{a}
After brining step (before smoking)	4.70 ± 0.90^{b}	0.71 ± 0.05^{a}	6.52 ± 2.56^{a}	1.15 ± 1.40^{a}	ND

[11]. The initial amount of putrescine was found between 0.42-0.88 mg/kg in different studies [42]. Tyramine was present throughout the storage period of hot smoked rainbow trout, and reached the maximum concentration of 5.17 ± 1.64 mg/kg. Results of cooked rainbow trout (R2) are given in table II. Total biogenic amine level of the cooked samples was higher than smoked samples. It can be due to the inhibitory effect of smoke components resulting in less microbial load. Histamine concentration in cooked samples was lower than limit values and this can be explained by the lack of amino acids in freshwater species for the decarboxylation or the inhibition of some strains of *Enterobacteriaceae* species. Each biogenic amine determined in our study seem to be too low to reach toxicological levels (> 50−100 mg/kg for histamine by FDA >100−800 mg/kg for tyramine) Various factors such as temperature, fish species, packaging type, processing phases and usage of antibacterial substances affect the type and amount of biogenic amines produced during storage.

Color Analysis

The color of smoked fish product is also reflected by different aspect of main importance from a consumer perspective. Quality of smoked rainbow trout meat is relatively identified through its color, which in turn is influenced by composition and quantity of smoke deposits, the carotenoid pigment content and their interactions with tissue components. The characteristic color of smoked fish is due to Maillard reactions between carbonyl and free amino groups. In smoking this is a combination of temperature and the freshness of the raw material, as spoilage will lead to the release of these compounds. Color values of all samples are given in table IV. a and b values of all samples decreased at the end of storage time. Significant differences were observed in cooked samples during 21 days of storage at 4°C. Smoking led to reddish color in final product which is proved by the difference in a value of smoked and cooked rainbow trout. Reference [45] found similar b results for vacuum packaged hot smoked rainbow trout. Higher L values and lower b values in vacuum packaged hot smoked rainbow trout [19]. L value was observed between 72.89-67.60 in rainbow trout during 7 weeks of storage [45].

Table IV. Changes in colour analysis of hot smoked rainbow trout fillets with vacuum packaging and cooked rainbow trout fillets with vacuum packaging (Mean ± SD of three replicates)

Storage time (d)	R1	R2	
0	63.17±3.18^{a}	67.16±2.65a	**L**
7	61.99±4.60^{a}	69.43±0.87a	
14	60.79±1.38^{a}	69.52±2.01a	
21	64.77±1.63^{a}	68.78±1.14a	
0	10.85±0.89^{a}	5.44±0.93^{b}	**a**
7	10.28±1.81^{a}	3.63±1.39^{ab}	
14	11.64±0.24^{a}	2.25±0.04^{a}	
21	10.49±0.95^{a}	4.46±0.88^{b}	
0	21.16±1.97^{b}	24.17±2.04^{b}	**b**
7	21.16±1.05^{a}	21.67±1.09^{ab}	
14	21.70±0.78^{a}	21.13±0.76^{a}	
21	22.13±0.70^{ab}	21.84±0.85^{ab}	

IV. Conclusion

The quality of smoked fish is affected by raw material, salting method, brining concentration, condition processing, composition of smoke, smoking method, smoke agents storage conditions. Raw material of the smoked fish used in this study was in good quality indicating low microbial load with the low TBA, TVB-N value. Smoking increased TBA value by the prooxidative effect of sodium chloride on fish lipids. TVB-N value rose significantly ($p<0.05$) with the smoking process, and continued this tendency throughout 21 days of storage at refrigerated temperature. The biogenic amine content in vacuum packaged hot smoked rainbow trout, cooked rainbow trout and rainbow trout flesh were lower than the allowed levels of FDA and EU. During the storage period, fluctuations were observed in the levels of biogenic amines.

21 days of storage at 4°C is recommended for the vacuum packaged hot smoked rainbow trout upon the chemical and microbiological quality.

This work showed that microbiology parameters are the main indicator of the shelf life of hot smoked rainbow trout therefore inhibition of the growth of microorganisms should be the future aim of this study.

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References

[1] H. H. Huss, A. Reilly and P. K. Ben Embarek. (2000). Prevention and control of hazards in seafood. *Food Control*, 11, 149-156.

[2] A. Stolyhwo and Z.E. Sikorski. (2005). Polycyclic aromatic hydrocarbons in smoked fish - a critical review. *Food Chemistry*, 91, 303-311.

[3] S. Arashisar, O. Hisar, M. Kaya and T. Yanik. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (Oncorynchus mykiss) fillets. *International Journal of Food Microbiology*, 97, 209-214.

[4] A. R. Shalaby. (1996). Significance of biogenic amines to food safety and human health. *Food Research International*, 29, 675-690.

[5] B. Tarladgis, B. Watts, M. Younathan and L. Dugan, Jr. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society*, 37, 44-48.

[6] L. Lehane and J. Olley. (2000). Histamine fish poisoning revisited. *International Journal of Food Microbiology*, 58, 1-37.

[7] Aoac, *Official methods of analysis (17th ed.)*, Association of official analytical chemists, Washington, DC.

[8] N. Antonacopoulos and W. Vyncke. (1989). Determination of Volatile Basic Nitrogen in Fish - a 3rd Collaborative Study by the West - European - Fish - Technologists Association (Wefta). *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 189, 309-316.

[9] T. Altug, Demirag, K., Kurtcan, U., Icibal, N., *Food Quality Control*, Aegean University Engineering Faculty Press, Bornova, Izmir

[10] S. Eerola, R. Maijala, A. X. R. Sagues, M. Salminen and T. Hirvi. (1996). Biogenic amines in dry sausages as affected by starter culture and contaminant amine-positive Lactobacillus. *Journal of Food Science*, 61, 1243-1246.

[11] M. Krizek, F. Vacha, P. Vejsada and T. Pelikanova. (2011). Formation of biogenic amines in fillets and minced flesh of three freshwater fish species stored at 3 degrees C and 15 degrees C. *Acta Veterinaria Brno*, 80, 365-372.

[12] S. Y. Tosun and O. Ozden. (2014). Survey of Inhibition of Listeria Monocytogenes in Hot-Smoked Rainbow Trout Fillets for Food Safety. *Journal of Food Processing and Preservation*, 38, 338-346.

[13] B. Tokur. (2007). The effect of different cooking methods on proximate composition and lipid quality of rainbow trout (Oncorhynchus mykiss). *International Journal of Food Science and Technology*, 42, 874-879.

[14] I. J. Clucas. (1982). Fish handling, preservation and processing in the tropics: part 2. *London: Tropical Development and Research Institute*.

[15] D. P., *Shelf life evaluation of foods*, Aspen Publishing Inc., Gaithersburg, MD, USA

[16] S. R. Javadian, M. Rezaei, M. Soltani, M. Kazemian and R. Pourgholam. (2013). Effects of Thawing Methods on Chemical, Biochemical, and Microbial Quality of Frozen Whole Rainbow Trout (Oncorhynchus mykiss). *Journal of Aquatic Food Product Technology*, 22, 168-177.

[17] B. Ersoy, E. Aksan and A. Ozeren. (2008). The effect of thawing methods on the quality of eels (Anguilla anguilla). *Food Chemistry*, 111, 377-380.

[18] F. Ozogul, B. Kus and E. Kuley. (2013). The impact of strawflower and mistletoe extract on quality properties of rainbow trout fillets. *International Journal of Food Science and Technology*, 48, 2228-2238.

[19 S. Cakli, B. Kilinc, T. Dincer and S. Tolasa. (2006). Comparison of the shelf lifes of map and vacuum packaged hot smoked rainbow trout (Onchoryncus mykiss). *European Food Research and Technology*, 224, 19-26.

[20] N. Kolsarici and O. Ozkaya. (1998). Effect of smoking methods on shelf-life of rainbow trout (Salmo gairdneri). *Turkish Journal of Veterinary and Animal Sciences*, 22, 273-284.

[21] N. Erkan. (2012). The Effect of Thyme and Garlic Oil on the Preservation of Vacuum-Packaged Hot Smoked Rainbow Trout (Oncorhynchus mykiss). *Food and Bioprocess Technology*, 5, 1246-1254.

[22] M. Abuznaid, A. S. Sallam, I. Hamdan, M. Al-Hussaini and A. Bani-Jaber. (2008). Diclofenac-bismuth complex: Synthesis, physicochemical, and biological evaluation. *Drug Dev Ind Pharm*, 34, 434-444.

[23] S. P. Aubourg. (1993). Interaction of Malondialdehyde with Biological Molecules - New Trends About Reactivity and Significance - Review. *International Journal of Food Science and Technology*, 28, 323-335.

[24] N. Pyrgotou, V. Giatrakou, A. Ntzimani and I. N. Savvaidis. (2010). Quality assessment of salted, modified atmosphere packaged rainbow trout under treatment with oregano essential oil. *Journal of Food Science*, 75, 406-411.

[25] M. Rezaei and S. F. Hosseini. (2008). Quality assessment of farmed rainbow trout (Oncorhynchus mykiss) during chilled atorage. *Journal of Food Science*, 73, 93-96.

[26] O. E. Coban and B. Patir. (2013). Antimicrobial and antioxidant effects of clove oil on sliced smoked Oncorhynchus mykiss. *J. Verbr. Lebensm.*, 8, 195-199.

[27] A. Nerantzaki, A. Tsiotsias, E. K. Paleologos, I. N. Savvaidis, E. Bezirtzoglou and M. G. Kontominas. (2005). Effects of ozonation on microbiological, chemical and sensory attributes of vacuum-packaged rainbow trout stored at 4 +/- 0.5 degrees C. *European Food Research and Technology*, 221, 675-683.

[28] S. Chytiri, I. Chouliara, I. N. Savvaidis and M. G. Kontominas. (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology*, 21, 157-165.

[29] M. G. Volpe, F. Siano, M. Paolucci, A. Sacco, A. Sorrentino, M. Malinconico and E. Varricchio. (2015). Active edible coating effectiveness in shelf-life enhancement of trout (Oncorhyn--chusmykiss) fillets. *Lwt-Food Science and Technology*, 60, 615-622.

[30] C. Ruiz-Capillas and A. Moral. (2001). Residual effect of CO2 on hake (Merluccius merluccius L.) stored in modified and controlled atmospheres. *European Food Research and Technology*, 212, 413-420.

[31] M. Sivertsvik, J. T. Rosnes and G. H. Kleiberg. (2003). Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (Salmo salar) fil-

-lets. *Journal of Food Science*, 68, 1467-1472.
[32] A. A. Khanipour and N. Mirzakhani. (2013). Effect of different packaging methods on shelf life of hot smoked rainbow trout (Oncorhynchus mykiss) during storage at 0-2 degrees C. *Iranian Journal of Fisheries Sciences*, 12, 620-628.
[33] M. Rezaei, S. F. Hosseini, H. E. Langrudi, R. Safari and S. V. Hosseini. (2008). Effect of delayed icing on quality changes of iced rainbow trout (Onchorynchus mykiss). *Food Chemistry*, 106, 1161-1165.
[34] S. F. Mexis, E. Chouliara and M. G. Kontominas. (2009). Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 degrees C. *Food Microbiology*, 26, 598-605.
[35] W. Zorn, E. Greuel, J. Kramer. (1993). Beurteilung des Hygienestatusgeräucherter, vakuumverpackter Forellenfillets. *Arch. Lebensmittelhyg*, 44, 81-104.
[36] T. Shimasaki, K. Miake, Y. Tsukamasa, M. Sugiyama, Y. Minegishi and H. Shinano. (1994). Effect of Water Activity and Storage-Temperature on the Quality and Microflora of Smoked Salmon. *Nippon Suisan Gakkaishi*, 60, 569-576.
[37] B. Gimenez, P. Roncales and J. A. Beltran. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture*, 82, 1154-1159.
[38] P. Oguzhan and S. Angis. (2012). Effect of Salting and Packaging Treatments on Fresh Rainbow Trout (Oncorhynchus mykiss) Fillets During Storage at Refrigerator Temperatures. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 18, 443-448.
[39] S. Chytiri, E. Paleologos, I. Savvaidis and M. G. Kontominas. (2004). Relation of biogenic amines with microbial and sensory changes of whole and filleted freshwater rainbow trout (Onchorynchus mykiss) stored on ice. *Journal of Food Protection*, 67, 960-965.
[40] C. J. Rodriguez, I. Besteiro and C. Pascual. (1999). Biochemical changes in freshwater rainbow trout (Oncorhynchus mykiss) during chilled storage. *Journal of the Science of Food and Agriculture*, 79, 1473-1480.
[41] W. X. Du, C. M. Lin, A. T. Phu, J. A. Cornell, M. R. Marshall and C. I. Wei. (2002). Development of biogenic amines in yellowfin tuna (Thunnus albacares): Effect of storage and correlation with decarboxylase-positive bacterial flora. *J Food Sci*, 67, 292-301.
[42] M. Rezaei, N. Montazeri, H. E. Langrudi, B. Mokhayer, M. Parviz and A. Nazarinia. (2007). The biogenic amines and bacterial changes of farmed rainbow trout (Oncorhynchus mykiss) stored in ice. *Food Chemistry*, 103, 150-154.
[43] E. Rauscher-Gabernig, R. Grossgut, F. Bauer and P. Paulsen. (2009). Assessment of alimentary histamine exposure of consumers in Austria and development of tolerable levels in typical foods. *Food Control*, 20, 423-429.
[44] N. Hamada-Sato, K. Usui, T. Kobayashi, C. Imada and E. Watanabe. (2005). Quality assurance of raw fish based on HACCP concept. *Food Control*, 16, 301-307.
[45] N. Erkan, S. Ulusoy and Y. S. Tosun. (2011). Effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout. *J. Verbr. Lebensm.*, 6, 419-426.

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