RESEARCH REGARDING SENSORY AND PHYSICOCHEMICAL CHANGES OF RAINBOW TROUT MEAT UNDER DIFFERENT STORAGE CONDITIONS

C. E. Nistor, C. T. Cîrmaciu (Florea)*, G. V. Hoha, A. Usturoi, P. Macovei, B. Păsărin

Faculty of Food and Animal Sciences, Iasi University of Life Sciences, Iaşi, Romania

Abstract

Rainbow trout (Oncorhynchus mykiss) is a highly perishable food, and its shelf life depends strongly on the preservation method. This study compared the sensory and physicochemical changes of rainbow trout stored on ice (+2…+4°C), frozen, salted in 15% brine and smoked. Quality was assessed by the Quality Index Method (QIM), pH, total volatile basic nitrogen (TVB-N), the qualitative Eber, Nessler and H_2S tests, and proximate composition. During ice storage, TVB-N increased from 12.21 to 32.43 mg/100 g over 16 days, the QIM score reached the rejection limit of 15 demerit points on day 10, and the qualitative spoilage reactions became weakly positive on day 12. In frozen storage (−18°C), TVB-N rose slowly from 13.28 to 21.56 mg/100 g up to month 10, but the sensory score exceeded the acceptability limit (18 points) between months 9 and 10, so the shelf life was limited by sensory rather than chemical changes; moisture decreased from 74.46 to 73.22 g/100 g. Brining (15% NaCl, 12 h) reduced moisture to 62.90 g/100 g (8.64 g NaCl/100 g), lowered the rate of TVB-N accumulation (0.74 versus 1.60 mg/100 g per day on ice) and delayed the first weakly positive spoilage reactions to day 20. Smoking reduced moisture to 58.74 g/100 g, increased protein (26.97 g/100 g) and fat (8.22 g/100 g) on a wet-weight basis, and delayed the first weakly positive reactions to day 28. Freezing allowed storage for about 9 months, whereas under refrigeration the first weakly positive spoilage reactions appeared on day 12 (ice), day 20 (brine) and day 28 (smoked), at similar TVB-N values (25.8–27.9 mg/100 g).

Key words: *rainbow trout, preservation, sensory analysis, QIM, TVB-N, shelf life*

INTRODUCTION

Fish and aquatic products, including trout, are among the most perishable food commodities because of their high water content, abundant polyunsaturated fatty acids (PUFA) and susceptibility to microbial contamination [1,2]. The flesh of rainbow trout (*Oncorhynchus mykiss*) is rich in PUFA and haem proteins and is therefore prone to lipid oxidation [3]. Preserving trout quality requires an understanding of the interactions between storage conditions, physicochemical parameters and sensory attributes [4].

Different preservation methods (refrigeration, freezing, salting and smoking) present distinct challenges and operate through different mechanisms. Refrigerated storage on ice slows metabolic and enzymatic reactions but does not stop microbial growth, which, together with autolysis, remains the main cause of quality deterioration in fresh fish [1,2,4]. In iced rainbow trout, *Pseudomonas*, *Shewanella* and other Gram-negative psychrotrophic bacteria dominate the spoilage microflora [5,6], and shelf lives of about 10–12 days for fillets and 15–16 days for whole ungutted fish have been reported [5]. The Quality Index Method (QIM) provides a rapid and objective sensory assessment of freshness: its demerit score increases

*Corresponding author: catalina.florea@iuls.ro

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linearly with storage time in ice and can be used to estimate the remaining shelf life [7,8]. Freezing greatly extends shelf life, but quality losses continue during frozen storage: formation and growth of ice crystals damage the muscle structure and cause water migration, drip loss on thawing, and protein and lipid oxidation, with consequent loss of texture and nutritional value [9–14]. Salting lowers water activity and modifies protein structure, water-holding capacity and the sensory characteristics (flavour and texture) of fish muscle [15], and the salt uptake and water loss depend on brine concentration and brining time [16,17]; however, high salt concentrations may accelerate lipid oxidation [18]. Smoking combines partial drying and salting with the deposition of smoke constituents, mainly phenolic and carbonyl compounds, which contribute to the characteristic flavour and colour and have antioxidant and antimicrobial effects [19], although it may also generate polycyclic aromatic hydrocarbons, depending on the smoking temperature and fuel [20]. Refrigerated shelf lives of hot-smoked rainbow trout between about 14 and 35 days have been reported, depending on salting, sauces and packaging [21–23].

The aim of this study was to assess the sensory and physicochemical changes that occur in rainbow trout meat stored and preserved by four methods (ice refrigeration, freezing, salting in brine and smoking), and to compare the shelf life obtained with each method on the basis of QIM scores, TVB-N and qualitative spoilage reactions.

MATERIALS AND METHODS

The biological material consisted of 80 rainbow trout (*Oncorhynchus mykiss*) of both sexes, randomly selected in September 2025 from a farm in Suceava County, with body weights between 257 and 321 g. The fish were transported to the laboratory in an isothermal box. For each preservation method (ice refrigeration, freezing, salting and smoking), 20 fish were randomly assigned and divided equally between sensory evaluation and physicochemical analyses.

Sensory evaluation was performed by 15 semi-trained panellists using a demerit Quality Index Method (QIM) with a maximum of 24 points, modified for each preservation method [8,24,25]. The scheme for refrigerated fish comprised 11 parameters of skin, eyes, gills and abdomen (Table 1), the same structure as the QIM schemes applied to rainbow trout and Arctic char [7,8], and that for frozen fish 8 parameters (Table 3); higher scores indicate lower quality. The rejection limit was set at 15 demerit points for refrigerated samples and 18 points for frozen samples; 15 points is the limit commonly used as the end of shelf life in QIM studies on rainbow trout [8,24]. Proximate composition was determined as described by Nistor et al. [26–28]. Moisture and dry matter were determined by oven drying at 105°C to constant weight. Crude protein was determined by the Kjeldahl method (digestion with sulphuric acid and catalyst, distillation and titration) using a Kjeltec Auto 2300 (Tecator, Sweden) and a nitrogen-to-protein factor of 6.25. Lipids were determined gravimetrically by Soxhlet extraction with petroleum ether (Velp Scientifica SER 148). Minerals (ash) were determined by incinerating 2 g of sample at 550°C for 5 hours. After cooling in a desiccator and weighing, the heating, cooling, and weighing cycle was repeated in 1–1.5 hour intervals until a constant weight was achieved. Sodium chloride was determined by the Mohr method. Composition is expressed in g/100 g of fresh (wet) sample.

Total volatile basic nitrogen (TVB-N) was determined by alkalinisation of the sample, extraction of the volatile bases by steam distillation and quantification of the distilled ammonia by acid–base titration, following the principle of the EU reference

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method [29]. Results are expressed as mg TVB-N/100 g of sample.

The Eber, Nessler and H_2S tests are classical qualitative assays for early spoilage of fish. The Eber test detects volatile alkaline compounds (ammonia and amines) through the white fumes formed with hydrochloric acid vapour; the Nessler reagent gives a yellow-brown colour with free ammonia; and the H_2S test detects the hydrogen sulphide released by the breakdown of sulphur-containing amino acids. Interpretation for fresh meat – negative reaction when, after 15 minutes, the filter paper remained white on its entire surface, relatively fresh meat – weak positive reaction when, after the same interval of time, the filter paper acquired a brownish tint, more pronounced at the edges; spoiled meat – positive reaction, when in the first few minutes the paper turned brown, and towards the end of the 15-minute interval, the colour turned brownish-black over its entire surface.

The pH was measured using a Hanna pH meter at 20°C after combining ten grams of homogenized meat with 100 mL of distilled water and allowing the mixture to sit for 30 minutes.

Ice refrigeration: whole gutted fish were kept on ice at +2…+4°C for 16 days, with first sampling after 4 h and then every 2 days for sensory analysis; pH, TVB-N and the qualitative reactions were determined on days 0, 4, 8, 10, 12 and 16. Freezing: whole gutted fish were placed in freezing bags, slowly frozen at −18°C in a commercial freezer and stored for 11 months. Salting: fillets were kept in 15% NaCl brine for 12 h at 4°C, washed with clean water, dried for 4 h, packaged and stored under refrigeration at 4°C (sampled every 4 days up to day 24). Smoking: pre-salted rainbow trout fillets were hot smoked with oak wood for 4 hours; after cooling, the fillets were packed in conventional storage bags for food products and stored in a refrigerator at 4°C for up to 32 days (sampled every 4 days).

Statistical analysis was conducted using SPSS program. Results are presented as mean ± standard deviation. Relationships between indicators were examined by Pearson correlation on the mean values of the sampling times (n = 5 for days 4–16 of ice storage; n = 8 for frozen storage); because these are time series with few points, the correlations are descriptive only. Rates of change were estimated as the slope of the linear regression of the mean value on storage time.

RESULTS

The sensory characteristics of the trout were assessed immediately after the fish were brought from the farm in an isothermal box (about 4 h post mortem) and then every two days during ice storage at +2…+4°C for 16 days; the results are presented in Table 1.

Table 1. Sensory analysis of rainbow trout during ice refrigeration period

Quality	Parameter	Days								
		Fresh	2	4	6	8	10	12	14	16
	Colour	0	0	0	1	1	1	2	2	2
	Mucus	0	1	1	1	1	2	2	2	2
	Odour	0	0	0	1	1	1	2	2	3
Skin	Texture	0	0	0	0	1	1	1	2	2
	Pupils	0	0	1	1	1	1	1	2	2
Eyes	Form	0	0	0	0	1	1	1	2	2
	Colour	0	0	1	1	1	2	2	2	2
	Mucus	0	1	1	1	1	2	2	2	2
Gills	Odour	0	0	1	1	1	2	2	2	3
	Blood in the abdomen	0	0	0	0	1	1	1	1	1
Abdomen	Odour	0	0	1	1	1	1	2	2	3
Total		**0**	**2**	**6**	**8**	**11**	**15**	**18**	**21**	**24**

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The physicochemical analyses included pH, TVB-N and the Eber, Nessler and H_2S tests, which are presented in Table 2.

Table 2. Freshness indicators of rainbow trout during ice refrigeration

Storage days	pH	TVB-N (mg/100 g)	Eber reaction	Nessler reaction	H_2S
0	6.71±0.02	12.21±0.45	negative	negative	negative
4	6.29±0.04	15.74±0.49	negative	negative	negative
8	6.75±0.07	19.46±0.56	negative	negative	negative
10	6.84±0.07	24.15±1.24	negative	negative	negative
12	7.12±0.09	27.89±1.86	weak positive	weak positive	weak positive
16	7.31±0.12	32.43±2.51	positive	positive	positive

The QIM score increased linearly with storage time (QI = 1.53 × days − 0.60; R^2 = 0.997). The qualitative spoilage reactions remained negative until day 10 and became weakly positive on day 12, when TVB-N reached 27.89 mg/100 g, whereas the QIM score reached the rejection limit of 15 demerit points on day 10 (11 points on day 8; Table 1).

The results from sensory analysis, physicochemical parameters and freshness of frozen rainbow trout during storage period are presented in tables 3-5.

Table 3. Sensory analysis of rainbow trout during freezing storage

Parameter	Month							
	0	2	4	6	8	9	10	11
Skin appearance	0	0	1	1	2	2	3	3
Eye condition	0	0	1	1	2	2	2	3
Gill colour	0	1	1	2	2	2	3	3
Fishy smell	0	0	1	1	2	2	2	2
Meat texture	0	1	1	2	2	3	3	3
Fat oxidation	0	0	0	1	1	1	2	2
Abdominal cavity	0	0	1	1	2	2	2	3
Thawing exudate	0	0	1	2	2	3	3	3
Total points	0	2	7	11	15	17	20	22
The overall state of the product	Fresh, optimal texture, juicy	Excellent quality; small ice crystals.	Good quality; first signs of colour change	Early changes appear (subtle oxidation).	Moderate decrease in water retention capacity.	Slightly more fibrous texture after thawing	Mediocre quality; significant juice loss during thawing.	Beyond consumption limit; slight rancid smell

Sensory quality of frozen rainbow trout meat declined progressively during the storage period. Until the 4th month the product remained in good to excellent condition, between months 6 and 9 the quality decreased visibly (texture and thawing exudate), and from month 10 onwards the rejection limit of 18 points was exceeded.

Table 4. Freshness state of trout meat during freezing storage

Storage period (month)	pH	TVB-N (mg/100 g)	Eber reaction	Nessler reaction	H_2S
Fresh	6.67±0.03	13.28±0.35	negative	negative	negative
2	6.89±0.02	14.37±0.29	negative	negative	negative
4	6.85±0.04	16.51±0.42	negative	negative	negative
6	6.82±0.03	18.64±0.38	negative	negative	negative
8	6.78±0.05	19.69±0.51	negative	negative	negative
9	6.84±0.04	20.11±0.65	negative	negative	negative
10	6.89±0.06	21.56±0.98	negative	negative	negative
11	6.95±0.08	24.65±1.42	negative	negative	negative

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Table 5. Chemical composition of rainbow trout during the freezing storage

Storage period (month)	Water	Proteins	Fats	Minerals
Fresh	74.46±0.22	19.04±0.22	5.12±0.10	1.38±0.05
2	74.44±0.34	19.04±0.24	5.12±0.13	1.40±0.07
4	74.35±0.30	19.14±0.11	5.16±0.10	1.35±0.03
6	74.25±0.27	19.24±0.29	5.18±0.09	1.33±0.05
8	74.20±0.18	19.25±0.27	5.21±0.14	1.34±0.06
9	74.05±0.18	19.30±0.14	5.30±0.07	1.35±0.02
10	73.61±0.16	19.55±0.14	5.47±0.09	1.37±0.06
11	73.22±0.32	19.81±0.14	5.59±0.10	1.38±0.03

For the evaluation of the storage period in a preserved form using salt, the trout fillets were kept in brine, then washed with clean water and dried for 4 hours, after which they were packaged and refrigerated. The results of the physicochemical parameters and freshness of brined trout meat during storage period are presented in tables 6 and 7.

Table 6. Chemical composition of fresh and brine-salted rainbow trout (15% NaCl, 12 h; g/100 g, mean ± SD)

Specification	Fresh	Salted in brine for 12 hours (15% NaCl)
Water	74.31±0.23	62.90±0.47
Proteins	19.04±0.25	21.20±0.54
Fats	5.28±0.26	5.81±0.24
Minerals	1.37±0.10	10.09±0.07
NaCl	-	8.64±0.12

Table 7. Freshness indicators of rainbow trout salted in 15% NaCl brine during refrigerated storage

Storage period (days)	pH	TVB-N (mg/100 g)	Eber reaction	Nessler reaction	H_2S
Initial	6.91±0.02	12.36±0.45	negative	negative	negative
4	6.90±0.03	14.81±0.62	negative	negative	negative
8	6.89±0.02	17.78±0.78	negative	negative	negative
12	6.87±0.04	20.20±0.95	negative	negative	negative
16	6.86±0.03	23.54±1.12	negative	negative	negative
20	6.83±0.05	26.22±1.35	weak positive	weak positive	weak positive
24	6.82±0.04	30.43±1.58	positive	positive	positive

Smoking enhances the sensory characteristics of fish while also providing protection against microorganisms responsible for spoilage and oxidative rancidity [19]. The results of the physicochemical parameters and freshness of smoked trout meat during storage period are presented in tables 8 and 9.

Table 8. Chemical composition of fresh and smoked rainbow trout (g/100 g, mean ± SD)

Specification	Fresh	Smoked
Water	74.53±1.02	58.74±1.45
Proteins	18.90±1.21	26.97±1.56
Fats	5.43±0.26	8.22±0.28
Minerals	1.14±0.11	6.07±0.12
NaCl	-	4.46±0.12

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Table 9. Freshness indicators of smoked rainbow trout during refrigerated storage

Storage period (days)	pH	TVB-N (mg/100 g)	Eber reaction	Nessler reaction	H_2S
Initial	6.21±0.03	12.14±0.40	negative	negative	negative
4	5.87±0.04	13.98±0.52	negative	negative	negative
8	5.95±0.02	15.86±0.61	negative	negative	negative
12	6.01±0.05	17.56±0.78	negative	negative	negative
16	6.08±0.04	19.65±0.84	negative	negative	negative
20	6.17±0.06	21.94±0.99	negative	negative	negative
24	6.24±0.05	23.97±1.12	negative	negative	negative
28	6.32±0.07	25.75±1.37	weak positive	weak positive	weak positive
32	6.54±0.09	28.64±1.65	positive	positive	positive

DISCUSSION

The evaluation of rainbow trout (*Oncorhynchus mykiss*) quality under different preservation technologies showed that sensory and physicochemical indicators evolved in parallel, but at very different rates depending on the method. Deterioration of fresh trout was slowed most by freezing and, to a lesser extent, by salting and smoking, which reduce water availability (water activity was not measured in this study).

During iced storage (+2…+4°C), pH fell from 6.71 to 6.29 by day 4 and then rose continuously to 7.31 on day 16. The initial decrease is consistent with post-mortem glycolysis and accumulation of lactic acid around rigor resolution, which in trout kept on ice occurs about 76 h after death; the course of rigor and of pH is influenced by the stunning method and the rearing temperature [30]. Because pH was not measured between 4 h and day 4, the minimum could not be located precisely. The subsequent increase from day 4 to day 16 reflects the accumulation of basic volatile nitrogen compounds produced by autolytic and bacterial deamination [2,4]. In Arctic char kept in ice for 18 days, pH varied only between 6.3 and 6.5 [7]; the much larger rise observed here (about 1 pH unit between day 4 and day 16) is consistent with faster accumulation of basic compounds at +2…+4°C.

TVB-N increased from 12.21 to 32.43 mg/100 g over 16 days (about 1.26 mg/100 g per day), at an accelerating rate (about 0.9 mg/100 g per day up to day 8 and 1.9–2.6 mg/100 g per day thereafter). The EU sets TVB-N limits only for certain species or categories (35 mg N/100 g for *Salmo salar*, Merlucciidae and Gadidae; 25 and 30 mg N/100 g for other categories), and rainbow trout is not among them [29]. A lower value of 25 mg N/100 g has been proposed as the highest acceptable level for rainbow trout [31,32]. TVB-N remained below 25 mg/100 g until day 10 (24.15 mg/100 g), exceeded it between day 10 and day 12 (27.89 mg/100 g; about day 10.5) and exceeded the 35 mg/100 g reference between day 12 and day 16 (38.43 mg/100 g; about day 15). The Eber, Nessler and H_2S tests remained negative until day 10, were weakly positive on day 12 and positive on day 16, in agreement with the TVB-N trend. The final TVB-N values were higher than those reported for iced rainbow trout elsewhere (20.16 and 26.06 mg N/100 g after 18 days in ice for whole ungutted and filleted trout, respectively [5]; 18.7 mg/100 g for trout stored at 4°C [6]), and in Arctic char kept in ice for 21 days TVN did not increase at all (15.6–19.8 mg N/100 g) and was considered unsuitable as a spoilage indicator [33]. TVB-N therefore varies considerably among salmonids and handling conditions, and the faster increase observed here suggests faster spoilage at +2…+4°C or differences in handling and in the analytical procedure. TVB-N is an indicator of freshness and spoilage, not a direct measure of safety, and no microbiological analyses were performed.

The QIM score increased linearly with storage time (QI = 1.53 × days − 0.60; R^2 =

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0.997), as expected for a valid QIM scheme, and reached the limit of 15 points on day 10 (11 points on day 8). The sensory rejection limit therefore coincided with the exceeding of 25 mg N/100 g (about day 10.5) and with the last day with negative qualitative reactions (day 10), and preceded the exceeding of the 35 mg/100 g reference (about day 15). Taking all indicators together, the shelf life of whole trout stored on ice at +2…+4°C was about 10 days. This is shorter than the 12–14 days reported for rainbow trout stored in melting ice at about 0–1°C [8,24,25], the 15–16 days found for whole ungutted trout in ice [5] and the 14–17 days reported for Arctic char [7,33]; gutted trout packed in ice are also considered acceptable for about 2 weeks [34]. The QIM score increased by about 1.5 points per day, faster than in ice-stored rainbow trout (about 1 point per day [8]) and Arctic char (0.7–0.8 points per day [7,33]). In Arctic char, even a single 24-h exposure to temperatures rising from 3 to 12°C increased the QI by 1.7 points and shortened the shelf life by 2 days [7], so the constant storage at +2…+4°C, differences in the QIM scheme and fish handling (gutted or whole fish [24]) probably explain the shorter shelf life. Filleting shortens the shelf life considerably (10–12 days in ice [5]; 4 days for aerobically packaged fillets stored at 4°C [35]). Early quality loss in iced fish is dominated by autolytic changes, whereas psychrotrophic spoilage bacteria such as *Pseudomonas* spp. and *Shewanella* spp. become dominant later [2,5,6]; these were not determined here.

Pearson correlations were calculated from the mean values of days 4–16 (n = 5). pH was strongly correlated with TVB-N ($r = 0.932$; $p = 0.021$), and so was the QIM score ($r = 0.984$; $p = 0.002$). With so few points and time-dependent data, these coefficients mainly reflect the common monotonic increase of all indicators with storage time; they show that sensory and chemical indicators were concordant in this experiment, but they neither prove a causal link nor validate the QIM scheme as a predictor of TVB-N.

In frozen storage, TVB-N increase slowly from 13.28 mg/100 g at the start to 21.56 mg/100 g in month 10 (about 0.8 mg/100 g per month) and to 26.65 mg/100 g in month 11, whereas the qualitative spoilage reactions stayed negative throughout; this is consistent with the stop of microbial growth at −18°C and a continuing slow autolytic and chemical activity [9], and with the higher TVB-N reported for frozen compared with fresh rainbow trout [14]. The sensory score, in contrast, increased steadily and exceeded the acceptability limit of 18 points between month 9 (17 points) and month 10 (20 points), so the sensory shelf life of frozen trout was about 9 months (Table 3). This lies between the first-class (about 5 months) and the acceptable (up to 18 months) storage lives reported for wrapped frozen trout at −20°C [34]. The divergence between low TVB-N and deteriorating sensory attributes is consistent with protein denaturation and lipid oxidation, which continue slowly at −18°C and are generally more limiting than microbial spoilage in frozen fatty fish [3,9,10]; lipid oxidation products promote protein oxidation during frozen storage [13]. The increasing scores for fat oxidation, texture and thawing exudate (Table 3) point in the same direction, but oxidation indices (peroxide value, TBARS) were not measured.

Moisture decreased from 74.46 to 73.22 g/100 g (a decrease of 1.24 g/100 g) during 11 months of storage, which can be attributed to sublimation and drip loss, because ice-crystal damage reduces the water-holding capacity of the muscle [11,12,36]; deformation of muscle fibres has been observed in frozen trout [14]. Protein and fat increased slightly (by 0.77 and 0.47 g/100 g, i.e. +4.0% and +9.2% in relative terms), in line with the +4.9% expected for the dry matter if water loss were the only change, so the changes in composition are consistent with a concentration by water loss. Across the eight sampling times, moisture content was negatively correlated with TVB-N ($r = -0.940$; $p < 0.001$) and with the QIM score

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($r = -0.869$; $p < 0.01$); these correlations are largely determined by the last sampling times and show only that the changes occurred together, not that water loss causes spoilage.

Brining in 15% NaCl for 12 h reduced moisture from 74.31 to 62.90 g/100 g and raised the mineral content to 10.09 g/100 g, of which NaCl accounted for 8.64 g/100 g, i.e. a water-phase salt content of about 12% (NaCl/(NaCl + water)). Salt uptake and weight loss of trout and salmon fillets depend on brine concentration and brining time [16,17]. The lower water availability is expected to restrict the growth of Gram-negative spoilage bacteria, although highly salted fish may instead be spoiled by halophilic bacteria [1]. During storage at 4°C, brined fillets accumulated TVB-N considerably more slowly than unsalted trout on ice (about 0.74 versus 1.60 mg/100 g per day, i.e. 54% lower). Their qualitative spoilage reactions remained negative until day 16, became weakly positive on day 20 (TVB-N 26.22 mg/100 g) and positive on day 24 (30.43 mg/100 g), i.e. 8 days later than in ice-stored trout (weakly positive on day 12 at 27.89 mg/100 g). TVB-N exceeded 25 mg/100 g at about day 18 (versus day 10.5 on ice) and did not reach the 35 mg/100 g reference within 24 days, so brining extended the chemical shelf life by about 6–8 days (16–18 versus about 10 days). The comparison with ice storage must nevertheless be made with caution, because brined fillets were packaged and kept at 4°C whereas whole gutted fish were iced at +2…+4°C, and no microbiological or sensory data were collected for brined fillets.

Hot smoking of pre-salted fillets reduced moisture to 58.74 g/100 g and increased protein (18.90 to 26.97 g/100 g, ×1.43), fat (5.43 to 8.22 g/100 g, ×1.5) and minerals (1.14 to 6.07 g/100 g) on a wet-weight basis; this mainly reflects water loss, since dry matter increased ×1.6. The moisture is close to that of hot-smoked rainbow trout brined in 15% NaCl for 4 h (60.3 g/100 g; protein 23.9, fat 7.8 and ash 4.1 g/100 g [22]), although protein and minerals were higher here, and lower than in traditionally smoked whole trout (moisture about 66, protein 22–23 and lipids about 6 g/100 g [20]); such differences are expected from different salting, drying and smoking schedules and from the use of fillets instead of whole fish. The NaCl content of 4.46 g/100 g corresponds to a water-phase salt content of about 7%, well above the 3% recommended for hot-smoked trout [34]. TVB-N accumulated at about 0.51 mg/100 g per day, the slowest rate among the refrigerated treatments (about one third of that on ice). The qualitative reactions remained negative until day 24 (TVB-N 23.97 mg/100 g), became weakly positive on day 28 (25.75 mg/100 g) and positive on day 32 (28.64 mg/100 g); TVB-N exceeded 25 mg/100 g at about day 26 and did not reach 35 mg/100 g within 32 days, so the chemical shelf life of the smoked product was about 24–26 days. This agrees with shelf lives of 21 days at 4°C [21] and 20–30 days in conventional packaging at 0–2°C [22], and the rate of TVB-N increase is within the range calculated for vacuum-packed hot-smoked trout fillets with different sauces at 4°C (about 0.5–0.9 mg/100 g per day [23]) and faster than in traditionally smoked whole trout stored at 2–4°C (10.5 to 19.5 mg/100 g in 30 days [20]). The shelf life was longer than the 1 week below 4°C indicated for a lightly processed hot-smoked product [34], which shows how strongly shelf life depends on salt content, smoking schedule, product form and packaging. As in hot-smoked trout stored at 0–2°C [22], pH fell after smoking (6.21 to 5.87 on day 4), attributed to absorption of CO_2 by the muscle, and then rose (6.54 on day 32) as volatile bases accumulated.

Across the three refrigerated treatments, the first weakly positive qualitative reactions coincided with similar TVB-N values (27.89, 26.22 and 25.75 mg/100 g in ice-stored, brined and smoked trout, respectively), which supports the use of about 25 mg N/100 g as a practical spoilage

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threshold for rainbow trout [31,32] and indicates that the 35 mg/100 g reference is too permissive for this species, as it was reached only in iced fish. The extended shelf life of smoked trout is attributed to the combined effects of partial desiccation, salt and smoke constituents, especially phenolic and carbonyl compounds with antioxidant and antimicrobial properties [19]; the contribution of each factor was not separated in this study. The main limitations of the study are that a single batch of fish from one farm was used, that the QIM was applied by semi-trained assessors whereas published schemes use smaller panels of trained assessors [7,8], that sensory and microbiological quality were not assessed in brined and smoked trout, that lipid oxidation, water activity and polycyclic aromatic hydrocarbons in the smoked product [20] were not determined, that fish form and storage temperature differed among treatments, and that the correlations are based on few time points. Natural antimicrobial and antioxidant sauces (14 days for vacuum-packed smoked fillets with olive oil, versus 28–35 days with garlic or garlic and thyme [23]) and packaging (vacuum, modified atmosphere or active packaging [37,38]) are further factors that can extend the shelf life of refrigerated trout and should be included in future research.

CONCLUSIONS

Under the conditions of this study, the following conclusions can be drawn for rainbow trout (*Oncorhynchus mykiss*) stored and preserved by different methods:

Ice refrigeration (+2…+4°C): the QIM score reached the rejection limit (15 demerit points) on day 10, TVB-N exceeded 25 mg/100 g at about day 10.5 and the reference value of 35 mg/100 g between day 12 and day 16, and the qualitative spoilage reactions became weakly positive on day 12. All indicators therefore converged on a shelf life of whole trout of about 10 days.

Frozen storage: TVB-N remained at or below 21.56 mg/100 g up to month 10, but the sensory score exceeded the acceptability limit (18 points) between months 9 and 10; the shelf life, about 9 months, was thus limited by sensory changes (texture, colour, thawing exudate and slight rancidity) rather than by chemical freshness. Moisture decreased from 74.46 to 73.22 g/100 g, with a relative concentration of protein (+4.0%) and fat (+9.2%).

Brining (15% NaCl, 12 h): moisture fell to 62.90 g/100 g and NaCl rose to 8.64 g/100 g; TVB-N accumulated more slowly than on ice (0.74 versus 1.60 mg/100 g per day), and the first weakly positive spoilage reactions appeared on day 20 instead of day 12, i.e. an extension of the chemical shelf life of about 6–8 days, but the product form and storage temperature differed from those of the iced fish.

Smoking: smoked fillets (58.74 g/100 g moisture, 4.46 g/100 g NaCl) accumulated TVB-N at about 0.51 mg/100 g per day, the slowest rate among the refrigerated treatments, with the first weakly positive reactions on day 28 and a chemical shelf life of about 24–26 days, the longest of the refrigerated treatments; the higher protein (26.97 g/100 g) and fat (8.22 g/100 g) contents mainly reflect water loss.

In all three refrigerated treatments the first weakly positive reactions coincided with TVB-N values of 25.8–27.9 mg/100 g, which supports about 25 mg N/100 g, rather than the 35 mg/100 g limit set for other salmonids, as a practical rejection threshold for rainbow trout; the 35 mg/100 g reference was reached only in iced fish.

Freezing was the most effective method for long-term storage, whereas smoking was the most effective method under refrigeration.

Future research should examine a larger number of batches or even different salmonid breeds, using smart or modified atmosphere packaging (MAP), integrating microbiological analyses, lipid oxidation indices, water activity, polycyclic aromatic hydrocarbons in smoked products, and sensory evaluation of brined and smoked products.

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