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PAPER

Effects of chitosan on the shelf life of marinated sardine (*Sardina pilchardus*) fillets during refrigerated storage

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Abstract

This study was carried out to evaluate the effect of chitosan on chemical, colour, sensory and microbial changes of marinated sardine (*Sardina pilchardus*) fillets. Marination solution consisted of 10% sodium chloride +1% chitosan (dissolved in 3% acetic acid) for the chitosan group, and 10% sodium chloride +3% acetic acid solution for the control group. After the marination process, sardine fillets were packed and stored at 4°C for 60 days. Thiobarbituric acid (TBA) values were found to be lower in the chitosan group than the control group (P<0.05). There was no difference in the total volatile basic nitrogen (TVB-N) value between groups and this remained low during storage. pH was under 4.5 in both groups during storage. Chitosan had no statistically significant effect on *L**, *a**, or *b** values of marinated sardine fillets. According to sensory analysis, shelf life of the chitosan group was found to be ten days longer than that of the control group. Total bacteria count of two marinated groups was found to be less than 1 log CFU/g. This study concluded that sardine marination with the addition of chitosan can delay undesirable chemical changes, retard lipid oxidation, improve sensory attributes and extend the shelf life of the product during refrigerated storage.

Introduction

Fishery products have high nutritional value but are also sensitive to microbiological, enzymatic and physical degradation. Therefore, they are included in the food group that should be considered the most important in terms of conservation from production to consumption. For this reason, they should be consumed soon after fishing or should be conserved by processing methods. For this purpose, different processing technologies are used that take into account the properties of the raw material in order to conserve fishery products without degradation and transport them according to health standards. Marination is one of the most important technologies used to achieve this.

Marination is a process of treating fish meat with acetic acid and salt. It is an alternative conservation method for fishery products (Poligne and Collignan, 2000). Vinegar and salt stop bacterial and enzyme action in fish and therefore extend the shelf life of the product. This method is used especially for anchovy, sardine, sprat, codfish, shad, *etc*., and this type of food conservation is a popular favorite in Turkey and in other European Countries (McLay, 1972; Schenderyuk and Byokowski, 1990; Erkan *et al.,* 2000). The quality and shelf life of marinated products change according to the freshness of fish, the conditions of marination and storage, and the addition of preserving substances (Dokuzlu, 1996). Previous studies on marinated fish usually investigated the direct effect of acetic acid and salt without any additive substance on shelf life during storage (Erkan *et al.,* 2000; Gökoğlu *et al.,* 2004; Kılınc and Caklı, 2005; Özden and Erkan, 2006). In fact, so far no studies have been published investigating the application of chitosan. This is known to have antioxidant and antimicrobial properties for the conservation of different fishery products (Jeon *et al.,* 2002; Kamil *et al*., 2002; Tsai *et al.,* 2002; Sathivel *et al.,* 2007). This study, therefore, aims to investigate the chemical, physical, sensory and microbiological changes in sardine during marination with the addition of chitosan and then during refrigerated storage. Sardine is one of the most commonly caught fish species. It is consumed either fresh or frozen and sold on domestic and foreign markets.

Materials and methods

Materials

Chitosan with low molecular weight (448869, Sigma Chemical Co., St Louis, MO, USA) was used in the study. Sardine samples (*Sardina pilchardus*) (average weight and length 19.59±1.55 g and 13.29±0.33 cm, respectively) were purchased from a local fish market. They were stored in ice in an insulated box and transferred to the laboratory. The head and viscera were removed from each fish and the fish were filleted.

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Marinating process

Chitosan was dissolved in acetic acid (3%) at 1% concentration. Sardine fillets (approximately 2 kg fillets for each group) were immersed into the following marinating solutions for 27 h (1:1.5 fish:solution ratio) in a refrigerator: the chitosan group containing 1% chitosan and sodium chloride (10%) and the control group containing only acetic acid (3%) and sodium chloride (10%). Samples were then removed from the treatment solution and placed in sterile bags (Baglight, 20×25 m, 400 mL, Interscience, Saint Nom, France). Samples were stored in the refrigerator (4±1°C) for 60 days. All analyses were performed in triplicate on Days 0, 30, 40, 50 and 60.

Chemical analysis

Samples were homogenized and subjected to moisture and ash analyses using AOAC (1990) methods. Crude protein content was calculated by converting the nitrogen content according to Kjeldahl's method (AOAC, 1990), and lipid content was determined according to the method of Bligh and Dyer (1959).

Thiobarbituric acid (TBA) number was determined using the method of Tarladgis *et al.* (1960), expressed as mg malondialdehyde/kg sample using a conversion factor of 7.8. The pH was determined from homogenates of minced fish and distilled water in a ratio of 1:10 (w/v) by using a digital pH meter (Testo 206, AG, Germany). Total volatile basic nitrogen (TVB-N) was determined on steam distillation using the Kjeldahl distillation apparatus and titration (Antonocopoulus, 1973).

Colourimetric analysis

Colourimetric measurements were taken according to the Calder (2003) method. Sample colour was measured using a portable Hunter Lab colour analyzer (Hunter Associates Laboratory Inc., Reston, VA, USA). The sensor was standardized with white and black tiles for the analysis. *L*, a** and *b** values were recorded. The *L** variable represents lightness (*L**=0 for black, *L**=100 for white), *a** scale represents the red/green, +*a** intensity in red and -*a** intensity in green. *b** scale represents the yellow/blue, +*b** intensity in yellow and -*b** intensity in blue. Colour was measured on three different parts of the fillet pieces, and then chroma, hue and whiteness values were calculated.

Sensory evaluation

Sensory characteristics of the marinated sardine fillets were determined by a panel of 7 experts, all members of staff of the Department of Fish Processing Technology, Faculty of Fisheries, Cukurova University. Panellists gave scores for sensory characteristics, such as appearance, odor, flavor and texture using a 9-point descriptive scale. On this scale, scores between 7.0 and 9.0 indicated extremely liked; scores between 4.0 and 6.9 indicated liked, and 3.9 was the limit of acceptability (Paulus *et al.,* 1979).

Microbiological analysis

For microbial counts, 10 g of samples were homogenized with 90 mL of 0.1% peptone water in a Stomacher (Bagmixer, Interscience) for 1 min at a normal speed. From the 10^{-1} dilution, other serial dilutions were prepared. Total viable count (TVC) was determined on plate count agar (Merck). Plates were incubated at 30ºC for 24-48 h and the number of viable microorganisms was counted, calculated and expressed as colony-forming units per gram (log cfu/g).

Statistical analysis

Data were subjected to analyses of variance at the 5% level using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software and the *t*-test was performed to separate differences among means.

Results and discussion

Proximate analysis

The proximate analysis results of fresh sardine fillets are shown in Table 1. Protein content of sardine fillets was determined as 17.01% while lipid content was 4.19%. Kılınc and Caklı (2005) found lower protein (13.20%) and lipid (3.60%) contents.

Effects of chitosan on chemical changes in marinated sardine fillets

Effects of chitosan on the chemical changes of marinated sardine fillets during refrigerated storage are shown in Table 2. TBA values were 1.22 and 1.26 minimum detectable activity (MDA)/kg at the beginning of the experiment. These increased to 8.47 in the chitosan group and 11.46 mg MDA/kg in the control group on the last day of storage. Considering the effect of chitosan on marinated sardine fillets, chitosan had a statistically significant effect on TBA value ($P<0.05$).

TBA value is another indicator commonly used to determine the level of lipid oxidation in fish (Sallam, 2007; Cakli *et al.,* 2008; Turhan *et al.,* 2009). Determination of TBA value is based on measuring malondialdehyde that indicates secondary oxidation products related to fish degradation (Al-Bandak *et al.,* 2009). TBA values were reported to be between 7-8 mg MDA/kg of consumable limit (Varlık *et al.,* 1993). According to this evaluation, the control group exceeded the consumable limit on the 50th day of storage, while in the present study the chitosan group exceeded this limit on the 60th day. TBA results are parallel with sensory results. Kılınc and Caklı (2005) found initial TBA values of pasteurized and non-pasteurized marinated sardine fillets to be 4.36 and 4.42, respectively, while these values became 9.25 and 9.49 at the 6th month of storage. In another study, TBA values of vacuum and oil packed marinated rainbow trout were found to be 0.45 and 2.8 mg MDA/kg, respectively, at the beginning of storage, while these values were 9.5 and 10.26 mg MDA/kg 90 day later (Özden and Erkan, 2006).

TVB-N was low in both groups during storage, and differences between values were not statistically significant ($P>0.05$). At the beginning of storage, TVB-N was 5.58 mg/100 g in the control group and 5.13 mg/100 g in the chitosan group, while 60 days later this value was 5.15 mg/100g and 5.40 mg/100g, respectively. Similar to the present study, Aksu *et al.* (1997) reported that the TVB-N value of 7.79 mg/100 g in anchovy marinated using 4% acetic acid increased to 13.48 mg/100 g after 150 days of storage. Özden and Erkan (2006) reported similar results in their study on trout fillets. The Authors found TVB-N contents in fresh fish and marinated trout to be 7.35 mg/100 g and 6.78 mg/100 g, respectively, and these values increased to 12.08 mg/100 g and 11.98 mg/ 100 g at the end of storage in vacuum and oil packed samples, respectively. TVB-N content in fish depends on bacterial deterioration and the activity of endogen enzymes; therefore, analysis of TVB-N is one of the most common methods used in determining freshness of fish (Lang, 1979; Vareltzis *et al.,* 1997). However, the results of the present and abovementioned

Table 1. The proximate composition in percentage of fresh sardine (*S. pilchardus*) fillets.

Moisture	Protein	Lipid	Ash
76.90±0.31	17.01±0.81	4.19±0.05	1.22±0.01

Data are expressed as means ± standard deviation.

Table 2. Effects of chitosan on chemical changes of marinated sardine (*S. pilchardus*) fillets during refrigerated storage.

		Storage days				
		0	30	40	50	60
TBA, mg MDA/kg	Control	1.26±0.00*	3.92±0.00	7.19±0.49*	9.36±0.10*	11.46±0.27*
	1% chitosan	1.22±0.00	3.33±0.60	5.27±0.69	6.38±2.02	8.47±0.28
TVB-N, mg/100 g	Control	5.58±0.16	7.35±0.02	5.88±0.31	6.28±0.04	5.15±0.41
	1% chitosan	5.13±0.49	6.80±0.26	6.21±0.13	5.47±0.31	5.40±0.20
pH	Control	4.25±0.00	4.15±0.01*	4.11±0.21*	4.34±0.01*	4.34±0.02
	1% chitosan	4.29±0.01	4.23±0.01	4.22±0.00	4.26±0.01	4.36±0.00

TBA, thiobarbituric acid; MDA, minimum detectable activity; TVB-N, total volatile basic nitrogen; *significant difference at $P<0.05$. Data are expressed as means ± standard deviation.

studies indicate that TVB-N can not be used as an indicator of shelf life in marinated fish.

The value of pH, an important parameter for marinated fish, remained under 4.5 in both groups during storage. pH was found to be lower in the control group compared to the chitosan group on the 30th, 40th and 50th days of storage ($P<0.05$); pH levels of the control and chitosan groups were 4.25 and 4.29 at the beginning of storage, and these values were 4.34 and 4.36 at the end of storage, respectively. Similarly, Kılınc and Caklı (2005) reported that pH levels of pasteurized and non-pasteurized sardine marinades increased from 3.76 to 4.06 and 3.78 to 4.19, respectively, at the end of the storage period (6 months). Gökoğlu *et al.* (2004) reported that there was no statistically significant difference in the pH level of marinated sardine (*S. pilchardus*) covered with sunflower oil and stored at 4°C during 150 days of storage ($P>0.05$) and pH was determined as 4.47 and 4.13 in the 2% and 4% acetic acid groups at the end of storage. Özden and Erkan (2006) reported pH levels of marinated trout to be 4.29 and 4.49 at the end of 90 days of storage.

Effects of chitosan on colour changes in marinated sardine fillets

Effects of chitosan on colour values of marinated sardine fillets during refrigerated storage are given in Table 3. Chitosan had no statistically significant effect on L^*, a^*, b^* values of marinated sardine fillets during 60 days of storage ($P>0.05$). Similarly, there was no significant difference in chroma, hue and whiteness values ($P>0.05$). At the beginning of the storage L^*, a^*, b^*, chroma, hue and whiteness values of the control group were 62.91, 1.79, 12.54, 12.67, 1.42 and 60.78, respectively, and for the chitosan added group, these values were 63.87, 1.98, 12.03, 12.19, 1.40 and 61.86, respectively. At the end of the 60-day storage period, L^*, a^*, b^*, chroma, hue and whiteness values of the control group were 65.78, 0.19, 13.00, 13.00, 0.92 and 63.37, respectively, and for the chitosan added group, these values were 64.03, 0.29, 12.79, 12.81, 0.29 and 61.79, respectively.

Table 3. Effects of chitosan on colour values of marinated sardine (*S. pilchardus*) fillets during refrigerated storage.

		Storage days				
		0	30	40	50	60
L^*	Control	62.91±3.52	64.47±2.45	65.32±3.87	65.69±2.30	65.78±1.50
	1% chitosan	63.87±1.05	65.51±2.09	65.80±1.63	65.53±3.16	64.03±1.91
a^*	Control	1.79±0.41	0.48±0.29	0.16±0.33	-0.01±0.47	0.19±0.44
	1% chitosan	1.98±0.09	0.53±0.45	0.24±0.96	-0.12±0.96	0.29±0.70
b^*	Control	12.54±0.48	12.18±0.70	11.28±1.15	13.09±1.34	13.00±1.00
	1% chitosan	12.03±0.80	11.61±1.55	10.71±0.16	12.51±0.86	12.79±1.68
Chroma	Control	12.67±0.48	12.19±0.70	11.28±1.15	13.10±1.35	13.00±0.99
	1% chitosan	12.19±0.80	11.63±1.56	10.74±0.15	12.54±0.84	12.81±1.68
Hue	Control	1.42±0.03	1.53±0.02	0.92±1.37	-0.31±1.69	0.92±1.38
	1% chitosan	1.40±0.00	1.52±0.03	0.29±1.63	-0.93±1.32	0.29±1.67
Whiteness	Control	60.78±3.19	62.42±2.30	65.37±3.40	63.23±1.80	63.37±1.39
	1% chitosan	61.86±1.18	63.56±1.97	64.15±1.53	63.29±2.96	61.79±1.81

Data are expressed as means ± standard deviation.

Table 4. Effects of chitosan on sensory attributes of marinated sardine (*S. pilchardus*) fillets during refrigerated storage.

		Storage days				
		0	30	40	50	60
Appearance	Control	8.60±0.54	8.60±0.54	6.40±0.89	3.80±0.54*	2.40±0.89
	1% chitosan	8.60±0.54	8.60±0.54	6.40±0.89	5.20±0.44	3.00±0.70
Odour	Control	8.80±0.44	8.80±0.44	6.80±0.83	3.80±0.44*	2.80±0.83
	1% chitosan	8.80±0.44	8.80±0.44	6.60±0.89	5.00±0.70	3.20±0.44
Flavour	Control	9.00±0.00	8.80±0.44	6.40±1.51	3.60±0.54*	2.60±0.54
	1% chitosan	8.80±0.44	8.60±0.54	6.20±1.09	4.60±1.14	3.00±0.70
Texture	Control	8.60±0.54	8.40±0.54	6.00±1.22	3.00±0.70*	2.60±0.54
	1% chitosan	8.40±0.54	8.60±0.54	6.00±1.22	4.60±0.54	3.00±0.70

Data are expressed as means ± standard deviation. *Significant difference at $P<0.05$.

Effects of chitosan on sensory changes in marinated sardine fillets

The results of the sensory analyses of marinated sardine fillets during refrigerated storage are presented in Table 4. Sensory analysis included the evaluation of appearance, odor, flavor and texture criteria, which were scored on a scale of 1 to 9. The initial sensory scores of marinated sardine fillets indicated good quality at the beginning of the storage period. No significant difference was observed between the sensory parameters of two groups during the initial 40 days of storage ($P>0.05$) and both groups were evaluated as good. However, the control group obtained lower scores than the chitosan group on the 50th day ($P<0.05$) and dropped under the consumable limit. On the other hand, the chitosan group fell under this limit on the 60th day. In conclusion, chitosan addition extended shelf life of marinated anchovy by ten days in terms of sensory evaluation. In addition to these studies, Agustini and Sedjati (2007) reported that the treatment of chitosan concentration and storage time had no significant effect on sensory analysis of salted-dried anchovy (*Stolephorus heterolobus*). The total viable bacteria count of raw anchovy fillets was 4.83 log CFU/g. Total viable bacteria count of all marinated groups were determined as less than 1 log CFU/g. Similarly, a decrease in total bacteria count after marination was reported by Aksu *et al.* (1997) for marinated anchovy, by Özden for marinated rainbow trout, and by Kılınc and Caklı (2005) for marinated sardine fillets.

Conclusions

Today, there is an increasing interest in food additives. Chitosan is of interest as a preservative in the food industry because of its antioxidant and antimicrobial properties. The results of this study can be useful to researchers and manufacturers for the long-term storage of fish and fish products. In particular, the results of TBA and sensory analyses indicated that marination group with chitosan addition deteriorated later than the control group, and it could be suggested that the addition of protective additives like chitosan, known to have antioxidant and antimicrobial effects in solutions, will be beneficial.

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