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Increasing the quality of cold-stored Atlantic Salmon (*Salmo salar* Linnaeus, 1758) via single and combined use of natural preservatives: chitosan, nisin and garlic essential oil

Steigerung der Qualität von kalt gelagertem Atlantischem Lachs (Salmo salar Linnaeus, 1758) durch einmalige und kombinierte Verwendung natürlicher Konservierungsmittel: Chitosan, Nisin und ätherisches Knoblauchöl

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Summary

It was aimed to reduce the economic and commercial damages caused by the loss of quality during marketing of salmon, a commercially popular fish around the world. Considering the trend of the consumer towards natural preservatives, the effects of low molecular weight chitosan (Ch, 2%), nisin (N, 0.5%), garlic essential oil (G, 1: 100 v:w) and their combinations (ChN, ChG, NG and ChNG) on the quality of cold-stored (2 ± 1 °C) salmon were investigated. The treatments containing chitosan gave successful results. When combined with nisin and garlic oil; chitosan was very effective to retard microbiological and physicochemical spoilage during storage. Chitosan-added samples also resulted in better sensory scores. The ChN samples had the lowest microbiological counts and pH, TVB-N and TMA-N values of this group remained below the acceptability limit during storage. Unlike other quality parameters, $TBAR_S$ values were found to increase more rapidly in chitosan-treated groups during storage period, but none of these groups reached the limit of acceptability during the study. The results indicated that chitosan significantly increases the shelf life and microbial quality of salmon, especially when combined with nisin. Increasing quality by using natural preservatives is important in terms of reducing economic losses, as well as delivering perishable foods such as salmon to consumers in remote areas and sustaining limited resources.

Keywords: Chitosan, nisin, garlic essential oil, salmon, cold storage

Introduction

Fish are rich in unsaturated fatty acids, high quality protein, and minerals. They are also highly digestible foods, having low connective tissue contents. These properties make them valuable and essential for humans, but they also lead fish to a very perishable nature. It is very common to store, transport and market fish under chilled conditions, but biochemical and bacteriological activities still continue. One of the most important species cultured in the world is the Atlantic salmon. As its popularity and trade has increased in the world, delaying quality changes during transport, storage and sale of salmon is important for economic reasons as well as for delivering healthy foods to people. Besides, the consumer may not eat the fish on the day of purchase, and may store it in the refrigerator for later consumption. So, it is important to reduce or delay quality losses, especially in the later stages of cold storage, and delayed the increase of main spoilage indicators such as the Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N), Thiobarbituric acid reactive substances (TBARs). So, the use of additional preservation methods is needed (Cao et al., 2009). However, in recent years, consumer do not prefer chemical preservatives and the use of natural preservatives gained importance (Schelegueda et al., 2012).

Chitosan is an animal-based natural antimicrobial, having biocompatible, biodegradable, nonantigenic, nontoxic, biofunctional, and antioxidant properties. It is derived from deacetylation of chitin, a component of crustaceans shells (No et al., 2007; Fan et al., 2009). Chitosan-based coatings or solutions have been studied to enhance quality and extend shelf life of various foods (Cao et al., 2009; Duan et al., 2010; Kanatt et al., 2013; Latou et al., 2014; Li et al., 2013; Wu et al., 2016; Yu et al., 2017a), and it has been reported that the antibacterial activity of chitosan can be increased by combining with other substances (Devlieghere et al., 2004). It was stated that the single use of chitosan-coated plastic films did not prevent the development of *Listeria monocytogenes* in red meats, but its antimicrobial effect increased when combined with various antimicrobial agents (Ye et al., 2008a). Similarly, chitosan has been reported to provide a more effective protection in smoked salmon, when combined with sodium lactate (Ye et al., 2008b).

Nisin is a natural preservative, produced by *Lactococcus lactis* subsp. *Lactis.* It is a commercially available food ingredient and former studies have demonstrated the antimicrobial activity of nisin in smoked salmon (Ye et al., 2008b), vacuum-packed smoked salmon (Neetoo et al., 2008), chilled shrimp (Shirazinejad et al., 2010) and minced fish meat (Abdollahzadeh et al., 2014). It has been reported that the antimicrobial effect of nisin is predominantly on gram positive bacteria (Guohua et al., 2016).

Herbal oils have also been used by many food manufacturers as natural food preservatives (Burt, 2004). Garlic oil can be used as a natural food additive, since it contains organic sulfur compounds with antimicrobial activity against a wide range of bacteria (Leyva et al., 2016). An in vitro study by Pranoto et al., (2005) stated that the addition of garlic oil increases the antimicrobial activity of chitosan film and emphasized that antimicrobial properties can be increased when different antimicrobials used together. Inadequate aspects of natural preservatives such as ineffectiveness against some bacterial groups or improper sensory characteristics at high doses may be supported by combining with another (Zeuthen and Bogh-Sorensen, 2003). The combination of preservation methods provides marketing advantages by increasing the shelf life of food, and it is called as „hurdle technology“ (Fellows, 2000). So, longer shelf life can be achieved and better applications can be made in terms of food safety.

In this context, it was aimed to determine the effects of single and combined use of chitosan, nisin and garlic oil on the quality of cold-stored salmon, considering the consumer tendency to natural preservatives. The use of natural preservatives to extend shelf life is important in reducing economic losses, distributing perishable foods such as salmon to consumer groups at greater distances; and sustaining limited food resources.

Materials and methods

Preparation of salmon fillets and treatment application

Imported (Norway) Atlantic salmon fillets were purchased from a multinational hypermarket in Istanbul, just after they were put on sale. Since salmons are generally sold as fillets or cuts in the market, the samples were purchased as fillets, then they were transferred to the laboratory in a chilled box within 30 minutes. The samples were prepared to be 100 grams (11×6×2 cm) from the dorsal part of fillets. Raw material analyses were performed immediately. Chitosan solution was prepared by dissolving low molecular weight chitosan (deacetylated chitin, Poly (D-glucosamine)) (Sigma Aldrich) in acetic acid (1%v/v glacial Merck) to a final concentration of 2% (w/v). Nisin solution was prepared by dissolving nisin (from *Lactococcus lactis*) (Sigma Aldrich) in sterile distilled water to the final concentration of 0.5% (w/v). Garlic essential oil was the commercial pure product (100%) of KRK FOOD Company, Istanbul-Turkey. Samples were divided as the following 8 groups: Control (C), Chitosan (Ch), Nisin (N), Garlic essential oil (G), Chitosan-Nisin (ChN), Nisin-Garlic essential oil (NG), Chitosan-Garlic essential oil (ChG), Chitosan-Nisin-Garlic essential oil (ChNG).

Control samples were immersed into sterile distilled water for 15 minutes. For the treatments with chitosan and nisin, samples were immersed in related solutions separately for 15 minutes. Then the samples were drained for 15 min. The ratio of fish samples to chemical solution volume was 1:2 (w/v). Garlic essential oil (1 ml) was added using a sterile micropipette onto the surface of 100 g fish samples, in order to achieve 1% oil volume per fish weight (v/w). Combined applications were performed by successive applications of these procedures. The samples belonging to different treatment groups were placed in polystyrene boxes separately, and stored at 2±1 ºC. Analyses were performed at 3-day intervals. Randomly chosen five fish samples (100 g) were taken from each group boxes for analyses.

Microbiological analyses

Ten grams of samples were homogenized with 90 mL peptone water (0.1%) (Merck) in stomacher (IUL Instruments, Barcelona, Spain) for 60 s. Appropriate serial dilutions (1:10 diluent) were prepared with 0.1% peptone water. One mL of the diluent was poured into petri dish, then plate count agar (PCA, Merck) was added. Total mesophilic aerobic bacteria count (TMC) was determined after incubation at 37 °C for 24-48 h. Total psychrophilic bacteria count (TPC) was determined after incubati-

Journal of Food Safety and Food Quality 73, Heft 3 (2022), Seiten 75–108

on at 7 °C for 10 days (Baumgart, 1986). Enumeration of yeast and mold (YM) was performed on Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck). Diluent (0.1 mL) was spread on the surface of DRBC agar. Yeast and mold was determined after incubation at 25 °C for 5 days (Tournas et al., 2001).

Physicochemical analyses

For the measurement of pH, fish flesh was homogenized in distilled water (1:10 w:v) and measured using pH meter (Hanna pH 211 Micro-processor pH meter, Ann Arbor, MI) (Vyncke, 1981). The Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) contents of fish samples were determined according to the methods described by Schormüller (1968). Fish samples (10 g) were mixed with catalyst (MgO, 1 g, Merck), then heated and vapor components were collected in a flask, where HCl (0.1 N, Merck) added. Then, NaOH (0.1N, Merck) was used to titrate this mixture and the TVB-N was expressed as mg/100 g fish. For the TMA-N estimation, samples were homogenized with trichloracetic acid solution (10%, Merck), filtrated, then mixed with toluene (Balmumcu, Ltd), formaldehyde (20%, Merck), and potassium hydroxide (50%, Carlo Erba). The upper phase was mixed with picric acid (0.2%) in another tube, then measured at 410 nm by spectrophotometer (PG Instruments, UV/VIS, T801, UK). Standard trimethylamine solutions were also prepared and measured similarly. Then, sample measurements were compared with the standard curve and trimethylamine content was expressed as mg/100g fish. Thiobarbituric acid reactive substances (TBARs) value was determined according to the method described by Varlık et al., (2007). Samples (5g), butylated hydroxytoluen (100 µl, SIGMA) and distilled water (50 mL) were shaken, mixed with HCl (2.5 mL, 4 N, Merck) and distilled water (97.5 mL). After heating and condensation, the liquid (5 mL) was mixed with 2-TBAR (5 mL, Merck) and heated at 70–80°C for 30 min. A water bath was used for heating. Then density was measured at 532 nm (PG Instruments, UV/VIS, T801, UK). The malondialdehit (MDA) concentration was calculated from a standard curve using solutions of the MDA precursor and the result were expressed as mg MDA/kg of fish.

Sensory analysis

The texture, odor, color and taste of salmon samples were assessed by 7 panelists, experienced to assess fish quality by sensory tests. For the taste evaluation, samples were placed in glass jars, the jars were closed then cooked in boiling water bath for five minutes. Then samples were randomly coded, placed in white plates and served to the panelists. The laboratory was well ventilated, daylight was used, and panelists' interaction was avoided. An acceptability scale between 0–10 was used (Chytiri et al., 2004). According to this, 10–9 was regarded excellent, 8–7 very good, 6–5 acceptable, and the scored below 5 unacceptable.

Statistical analysis

In this study, chemical analyzes were carried out in three replicates and microbiological analyzes were performed in duplicate. Statistical analyses were performed using IBM SPSS 21 software (SPSS Inc.; Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare the results of the groups. Data are presented as means and ± standard deviations. Differences were accepted as significant when $P<0.05$.

Results and discussion

Microbiological analyses

The initial microbial load of fish has been generally reported between 2–5 log cfu/g (Cao et al., 2009). In this study, the initial TMC and TPC of the untreated salmon samples were 4.18±0.09 and 4.61±0.12 log cfu/g, respectively. The TMC of C, N, G and NG samples were significantly higher ($P<0.05$) than the samples treated with chitosan (Ch, ChN, ChG and ChNG), during the 9 days of storage (Table 1). The samples without chitosan (C, N, and NG) exceeded the acceptability limit of 5.69 log cfu/g (ICMSF, 1986) at the 3th day, and G group samples exceeded this limit at the 6 day of storage. However, mesophilic aerobic bacteria count of the Ch, ChG, and ChNG samples exceeded this value after 9 days, and CN samples after 12 days of storage (Table 1). Mostly, the combination of chitosan with nisin resulted in the lowest mesophilic aerobic count. It was seen that the chitosan-treated samples, had lower mesophilic aerobic bacteria counts. Shahbazi and Shavisi (2018) showed that mesophilic aerobic bacteria count of untreated rainbow trout exceeded the acceptability limit on the 6th day of refrigerated storage, while chitosan-treated (2%) ones remained below that limit. Wang et al., (2018) reported a similar result for the grass carp treated with 2% chitosan. Reduced growth of mesophilic aerobic bacteria was also reported for chitosan-treated oysters during cold storage by Cao et al., (2009). The reduced micro-

TABLE 1: *Changes in the microbiological quality of salmon samples during storage at 2±1°C.*

	Treatment	Storage time (day) 3	6	9	12
TMC (log CFU/g)	C	6.25 ± 0.18^{aA}	7.92 ± 0.06^{aB}	9.03 ± 0.07^{aB}	7.87 ± 0.11^{acB}
	Ch	2.59 ± 0.10^{bA}	5.39 ± 0.02^{bB}	7.26 ± 0.03^{bC}	7.86 ± 0.19^{acC}
	N	6.17 ± 0.24^{aA}	8.13 ± 0.11^{aB}	8.21 ± 0.15^{cB}	7.99 ± 0.06^{acdB}
	G	5.38 ± 0.30^{cA}	8.15 ± 0.09^{aB}	8.50 ± 0.08^{cC}	7.77 ± 0.02^{aD}
	ChN	3.28 ± 0.23^{dA}	3.64 ± 0.09^{cA}	4.81 ± 0.05^{dB}	6.19 ± 0.11^{bC}
	NG	6.38 ± 0.02^{aA}	8.22 ± 0.01^{aB}	8.51 ± 0.03^{cC}	8.04 ± 0.06^{cD}
	ChG	3.44 ± 0.21^{dA}	4.88 ± 0.04^{dB}	6.26 ± 0.19^{eC}	7.86 ± 0.05^{acD}
	ChNG	3.37 ± 0.19^{dA}	4.76 ± 0.19^{bdB}	6.05 ± 0.22^{eC}	7.42 ± 0.03^{dD}
TPC (log CFU/g)	C	7.47 ± 0.08^{aA}	9.49 ± 0.04^{aB}	9.89 ± 0.05^{aC}	9.21 ± 0.08^{abD}
	Ch	4.32 ± 0.02^{bA}	6.69 ± 0.10^{bB}	8.37 ± 0.07^{bC}	9.20 ± 0.06^{aD}
	N	7.33 ± 0.04^{aA}	9.14 ± 0.03^{cB}	9.66 ± 0.16^{acC}	9.39 ± 0.06^{bB}
	G	6.12 ± 0.07^{cA}	9.01 ± 0.03^{cB}	9.37 ± 0.03^{cC}	9.79 ± 0.04^{cD}
	ChN	3.49 ± 0.10^{dA}	4.59 ± 0.10^{dB}	7.42 ± 0.06^{dC}	7.79 ± 0.03^{dD}
	NG	7.31 ± 0.10^{aA}	9.33 ± 0.15^{acB}	$9.53 \pm 0,11^{acBC}$	9.79 ± 0.01^{cC}
	ChG	3.75 ± 0.25^{bdA}	6.37 ± 0.02^{bB}	7.24 ± 0.11^{dC}	8.77 ± 0.09^{eD}
	ChNG	4.03 ± 0.20^{bdA}	6.33 ± 0.18^{bB}	7.65 ± 0.10^{dC}	8.83 ± 0.05^{eD}
YM (log CFU/g)	C	5.59 ± 0.14^{aA}	8.47 ± 0.03^{aB}	8.68 ± 0.11^{aBC}	9.03 ± 0.01^{aC}
	Ch	3.54 ± 0.45^{bA}	4.84 ± 0.89^{beB}	6.72 ± 0.07^{bC}	8.63 ± 0.06^{bD}
	N	6.01 ± 0.10^{aA}	8.28 ± 0.05^{cB}	9.10 ± 0.15^{cC}	9.46 ± 0.08^{cC}
	G	5.53 ± 0.10^{aA}	7.92 ± 0.04^{dB}	8.39 ± 0.13^{aC}	9.45 ± 0.09^{cD}
	ChN	2.50 ± 0.17^{cA}	4.75 ± 0.03^{bB}	5.59 ± 0.12^{dC}	7.49 ± 0.02^{dD}
	NG	6.01 ± 0.42^{aA}	8.37 ± 0.02^{acB}	9.23 ± 0.05^{cC}	9.43 ± 0.07^{cD}
	ChG	2.70 ± 0.29^{bcA}	4.83 ± 0.09^{beB}	6.20 ± 0.11^{eC}	8.76 ± 0.04^{bD}
	ChNG	3.48 ± 0.44^{bA}	4.94 ± 0.05^{eB}	7.45 ± 0.22^{fC}	9.00 ± 0.12^{aD}

C: Contol, Ch: Chitosan, N: Nisin, G: Garlic essential oil, ChN: Chitosan combined with nisin, NG: Nisin combined with garlic essential oil, ChG: Chitosan combined with garlic essential oil, ChNG: Chitosan combined with nisin and garlic essential oil. Different lower letters in the same column show significant differences ($P<0.05$). Different upper letters in the same raw show significant differences ($P<0.05$). ±: Standard deviation.

bial growth in chitosan-containing groups may occur due to cell membrane damage caused by the binding of positively charged chitosan to the negatively charged bacterial surface. This may cause leaking of nutrients out, which are necessary for the bacteria to feed. Other possible mechanisms of chitosan are the prevention of required nutrients entry to the cell by forming a polymer layer on cell surface, and inhibiting mRNA and protein synthesis by interacting with DNA (Demir et al., 2008; Hosseinnejad and Jafari, 2016; Kulawik et al., 2020). In this study, chitosan was found to be more effective in delaying microbial growth when used in combination with other antibacterials, and it was found to be more effective especially with nisin.

Similar results were also found for the total psychrophilic aerobic count in this study. Chitosan-treated samples (Ch, ChN, ChG and ChNG) resulted in significantly ($P<0.05$) lower psychrophilic counts when compared to other groups. Even though total psychrophilic aerobic count of C, N, G, NG samples exceeded acceptability limit of 6 log cfu/g (Mol et al., 2007) at the 3th day; Ch, ChG and ChNG samples were above this limit after 6 days, and ChN samples after 9 days of storage. Generally, the combination of chitosan with nisin gave the lowest psychrophilic aerobic count, during the study. Combined use of chitosan resulted in lower psychrophilic counts in similar studies. Combination of chitosan with 6-gingerol (Mi et al., 2017), haw horn flavonoid (Li et al., 2017), sumac (Fadıloğlu and Emir Çoban 2018), and pomegranate peel extract (Alsaggaf, et al., 2017) resulted in reduced growth of psychrophilic bacteria in various seafood products. Likewise, Kootenaie et al., (2017) combined chitosan with natural oil, and reported better results compared to the control and the chitosan-treated samples.

The initial yeast and mold (YM) count of salmon samples was 3.65±0.12 log cfu/g. Yeast and mold counts of chitosan-free groups exceeded 5 log cfu/g only after 3 days of cold storage (Table 1), and they were significantly higher ($P<0.05$) than chitosan-added groups. Similar to mesophilic and psychrophilic aerobic counts, combined use of chitosan and nisin resulted in significantly lower YM counts than all other treatments, especially at 9th and 12th days ($P<0.05$). Previous studies exhibited that chitosan coating reduced YM growth on different kinds of fish during the cold storage, similar to findings of our study (Feng et al., 2016; Bonilla et al., 2019; Kalkan et al., 2019).

Physicochemical analysis

The initial pH value of samples was determined as 6.34±0.01. The fish is generally accepted as spoiled when its pH is above 6.8 (Ludorf and Meyer 1973). In this study, it is observed that the pH values of the Ch, ChN, ChG and ChNG groups remained below this value even after 12 days of storage ($P<0.05$), as shown in Table 2. Similar to our results, Duan et al., (2010) reported that pH values of chitosan-coated lingcod fillets were significantly lower than those of control group during the cold storage. In other studies, it has also stated that chitosan treatment reduced pH values of different types of food (Mohan et al., 2012; Aşık and Candoğan 2014; Cai et al., 2018; İzci and Şimşek 2018; Pabast et al., 2018). This effect of chitosan on pH is based on it reducing the production of alkaline compounds by limiting bacterial growth (Yu et al., 2017b; Gürel İnanli et al., 2020).

The TVB-N is one of the important indicators to evaluate fish freshness (Masniyom & Benjema 2007). The limit of acceptability is 30 mg/100g TVB-N, according to Sikorski et al., (1990). In the present study, the initial TVB-N value of fish sample was 3.83±1.66 mg/100g, showing that the quality of the material was very good. This value increased and exceeded the limit of acceptability in groups without chitosan, but Ch, ChN and ChG samples did not reach this limit during the storage (Table 2). TVB-N values of ChNG group remained lower than samples without chitosan, but exceeded the limit on the 12th day. Guohua et al., (2016) reported significantly lower TVB-N values in chitosan-coated yellow croaker *(Pseudosciaena crocea)* than control group. Similar to our results, they also found that the combined use of nisin (0.2%) and chitosan (1%) provided lower TVB-N levels than chitosan alone. Chitosan treatment provided lower TVB-N values than control samples in cold stored shrimp (Aşık and Candoğan 2014), and grass carp (Yu et al., 2017b) as well. The antibacterial effect of chitosan may result in retarded increase of TVB-N (Sun et al., 2017; Gürel İnanli et al., 2020).

Trimethylamine nitrogen contributes to the characteristic ammonia-like off-odor in fish spoilage (Gram and Huss

TABLE 2: *Changes in the physicochemical quality of salmon samples during storage at 2±1 °C.*

	Treatment	Storage time (day) 3	6	9	12
pH	C	6.30 ± 0.00^{aA}	6.55 ± 0.00^{aB}	6.83 ± 0.01^{aC}	6.98 ± 0.00^{aD}
	Ch	6.12 ± 0.01^{bA}	6.05 ± 0.00^{bB}	6.27 ± 0.02^{bC}	6.31 ± 0.02^{bC}
	N	6.34 ± 0.02^{aA}	6.38 ± 0.01^{cB}	6.72 ± 0.01^{cC}	6.98 ± 0.00^{aD}
	G	6.40 ± 0.01^{cA}	6.54 ± 0.01^{aB}	6.90 ± 0.00^{dC}	7.08 ± 0.02^{cD}
	ChN	6.15 ± 0.00^{bA}	6.08 ± 0.01^{bB}	6.12 ± 0.01^{eC}	6.19 ± 0.00^{dD}
	NG	6.45 ± 0.02^{cA}	6.46 ± 0.00^{dA}	6.84 ± 0.00^{aB}	7.03 ± 0.00^{cC}
	ChG	6.15 ± 0.00^{bA}	6.07 ± 0.01^{bB}	6.26 ± 0.01^{bC}	6.21 ± 0.01^{dD}
	ChNG	6.23 ± 0.00^{dA}	6.14 ± 0.00^{eB}	6.24 ± 0.01^{bA}	6.49 ± 0.00^{eC}
TVB-N (mg/100g)	C	16.50 ± 1.32^{acA}	22.00 ± 0.56^{aB}	43.38 ± 2.49^{aC}	63.42 ± 1.41^{aD}
	Ch	15.81 ± 1.27^{aA}	17.39 ± 1.52^{bcA}	22.06 ± 0.84^{beB}	26.77 ± 1.38^{bC}
	N	20.85 ± 0.90^{bdA}	15.39 ± 0.23^{bB}	30.75 ± 1.08^{cC}	52.68 ± 3.20^{cD}
	G	19.23 ± 0.16^{cbdA}	24.32 ± 0.54^{aB}	47.02 ± 1.36^{adC}	71.06 ± 0.23^{dD}
	ChN	18.15 ± 1.28^{abAB}	16.09 ± 0.77^{bA}	19.42 ± 1.26^{bBC}	21.32 ± 0.55^{eC}
	NG	16.92 ± 1.38^{acA}	23.58 ± 0.83^{aB}	49.11 ± 1.68^{dC}	85.08 ± 1.74^{fD}
	ChG	21.64 ± 1.08^{dAC}	19.14 ± 1.46^{cAB}	18.17 ± 1.57^{bB}	23.30 ± 1.00^{beC}
	ChNG	18.73 ± 0.47^{abcdA}	18.08 ± 1.07^{bcA}	25.57 ± 2.13^{eB}	32.87 ± 1.44^{gC}
TMA (mg/100g)	C	1.65 ± 0.11^{acA}	3.99 ± 0.29^{aB}	5.43 ± 0.19^{aC}	7.54 ± 0.36^{acD}
	Ch	0.55 ± 0.06^{bdA}	0.52 ± 0.11^{bA}	0.55 ± 0.04^{bcA}	0.53 ± 0.06^{bA}
	N	0.28 ± 0.02^{bA}	1.74 ± 0.03^{cB}	4.41 ± 0.00^{aC}	6.81 ± 0.17^{aD}
	G	1.99 ± 0.05^{aA}	3.62 ± 0.18^{aB}	6.98 ± 0.66^{aC}	8.14 ± 0.12^{cdC}
	ChN	0.38 ± 0.18^{bdA}	0.84 ± 0.26^{bcB}	0.27 ± 0.07^{bA}	0.61 ± 0.02^{bAB}
	NG	0.71 ± 0.23^{bcdA}	3.31 ± 0.21^{aB}	6.82 ± 0.50^{aC}	10.72 ± 0.75^{dD}
	ChG	0.62 ± 0.02^{dAB}	0.53 ± 0.04^{bA}	0.69 ± 0.04^{cB}	1.06 ± 0.25^{beB}
	ChNG	0.56 ± 0.15^{bdA}	0.59 ± 0.19^{bA}	0.93 ± 0.13^{cA}	1.66 ± 0.14^{eB}
TBARs (mg MDA/kg)	C	1.37 ± 0.26^{acA}	1.03 ± 0.17^{aAC}	0.46 ± 0.05^{aB}	0.68 ± 0.01^{aBC}
	Ch	3.21 ± 0.37^{bA}	4.69 ± 0.17^{bB}	3.11 ± 0.26^{bA}	6.61 ± 0.10^{bC}
	N	0.51 ± 0.08^{aA}	1.62 ± 0.95^{abAB}	1.54 ± 0.25^{cB}	1.44 ± 0.20^{acB}
	G	2.34 ± 0.30^{cbA}	1.12 ± 0.11^{aB}	1.41 ± 0.14^{cBC}	1.92 ± 0.29^{cAC}
	ChN	1.51 ± 0.64^{aceA}	5.39 ± 0.27^{bB}	5.31 ± 0.56^{dB}	6.41 ± 0.42^{bB}
	NG	0.54 ± 0.08^{aA}	1.30 ± 0.08^{aB}	3.26 ± 0.21^{bC}	1.95 ± 0.18^{cD}
	ChG	6.94 ± 0.49^{dA}	5.05 ± 0.34^{bB}	4.32 ± 0.45^{eB}	5.10 ± 0.65^{dB}
	ChNG	2.49 ± 0.43^{beA}	5.00 ± 0.53^{bB}	5.66 ± 0.33^{dB}	3.18 ± 0.08^{eA}

C: Contol, Ch: Chitosan, N: Nisin, G: Garlic essential oil, ChN: Chitosan combined with nisin, NG: Nisin combined with garlic essential oil, ChG: Chitosan combined with garlic essential oil, ChNG: Chitosan combined with nisin and garlic essential oil. Different lower letters in the same column show significant differences ($P<0.05$). Different upper letters in the same raw show significant differences ($P<0.05$). ±: Standard deviation.

Journal of Food Safety and Food Quality 73, Heft 3 (2022), Seiten 75–108

1996), and the limit of acceptability for fish has been reported between 5–10 mg/100 g (Sikorski et al., 1990). In this study, the initial TMA-N value of salmon was 0.23±0.04 mg/100 g, showing a high quality of the material. Especially in the later stages of storage, the TMA-N levels of all chitosan-containing groups (Ch, ChN, ChG, ChNG) were significantly lower ($P<0.05$) than the others, and well below the limit value. Lower TMA-N values of chitosan-treated fish have also been reported in various studies (Souza et al., 2010; Mohan et al., 2012; Yu et al., 2017b). The antibacterial effect of chitosan may be responsible for delaying TMA-N formation in fish (Yu et al., 2017b).

The TBARs content is another widely used indicator of freshness (Wu et al., 2016). A value of 8 mg MDA/kg has been suggested by Schormüller (1968) as the acceptance limit for fish. In this study, the initial TBARs value of fresh salmon was 0.64±0.24 mg MDA/kg. Unlike other quality parameters, TBARs values were found to increase more rapidly in chitosan-treated groups (Table 2). However, it should be noted that TBARs values were below the acceptable limits in all groups during the storage. İzci and Şimşek (2018) reported that TBARs values did not exceed the limit value (8 mg MDA/kg) during the storage in chitosan-treated fish and untreated fish samples, similar to our study. Likewise, Alak (2012) and Yumuk et al., (2019) reported higher TBARs values of chitosan-coated fish during later stages of cold storage. However, Wang et al., (2018) reported that there were no differences in TBARs value between the untreated and chitosan-treated grass carp fillets during the cold storage. There are other studies reported that chitosan treatment reduced lipid oxidations in sea bass (Ahmed et al., 2017), silver carp (Kootenaie et al., 2017) and grass carp (Yu et al., 2017a,b; Cai et al., 2018; Wang et al., 2018). Although the TBARs values of the groups containing chitosan were higher than the others in our study, they were found to be of acceptable quality since they remained below the limit of 8 mg/100 g during storage.

Sensory evaluation

The initial sensory score of fresh salmon was 9.3±0.9, but this score decreased in all groups during cold storage. The C, G and NG samples spoiled on the 6th day, while chitosan added-groups (Ch, ChN, ChG and ChNG) and N samples still acceptable Table 3. The panelists stated that the tissues of chitosan-containing groups had more firmness than other groups, and the stickiness of other groups increased throughout the storage. In another study, sensory quality of chitosan-coated (2%) silver carp remained acceptable for 30 days, but control samples spoiled at the 25th day of frozen storage. This result was attributed to the functional properties of chitosan (Fan et al., 2009). Chitosan coating has also been reported to be effective in preserving the sensory properties of grilled pork during cold storage (Yingyuad et al., 2006). Mohan et al., (2012) reported that sardine fillets coated with 1% and 2% chitosan had the highest acceptability scores. Likewise, Wang et al., (2018) found that the fish samples coated with chitosan or chitosan & essential oil combination had highest sensory scores during storage. In another study, the sensory scores of grass carp fillets in which chitosan and essential oils were used together showed the highest sensory acceptability (Cai et al., 2018).

TABLE 3: *Changes in the sensory properties of salmon, during storage at 2±1°C.*

Treatment	Storage time (day) 3	6	9	12
C	9.06 ± 0.87^{aA}	3.87 ± 0.66^{aB}	1.91 ± 0.95^{aC}	1.91 ± 0.46^{aC}
Ch	8.77 ± 0.83^{aA}	5.00 ± 1.06^{aB}	3.24 ± 1.21^{aC}	2.72 ± 0.96^{aC}
N	8.70 ± 0.88^{aA}	5.25 ± 1.36^{aB}	2.62 ± 0.81^{aC}	2.34 ± 0.78^{aC}
G	8.35 ± 1.27^{aA}	4.66 ± 1.26^{aB}	2.27 ± 0.80^{aC}	1.73 ± 0.74^{aC}
ChN	8.47 ± 1.43^{aA}	5.57 ± 1.45^{aB}	2.89 ± 1.08^{aC}	2.97 ± 1.18^{aC}
NG	8.66 ± 0.88^{aA}	4.67 ± 0.78^{aB}	2.20 ± 0.62^{aC}	2.15 ± 0.61^{aC}
ChG	8.54 ± 1.01^{aA}	5.22 ± 1.43^{aB}	3.20 ± 1.11^{aC}	2.64 ± 1.09^{aC}
ChNG	8.37 ± 1.56^{aA}	5.11 ± 0.99^{aB}	3.35 ± 0.93^{aBC}	3.25 ± 0.70^{aC}

C: Contol, Ch: Chitosan, N: Nisin, G: Garlic essential oil, ChN: Chitosan combined with nisin, NG: Nisin combined with garlic essential oil, ChG: Chitosan combined with garlic essential oil, ChNG: Chitosan combined with nisin and garlic essential oil. Different lower letters in the same column show significant differences ($P<0.05$). Different upper letters in the same raw show significant differences ($P<0.05$). ±: Standard deviation.

Conclusion

Considering the effects of chitosan, nisin and garlic essential oil on the quality changes of salmon during cold storage, the treatments containing chitosan gave the most successful results regarding sensory, microbiological and pH, TVB-N and TMA-N analyses. It was seen that the combined use of chitosan and nisin generally gave the lowest microbiological counts during storage. The results of our study showed that chitosan is an effective natural preservative in cold-stored salmon, especially when used in combination with nisin. This process can help to reduce economic losses and increase microbial safety of cold-stored fish and can be a consumer preferred application due to its natural properties.

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Conflict of interest

The authors declare no conflict of interest.

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