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Effect of lemon juice on the chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) under refrigerated storage

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Abstract: In recent years, there has been an increasing interest in using food additives from natural sources to improve taste, texture and extend the shelf-life of foodstuffs. The aim of this study was to examine the quality changes in trout treated with lemon juice for 21 days. Fish were divided into T0, T1, T2 and T3 and treated with 0, 1%, 2% and 3% lemon juice respectively. The lowest pH was found in T3 followed by T2, T1 and T0. A significant difference in total volatile base nitrogen (TVBN), peroxide value (PV) and free fatty acid (FFA) was observed between the control and treatments, with the lowest increase in T3 followed by T2. Total plate count (TPC) crossed the limit in T2 and T3 on the 21st day, whereas in control and T1, the permissible limit crossed on the 12th and 18th days respectively. The sensory panel rejected the control on the 12th day and T1 and T3 on the 18th day; however, T2 remained acceptable till the 21st day, thus showing the promising effect of lemon juice on the quality of trout fillet and thereby increasing the shelf-life of the product by nine days.

Keywords: trout; lemon juice; quality; chilled storage

1. Introduction

The rainbow trout is a commercially important cold-water fish closely related to salmon, containing higher polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid compared to other fish [1]. Due to its consistent productivity, it is also the most cultivated freshwater fish in some parts of the world [2]. It contains polyunsaturated fatty acids that decrease the risk of breast and colon cancer as well as inflammatory and cardiovascular diseases [3]. However, high protein and fat content makes it highly perishable. Lipid oxidation and microorganisms are mainly responsible for the quality deterioration of such fatty fish [4]. Lipid oxidation causes sensory quality degradation, nutritional value decline, loss of essential polyunsaturated fatty acids (PUFA), and production of harmful oxidation products [5]. Method of catching, handling, transportation, storage and processing, besides intrinsic properties like biochemical composition, affect the quality of the fish [6]. Consumer acceptability of the product is affected by the pattern of industrial transformations, besides organoleptic features and nutritional quality [7].

Ice is mostly used to transport and store the fish for a short duration [8]; however, it is still very much susceptible to oxidation due to higher poly-unsaturated fatty acids. Therefore, the extension of the shelf life without compromising the quality would be a priority for the food industry. The use of synthetic antioxidants is a common practice followed by the processing industry, but the usage of synthetic antioxidants has

sparked intense debates on toxicity and food safety issues [9]. Therefore, it is important to look for alternative safe natural antioxidants which do not affect consumer acceptability.

Citrus limon (L.) Burm.f., commonly called lemon belongs to the Rutaceae family, originating from Asia. Lemon is known for its nutritional benefits as well as antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer and cardio-protective properties [10]. Lemon is rich in various bioactive components like flavonoids, flavanones, flavones, flavonols and their derivatives, phenolic acids like ferulic acid, synaptic acid, amino acids, vitamins and their metabolites (choline, pantothenic acid, trigonelline and ascorbic acid) [11]. Some organic acids have been extensively studied and known to improve the shelf life and sensory quality in fish [12], however, there are limited studies on the use of citric acid as a preservative in seafood. Lemon juice has been used for marination of the seafood [13] and is found to be rich in citric acid which is an excellent choice of natural additive for fish maturation and preservation as it controls the acidity of the product, softens the consistency of muscle and gives it a lighter colour. Since there is little information on using lemon juice as a preservative in seafood, this study looked at the effects of different lemon juice concentrations on the qualitative attributes of rainbow trout stored at 4 °C.

2. Materials and methods

2.1. Chemicals

Chemicals of analytical grade were acquired from Merck, India.

2.2. Collection and preparation of fish sample

The experiment was performed in the Post-Harvest Technology laboratory of the division of Post-Harvest Technology, Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir in May 2022. Table size (250–270 g) trout fish was collected from the instructional farm of the Faculty of Fisheries, Rangil-Ganderbal and transported to the laboratory in the live state in a water-filled tank with aeration. Upon reaching the laboratory, fish was slaughtered by stunning, thoroughly washed, gutted, and filleted and divided into 4 batches of equal weight. The first batch was given no treatment and kept as control (T0). The second batch (T1) was given the treatment of 1% (w/w) lemon juice by rubbing on all surfaces. Similarly, 3rd and 4th batches were treated with 2% and 3% (w/w) lemon juice respectively. All samples were put in separate PE bags and kept under chilled conditions at 4 °C. Sampling was done at 3 days intervals up to 21 days.

2.3. Proximate composition

AOAC protocol [14] was followed for determining moisture, crude protein, fat and ash of samples.

2.4. Biochemical parameters

2.4.1. pH determination

Fish flesh (10 g) was weighed and completely homogenized in 40 mL of water

for 10 min. A pH meter was used to measure the pH of the supernatant [15].

2.4.2. Total volatile base nitrogen (TVBN)

Estimation of TVBN was performed following official European Steam-Distillation method with a few modifications [16]. The method is based on the extraction of TVBN using an alkaline solution and the titration of the recovered ammonia as follows: 50g of fish was weighed and homogenized with 100 mL of 7.5% trichloroacetic acid (TCA) for 2 min. After that, the mixture was filtered using Whatman no. 1 filter paper to obtain a clear solution. 25 mL of fish extract and 6 mL of 10% NaOH solution was taken in the distillation flask for steam distillation. The steam distillate was collected in a flask containing 15 mL of 4% boric acid and a few drops of mixed indicator (methyl red and methylene blue in 2:1 ratio). The steam distillation procedure was continued until 100mL of distillate was collected. The distillate was titrated against 0.025 N H₂SO₄ to the endpoint indicated by pink colour.

TVBN is calculated as:

$$\text{TVBN (mgN/100g)} = 14 \times N \times X \times 100/W$$

where, *N*—normality of H₂SO₄ used, *X*—mL of H₂SO₄ used for titration and *W* is the weight of sample taken.

2.4.3. Free fatty acid

The acid number and free fatty acid of oil was determined in accordance with the AOAC protocol [17], with the results represented as oleic acid percentage. A known weight of fat or oil is taken in a clean and dry iodine flask. 25mL neutral alcohol was added to it followed by few drops of phenolphthalein indicator. The contents were titrated against 0.1N KOH till the first permanent pink colour appears.

Free fatty acid as oleic acid, per cent by weight = $(28.2 \times V \times N)/W$

where, *V* = volume of potassium hydroxide used, *N* = normality of potassium hydroxide, *W* = weight of the sample in g.

$$\% \text{ FFA} \times 1.99 = \text{Acid value}$$

2.4.4. Peroxide value

Peroxide was measured following the AOCS standard methodology [18], and it was denoted as milliEquivalentsO₂/kg of fat. 10g fish muscle was taken and homogenized in a mixture of solvents (acetic acid and chloroform) to extract the fat. To this, saturated potassium iodide solution was added along with distilled water to liberate iodine from the peroxides. The mixture was then titrated against 0.01N sodium thiosulfate solution using starch solution as an indicator during the titration.

Peroxide value = $\text{Volume of 0.01N Na}_2\text{S}_2\text{O}_3 \text{ used} \times N \times 100/\text{Weight of fat in chloroform extract}$

where, *N*—Normality of sodium thiosulfate.

2.5. Total plate count

Microbiological analysis was performed using nutrient agar plate by spread plate technique. Plates were maintained at 35 °C for 48±2 h for total microbial count following bacteriological analytical method [19]. The samples (10 g) were aseptically homogenized with physiological saline (0.85% NaCl) 90 mL. The obtained homogenized sample were serially diluted to (1:10) in physiological saline as needed.

Total plate count (TPC) was enumerated by spreading (1 mL) of homogenate on plate count agar and incubated at 35 °C for 48 h.

$$\text{Total plate count (CFU/mL)} = \text{Dilution factor} \times \text{Number of colonies}$$

2.6. Sensory evaluation

Sensory evaluation was done by ten panellists according to 10-point scale [20]. Where, out of ten, one is the most disliked, three is moderately disliked, five are neither like nor dislike, and ten is the most liked. Texture, colour, odour, and general acceptance were all asked to be evaluated by the panel.

2.7. Statistical analysis

Obtaining a properly randomised design, each experiment was conducted in triplicate. SPSS software was used to conduct the analysis and paired-test. Statistically significant was having *P* value of 0.05 or less.

3. Results

3.1. Proximate composition

Table 1 shows the proximate composition of fresh rainbow trout (*Oncorhynchus mykiss*). In the present study moisture, protein, fat and ash were found to be 74.14%, 20.93%, 4.71% and 1.14% respectively.

Table 1. Proximate composition of fresh rainbow trout fillet.

Parameter	Percentage
Moisture	74.14 ± 0.07
Protein	20.93 ± 0.96
Fat	4.71 ± 0.12
Ash	1.14 ± 0.03

*Data (*n*=3) is expressed as mean ± SD.

3.2. Biochemical parameters

3.2.1. pH determination

The changes in pH value of rainbow trout with the effect of lemon during refrigerated storage are given in **Table 2**. The initial pH value of control, T1, T2, T3 were 6.12, 5.16, 5.16, 5.01 respectively and showed increase during storage period. At the end of storage period pH of control, T1, T2, T3 were found to be 6.77, 6.40, 6.30 and 6.20 respectively. pH of control remained more than the treatments throughout the study. The increase in the pH of the treatments was lower than the control.

3.2.2. Changes in peroxide value

Peroxide value is a measure of the primary degree of oxidation. The changes in peroxide value of the rainbow trout with the effect of lemon during refrigerated storage are presented in **Table 2**. The results indicated that PV value of rainbow trout increased throughout the storage. It was observed that PV increased from 2.11 to 16.09 milliEquivalentsO₂/kg in the control, while T1, T2 and T3 had a significantly (*P*<0.05)

lower final value of 11.21, 10.03 and 9.73 milliEquivalentsO₂/kg respectively (**Table 2**). The results of the peroxide value indicated that lemon was more effective in limiting hydroperoxide formation.

Table 2. Changes in pH and PV of rainbow trout fillet under refrigerated conditions.

Day	Treatment	pH	PV(milliEquivalentO ₂ /kg)
0	Control	6.12 ± 0.01 ^c	2.14 ± 0.02 ^a
	T1	5.16 ± 0.01 ^b	2.15 ± 0.01 ^a
	T2	5.16 ± 0.01 ^b	2.15 ± 0.02 ^a
	T3	5.01 ± 0.01 ^a	2.18 ± 0.01 ^a
3	Control	6.18 ± 0.01 ^d	5.22 ± 0.01 ^d
	T1	5.3 ± 0.02 ^c	4.18 ± 0.02 ^c
	T2	5.18 ± 0.01 ^b	3.79 ± 0.01 ^b
	T3	5.02 ± 0.02 ^a	3.71 ± 0.02 ^a
6	Control	6.25 ± 0.01 ^d	5.48 ± 0.01 ^d
	T1	5.77 ± 0.01 ^c	4.19 ± 0.01 ^c
	T2	5.52 ± 0.01 ^b	3.56 ± 0.02 ^a
	T3	5.01 ± 0.01 ^a	3.73 ± 0.02 ^b
9	Control	6.03 ± 0.02 ^d	6.14 ± 0.02 ^d
	T1	5.86 ± 0.01 ^c	3.97 ± 0.01 ^c
	T2	5.67 ± 0.02 ^b	3.26 ± 0.02 ^a
	T3	5.11 ± 0.01 ^a	3.66 ± 0.01 ^b
12	Control	6.14 ± 0.02 ^{da}	9.38 ± 0.03 ^d
	T1	5.89 ± 0.01 ^c	3.97 ± 0.01 ^c
	T2	5.71 ± 0.01 ^b	3.28 ± 0.01 ^a
	T3	5.25 ± 0.02 ^a	3.62 ± 0.01 ^b
15	Control	6.24 ± 0.01 ^d	11.53 ± 0.05 ^c
	T1	6.01 ± 0.01 ^c	5.55 ± 0.02 ^b
	T2	5.8 ± 0.01 ^b	4.83 ± 0.03 ^a
	T3	5.41 ± 0.01 ^a	4.82 ± 0.02 ^a
18	Control	6.4 ± 0.01 ^d	13.2 ± 0.01 ^d
	T1	6.29 ± 0.01 ^c	7.85 ± 0.03 ^c
	T2	5.98 ± 0.02 ^b	6.86 ± 0.03 ^b
	T3	5.66 ± 0.03 ^a	6.59 ± 0.03 ^a
21	Control	6.77 ± 0.02 ^d	16.04 ± 0.02 ^d
	T1	6.4 ± 0.02 ^c	11.19 ± 0.01 ^c
	T2	6.3 ± 0.02 ^b	10.04 ± 0.02 ^b
	T3	6.2 ± 0.01 ^a	9.69 ± 0.02 ^a

Means in the same column with different superscript are significantly different ($P < 0.05$).

3.2.3. Total volatile base nitrogen content

TVBN is an important indicator for freshness or frozen fish quality. Data pertaining to the changes in the TVBN of rainbow trout fillet treated with the lemon juice under refrigerated conditions is presented in **Figure 1**. The starting value of TVBN in all the groups was as low as 5 mgN/100 g. The value of TVBN had

significant difference ($p < 0.05$) across various groups. The final value of 32.82 ± 0.04 mgN/100 g was found in control on 21st day.

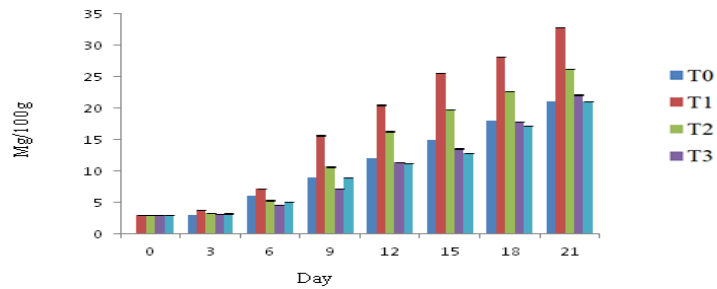


Figure 1. Changes in TVBN content of rainbow trout fillet under refrigerated conditions.

3.2.4. Changes in Free fatty acid content

The hydrolytic changes were evaluated by free fatty acid (FFA) value. Data pertaining to change in FFA of treated rainbow trout fillet with the lemon juice under refrigerated conditions is presented in **Figure 2**. Significant difference ($P < 0.05$) in FFA concentrations was found in control and treatment groups throughout the study. Highest increase in FFA was recorded in the control with initial value of 0.56 to the final value of 1.61. Lowest final value (1.06) was found in the treatment T3. These results indicate lemon inhibits enzymatic action to liberate free fatty acid.

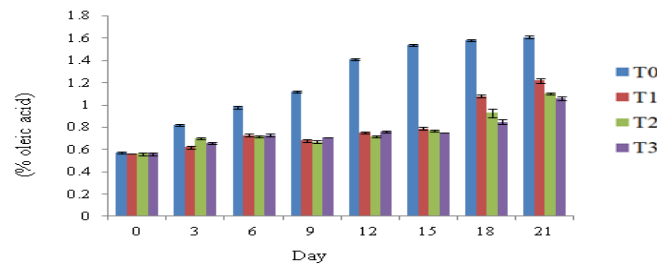


Figure 2. Changes in TVBN content of rainbow trout fillet under refrigerated conditions.

3.3. Changes in total plate count

Figure 3 displays changes in the overall plate count over the course of the chilled storage. Initial microbial count of log 3.5 CFU/g in the current investigation suggests good fish quality [21]. For all slots, bacterial count on the third day ranged from log 3.5 to 3.8 CFU/g. On 12th day, the TPC of control group increased to log 7.56 CFU/g and beyond the critical consumable limit of 7 log CFU/g recorded by IFST [22]. T1 displayed log 6.16 CFU/g on the 12th day that was considerably different ($P < 0.05$) than the control sample and crossed limit on 15th day, whereas T2 and T3 treatments the limit exceeded on 21st day of storage.

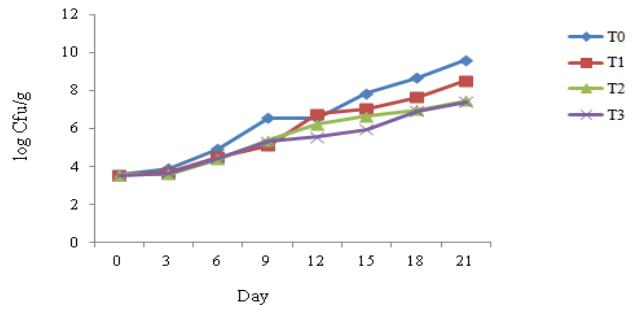


Figure 3. Changes in TPC of Rainbow trout fillet under refrigerated conditions.

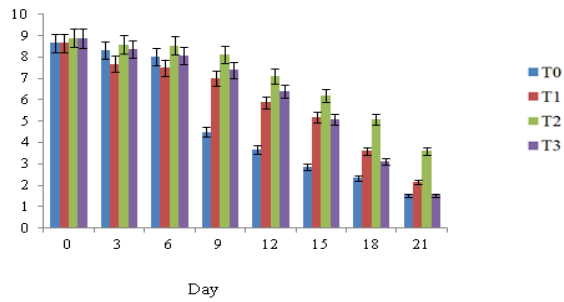
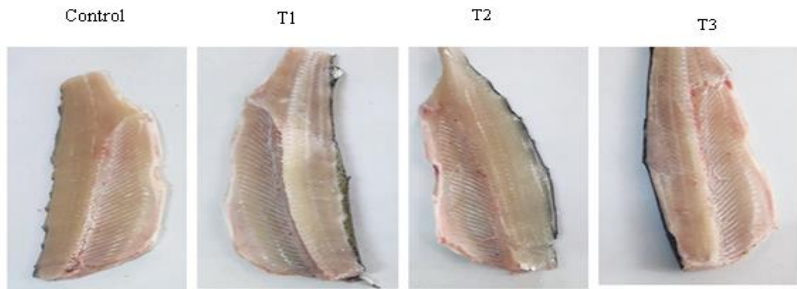
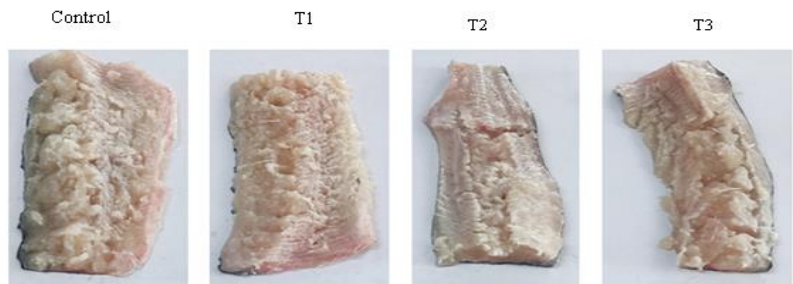


Figure 4. Changes in sensory score of rainbow trout fillet under refrigerated conditions.



Effect of lemon juice treatment on sensory characteristics of rainbow trout treated fillet under refrigerated conditions on zero day



Effect of lemon juice treatment on sensory characteristics of rainbow trout treated fillet under refrigerated conditions on 21st day

Figure 5. Changes in sensory characteristics of rainbow trout fillet under refrigerated condition.

3.4. Sensory evaluation

Sensory score and pictures of rainbow trout fillet treated with the lemon juice under refrigerated conditions are presented in **Figures 4** and **5**. Initially, all samples had scores close to 9, and this similarity was seen across all treatments ($P < 0.05$). The

score of treated samples remained preferable than the control ($P < 0.05$) throughout the study. Control was rejected by the panel on 12th day. T1 and T3 were acceptable until 15th to 18th day of the study whereas T2 was acceptable until 21st day.

4. Discussion

4.1. Proximate composition

The proximate composition of trout was observed and it was found that the outcomes concur with those of other studies [23]. Korkmaz and Kirkagac also reported the crude protein, lipid, ash and moisture of rainbow trout as 20.33%, 4.1%, 1.22% and 74.18% respectively which coincides with our study [24].

4.2. Biochemical parameters

4.2.1. pH determination

The increase in pH indicates the loss of quality. Rise in pH is caused by the formation of volatile substances like ammonia [25]. In our study, lowest overall pH was recorded in the treatment T3 throughout the experimental period while control recorded a pH of 6.77 at the end. Since treatments were treated with the acid, it might have slowed down the bacterial growth [26] as is indicated by the total plate count. Hence, present study found that lemon treatment lowers the pH value during refrigerated storage which also resulted in low bacterial count in the treated samples compared to the control slot.

4.2.2. Changes in peroxide value

Due to fatty acids in fish, it is very prone to lipid oxidation and hydro-peroxides are formed. PV gives the measure of lipid hydro-peroxides or lipid oxidation products [12]. Similar patterns of hydroperoxide levels in rainbow trout fillets stored in ice have also been found in other studies [27] which are comparable to the results obtained in our study. The findings of our study are also in line with research of other scientists [21], in which the preservation of rainbow trout fillets using oregano essential oil at 4 °C resulted in decreased production of lipid oxidation products.

4.2.3. Total volatile base nitrogen content

TVBN is an important indicator of fish spoilage and is frequently used as a benchmark for fresh or frozen fish quality. According to EU/EC [28], TVB-N value denotes “excellent quality” up to 25 mg/100 g, “good quality” up to 30mg/100 g, and “spoilt” above 35 mg/100 g. The initial value of TVB-N in all the groups was low in every group. In all of the treatments, TVBN showed an upward trend, but the rate at which control climbed was fast than that of other treatments. The value of TVBN had significant difference ($p < 0.05$) across various groups. The decrease in the TVBN value in the treatments compared to the control can be attributed to the effect of lemon juice. A marinating bath with citric acid (2%) and salt (4%) decreased TVBN value from its initial 27.5 mgN/100 g shrimp flesh to little as 7mgN/100 g [29]. Coherent result by lemon juice and olive oil mixture on the TVBN value of anchovies has been reported by others [13].

4.2.4. Changes in free fatty acid content

Free fatty acids are developed during storage as the result of oxidative hydrolysis of lipids which deteriorate the quality of meat [30]. Free fatty acids are produced when lipids are oxidized and hydrolyzed, and their presence is undesirable since they could be transformed into pungent volatiles [31]. The lower content of FFA in the treatments compared to control group might be due to the antioxidant properties of lemon juice. Kolakowska et al. [32] reached similar conclusions, finding increasing value of FFA in rainbow trout fish after 14 days of storage. FFA emission and deterioration of freshness have been found to be related [27]. Freshness of the fish and the release of FFA content are inversely correlated.

4.3. Changes in total plate count

Total plate count of bacteria present in the food is the most important part of any proposed microbiological standard. A low bacterial count indicates enhanced shelf life. The initial load of bacteria was similar for all the treatments initially among which T1 displayed log 6.16 CFU/g on the 12th day that was considerably different ($P < 0.05$) than the control sample and crossed limit on 15th day while as in T2 and T3 treatments the limit exceeded on 21st day of storage which showed the antimicrobial effect of lemon juice on the fish muscle. Treating fish with organic acid increased its shelf life by decreasing bacterial count [33]. Del Nobile and others [34] noted use of modified atmospheric packaging (MAP) along with thymol and lemon extract significantly ($P < 0.05$) decreased the bacterial load in blue fish burgers. The addition of oregano oil in different concentrations was also seen to decrease the bacterial count of the crab meat samples [35] showing the potential of natural extracts as preserving agents. Fresh lemon juice contains sugars, ascorbic acid, and phenolics, which have antimicrobial and antioxidant properties [36]. The antimicrobial effects of plant extracts have been attributed to their ability to disrupt cell walls/membranes, inhibit adenosine triphosphate (ATP) production, and disrupt protein synthesis and intracellular pH imbalances. The plant-derived essential extracts have shown excellent antimicrobial and/or antioxidant activities in fish preservation [37,38].

4.4. Sensory evaluation

The objective of sensory analysis is to measure the intrinsic sensory attributes of a sample through the analytic sensory perception of human assessors. When sensory characteristics like off-odour and flavour turn putrid or rotten due to microbial load, the shelf life of a product ends. The score of treated samples remained preferable to the control ($P < 0.05$) throughout the study. Control was rejected by the panel in the second week of observation while only T2 remained acceptable until the 21st day. Similar results were observed by Sengor and others [39] in the treatment of the spiny dogfish fillet with salt, ascorbic acid and citric acid. The most important group of secondary metabolites in the lemon includes flavonoids and other compounds, such as phenolic acids, coumarins, carboxylic acids, amino acids, and vitamins. The sensory-derived effects of lemon EO addition to salted fishes were positively registered by panellists and no off-odours were recognized in the experimental salted sardines after 75, 120 and 150 days of ripening [40]. The present study revealed that the application

of lemon juice significantly impedes sensory changes in rainbow trout fillets at 4 °C.

5. Conclusion

The shelf life of rainbow trout at 4 °C is effectively extended by treating it with lemon juice. Compared to control, treated samples showed lower TVBN, PV, FFA, TPC values and high sensory scores and thus increased shelf-life by preventing oxidation and delaying microbial spoilage. Among the treatment groups, based on TPC and sensory evaluation, the acceptability of T1 was up to 12–15 days while as T2 showed good quality characteristics till 18–21 days and T3 was considered spoiled on the 18th day. The current study also shows the effectiveness of lemon juice as a natural alternative to synthetic chemicals/ preservatives for fish products. The preservative effect of lemon juice may be due the presence of antioxidant and antimicrobial compounds which led to a significant decrease in the spoilage indices during cold storage.

Author contributions: Conceptualization, TH and FR; methodology, AF and TS; software, TH; validation, TH, FR and AF; formal analysis, TH; investigation, AF; resources, TH; data curation, TH and NH; writing—original draft preparation, AF and TS; writing—review and editing, TH; visualization, TH and FR; supervision, TH; project administration, TH and FR; funding acquisition, TH. All authors have read and agreed to the published version of the manuscript.

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