Effect of Ascorbic Acid Utilization on Cold Smoked Fish Quality (Oncorhynchus mykiss) during Process and Storage

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Received March 21, 2013; Accepted May 16, 2013

The objective of the study is to investigate the effects of ascorbic acid on the quality of cold-smoked trout (Oncorhynchus mykiss), with or without sodium nitrite (NaNO2). For this purpose, fish samples (~250 g) were treated with six forms of brine prepared with 10% salt which is the control, treated with 10% salt and 0.4%NaNO2, 10% salt and 0.2%NaNO2, only 2.5% ascorbic acid (Dry Salt Basis) and their combinations, and stored at +4°C. The groups were analyzed after 4 days interval for microbiologic quality, biochemical quality and sensory quality. According to the results, the samples treated with only ascorbic acid increased the shelf-life of smoked fish about > 8 days. TVB-N value of the control group was average 41.72 mg/100 g after 16 day; the group containing ascorbic acid was on average 30.53 mg/100 at this time. Ascorbic acid reduced the residual nitrite in all groups. The residual nitrite disappeared in the ascorbic acid including groups after 20 day.

Keywords: cold-smoked fish, ascorbic acid, nitrite, fish quality, fish shelf life

Introduction

Ascorbic acid is an antioxidant and antimicrobial preservative. Acting as an antioxidant, ascorbic acid can improve the color and palatability of many kinds of food products. Recent studies have presented that the maximum addition of reducing agents including sodium ascorbate and erythorbate provide a drastic decline of residual nitrite in meat products (Ahn et al., 2004). One of the most effective inhibitors of nitrosation is ascorbic acid. This vitamin as an alternative preservative or a reduction agent of the residual nitrite rapidly reacts with nitrite to form nitric oxide and dehydroascorbic acid. It can inhibit the formation of dimethyl nitrosamine by more than 90% (Kataoka et al., 1996). Ascorbic acid can prevent nitrosamine formation in cured meats by reducing nitrate to nitrogen oxide, which will not be able to react with the amines to form nitrosamines. On the other hand, ascorbic acid is a free radical scavenger that blocks nitrosation by scavenging NO radicals and therefore preventing nitrosation (Mercogliano et al., 2012). Ascorbic acid may also be able to inhibit carcinogens by blocking the conversion of precursors (procarcinogens) into carcinogens and carcinogenic metabolites (Miyauchi et al., 2002; Radcliffe et al., 2003). Subjects with a high intake of both nitrate and ascorbic acid tended to have the lowest risk of cancer (Roger et al., 1995; Mirvish et al., 1972). In addition to its benefits as a processing aid and preservative, ascorbic acid has a nutritional value in food products.

Nevertheless, cold-smoked fish is a lightly salted, lightly-preserved and highly perishable fish product, which is typically vacuum-packed and stored at chill temperatures. The smoking process increases the shelf-life of fish as a result of the combined effects of dehydration, antimicrobial and antioxidant activity of smoke (Hornero et al., 1997; Leroi and Joffraud, 2000; Rorvik, 2000). While, cold smoked fish products are not a satisfactory preserved product; the amounts of salt and smoke used are not sufficient to prevent spoilage. Some preservatives are occasionally used to act as a preservative in cold smoked fish. However, there are varying opinions among experts as to the best method of application and effectiveness of the preservatives. Some antimicrobial and antioxidants, such as nitrates are added to retard microbial spoilage in processed fish (Rogers et al., 1995; Rørvik, 2000).

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Nitrite is an effective antimicrobial and antioxidant preservative, which is added to the curing salt mixture in order to delay spoilage and the control of microbial activity in processed fish (Rogers et al., 1995; Duncan et al., 1997; Lyhs et al., 1998). It is also known that healthy adults are able to consume large quantities of the natural nitrate during the nutrition. The oral micro flora can reduce nitrate to the nitrite. Nitrates are also found in the water of foods and raw material naturally (Duncan et al., 1997). Nitrosamines may be formed in the human stomach due to the reaction of nitrite with amides. There have been reports of the relationship between the high incidence of nasopharyngeal cancer and the consumption of nitrite containing smoked fish (Lijinsky, 1999). Residual nitrite ratio may be an indicator of the amount of nitrosation (Bruce, 2005).

In this regard, nitrite deductible additives such as ascorbic acid may be a suitable alternative preservative for use in the cold smoked fish industry. Ascorbic acid is used extensively in the food industry, not only for its nutritional value but for its many functional contributions to product quality.

Consequently, there are some reports in the literature on the benefits of supplementing ascorbic acid but there is no study regarding the effects on cold-smoked fish quality, shelf life, sensory properties and residual nitrite content. Taking into account these important explanations, the main objective of the present study is to experimentally investigate the effects of ascorbic acid utilization as an antioxidant, antimicrobial and residual nitrite scavenger on the quality, shelf life and residual nitrite content of vacuum packaged cold-smoked rainbow trout was investigated.

Materials and Methods

Preparation of the experimental groups  Cultured fresh water rainbow trout (Oncorhynchus mykiss) was used as a raw material. The mean weight of the trout approximately ~250 g (Condition factor = 1.28 ± 0.013) (one year old on average) was slaughtered, gutted, washed and packaged in polyester boxes in ice and have been transported to the laboratory by Bage Fishery Co. (in Turkey). The selected fish size (~250 g/year) has an average weight for the market. Five fish samples for each experimental analysis, randomly selected, trimmed and homogenized as a minced fish meat. This minced fish samples are analyzed for their moisture, crude lipid, protein, ash, pH, salt, aw (Water activity), condition factor, titrated acid, TVB-N (total volatile basic nitrogen), TBARS (thiobarbituric acid reactive substances) content, TVC (total viable count), LAB (lactic acid bacteria), TPB (total psychrotropic bacteria) and TYM (total yeast and mould). The remaining fish were further processed for brining and cold smoking. Weights were recorded in all the processes.

All these experimental analyses are also made for the all experimental groups and all process steps. These experimental analyses are made for the control of process. In this contest, all these experimental data are used for the control of process cycle. Abbeumé meter was used for the salting process control during the fish brining. On the other hand, the standard Mohr method was used for the identification of salt content in the processed fish samples.

Brining  ~250 g each of trout were treated with six forms of brine prepared with 10% salt which is the control group C), treated with 10% salt, 0.4% NaNO2 (N4), 10% salt and 0.2% NaNO2 (N2), only 2.5% ascorbic acid (A) and their combinations (N4A, N2A). In this regard, the groups were cold smoked, vacuum packaged with polyamide bags, then stored at +4°C for 40 days. The experimental group was analyzed after each 4 day of the storage.

Six different brine samples were prepared for the fish groups. Experimental brine series of fish groups were given in Figure 1.

100 g of dry salt was dissolved in a litre for all experimental groups. NaNO2 (0.4 and 0.2 g) was dissolved in a litre of 10% brine for group N4, N2, N4A and N2A. 2.5 g of ascorbic acid was dissolved in a litre of 10% brine for group A. 2.5 g of ascorbic acid was also dissolved in a litre of 10% brine that was prepared for group N4A and N2A. The ratio of brine to fish in each of brine was 2:1 (v:w) during brining. The salt was a commercially refined dry salt. NaNO2 was purchased from Merck Chemical Co. Ltd. (Darmstadt, Germany) and ascorbic acid (L-ascorbic acid) was purchased from Sigma Aldrich Co. Ltd. (Darmstadt, Germany). The fish were filleted mechanically and manually before brining. Fish samples were brined at +4°C for 6 h and then rapidly rinsed in fresh water (+15°C); the brining process was continuously followed by a baumé meter (Hi-Tech Instruments Co., Ltd, China). During the brining, only ~0.25 g/l ascorbic acid transition per kg fish was observed. All these experimental parameters are selected according to preliminary experiments.

Cold smoking procedure  The fillets were dried for 30 min and then rapidly placed in a smoking oven (Ari Torna Makineleri Sanayii Co., Istanbul, Turkey). The smoking oven had a smoke funnel of 20 cm φ, and 2 m² capacity, with a vertical smoke flowing and was automatically controlled. The fillets were cold-smoked for ~8 h. This was done to ensure that the fillets were processed under equivalent conditions as much as possible. The drying and smoking temperatures, along with the relative humidity were set to +28°C and 70% during the process, respectively. The NaCl concentration in the fish after curing and smoking was approximately 2.5% NaCl (water phase salt, WPS). After smoking, the fil-
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- **Harvesting** Raw fish were harvested in a farm.
- **Transporting** Harvested fish samples were transported to process laboratory and stored.
- **Trimming and filleting** Fish headed, gutted and filleted (+4°C).
- **Brining** Trout fillets were immersed in 6 different liquid brines at +4°C for 6 h.
- **Experimental groups, brine contents and abbreviations**
  1. C; trout fillets treated with 100 g l⁻¹ salt
  2. N4; trout fillets treated with 0.4 g l⁻¹ nitrite and 100 g l⁻¹ salt.
  3. N2; trout fillets treated with 0.2 g l⁻¹ nitrite and 100 g l⁻¹ salt.
  4. A; trout fillets treated with 2.5 g l⁻¹ ascorbic acid and 100 g l⁻¹ salt.
  5. N4A; trout fillets treated with 0.4 g l⁻¹ nitrite, 2.5 g l⁻¹ ascorbic acid and 100 g l⁻¹ salt.
  6. N2A; trout fillets treated with 0.2 g l⁻¹ nitrite, 2.5 g l⁻¹ ascorbic acid and 100 g l⁻¹ salt.
- **Rinsing with fresh water and Drying** Drying at +28°C for 30 minute (Average drying loss 10%).
- **Smoking** Smoking at +28°C for 8 h.
- **Packaging** Vacuum packed at PE/PA laminate.
- **Storage** Storage at +4°C.

**Fig. 1.** Flow diagram of the cold-smoked trout production.

- let's were stored at +4°C and trimmed by hand. Five fishes from each treatment were weighed just before being placed in the smoking oven. The same fish were then reweighed after brining, smoking and prior to cutting. The water loses of samples were expressed as a percentage.

**Packaging** Each of the cold-smoked fish fillets was packed with polyethylene and polyamide laminate whose water vapour permeability was 10 – 15 g/ m²/ 24 s. (38°C, 50% RH)- (1 atm/101325 pa), where the oxygen permeability was 29 – 45 mL O₂/m² / 24 s/ atm (23°C, 50% RH) in a vacuum machine (Abant Machine Co., Turkey). Packaged samples were stored at +4°C.

**General chemical analysis** The moisture, protein, ash, fat and salt contents (Mohr method) were determined by the standard methods of AOAC (1995).

**Weight loss and aw** Weight loss was measured as percentages of the original weight and aw values were measured with an Aqua Lab Model cx2 (Sensitivity = ± 0,003) at the first day for tree samples.

**pH** A 10 gram fish sample was blended with 90 mL distilled water. After filtering, pH measurements were taken with an Inolab ba 12217e model digital pH meter for four days period (AOAC, 1995).

**Total volatile basic nitrogen (TVBN)** The TVBN content was measured according to the method introduced by Antonacopoulos and Vyncke (1989) and expressed as mg TVB-N per 100 g fish for four days periods.

**Residual nitrite levels** Residual nitrite contents in fish
samples were determined (UV-2102 PC; UV-VIS scanning spectrophotometer, Germany) at 540 nm absorbance as colorimetrically. The results were calculated according to standard curve. The standard curve was a straight line up to 1 ppm NaNO₂ in final solution (AOAC, 1995).

Thiobarbituric Acid Reactive Substances (TBARS) The value of thiobarbituric reactive substances (TBARS) was determined for each fresh, brined and cold smoked trout to evaluate the oxidation stability, during processing and storage of the fillets for a four day period (Tarladgis et al., 1960).

General microbiological analysis Each series of experiments was examined microbiologically after a four day period. Twenty-five grams of trout were homogenized in 225 mL of 0.1% (w/v) of sterile peptone solution in a Stomacher 400 Lab Blender for 2 min. The homogenized sample was diluted, prepared onto Plate Count Agar (PCA, Merck, Germany), and incubated at 37°C for 48 h (0.1% peptone). Furthermore, decimal dilutions were made in the same diluents. Psychrophyl were determined on Plate Count Agar at an incubation temperature of 7°C for 10 days following the pour plate method (ICMSF, 1978). Lactic acid bacteria were determined in MRS (Merck, Darmstadt, Germany) incubated at 30°C for 72 h (de Man et al., 1960; FDA, 1998).

Sensory analysis The analysis was made using a scoring system and six descriptive parameters such as texture, odor, taste, internal color, external color, appearance, after taste and overall acceptability each four day after preparation. A quality scale from 0 to 9 was used and all scores were calculated as an average. The panel consisted of ten well-trained panelists.

Statistical analysis of data The experiment was repeated twice and the data were statistically analyzed by multiple variance analysis using SPSS-11.0 software (Lead technologies Inc., USA). All experimental values were analyzed by using ANOVA among the fillets and time periods. The differences between mean values were analyzed and presented using the Duncan test for comparison and the effects were considered to be significant at $p < 0.05$.

Results and Discussion

Physical and chemical characteristics of the raw and processed products In order to determine the effects of ascorbic acid utilization on cold smoked fish quality, the experiments were carried out in the Food Engineering laboratory of Hacettepe University. Figure 1 presents the flow diagram of the cold-smoked trout. The properties indicating the cold smoked fish quality were determined to the raw, brined, smoked fish of rainbow trout (O. mykiss) at the beginning of storage.

Experimental analysis performed before the samples spoiled (TVBN > 40 mg/100 g). The chemical composition of fish samples may vary according to age, species, sex, environment and season. Cold smoking process caused an average 10.15% wet bases (wb) reduction in moisture content of the fish samples. This value was reported to be higher than that found by Catteneo and Cantoni (1987), but this value is well in agreement with the results obtained as 21.5% and 10.8% by González- Rodríguez et al. (2002). Water phase salt (WPS) was found to be 3.5% in average. WPS was homogeneity in experimental groups. González- Rodríguez et al. (2002) have reported that this value is between 4.1 – 4.7 while these values were given as 3.5 in FDA (2001).

Residual nitrite In general the amount of nitrite and the depletion rate differs from one system to another, since the situation involves several different depletion pathways plus a number of variables, including product formulation, pH; and temperature relationships during processing and storage (Bruce, 2005; Hyytiä et al., 1997). The depletion of nitrite was detected in cold-smoked fillets during process and storage at +4°C. At the beginning of the storage, the residual nitrite content of the groups (N4, N2, N4A and N2A) were found to be as ~50, 45, 45, 40 ppm respectively (Table 1). However, these values diminish throughout the storage. Ascorbic acid treatment reduced the nitrite contents for the cold smoked N4, N2, N4A and N2A groups. At the end of 16th day of the storage of samples to decreased by ~30, 20, 20, 0 ppm respectively, and then after 32 days the nitrite had completely disappeared.

Intermediates in the reaction between nitrite and ascorbic acid in muscle food are reactive nitrosating agents. Nitrite and ascorbic acid can produce nitrite oxide (NO), dinitrogen monoxide (N₂O) or volatile nitrogen gas (N₃) (Leif, 2011). Added ascorbic acid becomes oxidized to yield NO as below;

Ascorbic acid + 2HNO₂ → Dehydroascorbic acid + 2NO + 2H₂O

NO⁺ is a strong electrophile and, N₂O₃ as a reactant, depend on the disappearance ratio of ascorbic acid. During the curing process, ascorbic acid reacts faster than secondary amines with the nitrosating agent N₂O₃. All these biochemical reactions can decrease the free nitrite and ascorbic acid content of the complex muscle matrix. N-nitrosamine formation can be blocked or reduced by ascorbic acid (Mirvish et al., 1972; Leif, 2011). However, residual nitrite in N2, N4 groups disappeared for a long period. These means that, the residual nitrite in ascorbic acid free N2, N4 groups may reacted with any free reactants such as amine groups and myoglobin in the complex muscle matrix. However, residual nitrite can also react to form minor volatile components during a long time storage period. Six factors influence nitrite loss in muscle matrix. (Weng et al., 1992; Hotchkiss, 1985).
have been determined and are given in Table 3.

Ascorbic acid allowed the nitrite to be reduced into nitric oxide, resulting in a gradual reduction of residual nitrite during storage. Groups N4 and N2A were the best in reducing residual nitrite levels, whereas Group N4A was in a middle position, and Group N2 was the worst. The results indicate that ascorbic acid has a significant effect on the stability of cold-smoked fish, whereas this effect was negative in groups with sodium nitrite. These results indicated that combinations of ascorbic acid and vacuum packaging could be effective for reducing residual nitrite levels in cold-smoked fish during storage.

Total volatile basic nitrogen (TVB-N) TVB-N values, which are quality indicators of cold smoked fish samples, have been determined and are given in Table 3.

It is recommended that samples including 25 mg N/100 g TVB-N value are in “perfect quality”, samples including 30 mg N/100 g TVB-N value are in “good quality”, samples including 40 mg N/100 g TVB-N value are in “marketable quality” and the samples including more than 40 mg N/100 g TVB-N value are in “spoiled” (Schormuller, 1968). TVB-N values have shown significant statistical differences among all of the experimental groups during storage. These values correlate with TVC and the increase in TVB-N quality and the samples including more than 40 mg N/100 g TVB-N value are in “spoiled” (Schormuller, 1968).

Table 1. Some quality properties of the raw, the brined and the cold-smoked trout groups at the beginning of storage.

<table>
<thead>
<tr>
<th>Experimental Analysis</th>
<th>Experimental Groups (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>N4</td>
</tr>
<tr>
<td>Salt % (WPS)</td>
<td>71.52 ± 0.09c</td>
</tr>
<tr>
<td>TVB-N (mg/100g)</td>
<td>16.07 ± 0.22c</td>
</tr>
<tr>
<td>TVC (log cfu/g)</td>
<td>4.53 ± 0.11e</td>
</tr>
<tr>
<td>TPC (log cfu/g)</td>
<td>4.26 ± 0.03e</td>
</tr>
<tr>
<td>LAB (log cfu/g)</td>
<td>3.83 ± 0.06b</td>
</tr>
</tbody>
</table>

a-g mean values within a row with different capital letter are significantly different (p < 0.05).

Table 2. Changes in residual nitrite content of the samples during the storage of cold-smoked vacuum-packed trout at +4°C

<table>
<thead>
<tr>
<th>Groups</th>
<th>Residual nitrite in the groups (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N4</td>
<td>0 4 8 12 16 20 24 28 32 36 40</td>
</tr>
<tr>
<td>N2</td>
<td>50 ± 0.0 50 ± 0.0 40 ± 0.0 40 ± 0.0 30 ± 0.0 30 ± 0.0 30 ± 0.0 20 ± 0.0 20 ± 0.0 10 ± 0.0 0 ± 0.0 0 ± 0.0</td>
</tr>
<tr>
<td>N4A</td>
<td>45 ± 0.0 45 ± 0.0 30 ± 0.0 30 ± 0.0 20 ± 0.0 20 ± 0.0 10 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0</td>
</tr>
<tr>
<td>N2A</td>
<td>40 ± 0.0 40 ± 0.0 15 ± 0.0 15 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0 0 ± 0.0</td>
</tr>
</tbody>
</table>

*Average of double analysis (n = 4).

Table 3. Changes in TVB-N (mg/100g) during storage of cold-smoked vacuum packaged trout at +4°C.

<table>
<thead>
<tr>
<th>Group</th>
<th>TVB-N (mg/100 g) of the groups and storage time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>16.66 20.86b 27.15b 31.29b 41.72d 48.92c</td>
</tr>
<tr>
<td>N4</td>
<td>16.20 17.35a 18.18a 19.86a 20.39b 21.70a 27.40a 26.27a 27.36a 25.80a 27.51a</td>
</tr>
<tr>
<td>N2</td>
<td>16.31 17.82ab 18.24a 21.72a 20.74a 24.52a 27.86a 31.15b 31.97b 32.69b 33.29b</td>
</tr>
<tr>
<td>A</td>
<td>16.47 20.53b 24.63b 27.59b 30.53b 35.60b 40.27b</td>
</tr>
<tr>
<td>N4A</td>
<td>16.22 18.48ab 19.74a 22.32a 27.77b 32.91b 33.58b 35.73b 36.78c 40.48c 39.97</td>
</tr>
<tr>
<td>N2A</td>
<td>16.46 18.39b 20.04a 22.38a 29.55bc 35.09b 34.68b 37.77c 40.65c 46.54d 51.26d</td>
</tr>
</tbody>
</table>

a-d Mean values within a column with different letter are significantly different (p < 0.05). *Average of double analysis (n = 6).

Nordin (1969), Petäjä et al. (2000) and Christiansen (1980) moreover have observed similar results, finding that the residual nitrite level was quite low after six weeks. Ascorbic acid allowed the nitrite to be reduced into nitric oxide, resulting in a gradual reduction of residual nitrite during storage (Ahn et al., 2004). These results indicated that combinations of ascorbic acid and vacuum packaging could be effective for reducing residual nitrite levels in cold smoked fish during storage.
the groups containing nitrite. The differences in these values are significant from the statistical point of view. High differences between 4 and 20 days have been noticed statistically ($p < 0.05$). Group N4A and N2A increased TVB-N quickly than groups N4 and N2 without ascorbic acid. This means that, the free residual nitrite is reduced by the combination ascorbic acid with nitrite in these groups, because, ascorbic acid in aqueous media rather rapidly interacts with free nitrite ions to form dehydroascorbic acid (Anatoli et al., 1996), and the addition of reducing agents provides a drastic decline of residual nitrite (Sen et al., 2001; Cassan 1997; Ahn et al., 2004).

**Thiobarbituric acid reactive substances (TBARS)** TBA is a widely used indicator for the assessment of degree of lipid oxidation and secondary oxidation metabolite. Table 4 presents variations of TBARS. TBARS value was 0.24 mg MA/kg for group F and has increased during brining and smoking process. The values were significantly different in C, N4, N2, A, N4A and N2A groups ($p < 0.05$). Bhuiyan et al. (1986) have also found an increase in TBARS values during the process that gradually have increased during the 32 days storage (Table 4).

There were differences in these values with respect to storage time statistically ($p < 0.05$). A positive effect for nitrite and ascorbic acid was found but this effect was diminished when they were used together. It was also found that ascorbic acid to be reduced residual nitrite. Alternatively, vitamin C is a remarkable antioxidant which has been shown to be a strong reducing agent in inhibiting or reversing met-myoglobin formation (Umah et al., 2003). These results show that TBARS values are much lower than an acceptable limit (3–mg malonaldehyde/kg) after 20 days storage. Shahidi and Wanasundara (1998) have reported that TBARS values give more sensitive results at higher values. Sánchez-Escalante et al. (2001) have found that rosemary powder and rosemary containing ascorbic acid were the most effective in inhibiting oxidation of both lipid and myoglobin.

**Microbiological characteristics of the experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.49×</td>
<td>0.82×</td>
<td>1.02×</td>
<td>1.54×</td>
<td>1.70×</td>
<td>1.99×</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>0.40×</td>
<td>0.45×</td>
<td>0.65×</td>
<td>0.70×</td>
<td>0.86×</td>
<td>0.95×</td>
<td>0.90×</td>
<td>0.91×</td>
<td>1.05×</td>
<td>1.09×</td>
<td>1.30×</td>
</tr>
<tr>
<td>N2</td>
<td>0.47×</td>
<td>0.58×</td>
<td>0.66×</td>
<td>0.82×</td>
<td>0.83×</td>
<td>1.21×</td>
<td>1.20×</td>
<td>1.16×</td>
<td>1.33×</td>
<td>1.33×</td>
<td>1.36×</td>
</tr>
<tr>
<td>A</td>
<td>0.65×</td>
<td>1.03×</td>
<td>1.06×</td>
<td>1.14×</td>
<td>1.36×</td>
<td>1.48×</td>
<td>1.88×</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4A</td>
<td>0.40×</td>
<td>0.51×</td>
<td>0.71×</td>
<td>0.97×</td>
<td>1.13×</td>
<td>1.12×</td>
<td>1.29×</td>
<td>1.19×</td>
<td>1.42×</td>
<td>1.46×</td>
<td>1.41×</td>
</tr>
<tr>
<td>N2A</td>
<td>0.52×</td>
<td>0.65×</td>
<td>0.78×</td>
<td>0.82×</td>
<td>1.19×</td>
<td>1.36×</td>
<td>1.38×</td>
<td>1.47×</td>
<td>1.41×</td>
<td>1.32×</td>
<td>1.92×</td>
</tr>
</tbody>
</table>

*a-e* Mean values within a column with different letter are significantly different ($p < 0.05$). *Average of double analysis (n = 6).

Figure 2 presents the TVC of cold-smoked vacuum packaged rainbow trout during storage at 4°C. TVC of the group C was 4.19 to 8.57 log cob/g at the 20th day of storage, higher than the entire other experimental groups. The TVC value of group A increased more slowly than group C. This was probably due to the preservation effect of ascorbic acid on TVC. The TVC values were reported as 5.62 – 8.39 log cob/g after a two-week storage at +2 +1°C by González-Rodríguez et al. (2002). Hyytiä et al. (1997) found to be similar to our results.

Significant differences were found in variance analysis between all of the groups during storage. Those groups containing nitrite were more stable with respect to the other groups ($p < 0.05$). Ascorbic acid has affected nitrite groups negatively while it has decreased the TVC when used alone. TVC values found in all of the groups studied have shown correlations for TVB-N values. However, these values in the N2A and N2 groups were found to be very similar. Hyytiä et al. (1997) establish TVC as 7.93 log cob/g in 3.4% salty samples during 4 weeks of storage at +4°C. This value was
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5.08 log cob/g in-group include nitrite. Our values in all of the groups containing nitrite were found to be higher than the values reported by Hyytiä et al. (1997). This is probably due to differences in the salt content, \( a_w \), total nitrite.

Figure 3 presents the evaluation of TPC of cold-smoked vacuum packaged rainbow trout during storage at 4°C.

TPC of group C has increased more rapidly compared to the other groups, where the TPC has increased much more slowly than that in groups containing nitrite; and ascorbic acid has negatively affected the groups containing nitrite and positively when used alone.

Figure 4 presents the evaluation of TLAB of cold-smoked vacuum packaged rainbow trout during storage at 4°C.

LAB values were the highest in group C as compared with the other microbiological results. However, no clear correlation exists between total LAB level and sensory rejection. These results are similar to the values reported by Hyytiä et al. (1997) and Lyhs et al. (1998). Nevertheless, a positive or negative effect of ascorbic acid on LAB was not observed. Whereas, it was demonstrated that fermentation with LAB can be applied to cold-smoked fish products (Petäjä et al., 2000). Hyytiä et al. (1997) have found the LAB value to be 7.08 log cob/g in the control group that has 3.4% salt during the 4 week storage at +4°C. This value was 5.17 log cob/g in groups containing nitrite.

Figure 5 presents the evaluation of TYM count of cold-smoked vacuum packaged rainbow trout during storage at 4°C.

We observed an improved preservative effect for TYM, where the TYM value of group A was lower than group N4A and N2A at the conclusion of the storage.

Sensory quality Sensory analysis was performed by a panel with proven skills and experience in the sensory assessment of meat and fish products. The panel had no information about the samples before. The results of sensory analysis correlated with the other results. Sensory score as
the appearance were the least in group C. The scores of group C were significant less than the other groups (p < 0.05). Therefore we can say that ascorbic acid did not also effect to product in appearance. The external colour of the all experimental group correlated with the appearance. These values were significant after 12 day (p < 0.05).

Figure 6 presents the evaluation of average sensory scores during the storage of cold-smoked vacuum packaged rainbow trout at ±4°C*.

Group C lost all sensory properties on the 20th day and acceptability of sample disappeared. Internal colour was also improving significant differences in all groups and between groups after 8 days. The effect of the additives was not found in the internal colour (p > 0.05). There were some significant differences in texture properties in groups throughout the storage (p < 0.05). The texture properties were correlated with spoiling. The odour of samples improved the most significant differences in all sensory properties. Odour scores were rapidly decreased in group C than the other groups. As a result, ascorbic acid did not reveal any negative effect on total quality of cold-smoked rainbow trout as sensory.

Conclusions
This study reveals that ascorbic acid has a positive effect on the quality of cold-smoked rainbow trout. This result is based on a comparison of group C to the ascorbic acid including group. On the other hand, by the utilization of ascorbic acid to the nitrite including groups (N4A and N2A), the total residual nitrite content in fish muscle decreased without reducing the quality too much. So, based on the results of this investigation, the following conclusions are drawn.

- It is suggested that the utilization of ascorbic acid be applied to increase total quality of fish samples partially.
- That the utilization of ascorbic acid in cold smoking fish with nitrite can be taken into consideration for the practical applications because of the lower residual nitrite and acceptable TVB-N, TBARS, TVC, TPC and TYM stability.
- That ascorbic acid can be used as a scavenger residual nitrite without reducing the quality.
- That ascorbic acid can be used for all the positive functional properties, such as oxygen scavenger, antimicrobial, antioxidant agent and nitrite scavenger.

As a result of this work, it can be concluded that, when comparing to the microbiological and chemical properties of fresh and processed fishes in the literature, ascorbic acid utilization of cold smoked fish contributes improving safety without reducing the quality.

Acknowledgements This study was supported by Hacettepe University, Nigde University and Bagci Seafood Co. in Turkey. The authors would kindly like to thank Mustafa Bagci providing the raw material and other supplies.

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