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Significant decreases in chemical factors were observed in the coated samples compared to the control samples ($p<0.05$).

This research investigates the effects of whey protein coating on the chemical, bacterial, and sensory properties, proximate analysis, and shelf life of common kilka fish during frozen storage.

For this experiment, common kilka were coated with 9% whey protein while non-coated kilka were used as a control sample. Both the coated and non-coated samples were stored at -18 °C for six months. Results showed that total bacterial counts (1.47–2.49 log CFU/g) and S*taphylococcus* bacteria (1.02–1.71 log CFU/g) were lower in the coated samples compared to the control samples ($P > 0.05$). Coliform, *E. coli*, and *Pseudomonas* bacterial contamination were undetectable in both the coated and control samples throughout the storage period. Humidity (73.92–46.18%), protein (18.24–19.07%), lipid (4.27–4.01%), ash (1.82–2.12%), and calories (108.85–120.78 kcal/kg) measured higher in the test samples compared to the control samples. Values for peroxide (0.15–5.12 meq/kgoil), free fatty acids (1.32–12.40 g/100), thiobarbituric acid (0.14–0.98 mg/kg), total volatile basic nitrogen (TVB-N) (6.32-21.79 mg/100 g), and pH (6.32-7.45) were lower in the test samples.

Significant decreases in chemical factors were observed in the coated samples compared to the control samples ($p<0.05$). The overall acceptance score had a better quality in the coated samples (80) compared to the control samples (113) ($p<0.05$). According to

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the results of experiments and statistical analysis, the coated samples had a favorable quality until the end of the storage period but the control samples had lost their quality. Therefore, a 9% whey protein coating is recommended for kilka fish as a better alternative to using disposable packaging dishes with a cellophane coating.

Introduction

Kilka fish belong to the genus *Clupeonella*, in the family Clupeidae. These fish are composed of three species consisting of *Clupeonalla delicatula, C. engrauliformis,* and *C. grimmi* (Coad, 2017). They can be processed into salted, smoked, pickled conserved, dried, and frozen fish. In Iran, kilka products are sold fresh, canned, or in frozen packaging. From 2016 to 2021, the annual catch ranged from 20,138 to 22,429 tons. Approximately 10-12% of this catch was used for human consumption, and the remaining 88-90% was used for animal feeds. Between 6,307 and 6,626 tons of the fish were caught in the Guilan Province, of which 5-12% was used for human consumption and the remaining 88-95% for animal feeds (Program and Budget Office, 2022). Consumption of fresh kilka fish dropped from 6% to 2.20% during the period of 2004 to 2009. Consumption of canned kilka also dropped during the same period, whereas consumption of frozen kilka rose during the same years (Seifzadeh, 2014). The frozen fish packs had a much higher sales rate in comparison to the sales of fresh fish because of their longer storage time and wider distribution. Sales of frozen fish were also higher. The fish packs were frozen for less than three months because longer frozen-storage time may lead to color changes, surface dryness, and peroxide accumulation. Even so, the first indication of a decline in quality after only one month of frozen storage was a reduction in the weight of frozen packed fish, which in turn had a deteriorating effect on the texture and taste of the small-sized fish. There was a 3.5% decline in fish weight after three months of frozen storage (Moeini, 2009).

Kilka fish, which have valuable protein and digestible fats, rich vitamins, and minerals, have attained an important position in the food-product market. Overall, the value of food products, such as kilka, depends on their nutritional specifications and acceptability in society; therefore, accurate processing and the preparation of appealing product varieties are crucial for the market (Khanipor et al., 2017).

The final step of the food-production chain is packaging, which occupies the middle ground of production, distribution, and consumption. Many small and large companies all over the world are very active in the packaging industry. Competition among packaging manufacturers has led to improved quality and more types of packaging. Currently, numerous packaging and preservation methods, including nonbiological decomposable synthetic chemicals, are used for food preservation. Recently, new packaging materials—such as edible films that are biologically decomposable—have entered the market (Aguilar-Rivera et al., 2023; Kandasamy et al., 2018).

Consumer demand is high for high-quality seafood products, especially those that can retain their superior quality of taste, texture, and general fresh appearance following a prolonged period of cold or frozen storage (Bayram et al., 2021). The use of edible films for packaging kilka seems to be an ideal method for proper preservation during periods of long storage.

Edible coatings are completely water soluble and glossy; they perform just like a secondary skin and have favorable properties such as rapid attachment to foodstuff, label attachment, antibacterial, and antioxidant properties (Seifzadeh, 2022). These types of coatings protect the aroma, taste, and food color as well as help to maintain the nutritional components. Coating food products with these films can lead to preservation of food moisture and the lowering of oxygen absorption, which can substantially improve the appearance of food products. These coatings are invisible to the naked eye (Londoño-Hernandez et al., 2018; Yu et al., 2019).

Whey protein is derived from milk and is composed of protein, lactose, and inorganic salts. It is anti-bacterial, antiproteolysis, and preserves food moisture (Setiadi and Sauria, 2020). Edible films made of whey protein have been used for packaging salmon, hotdogs, sausages, crackers, and frozen fish filets, and have improved their quality and shelf life (Seifzadeh, 2014). The objective of this study is to investigate the effects of a whey protein coating on the chemical, bacterial, and sensory properties, proximate analysis, and shelf life of frozen kilka.

Materials and Methods

For this study, 180 kg of common kilka were caught at 5 a.m. in spring. The fish were chilled to 0 °C under ice at a fish-to-ice ratio of 2:1 in chilled seawater (CSW) under hygienic conditions. Before processing, the fish were washed with chlorinated water. The heads were then cut, and organs were taken out. The fish were washed again. Two treatments were used for this research: coated kilka and non-coated kilka. The cleaned fish were immersed in a 9% whey protein solution at the start of the experiment (time = 0) (Socaciu et al., 2018).

To prepare the control sample, the kilka were washed, the heads were cut, and then the organs were removed. After cleaning, the fish were washed again and packaged in disposable dishes with cellophane covers that were 500 g in weight. Both the coated and control samples were stored at -18 ˚C in for six months. The quality of these samples was compared to the test samples at specific intervals using microbial, chemical, and sensory tests. The samples were processed three times, and each test step was repeated three times.

Microbial quality determination was conducted for both the test and control samples (48 packages) by measuring total bacterial counts (Andrews and Hammak, 2003; Maturin and Peeler, 2001), and testing for *Staphylococcus* (Bennet and Lancette, 2001, and Holt et al., 1994), coliform (Feng et al., 2002), *E. coli* (Feng et al., 2002), and *Pseudomonas* (Hasegawa, 1987). Microbial tests were carried out in seven steps. The first microbial tests on fish samples were conducted one day after processing, followed by monthly tests at predetermined intervals for six months. Each test step was repeated three times.

The chemical tests for the packaged samples with edible coating and control (42 packages) measured calories (Iranian National Standard No. 8867, 2005), protein (FAO, 1986), humidity (FAO, 1986), lipid (FAO, 1986), ash (FAO, 1986), peroxide value (FAO, 1986), TVB-N (FAO, 1986), TBARS (FAO, 1986), FFA (FAO, 1986), and pH (FAO, 1986). These tests were conducted in eight steps that included the raw fish samples one day after processing, and additional testing at specific times once a month from the first month after processing up to the sixth month. Each test step was repeated three times.

TABLE 1
Microbial Results in Coated and Control Kilka During Frozen Storage (Log CFU/G)

Index Sampling Time	Total Bacterial Counts		*Staphylococcus* Bacteria	
	Control Samples	Coated Samples	Control Samples	Coated Samples
First day	3.69±0.23^{a}	3.45±0.35^{a}	2.87±0.12^{a}	2.27±0.14^{a}
First month	3.48±0.12^{b}	3.36±0.27^{b}	2.59±0.42^{b}	2.12±0.26^{b}
Second month	3.34±0.26^{c}	3.27±0.46^{c}	2.34±0.32^{c}	1.86±0.32^{c}
Third month	3.22±0.33^{d}	3.09±0.19^{d}	2.19±0.17^{d}	1.61±0.19^{d}
Fourth month	2.97±0.145^{e}	2.71±0.15^{e}	2.02±0.18^{e}	1.37±0.13^{e}
Fifth month	2.79±0.128^{f}	2.02±0.12^{f}	1.77±0.22^{f}	1.22±0.17^{e}
Sixth month	2.49±0.25^{g}	1.47±0.18^{f}	1.71±0.28^{g}	1.02±0.14^{e}

Letters within the same column indicate significant differences ($p<0.05$) between storage times.
Values are mean + standard deviation.

TABLE 2
Chemical Results in Coated and Control Kilka Samples During Frozen Storage

Index Sampling Time	PV Value meq/kgoil		FFA g/100		TBARS mg/kg		pH		TVB-N	
	Control	Coated	Control	Coated	Control	Coated	Control	Coated	Control	Coated
First day	0.22±0.03^{g}	0.10±0.04^{a}	4.12±0.78^{f}	1.12±0.39^{a}	0.05±0.07^{b}	0.02±0.04^{a}	6.21±0.19^{d}	6.12±1.22^{a}	9.83±1.19^{g}	9.82±1.04^{a}
First month	1.72±0.28^{f}	0.10±0.07^{a}	6.85±0.35^{e}	1.12±0.28^{a}	0.09±0.24^{b}	0.02±0.01^{a}	6.21±0.21^{d}	6.12±1.33^{a}	12.21±1.21^{f}	9.82±1.01^{a}
Second month	3.22±0.13^{e}	0.10±0.05^{a}	8.36±0.93^{d}	1.12±0.22^{a}	0.12±0.59^{b}	0.03±0.08^{a}	6.42±0.49^{cd}	6.18±1.77^{a}	14.42±1.49^{e}	9.82±1.08^{a}
Third month	4.52±1.11^{d}	0.10±0.09^{a}	9.54±0.85^{c}	1.12±0.14^{a}	0.24±0.47^{b}	0.06±0.02^{a}	6.73±0.58^{bc}	6.19±1.82^{a}	16.73±1.58^{d}	9.82±1.02^{a}
Fourth month	6.02±0.26^{c}	0.11±0.06^{a}	10.98±0.75^{b}	1.23±0.17^{a}	0.53±0.44^{ab}	0.07±0.11^{a}	7.04±0.64^{ab}	6.24±1.73^{a}	18.54±1.64^{c}	9.94±1.11^{a}
Fifth month	6.52±0.61^{b}	0.11±0.03^{a}	12.39±0.69^{a}	1.23±0.18^{a}	0.78±0.32^{a}	0.12±0.09^{a}	7.25±0.61^{a}	6.27±1.85^{a}	20.85±1.61^{b}	9.97±1.09^{a}
Sixth month	5.12±0.31^{a}	0.15±0.09^{a}	12.40±0.68^{a}	1.32±0.09^{a}	0.98±0.35^{a}	0.14±0.07^{a}	7.45±0.67^{a}	6.32±1.92^{a}	21.79±1.67^{a}	9.99±1.07^{a}

Letters within the same column indicate significant differences ($p<0.05$) between storage times. Values are mean + standard deviation.

TABLE 3
Proximate Analysis Results in Coated and Control Samples During Frozen-Storage Period

Index Sampling Time	Moisture %		Protein %		Fat %		Ash %		Calorie kcal/kg	
	Control	Coated	Control	Coated	Control	Coated	Control	Coated	Control	Coated
First day	72.23±0.36[a]	73.95±0.24	18.24±0.78	19.07±0.38	4.01±0.37	4.27±0.22	1.82±0.19	2.12±0.21	108.85	120.78
First month	67.37±0.25[b]	73.95±0.17	18.24±0.35	19.07±0.29	4.01±0.23	4.27±0.29	1.82±0.21	2.12±0.32	108.85	120.78
Second month	63.22±0.11[c]	73.95±0.15	18.24±0.93	19.07±0.21	4.01±0.57	4.27±0.48	1.82±0.49	2.12±0.76	108.85	120.78
Third month	58.92±1.62[d]	73.93±0.09	18.24±0.85	19.07±0.13	4.01±0.41	4.27±0.53	1.82±0.58	2.12±0.81	108.85	120.78
Fourth month	54.17±0.17[e]	73.93±0.46	18.24±0.75	19.07±0.16	4.01±0.39	4.27±0.61	1.82±0.64	2.12±0.72	108.85	120.78
Fifth month	50.45±0.15[f]	73.92±0.05	18.24±0.69	19.07±0.17	4.01±0.31	4.27±0.72	1.82±0.61	2.12±0.84	108.85	120.78
Sixth month	46.18±0.18[g]	73.92±0.07	18.24±0.68	19.07±0.08	4.01±0.34	4.27±0.46	1.82±0.67	2.12±0.91	108.85	120.78

Letters within the same column indicate significant differences ($p<0.05$) between storage times. Values are mean + standard deviation.

Sensory tests for the test sample and control sample (14 packages) consisted of evaluation of tissue, odor, color, and taste with the method of ranking and through execution of the Friedman test (ISO 85–87, 1988). The sensory tests on the packaged samples with edible coating and the control samples were repeated once in each step. The results of the microbial and chemical tests were analyzed using SPSS Software and Two-Way Variance Analysis, while the sensory test results were evaluated using a ranking method and the Friedman Test.

TABLE 4
Sensory Results in Coated and Control Samples During Frozen Storage

Index Samples	Color	Odor	Tissue	Taste	Overall Acceptance
Coated samples	76[a]	71[a]	80[a]	80[a]	80[a]
Control samples	111[b]	107[b]	97[b]	105[b]	113[b]
Score	111 - 76	107 - 77	100 - 70	105 - 65	113 - 69
LSD	36>19.6	37>19.6	24>19.6	41>19.6	45>19.6

Letters within the same column indicate significant differences ($p<0.05$) between storage times. Values are mean + standard deviation.

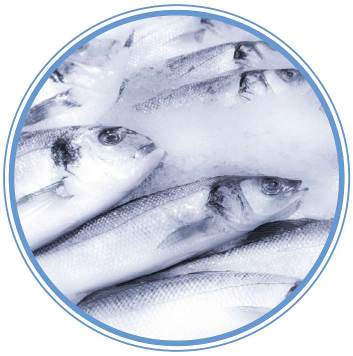
Results

As indicated, no coliform, *E. coli*, and *Pseudomonas* bacterial contamination was observed in the coated and control samples during storage time. The mean total bacterial counts and *Staphylococcus* bacteria count in processed samples by whey protein were 2.49 and 1.63 log CFU/g and in control samples 3.15 and 2.20 log CFU/g from 1 day until six months after processing, respectively. There was a meaningful difference in the microbial results in the control samples and coated samples from the first day up to the sixth month ($p<0.05$). Analysis of results obtained using a two-way ANOVA at 95% confidence level indicate significant differences in total bacterial counts and *Staphylococcus* bacteria count both in the coated samples as well as in the control samples ($p<0.05$) **(Table 1)**. According to the Kolmogrof-Smirnof test, distribution of microbial data was normal.

According to the Kolmogorov-Smirnov test, distribution of chemical data was normal. Variations of free fatty acids were significant from one day after keeping up to five months ($p<0.05$) and at sixth months. It was not notable in control samples. Variations of pH and TBARS showed significant differences between the first day with the sixth month after processing in control samples ($p<0.05$). No significant differences in moisture, free fatty acids, thiobarbitouric acid, TVB-N, and peroxide valuable in the coated samples ($p>0.05$) compared with the control ones ($p<0.05$) **(Table 2)**.

Significant decreases were not observed in proximate analysis in the coated samples compared to the control samples ($p>0.05$) **(Table 3)**.

The sensory results of the samples are shown in **Table 4**. Based on the Friedman test in sensory properties, there was a meaningful difference between coated samples and the control samples ($p<0.05$). According to the results of the tests and statistical analysis, better general quality was observed in the coated samples compared to the control samples. Based on the statistical test, the coated samples had preserved their quality up to the end of the storage period while the control samples had lost their quality.



The use of edible films for packaging kilka seems to be an ideal method for proper preservation during periods of long storage.

Discussion

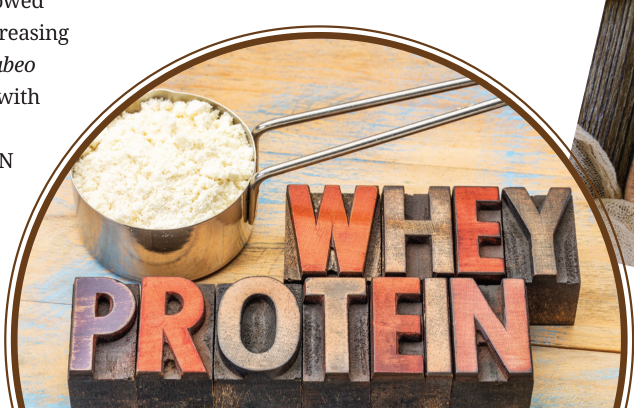
The total bacterial counts and *Staphylococcus* bacteria in the coated samples have been decreased **(Table 1)**. This may be because of lactic acid, acetic acid, probiotic bacteria, and production of ethanol and nisin. Probiotic micro-organisms of whey protein can produce organic acids such as lactic acid, acetic acid, nisin bacteriocin, hydrogen peroxide, ethanol, acetaldehyde, ammoniac, and di-acetyl. These contribute to the reduction of both the redox potential (Eh) and the pH, enhancing food preservation. These compounds have antimicrobial specifications and can prevent the growth of *Staphylococcus, Pseudomonas,* coliform and *E. coli*. But, the presence of air in the storage environment can promote the growth and proliferation of microorganisms (Wani, et al., 2023). The acceptable values for total bacterial counts and *Staphylococcus* bacteria were 7 log CFU/g and 3 log CFU/g, respectively (Centre for Food Safety, 2014). Furthermore, values for the same bacteria were 3.43–1.45 and 2.25–1.00 log CFU/g, respectively during storage for coated fish. Therefore, in the present study, the coated Kilka had favorable microbial characteristics during 15 days of storage at 4 °C. Yildiz and Yangilar (2016) determined the total bacterial counts in coated rainbow trout using whey protein concentrate (8%) at 3.04 – 10.36 log CFU/g after 15 days at 4 °C, which was inconsistent with the results of our study (3.43 – 1.45 log CFU/g[1]). In a 2016 study, Karakaya Tokur et al. examined the effect of a 5% whey protein isolate coating on the quality of trout in cold storage. They found that total bacterial counts of the coated samples were lower (1.74 log CFU/g) than the control samples' (4.10 log CFU/g), log CFU, which is in line with the results of the present study (2.47–1.45 log CFU/g). Erbay et al. protected fresh fish filets of rainbow trout (*Oncorhynchus mykiss*) using whey protein isolate (WPI) with 96 g/100 g protein content as the coating-forming agent for 15 days at 4 °C. Protected fish filets exhibited higher antimicrobial efficiency against mesophilic, psychrophilic, and lactic acid bacteria as well as Enterobacteriaceae. Khezri Ahmadabad et al. in 2015 determined the effect of whey protein edible coating (8.5%) on the total bacterial counts of rainbow trout filet during cold storage. They reported the number of mesophilic bacteria at the start of the experiment until 8 days after storage ranging from 3.63 to 6.85 log CFU/g, which differs from the findings of the present study. This difference can be due to handling, processing, storage temperature, and fishing location.

The improved quality of the coated fish can be attributed to the preservation of the water in the fish tissue during freezing, which provided a protective layer from the edible film, reduced oxidation, and limited the production of free radicals.

The frozen uncoated kilka showed an increase in the amount of TVB-N **(Table 2)**. This may be because of the decrease in humidity and production of free fatty acids on the denaturizing of the protein. These factors decreased in coated samples compared with the control sample. This effect can be attributed to the properties of whey protein, which help prevent proteolysis, reduce humidity loss, and limit the production of free fatty acids during protein denaturation, which then naturally increases TVB-N levels (Chaijan et al., 2020). In a 2016 study, Karakaya Tokur et al. reported the TVB-N value significantly increased to 31.49 mg N/100 g from 19.60 mg N/100 g in control and 30.85 from 18.60 mg N/100 g in trout coated with whey protein isolate (5%) at refrigerated storage (3–6 days). In a 2015 study, Khan et al. showed the effect of whey protein 8% on decreasing TVB-N production of coated rohu (*Labeo rohita*) filets (8mg/100 g) compared with uncoated samples (12.60 mg/100 g) during 40 days of cold storage. TVB-N was 9.8–9.9 and 9.8–21.7 mg N/100 g in control and coated samples in present study, respectively, which differs from the findings of the present study. This difference can be related to temperature of storage and microbial growth.

Based on the conducted experiments, the amount of the free fatty acids has been measured less in the samples coated by whey protein **(Table 2)**, compared with the control sample. This may be due to the prevention from losing the water from the surface and inside the body by the fine holes present at the body surface, prevention from the oxygen contact with the fish tissue and combination with non-saturated fatty acids and oxidation, and lack of absorption of light by the fish body surface (Galus et al., 2015). Freezing caused the fish tissue to denaturize and lose moisture, leading to weight loss that may be due to the oxygen penetration into the fish tissue and oxidation increasing of free fatty acids and peroxide value. But peroxide will start to induce decomposition, leading to the aldehydes and ketone production, subsequently, in peroxide value reduction to over time. The first products of lipid oxidation are hydroperoxide, and they are broken to secondary products such as aldehydes that can react with proteins. This compound also reacts with pigments and other molecules in the fish. This reaction leads to loss of color and a bad smell (Mohamed et al., 2020). Seifzadeh in 2013 showed the effect of whey protein 12% on decreasing FFA value of coated kilka filets (1.30 g/100) compared with uncoated samples (12.38 g/100) the end of freezing storage.

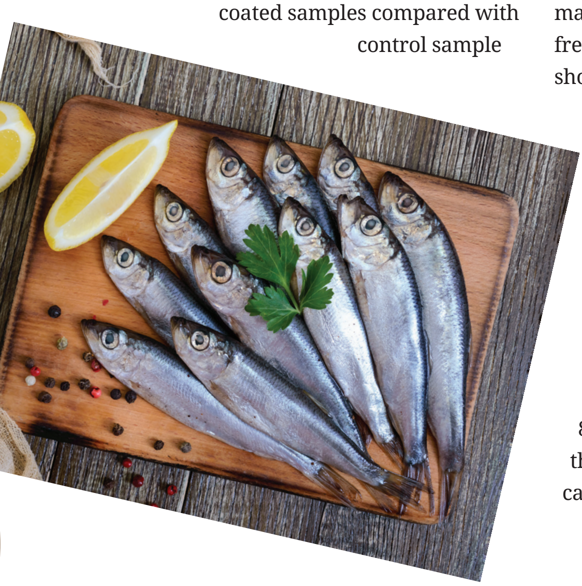
The measurement of Thiobarbituric Acid Reactive Substances (TBARS) is a suitable index for determining of the progress in fat oxidation and producing of carbonyl compounds **(Table 2)**. The presence of such compounds in fish meat causes some changes in its sensory specifications such as taste and smell. The amounts of peroxide and TBARS were lower in the coated samples compared with the control sample. The improved quality of the coated fish can be attributed



to the preservation of the water in the fish tissue during freezing, which provided a protective layer from the edible film, reduced oxidation, and limited the production of free radicals. The film prevented oxygen absorption and maintained humidity, which then reduced the oxidation and formation of secondary oxidation products, such as aldehydes (Bamdad et al., 2017). In the current research, the amount of TBARS increased toward the end of the cold storage in the control kilka. This may be because freezing decreased the humidity and the water-activity factor, and increased the low-water activity oxidation, the production of free radicals and free fatty acids, and being ready for oxidation (Alberto et al., 2018). In 2015, Khan et al. showed that an 8% whey protein coating significantly reduced TBARS production in coated rohu fillets (0.352 mg MDA/kg) compared to uncoated samples (0.820 mg MDA/kg) over 40 days of chilled storage.

The pH levels showed an increase in the coated samples compared with control sample **(Table 2)**. Over time, lipid-oxidation products such as hydroperoxides have been analyzed, and some compounds such as aldehydes and others have been produced in the control sample. These compounds have alkali specification and cause an increase in the pH of the product. Therefore, in the coated samples, changes in pH levels during the storage were not significant. In 2016, Yildiz and Yangilar reported that the pH of rainbow trout coated with 8% whey protein concetrate was 6.31 after 15 days at 4°C. Similarly, in 2013, Seifzadeh demonstrated that a 12% whey protein coating resulted in a pH of 6.30 in kilka filets, compared to 7.30 in uncoated samples at the end of frozen storage.

The amount of ash increased in the coated samples compared with control sample



(Table 3). This is due to the presence of sodium, potassium, calcium, manganese, and phosphate ions, as well as sulfur-containing amino acids in whey protein. These amino acids are considered mineral elements in food products (Sanches–Silva et al., 2016). Protein levels increased in coated samples compared with the control sample, as show in **Table 3**. This may be because of some protein compounds in the film such as α-lactalbomine and β-lactoglobine (Seifzadeh, 2014).

Lipid concentrations also increased in the coated samples compared with control sample **(Table 3)**. That is because of the presence of glycerides of milk lipid, phospho-lipids, and lipoproteins in whey protein. The lipid oxidation arising from the reaction of lipid with oxygen and its hydrolysis is influenced by the action of lipolytic enzymes (Cazón et al., 2017). The lipase from tissue, the lipolytic enzyme excreted from the *Staphylococcus* bacteria, and enzymes released from the dead bacteria remain active at low-water activity, which may cause the hydrolysis of lipids and production of unsaturated fatty acids through the lipolysis process. Although releasing the long-chain fatty acids by lipase may not immediately affect the taste, their accumulation over time can interact with the muscle proteins, causing an undesirable taste and damaging the tissue texture. In addition to fatty acids, protein denaturation caused some tissue changes and a drop in quality. In the control samples, the acid concentration was increased from the first month to the fifth month. However, the concentration of these acids remained nearly consistent toward the end of the storage period. This stability is likely because of the decrease of available raw materials and an increase of oxidation in free fatty acids. In a 2013 study, Seifzadeh showed using a 12% whey protein on protein, fat, and minerals (measured as ash) of coated kilka filets with values of 19, 4.25, and 2.10% respectively, compared with values of 18.2, 4, and 1.80% in uncoated samples at the end of freezing storage, which is consistent with the results of the current study.

The moisture content showed a significant increase from 79.05% to 81.06%, as shown in **Table 3**. Although the change was not statistically significant, an upward trend was observed in the ash content, which increased from 1.88% to 2.13%. Furthermore, a significant decrease was recorded in fat content, from 2.33 to 1.16%, and in protein content, from 16.74 to 15.655%. The increased moisture can be attributed to the absorption of water from the environment. Yildiz and Yangilar (2016) determined the moisture in coated rainbow trout by whey protein concentrate (8%) at 68.05 – 64.33% after 15 days at 4 °C, while it was (73.90 – 73.93%) in present study and was inconsistent with our results. This difference can be due to the change in the edible coating's concentration.

The calories increased in the samples coated with edible film compared with the control samples (**Table 3**) because of the increase of the calories in the edible coating. Based on the experiments, the amounts of protein, lipids, and carbohydrates in the coated samples are higher than the control sample and with consideration to the conversion coefficient of each gram of protein, carbohydrate, and lipid to energy. The number of calories in these samples increases.

The coated samples have better taste and smell compared with the control sample (**Table 4**), that is because of the di-acetyl and lactose in the film. Naturally, the whey proteins have a globule structure and high solubility and emulsification. This film has protein, lactose, and minerals and can increase the ability of connection to water in kilka. Absorption of water by the protein, and the interconnection and linking of protein chains, increases the size of protein, which may lead to higher viscosity, humidity, and altered texture in the coated samples compared with the control sample (Erbay et al., 2017). The proteins αlactalbomine and β-lactoglobine present in whey protein contribute to the formulation and accumulation of large aggregates. This process may result in the production of small sediment deposits. These deposits and their connection with water improved the taste of the product. In the control samples, because of presence of empty space between fish filets and also changes in temperature during storage, the kilka in the packages lost the humidity and will be dried; this leads to decrease in weight of about 3.5% after three months (Bakhy et al., 2018). This change may be because of production of ice crystals at the products, too. Freezing is considered a fundamental process of dehydration, as it causes moisture to leave the frozen food in the form of vapor.

The coated samples have better taste and smell compared with the control sample, that is because of the di-acetyl and lactose in the film.

This process may quicken the protein degradation and lipid oxidation, leading to a decline in taste quality and color changes in uncoated kilka (Aguirre-Joya et al., 2018). Yildiz and Yangilar (2016) showed the effect of 8% whey protein concentrate on improvement of sensory characteristics of farmed rainbow trout during chilled storage. Similar results were obtained in the current study, and the coated samples had good sensory characteristics after freezing.

Conclusion

The experimental results show a significant difference and overall acceptance among the coated samples compared with the uncoated (control) samples. No statistically significant differences were observed in the chemical properties of the coated samples during the storage period, while the control sample did exhibit significant changes. In addition, the bacterial count was lower in the coated samples compared with the uncoated samples. The coated samples maintained desirable quality until the end of the storage period in the cold conditions, but the control samples showed a decline in quality. Therefore, using a 9% whey protein concentration is suggested for the aquatic processing industry to coat kilka fish, as an alternative to packaging it in disposable dishes with cellophane coating. ❊

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