

The effects of chitosan, sodium lactate and sodium diacetate on the shelf life of hot smoked and vacuum packed rainbow trout fillets

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Summary: This study aims to determine the shelf life of hot smoked and vacuum packed rainbow trout fillets after the application of chitosan, sodium lactate and sodium diacetate. In the study, control group, and rainbow trout fillet sample groups (average weight 100-120 g) each processed with 2% chitosan, 2% sodium lactate and 0.2% sodium diacetate. Each group containing 20 samples, were examined in plate count agar for total mesophilic and psychrophilic microorganisms. Total volatile basic nitrogen (TVB-N) and pH values were also determined. As a result, the shelf life of trout fillets was extended by the use of consumable additives used in the process, particularly chitosan.

Keywords: Chitosan, rainbow trout, shelf life, sodium diacetate, sodium lactate.

Sıcak dumanlanmış ve vakum paketlenmiş gökkuşuğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetat'ın raf ömrüne olan etkisi

Özet: Bu çalışma, sıcak dumanlanmış ve vakum paketlenmiş gökkuşuğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetat işlemlerinden sonra bu ürünlerin raf ömürlerini belirlemek amacıyla yapılmıştır. Çalışmada, kontrol grubuna ait 20 örnek ile 20'şer adet %2 kitosan, %2 sodyum laktat ve %0,2 sodyum diasetat ile işlem görmüş alabalık fileto örnekleri (ortalama 100-120g), toplam mezofilik ve psikrofilik canlılar için plate count agarda incelendi. Aynı zamanda toplam uçucu bazik azot (TVB-N) ve pH değerleri belirlendi. Sonuç olarak, başta kitosan olmak üzere uygulamada kullanılan tüketilebilir katkı maddeleri etkisi ile balık filetolarının raf ömürleri uzatılabilmektedir.

Anahtar sözcükler: Gökkuşuğu alabalığı, kitosan, raf ömrü, sodyum diasetat, sodyum laktat.

Introduction

Fish products, especially those produced from fish and fish meat, have a greater importance than other animal products in today's market in terms of both health properties and their fast perishable characteristics. For a maximum benefit, these products must be consumed in a short time or delivered to the consumers in the best possible quality by storing them in optimum conditions after being caught (38). Additionally, due to high quality consumer food demands, products without chemical preservatives will not be as appealing. Studies move forward in the direction of the extending shelf life of products by using natural food preservatives (7). Chitosan is one of the new generation of additives, and is an important preservative on which research is being conducted today (32). Due to its lack of toxicity, antibacterial and antioxidant characteristics, the formation of a film layer on foods and its biodegradability, chitosan is widely used as a food preservative (19). In order to maintain the microbiological safety and increased shelf

life of the foods, organic acids and their derivatives are indicated as natural alternatives (10, 18). Lactic acid and its salts, lactates are prominently suggested for food additives which extend shelf life. The safety of food which must be a prime consideration is due to the highly effective microbiological properties that result in no health risks for consumers and does not change the sensory properties of the products (18). Today, in the decontamination of foods ready for use, the addition of sodium lactate is allowed up to 4.8% and sodium diacetate is sanctioned up to 0.25% (14).

This study aims to determine the shelf lives of hot smoked and vacuum packed rainbow trout by using chitosan, sodium lactate and sodium diacetate.

Materials and Methods

Microbiological examinations: For the preparation of chitosan (minimum 85% degree of deacetylation, Sigma-Aldrich) used in the study, 20 g chitosan was added to 900 mL distilled water in order to prepare 2% chitosan

solution in 1% acetic acid. This mixture was stirred for 10 minutes and 10 mL acetic acid was added to the mixture and stirred overnight with a magnetic stirrer. The next morning the mixture was completed to 1000 mL with distilled water.

The study consisted of control group, and rainbow trout fillet sample (average weight 100-120 g) groups processed with 2% chitosan, 2% sodium lactate and 2% sodium diacetate. Each study group contained 20 samples of fish, which were provided by a manufacturing company in Aydın/Bozdoğan. The trout fillets were kept in the chitosan solution mentioned above, in 2% sodium lactate and 0.2% sodium diacetate for 1 minute and were subjected to smoking process at 71°C for 25 minutes. Following the smoking process, fillets were vacuum packed and taken to storage containment at -18°C. These fillet samples were brought to the laboratory the next day in cold chain for weighing. The vacuum packed, smoked fillets that were selected for the study were randomly taken from the refrigerator at +4°C. Following the weighing of the samples, no other sample was taken from the same trout. For subsequent microbiological analyses, the selection of trout fillet samples were also done randomly. During this time, samples were kept in storage at +4°C. For the analyses, 25 g samples were rapidly weighed and homogenized in 225 mL peptone salt solution (0.85% salt + 0.1% peptone) for 2 minutes. Ten-fold serial dilutions were prepared from these homogenized samples (10^{-1} - 10^{-6}) and inoculated in the related media according to pour plate method. Smoked and vacuum packed trout fillet samples from control group, 2% chitosan, 2% sodium lactate and 0.2% sodium diacetate processed groups were inoculated to Plate Count Agar (Oxoid CM325) on day 0, 3rd, 5th, 12th, 14th, 16th, 18th,

20th, 22nd and 24th days. The resulting inoculations were left in incubation at 35°C (mesophilic) for 48 hours and 4 °C (psychrophilic) for 7 days (12).

Total volatile basic nitrogen (TVB-N) determination: 10 g samples homogenized with applied method were taken to Kjeldahl tubes. Then 2 g MgO and 100 mL distilled water were added to the sample. 100 mL water and 10 ml 3% boric acid and 7-8 drops of Tashiro's indicator were added to 250 mL flasks. Then, the tube and the flask were placed in Kjeldahl device and distillation was carried out until 200 mL distillate is obtained in the flask. The obtained distillate was titrated with 0.1 N HCl until the solution turned pink to indicate a change in pH. The calculation of TVB-N amount was conducted according to the following formula (4).

Formula: Calculation of the amount of TVB-N

$$\text{TVB-N mg / 100 g} = \frac{\text{The amount of consumed 0.1 N HCl (ml)} \times 1.4 \times 100}{\text{The amount of sample (g)}}$$

pH analysis: pH changes in fish meat was determined according to Soares et al. (33) and the analysis was conducted by using a digital pH meter. Accordingly, a 5 g fish sample was taken and stirred in 50 mL purified water for 30 seconds. pH meter was dipped into the solution and pH value of the fish sample was measured.

Results

In order to extend the shelf lives of smoked and vacuum packed rainbow trout fillets, 2% chitosan, 2% sodium lactate and 0.2% sodium diacetate trials were conducted and the related microbiological results are given in Table 1 and Table 2. Also, regarding the chemical analyses of these fillets, pH and TVB-N results are given in Tables 3 and 4.

Table 1. The effects of chitosan, sodium lactate and sodium diacetate applied to the smoked and vacuum packed rainbow trout fillets on the microbiological quality for Total Mesophilic Counts (TMC).

Tablo 1. Dumanlanmış ve vakum paketlenmiş Gökkuşluğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetatın muhafaza süreleri boyunca Toplam Mezofilik Canlı (TMC) sayısına etkileri.

| Application | Storage Time (days) | | | | | | | | | | | |
|-----------------------|---------------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0 ^a | 3 ^a | 6 ^a | 9 ^a | 12 ^a | 14 ^a | 16 ^a | 18 ^a | 20 ^a | 22 ^a | 24 ^a | 26 ^a |
| Control Group | 2.70 | 3.68 | 4.32 | 4.98 | 6.32 | 6.99 | 7.58 | | | | | |
| 2% Chitosan | 2.54 | 3.07 | 3.72 | 3.99 | 4.34 | 4.72 | 5.04 | 5.38 | 5.84 | 6.16 | 6.68 | 7.07 |
| 2% Sodium lactate | 2.62 | 3.20 | 3.85 | 4.26 | 4.58 | 4.98 | 5.44 | 5.88 | 6.72 | 7.04 | ANC | |
| 0.2% Sodium diacetate | 2.68 | 3.64 | 4.12 | 4.70 | 5.38 | 5.72 | 6.18 | 6.84 | 7.02 | ANC | | |

a: Log₁₀ cfu g⁻¹ (colony forming unit, per gram).

a: Log₁₀ kob g⁻¹ (koloni oluşturan birim, gramda).

ANC: Analiz Yapılmadı.

ANC: Analysis Not Conducted.

Table 2. The effects of chitosan, sodium lactate and sodium diacetate applied to the smoked and vacuum packed rainbow trout fillets on the microbiological quality for Total Psychrophilic Counts (TPC).

Tablo 2. Dumanlanmış ve vakum paketlenmiş Gökkuşluğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetatın muhafaza süreleri boyunca Toplam Psikrofilik Canlı (TPC) sayısına etkileri.

| Application | Storage Time (days) | | | | | | | | | | | |
|-----------------------|---------------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0 ^a | 3 ^a | 6 ^a | 9 ^a | 12 ^a | 14 ^a | 16 ^a | 18 ^a | 20 ^a | 22 ^a | 24 ^a | 26 ^a |
| Control Group | 2.68 | 3.94 | 4.92 | 5.78 | 6.82 | 7.64 | | | | | | |
| 2% Chitosan | 2.74 | 3.27 | 4.02 | 4.32 | 4.58 | 4.92 | 5.34 | 5.88 | 6.34 | 6.76 | 7.28 | ANC |
| 2% Sodium lactate | 2.60 | 3.50 | 3.92 | 4.48 | 4.96 | 5.42 | 6.02 | 6.68 | 7.24 | ANC | | |
| 0.2% Sodium diacetate | 2.68 | 3.84 | 4.42 | 4.96 | 5.62 | 6.02 | 6.54 | 6.94 | 7.42 | ANC | | |

a: Log₁₀ cfu g⁻¹ (colony forming unit, per gram).

a: Log₁₀ kob g⁻¹ (koloni oluşturan birim, gramda).

ANC: Analiz Yapılmadı.

ANC: Analysis Not Conducted.

Table 3. The effects of chitosan, sodium lactate and sodium diacetate on the TVB-N values of smoked and vacuum packed rainbow trout fillets.

Tablo 3. Dumanlanmış ve vakum paketlenmiş Gökkuşluğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetatın TVB-N sayılarına olan etkisi.

| Application | Storage Time (days) | | | | | | | | | | | |
|-----------------------|---------------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 0 ^a | 3 ^a | 6 ^a | 9 ^a | 12 ^a | 14 ^a | 16 ^a | 18 ^a | 20 ^a | 22 ^a | 24 ^a | 26 ^a |
| Control Group | 8.43 | 11.9 | 14.2 | 19.8 | 23.8 | 29.4 | | | | | | |
| 2% Chitosan | 8.43 | 9.22 | 9.96 | 12.3 | 14.8 | 16.3 | 18.2 | 19.4 | 21.1 | 23.6 | 27.1 | 30.0 |
| 2% Sodium lactate | 8.43 | 9.50 | 10.9 | 14.4 | 17.6 | 20.2 | 23.0 | 26.8 | 29.8 | 32.4 | | |
| 0.2% Sodium diacetate | 8.43 | 9.80 | 12.2 | 14.9 | 17.2 | 19.0 | 22.4 | 26.9 | 30.2 | 34.1 | | |

a: mg TVB-N/100g

Table 4. The effects of chitosan, sodium lactate and sodium diacetate on the pH values of smoked and vacuum packed rainbow trout fillets.

Tablo 4. Dumanlanmış ve vakum paketlenmiş Gökkuşluğu alabalık filetolarına uygulanan kitosan, sodyum laktat ve sodyum diasetatın pH üzerine olan etkisi.

| Application | Storage Time (days) | | | | | | | | | | | |
|-----------------------|---------------------|------|------|------|------|------|------|------|------|------|------|------|
| | 0 | 3 | 6 | 9 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 |
| Control Group | 5.98 | 5.90 | 6.18 | 6.20 | 6.46 | 6.94 | | | | | | |
| 2% Chitosan | 5.97 | 5.95 | 6.04 | 6.09 | 6.14 | 6.20 | 6.29 | 6.33 | 6.39 | 6.48 | 6.56 | 6.89 |
| 2% Sodium lactate | 5.98 | 5.92 | 6.12 | 6.17 | 6.23 | 6.28 | 6.41 | 6.49 | 6.55 | 6.68 | | |
| 0.2% Sodium diacetate | 5.98 | 5.92 | 6.12 | 6.21 | 6.26 | 6.30 | 6.39 | 6.49 | 6.56 | 6.80 | | |

Discussion and Conclusion

As a result of the analyses conducted with the addition of chitosan, sodium lactate and sodium diacetate, smoked and vacuum packed rainbow trout fillets, the total mesophilic aerobic counts (TMC), total psychrophilic counts (TPC) in the control group at the beginning of the trials were 2.70 and 2.58 log₁₀ cfu/g, respectively. TMC counts exceeded 7.00 log₁₀ cfu/g on the 14th and 16th days which is the 'best before' date for fish products. TPC count was 7.64 log₁₀ cfu/g on the 14th day. TMC count in 2% chitosan test samples exceeded this limit level on the 26th day. For 2% sodium lactate and 0.2% sodium

diacetate, the limit levels were surpassed by the 22nd and the 20th days, respectively. Limit levels for TPC counts in 2% chitosan, 2% sodium lactate and 0.2% sodium diacetate applications were surpassed on the 24th, 20th and 20th days, respectively. The chitosan, sodium lactate and sodium diacetate levels used in this study, similar to levels applied in previous studies (20, 22, 31, 35) have been preferred.

Sodium salts of the low molecular weight organic acids; such as acetic, lactic, and citric have been used to control microbial growth, improve sensory attributes and extend the shelf life of various foods. In addition to their suppressing effect on the growth of food spoilage bacteria,

organic salts of sodium acetate, lactate, and citrate were shown to possess antibacterial activities against various food-borne pathogens including *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli*, as well as *Clostridium botulinum*. Besides this, these salts are also widely available, economical, and generally "recognized-as-safe" (GRAS) (31).

TMC and TPC counts, are of prime importance for maintaining quality of food consumption in addition to the determination of shelf life. Mohan et al. (23) coated sardine fish with 1% and 2% chitosan and stored them at 2°C for 12 days and found the TMC counts in samples coated with 1% chitosan 7.6 log₁₀ cfu/g and in samples coated with 2% chitosan 7.3 log₁₀ cfu/g at the 11th day. Fan et al. (13), coated the carp fillets with 3% chitosan and stored at -3°C. At the end of 30th day, TMC counts in the application group were below the specified value. Duan et al. (12) coated lingcod (*Ophiodon elongatus*) fillets, which is a commercial fish species found in North American Pacific, with chitosan and stored the samples. This study yielded TPC counts in excess of 7 log₁₀ cfu/g in the 3rd week and reached 7.5 log₁₀ cfu/g in control group samples at the end of the 1st week. The increase in TMC and/or TPC counts during shelf life were reported by many researchers (3, 8, 26, 33, 34). Bacterial growth in our study was inhibited with chitosan, sodium lactate and sodium diacetate which have antimicrobial activities and extended the shelf life of trout fillets up to 24 days without compromising quality of product or taste. Hisar et al. (16) examined the microbiological (psychrotrophic, mesophilic aerobic bacteria and Enterobacteriaceae counts) and chemical effects (pH, TVB-N, TBA) of normal atmosphere (control), vacuum packaging and packaging under modified conditions containing gas mixtures (100 CO₂%, 2.5% O₂+ 7.5% N₂ + 90% CO₂ and 30% O₂+30% N₂+ 40% CO₂) at 4±1 °C on rainbow trout fillets. Psychrotrophic bacterial counts in samples stored in 10% CO₂ exceeded 7 log₁₀ cfu/log by the 12th day of storage. However, mesophilic bacterial counts reached 6 log cfu/g at the end of the storage time (14th day).

TVB-N value is a freshness indicator for all fish and fish products whereby TVB-N value increases with degradation of product. In the determination of quality of the fish, Connell (9) limited the TVB-N value to 35-40 mg TVB-N/100 g, while Hisar et al. (16) limited this value to 25 mg TVB-N/100 g in rainbow trout. Varlık et al. (36) evaluated the quality classification in TVB-N values as; "very good" up to 25 mg/100 g, "good" up to 30 mg/100 g, "marketable" up to 35 mg/100 g and "spoiled" for more than 35 mg/100 g. Also the researchers stated that the consumable limit value for TVB-N value in freshwater fish as 32-36 mg/100 g. According to Pezeshk et al. (27), TVB-N primarily consists of trimethylamine,

dimethylamine, ammonia and other volatile basic nitrogen compounds. TVB-N is one of the main fish quality index markers and an increase in TVB-N value can occur due to the activity of the microorganisms that cause degradation and endogenous enzymes (30). Changes in TVB-N values in our samples during analyses are given in Table 3. TVB-N value on day 0 in control group, 2% chitosan, 2% sodium lactate and 0.2% sodium diacetate applied group was 8.43 mg/100 g, while this value on the 14th day was 29.4 mg/100 g in control group, 30.0 mg/100 g in 2% chitosan applied group on the 26th day, 32.4 mg/100 g in 2% sodium lactate applied group on the 22nd day and 34.1 mg/100 g in 0.2% sodium diacetate applied group on the 22nd day. The initial TVB-N value determined on day 0 was lower than those reported in some studies (12, 15, 24, 28, 29). Jeon et al. (17), reported 35-50% decrease in TVB-N formation in morina fish coated with chitosan at the end of 12 days of storage. For sardine fillets that were frozen at -18 °C and stored for 6 months, Ozbay and Ayas (26) reported that 19.39 mg/100 g initial TVB-N values increased during storage and reached to 33.24 mg/100 g, 29.41 mg/100 g and 27.81 mg/100 g in control, acetic acid and chitosan groups, respectively. Comparing the TVB-N values of all groups, TVB-N value of the chitosan group was found to be lower compared to all other groups.

Sodium lactate (E325) and sodium diacetate (E262) are additives that are used in foods for their beneficial properties such as extending shelf life and improving the aroma properties. In the literature, there are studies in recent years for the extension of the shelf lives of fish products, mostly on chitosan, whereas the studies on sodium lactate and sodium diacetate are very limited. Some researchers have explained that the preservative action relied on two main principles as inhibiting microorganism growth by reducing the water activity, and the specific effect of the lactate ions (18, 37). Cetin and Bostan (11), in their study on 'ready to serve' fish balls, found the average water activity in control group samples 0.978, while the water activity in 2% sodium lactate added samples was 0.903. Oksuztepe et al. (25) reported that the sensory properties (color, odor and taste) of fish balls prepared from fresh rainbow trout were not affected by using sodium lactate, however it reduced the microbial growth and increased the shelf life. Additionally of 2% sodium lactate caused no unwanted changes in the sensory properties of the product. Also, it was observed that 2% sodium lactate added samples maintained their edible properties for 16 days at 4±1°C. It was reported that the shelf life increased from 6 days to 16 days.

Alak et al. (1) in their study on determining the effects of chitosan coating or modified atmosphere packaging and vacuum packaging at 4±1°C for 15 days on the microbiological and chemical (pH, TVB-N and

TBARS) properties of stored bonito fillets, found that the aerobic bacterial growth in fillets coated with chitosan was slower than those of control and vacuum packed groups. The lowest pH value in all groups were determined in samples coated with chitosan.

pH measurements were slightly lower than the results found on the 1st day and increasingly higher in the analyses carried out on subsequent days. The average initial value was 5.98 and this value first decreased in all groups and increased in the following days of the storage. pH range of the fish varied between 5.7 and 6.6 pH value of the fresh fish is close to neutral. However, pH decreases after death due to the formation of lactic acid and increases again with the start of degradation (5). In this study, the results of pH measurements following the 1st day analyses were slightly lower than the results of the 1st day, however the analysis results in the following days were increasingly higher. The average initial value was 5.98 and this value first decreased in all groups and increased in the following days of the storage. Similarly, this was also observed by some researchers (2, 6, 13, 21). The decrease in fish samples in the initial results was associated with the increase in soluble CO₂ and the subsequent increase in pH was associated with the increase in volatile bases (TVB-N) (6). Mohan et al. (23) found the pH values in sardine fish coated with 2% chitosan and stored in the cold as 6.1 on day0 and 6.6 at the 11th day.

The average storage time of chitosan, sodium lactate and sodium diacetate added, smoked and vacuum packed rainbow trout fillets was extended from the original of 14-15 days up to 24-26 days. This type is stored at -18°C while the satisfactory expiry time for the products that are stored at +4°C before offered to marketing is 18 days. This period was extended with these applications. According to the data obtained in this study, chitosan showed better results in terms of extending shelf life compared to sodium lactate and sodium diacetate.

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