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Prevention of Lipid Oxidation in Kilka Fillet (*Clupeonella Cultriventris*) using Biodegradable Packaging in the Refrigerator (3°C)

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Abstract:

Kilka Fish is classified in the fatty fish group and is more perishable than other aquatics. It is very sensitive to oxidation during cold storage. Fat oxidation reduces the quality of Kilka, a decrease in shelf life and economic losses. Because it leads to color change (yellow) of Kilka. Therefore, the present study aimed to evaluate the effects of sodium alginate and whey protein coating on the prevention of fat oxidation of Kilka fillets during 12 days of refrigerated storage. Whey protein 15% and sodium alginate 1% were used at time= 5 min. Uncovered samples were considered as controls. Humidity, peroxide, FFA, TBA, pH, and fat were not significant in the samples covered with whey protein (73.62%, 0.08 meq kg oil^{-1}, 1.01 g 100^{-1}, 0.06 mg kg^{-1}, 6.37 and 4.45%) compared with those covered by sodium alginate (73.91%, 0.05 meq kgoil^{-1}, 1.09 g 100^{-1}, 0.01 mg kg^{-1}, 6.49, and 4.00%) (P>0.05). However, the index chemicals were lower in these samples compared with the control (59.16%, 3.07 meq kgoil^{-1}, 5.73 g 100^{-1}, 2.14 mg kg^{-1}, 6.81 and 3.99%. Unlike fat, the other chemical tests were significant between control and covered samples ($p<0.05$). Covered samples had good quality at the end of the storage period. However, the control samples lost their quality after 6 days. Fish soaked with sodium alginate had better quality compared with the other samples. The use of sodium alginate is recommended by the seafood processing industry to maintain the quality of kilka during refrigeration.

INTRODUCTION

Kilka fish belong to the genus *Clupeonellia*, and the family *Clupeidae*. This fish has formed of species *Clupeonalla delicatula, Clupeonalla engrauliformis* and *Clupeonella grimi* [1]. They can be processed into salted, smoked, dried, and frozen fish. But in Iranian markets, Kilka products are canned- frozen and fresh. Consumption of fresh kilka fish decreased over time. It can be due to the low shelf life of Kilka because it is rich in unsaturated fatty acids and is classified in the fatty fish group, this may lead to oxidation, color changes, and a decrease in the marketability of Kilka fish [2].

Currently, several methods such as vacuum, modified atmosphere, aluminum foil, etc. are used for packaging seafood, and product quality protection is done with synthetic and non-biodegradable chemical compounds such as nitrite, nitrate, sorbate, etc. In addition, plastic is considered the main packaging material for food products. So almost 70% of plastic waste comes from food packaging. Plastics are durable, cheap, strong, and a good barrier against moisture and light and are produced in various forms [3]. However, there is controversy regarding the use of synthetic plastics, as they lead to the depletion of oil reserves, global warming, and environmental pollution. In addition, petrochemical-based packaging is considered a risk to the safety and health of humans and aquatic animals. So that the continuous use of plastic materials cause an increase of nano and microplastic pollution in all of food products [4]. Therefore, their potential residual effects on human health in the future are uncertain. Also, synthetic plastics have little recycling capability. Although heat and hydrothermal processing decomposes plastic waste and turns it into fuel, the release of toxic gases during the combustion of plastic at high temperatures limits its use. In recent years, enzymatic and microbial biological degradation of synthetic plastics has also begun [5]. However, due to the difficulty of sticking microbes on the plastic surface, it was impossible to apply biological treatment. In general, recycling, land filling, and incineration are ineffective for plastic waste management. Plastic materials for food packaging are disposable and non-degradable [6-8]. As a result, large amounts of plastic accumulate in terrestrial and aquatic ecosystems, thus endangering various sectors such as fisheries, agriculture, hydropower generation, maritime transport, health, and tourism [9].

It is increasing consumer awareness about the environmental risks of food packaged with synthetic materials, consumer demand for high-quality seafood products, and the absence of synthetic additives that lead to the need for the food industry to use degradable materials such as edible coatings in the seafood processing industry [10,11]. Edible coating seems to be an ideal method for increasing the shelf life and fish preservation. Edible coatings are completely water soluble, and glossy, act just like a secondary skin, and have the following favorable proportions as label attachment, anti-bacterial potential, improve the overall quality of food, raise the appearance of food products, prevent moisture loss in frozen products increasing product supply in retail stores, reducing rancidity, spoilage, increase product safety, provide economically viable packaging materials, maintain the nutritional value of frozen foods, reduce the absorption of fat during the frying process and have antioxidant properties [2,12,13]. Also, these coatings have various advantages, including degradability, compatibility with the environment, suitable appearance, transparency and changeability. In addition, food coatings can be used together with antimicrobial compounds. This packaging method has a high commercial potential, guarantees the quality without the use of preservatives and reduces waste, poisoning, and food allergic reactions in the consumer [14,15]. These coatings are invisible to the naked eye. Sodium alginate is derived from brown seaweeds. Whey protein is derived from milk and is composed of protein, lactose, and inorganic salts. It is anti-bacterial, anti-proteolysis, and preservative of food moisture [16,17]. However, no research has been carried out using whey protein and sodium alginate films for packaging fish in Iran. This study aimed to determine the effects of whey protein edible coating on the chemical characteristics of frozen common kilka.

MATERIAL METHODS

Sampling

10 kg of common kilk a was caught in spring. Fresh fish were obtained early in the morning (5 a.m.), and the characteristics of fresh fish were followed by the national standards of Iran. The fish were chilled down under a cover of ice at a fish: ice ratio of 2:1 and then transferred to the Iranian National Fisheries Research Processing Center.

Processing

Before filleting fish were washed with chlorinated water. Then the heads and viscera were removed and fillets

were washed. Three treatments were considered in this study: coated samples with whey protein 15%, coated samples with sodium alginate 1%, and uncoated samples (control samples). Samples were submerged in these solutions at time equal to 5 min. The control samples were packaged in disposable dishes and covered with cellophane (500 g). These samples were kept in a refrigerator (3^{o}C) for 12 days. Chemical tests were carried out for the quality evaluation of samples during the storage period. All of the samples were treated in three replicates.

The chemical tests for the packaged samples with edible coating and control (24 packages per treatment) were fat, humidity, lipid, peroxide value (PV Value), free fatty acids (FFA), thiobarbituric acid (TBARS), and pH. These tests were conducted according to [18] during eight steps including raw fish, after processing, and other tests were performed on days 2, 4, 6, 8, 10, and 12 days after treatment. Each step of the test was repeated three times.

Chemical results were analyzed by $SPSS_{25}$ Software. One-way ANOVA variance analysis and two-way ANOVA variance analysis were used to determine the significant difference between treatments and to examine the changes in the experimental and control treatments during storage time, respectively. Also, post-hoc and Dun-Ken tests were used to assess significant differences at a level of 5%.

RESULTS

The mean of moisture, peroxide value, free fatty acids, thiobarbituric acid value, pH, and fat in the fish samples covered with whey protein was 73.62%, 0.08 meq kgoil^{-1}, 1.01 g 100^{-1}, 0.006 mg kg^{-1}, 6.37 and 4.45% respectively (Table **1**), the covered samples with sodium alginate 73.91%, 0.05 meq kgoil^{-1}, 1.09 g 100^{-1}, 0.01 mg kg^{-1}, 6.49, and 4.00%, respectively (Table **2**) (P>0.05), and the control samples 59.16%, 3.07 meq kgoil^{-1}, 5.73 g 100^{-1}, 2.14 mg kg^{-1}, 6.81and 3.99%, respectively (Table **3**). Unlike fat chemical analysis showed significant differences between covered and control samples (P<0.05).

According to the Kolmogrov-Smirnov Test, the distribution of chemical data was normal.

Variations of moisture, peroxide value, pH, free fatty acids, thiobarbituric acid, and fat were not meaningful from one day after storage until the end of storage in the covered samples (p>o.o5). However, they showed significant differences in the control samples (p<o.o5).

Better general qualities were observed in the covered samples compared with the control ones. The covered samples had preserved their quality up to the end of the storage period whereas the control samples had lost their quality after 6 days (Tables **1**, **2**, and **3**). The fish samples soaked with sodium alginate had better quality compared with the ones covered with whey protein.

The different letters (For example a, b, c, and so on) in the same column indicate significant differences (p<0.05).

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In this treatment, no significant changes were observed during storage time.

The different letters (For example a, b, c, and so on) in the same column indicate significant differences (p<0.05).

Table 1: Chemical Results in Covered Samples by whey Protein During Storage Period in the Refrigerator (Mean + Standard Deviation)

Experiments Sampling time	Moisture%	PV value meq/kgoil	FFA gr/100	TBARS mg/kg	pH	Fat (%)
After processing	73.63±0.32^{a}	0.08±0.03^{a}	0.75±0.83^{a}	0.006±0.004^{a}	6.20±0.22^{a}	4.54±0.35^{a}
2 day	73.63±0.59a	0.08±0.06^{a}	0.77±0.67^{a}	0.006±0.003	6.20±0.19^{a}	4.52±0.20^{a}
4 day	73.63±0.56^{a}	0.08±0.07^{a}	0.83±0.45^{a}	0.006±0.002	6.28±0.65^{a}	4.49±0.25^{a}
6 day	73.62±0.73^{a}	0.08±0.11^{a}	0.89±0.02^{a}	0.007±0.02	6.37±0.33^{a}	4.45±0.55^{a}
8 day	73.63±0.81^{a}	0.09±0.26^{a}	0.92±0.09^{a}	0.007±0.001	6.39±0.29^{a}	4.42±0.41^{a}
10 day	73.61±0.95^{a}	0.09±0.15^{a}	1.28±0.15^{a}	0.007±0.11	6.48±0.51^{a}	4.39±0.31^{a}
12 day	73.61±0.99^{a}	0.10±0.25^{a}	1.64±0.35^{a}	0.008±0.025	6.67±0.15^{a}	4.38±0.49^{a}

Table 2: Chemical Results in Covered Samples by Sodium Alginate during the Storage Period in the Refrigerator (Mean + Standard Deviation)

Experiments Sampling time	Moisture%	PV value meq/kgoil	FFA gr/100	TBARS mg/kg	pH	Fat (%)
After processing	73.93±0.94 [a]	0.05±0.14 [a]	0.75±0.20 [a]	0.004±0.001 [a]	6.20±0.15 [a]	4.54±0.20 [a]
2 day	73.93±0.73 [a]	0.05±0.13 [a]	0.78±0.17 [a]	0.004±0.002 [a]	6.20±0.16 [a]	4.54±0.38 [a]
4 day	73.93±0.79 [a]	0.05±0.27 [a]	0.99±0.37 [a]	0.004±0.03 [a]	6.22±0.20 [a]	4.52±0.40 [a]
6 day	73.91±0.52 [a]	0.06±0.12 [a]	1.12±0.30 [a]	0.005±0.02 [a]	6.29±0.10 [a]	4.51±0.32 [a]
8 day	73.91±0.41 [a]	0.06±0.34 [a]	1.24±0.26 [a]	0.005±0.002 [a]	6.30±0.14 [a]	4.48±0.35 [a]
10 day	73.90±0.84 [a]	0.06±0.05 [a]	1.32±0.25 [a]	0.006±0.04 [a]	6.31±0.15 [a]	4.47±0.39 [a]
12 day	73.90±0.76 [a]	0.07±0.33 [a]	1.49±0.20 [a]	0.006±0.47 [a]	6.31±0.13 [a]	4.45±0.23 [a]

Table 3: Chemical Results in Control Samples during the Storage Period in the Refrigerator (Mean + Standard Deviation)

Experiments Sampling time	Moisture%	PV value meq $kgoil^{-1}$	FFAgr 100^{-1}	TBARS mg kg^{-1}	pH	Fat%
After processing	73.93±0.35[a]	0.20±0.01[e]	0.75±0.25[g]	0.004±0.024 [f]	6.20±0.10[d]	4.54±0.36[a]
2 day	67.35±0.25[b]	1.73±0.10[d]	1.83±0.32[f]	0.47±0.18[e]	6.20±0.20[d]	4.45±0.28[a]
4 day	63.20±0.10[c]	3.20±0.10[c]	2.34±0.25[e]	1.13±0.03[d]	6.43±0.10[cd]	4.26±0.43[ab]
6 day	58.90±1.61[d]	4.50±0.10[a]	4.52±0.28[d]	1.84±0.07[c]	6.86±0.15[bc]	3.98±0.14[bc]
8 day	54.15±0.16[e]	4.12±0.25 [ab]	7.96±0.46[c]	2.17±0.09[c]	7.13±0.15[ab]	3.74±0.29[c]
10 day	50.43±0.14[f]	3.90±0.6[b]	11.37±1.31[b]	3.55±0.03[b]	7.32±0.26[a]	3.58±0.18[cd]
12 day	46.16±0.17[g]	3.85±0.30[b]	11.39±0.10 [a f]	5.87±0.02 [a]	7.58±0.15[a]	3.39±0.39[d]

In this treatment, significant changes were observed during storage time.

DISCUSSION

According to Tables **1**-**3**, changes in humidity were not significant in the covered samples during the storage period but, was significant in control samples. Also, this feature was more prominent in samples coated with sodium alginate compared to coated samples with whey protein. The difference in moisture content between coated samples depends on the structure of the used coatings. Lower moisture content loss in samples covered by sodium alginate was attributed to the plasticizing nature of sodium alginate and its acts as an effective barrier for preventing water loss [19]. Also, the formation of gel by this component helped to prevent of decrease in moisture. Sodium alginate is a calcium ion-chelating agent and forms a calcium ion bridge. Therefore, sodium alginate had the effects of increasing the monolayer and multilayer water around myofibril, and inhibitory effects on their dehydration. Therefore, humidity was kept preserved to a greater extent in the samples covered by this coating, compared with the control sample [20,21]. Naturally, the Whey Protein has a globule structure and high solubility and emulsification. This coating has protein, lactose, and minerals and can increase the ability to connect to water in Kilka. Absorption of water by protein, and adhesion and linking of protein chains to each other increases the size of protein and may cause increase in the viscosity, and humidity of the covered samples, compared with the control sample [22,23]. In the control samples, because of the presence of space between fish fillets and also the changes in temperature of the cold storage, the kilka in the packages lost their humidity. This condition may quicken the oxidation of lipids, and cause color change in Kilka without cover. Humidity was higher in fish samples soaked with whey protein (73.62%) and sodium alginate (73.91%) compared with the control samples (59.16%). Similar results were obtained by [19]. These researchers stated that after 25 days of storage, coated *C. macropomum* fillets by sodium alginate bilayer had lost nearly 16% of moisture, whereas the control group had lost 30% of moisture.

[22] determined the moisture in covered Rainbow trout by whey protein concentrate (8%) at 68.05 – 64.33% after 15 days at4^{o}C. It was inconsistent with our results. This difference can be due to the change in the concentration of the edible coating.

Based on Tables **1**-**3**, the lipid content showed an increase in the whey protein-covered samples (4.55%) compared with the sodium alginate-covered samples (4.00%) and control samples (3.99%). That is because of the presence of glycerides, phospholipids, and lipoproteins in whey protein [24]. Also, this may be retarded by the presence of free fatty acids consisting of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid in sodium alginate [25]. The presence of more fat in the samples covered by whey protein allows more fat oxidation and quality reduction in these samples. Fat was variable in the control samples compared with the covered samples. This can be due to penetration of oxygen, and fat oxidation [26].

As shown in Tables **1** to **3**, free fatty acids were lower in coated samples compared to control samples. This may be due to the prevention of water loss from the surface and inside the fish body by the presence of fine holes, oxygen contact with the fish tissue and its reaction with saturated fatty acids and oxidation, and lack of absorption of light by the fish body surface and its effects on accelerating oxidation [27]. In addition, the enzyme of lipase of the tissue, the excreted lipolysis enzyme from the *Staphylococcus* bacteria, and enzymes released from the dead and decomposed bacteria have been able to lead to this condition, and may cause hydrolysis of lipids and production of non-saturated fatty acids through the lipolysis process [28]. Release of fatty acids containing high numbers by lipase enzyme may not provide a bad taste but over time, the effects of accumulation of free fatty acids in the muscles of fish may cause unfavorable taste and tissue damage. Also, protein denaturation caused some tissue changes and lowering of quality [29,30]. In the control samples, the concentration of these acids increased from the first day up to the 12 days. However, the nearly constant concentration of free fatty acidsat the end of keeping time can be due to the decrease of raw materials and the increase of oxidation these acids [31]. As can be seen in Tables **1** to **3**, the free fatty acids were measured in the covered samples by whey protein (1.01 g 100^{-1}), sodium alginate (1.09 g 100^{-1}), and the control sample (5.73 g 100^{-1}). Given that the limit of free fatty acids in fish oil is below 5 g100^{-1}, this factor was acceptable in coated samples during the storage period. However, in the control samples, after 6 days, it was removed from the acceptance state [32] evaluated Rainbow Trout (*Oncorhynchus mykiss*) treated with sodium alginate coating for 16 days at 4 ^{o}C. They reported FFA 4.69 - 21%. [33] detected the effect of sodium alginate coating with lacto peroxidase system and *Zataria multiflora Boiss* 1% essential oil on the quality of Rainbow Trout fillet under refrigerator conditions. The lowest amount of FFA was 1.84% of oleic acid in these samples at 8 days. [34] assessed the effect of whey protein isolate (1%) on the FFA of *Scomberoides commersonnianus* fillet during storage in a refrigerator. FFA of control and covered samples by whey protein isolate were 5.16 g 100^{-1} and 5.66 g 100^{-1} respectively, after 16 days. In the present study, the results obtained from the measurement of FFA were lower compared to the control sample. Therefore, the results of present study are consistent with the results obtained from these previous studies.

According to Tables **1**-**3**, PV and TBARS were lower in covered samples compared with control samples. TBARS is a suitable index for the determination of progress in fat oxidation and the production of carbonyl compounds. The presence of such compounds in fish meat causes some changes in its sensory specifications such as taste and smell. Debarment of TBARS development in Experimental samples can be explained by the prevention of a humidity decrease, oxygen absorption, oxidation, and production of secondary products of oxidation [35,36]. In the present research, amounts of TBARS showed an increasing trend up to the end of cold storage in the control Kilka. This may be due to the effect of the increase of free radical's production, and readiness for oxidation. Over time peroxide starts to induce decomposition, leading to aldehydes, ketones, and seton production. The protection against lipid oxidation might be due to the whey protein and sodium alginate that acted as oxygen barriers [37-39]. Peroxide value and TBARS contents in the samples covered by whey protein were(0.08 meq kg oil^{-1} and 0.006 mg kg^{-1}), sodium alginate (0.05 meq kg oil^{-1} and 0.01 mg kg^{-1}), and the control sample (3.07 meq kg oil^{-1} and 2.14 mg kg^{-1}) (Tables **1**-**3**). In fish products, TBARS values are 1 - 2 mg kg^{-1} to show good quality and with normal odor and taste [40], and peroxide values from 5 -10 meq $kgoil^{-1}$ are acceptable [2]. Therefore, the peroxide value and TBARS were acceptable in the coated samples during the storage period. However, these were acceptable for the control samples for 6 days. Cruz *et al.* [19] proved that TBARS

was1.17 mg kg^{-1} during 15 days in the coated *C. macropomum* fillets by sodium alginate bilayer incorporated with green propolis, whereas the uncoated group determined 2.04 mg kg^{-1} after 10 days of storage. This coating extended the *C. macropomum* fillets shelf-life by 7 days in terms of acceptable TBARS values. Hashemi *et al*. [32] evaluated Rainbow Trout fillets treated with sodium alginate coating. They determined peroxide values 2.03 – 5.28 meq $kgoil^{-1}$ and TBARS 0.09 – 0.17 mg kg^{-1} during 16 days of storage at 4°C. Bazargani-Gilani [41] studied sodium alginate-based edible coating to increase the shelf life of rainbow trout fillets during refrigerated storage for 15 days. The thiobarbituric acid value was significantly lower in covered treatments 0.195 – 1.795 mg kg^{-1} compared to the control (0.205 – 2.11 mg kg^{-1}). Nie *et al.* [42] evaluated the effect of sodium alginate 1.5%, coating with tea polyphenols 0.5% on the quality of fresh Japanese sea bass (*Lateolabrax japonicas*) fillets at 4 °C. TBARS and PV were 0.75 mg kg^{-1} and 3 meq kg oil^{-1}in covered samples at 20 days. They were lower compared with control (5.85 meq kg $oil^{-1}$and 0.85 mg kg^{-1}). Raeisi *et al.* [43] determined the effect of a sodium alginate 0.2 % coating incorporated with *Mentha piperita, Artemisia dracunculus*, and *Zataria multiflora* essential oils on chemical attributes of rainbow trout meat during 12 days of storage at 4°C. TBARS in these samples was 0.20 – 1.14 mg kg^{-1}. But, TBARS in control samples was 0.19 – 1.29 mg kg^{-1}. Barkhori Mehni *et al.* [44] detected the effect of sodium alginate coatings with lactoperoxidase system and *Zataria multiflora Boiss* 1% essential oil on the quality of Rainbow Trout fillet under refrigerator conditions. PV values ranged from 0.32 – 0.41 meq kg oil^{-1} during 4 days, and 1.97 meq kg oil^{-1} on day 8. Also, the lowest amount of TBARS was1.54 mg kg^{-1} in these samples. Khan *et al.* [45] found that whey protein 8% in combination with glycerol (1:2) led to a decrease in thiobarbituric acid of coated rohu fillets (0.78 meq kg oil^{-1}) compared with uncoated samples (1.48 mg kg^{-1}) during 40 days of chilled storage. Also, Khan *et al.* [45] reported that this compound decreased the peroxide value of coated rohu fillets (7.19 mg kg^{-1}) compared with uncoated samples (4.86 meq kg^{-1}). Farsanipour [34] assessed the effects of whey protein isolate 1% on TBARS of *Scomberoides commersonnianus* fillet. TBARS of control and covered samples by whey protein isolate were 2.25 and 0.74 mg kg^{-1} respectively, after 16 days of storage in the refrigerator. The results of present study are consistent with the results obtained from these studies.

Changes in pH were not significant in the covered samples during the storage period (Tables **1**-**3**). Over time, lipid oxidation products such as hydro-peroxides have been analyzed and some compounds such as aldehyde and others have been produced in the control sample. These compounds have alkali specifications and cause an increase in the pH of the product. In addition, the increase in pH during storage is associated with the release of nitrogen-containing metabolites produced by bacterial activity [40,46]. The pH levels in the samples covered with whey protein were higher compared with the samples covered with sodium alginate. This may be due to the production of free fatty acids by lactic acid bacteria in these samples and their oxidation [47]. The mean pH was measured lower in the covered samples with whey protein (6.37) and sodium alginate (6.49) compared with the control sample (6.81). pH in control samples was not acceptable after 6 days (7.13). Cruz *et al*. [19] showed pH values (6.04 - 7.03) in coated *C. macropomum* fillets by sodium alginate bilayer coating incorporated with green propolis sustained optimum after 20 days of cold-storage, whereas the uncoated group (control) was able to sustain neutral pH (7.01) for only 10 days. Therefore, the sodium alginate bilayer extended *C. macropomum* fillet shelf-life by 10 days in terms of optimum pH. Hashemi *et al.* [32] evaluated Rainbow Trout fillets treated with sodium alginate coating. They reported the pH (6.41 – 6.67) during 16 days of storage at 4 °C. Nie *et al.* [42] evaluated the effect of sodium alginate (1.5%, w/v) coating with tea polyphenols (0.5%, w/v) on the quality of fresh Japanese sea bass (*Lateolabrax japonicas*) fillets at 4 °C. pH was 6.6 and 7.1 in covered and control samples at 20 days. Yildiz and Yangilar [22] proved that the pH was 6.31 in covered Rainbow trout with whey protein concentrate 8% after 15 days of storage at 4°C. Farsanipour [34] assessed the effect of whey protein isolate (1%) on the pH of *Scomberoides commersonnianus* fillet during storage (16 days) in a refrigerator. pH of control and covered samples by whey protein isolate were 7.96 and 7.92 respectively. In the present study, the results obtained from the measurement of pH in the covered samples were lower compared to the control sample. Therefore, the results of these studies are consistent with the results obtained from the present study.

CONCLUSION

The Kilka samples covered by sodium alginate had better quality compared with the samples covered by whey protein. This can be due to the lower rates of

chemical factors in these samples. No statistically significant differences were observed in the results of chemical experiments of the covered samples. However, a meaningful difference was noted in the results of the chemical analysis in the control sample. Therefore, the covered samples had a favorite quality up to the end of the storage period at the refrigeration but, the control samples had lost their quality after 6 days.

CONFLICT OF INTEREST

There is noconflict of interest in this study.

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