

# Effects of whey protein edible coating on bacterial, chemical and sensory characteristics of frozen common Kilka (*Clupeonella delitula*)

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## Abstract

The objective of the current study was to investigate the effects of whey protein coating on the quality of common Kilka during frozen storage. For this study, common Kilka was coated with 20% whey protein concentration. Non-coated Kilka also was used as a control. Coated and non-coated samples were then stored at -18 °C for six months. Bacterial, chemical and sensory properties of the samples were determined in each month. Results showed that *Coliform*, *Escherichia coli* and *Pseudomonas* bacteria contaminations were negative until the end of storage period in the covered samples. Total bacterial counts and *Staphylococcus* bacteria count were lower in the test samples compared with the control samples. Humidity, protein, lipid, ash and calorie contents were higher in test samples as compared with the control samples, while peroxide value, free fatty acids, thiobarbitoric acid, TVN and pH were lower in test samples. Taste, odor, color, tissue and overall acceptability were studied in the test and control samples. These specifications had a better quality in test samples compared with the control ones. These factors showed a significant difference in the uncoated samples (control) ( $p < 0.05$ ). Significant decrease was observed in bacterial counts in the covered and control samples. According to the statistical analysis in sensory specifications, there was a significant difference between the covered samples and the control ones ( $p < 0.05$ ). According to the results of experiments and statistical analysis, the covered samples had a favorable quality until the end of storage period but the control samples had lost their quality after three months.

**Keywords:** Edible film, Whey protein, Chemical analysis, Sensory analysis, Kilka fish

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## Introduction

Kilka fish belong to the genus *Clupeonellia*, the order *Clupeiformes*, the phylum *clupeiform*, and the family *Clupeidae*. These fish are composed of three species consisting of *Clupeonella delicatula*, *Clupeonella engrauliformis* and *Clupeonella grimi* (yasemi, 2007). They can be processed into salted Kilka fish, smoked, pickled, conserved, dried and frozen fish. But in Iran, Kilka products in the market are canned, packaged Kilka in frozen shape and fresh Kilka (Martin, 1994).

The overall yearly Kilka fish catch has increased from 19610 tons (in the year 2004) to over 25483 tons in the year 2009. Around 4742 – 9350 tons of the yearly catch occurred in Guilan province. Around 9– 3% of this catch is used for human consumption and the remaining 88 – 90 % is used for animal feeds. About 10260 to 20741 tons of the fish catch occurred in Mazandaran Province, of which 5–12 % is used for human consumption and the remaining 88–95% for animal feeds (fisheries studies and statistical group, 1386). Consumption of fresh Kilka fish dropped during the years 2004-2009 (from 6% to around 2.2%). Consumption of canned Kilka fish also dropped from 5.2% to about 0.76% during the same period, whereas consumption of the frozen fish rose from 1% to 6.25% during these years (seifzadeh, 2010). The frozen fish packs, due to their longer storage time as well as wider countrywide distribution, had much higher sale rates in comparison to the sales of fresh fish. Sales of frozen fish were also higher. The frozen fish packs were mostly frozen for less than three months because

longer frozen storage time may lead to color changes, surface dryness and peroxide accumulation (Moeini et al., 2009). Despite this, the first sign of quality decline, even after only one month of frozen storage, was a decline in the weight of frozen packed fish. This will in turn have 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 (Kochakian, 2002 ; Moradi, 2010).

It seems as though consumer market demand is fairly high for high quality seafood products especially those which can retain their superior quality of taste, texture and general fresh appearance following prolonged period of cold or frozen storage (Ahvenainen, 2003). Use of edible films for packaging of Kilka fish seems to be an ideal method for proper long storage fish preservation (Shariatifar, 2007). Edible coatings are completely water soluble, glossy, act just like a secondary skin and have the following favorable proportion such as rapid attachment to foodstuff, label attachment, anti-bacterial and anti-oxidant properties (Marsh and Bugusu, 2007). These coatings are protective of the aroma, taste and food color and help to maintain the nutritional components such as their vitamin, amino acid and fatty acid ingredients. Covering of food products with these films can lead to preservation of food moisture, and oxygen absorption lowering, which can substantially improve the appearance of food products. These coatings are invisible to the naked eye (Seifzadeh, 2007). Whey protein is derived

from milk and is composed of protein, lactose and inorganic salts. It is anti-bacterial, anti proteolysis and preservation of food moisture (Morrissey et al., 2009).

Edible film whey protein has been used for packaging of Salmon, Hot-dog, sausage, cracker and frozen fish-fillet (Stuchell and Krockta, 1995). In Iran using whey protein films for packaging of fish has been carried out by Motallebi (2010) and Hassan Zati (2010). In other countries, films consisting of whey proteins have been used for food packaging by Cagri (2002, 2003), Crapo (1999), Min (2006 and 2007), Krockta (1996), Shah (1999), Stuchell (1995) and Piyachonkwan (1995). These results indicate prevention in deterioration of sensory quality, lipid oxidation decrease and shelf life extension in cold storage.

The aim of this study was to determine the effects of whey protein edible coating on the bacterial, chemical and sensory characteristics and shelf life of frozen common Kilka.

### Materials and methods

180 kg of common Kilka caught in spring were used for this study. Fresh fish landed from Kilka fishing vessels were obtained early in the morning (5 a.m.), and the characteristics of fresh fish following the Iranian National Standard (INS, 2002b) were recorded. The fish were chilled down to 0 °C under a cover of ice: ice ratio of 2:1 in insulated tubs and then they were transferred to the production line of the National Fish Processing Center under complete hygienic conditions. The method used in this study followed the protocol

described by other researchers with a few modifications.

Processing fish, which were already washed with chlorinated water, were then cut and viscera were taken out. The cleaned fish were washed again. Two treatments were employed: coated samples and uncoated samples (control samples).

Prepared samples were submerged deep in 12% concentration of whey protein. Following this, the fish were packaged in disposable dishes and covered with cellophane. These samples were kept at -18 °C for six months. Three replicates were used in this study. Microbial, chemical and sensory tests were carried out to test the quality of samples after storage.

The microbial tests for experimental and control samples (35 packages) consisted of total bacterial counts (Maturin, 2001; Andrews and Hammak, 2003) for *Staphylococcus* (Holt et al., 1994; Bennet and Lancette, 2001), *Coliform* (Feng et al., 2002), *Escherichia coli* (Feng et al., 2002) and *Pseudomonas* (Hasegawa, 1987). Microbial tests were carried out through nine steps. The first microbial tests on fish samples were conducted immediately after receiving the raw fish samples, the second was conducted after cleaning, and the third was conducted one day after processing. Further tests for microbial buildup on fish samples were conducted once a month after processing at determined times for a period of six months. In each stage, these tests were repeated three times.

The chemical tests for the packaged samples with edible film and

control (35 packages) included measurement of calorie (INS, 2005), protein (INS, 1994) humidity (INS, 2002), lipid (INS, 2002a), ash (INS, 2001), peroxide value (INS, 2003), TVN (INS, 2002), TBA (INS, 2006), FFA (INS, 2003) and pH (INS, 2007). These tests were conducted through eight steps; the first on raw fish samples, the second one day after processing and the remaining tests once a month from the first month after processing up to the sixth months, at specific times. Each step of the tests was repeated three times.

Sensory tests for the test sample and control sample (14 packages) consisted of evaluation of tissue texture, odor, color, taste and overall acceptability with the method of ranking and through the execution of the Freedman test (Iso 85–87, 1988). The sensory tests on the packaged samples with edible film and the control samples were repeated once in each step.

For controls, 500 g of prepared samples were packaged in disposable dishes with cellophane covers. These samples were kept at  $-18^{\circ}\text{C}$  in cold-storage for a period of six months. The quality of these samples was studied similar to the test samples at the determined times by microbial, chemical and sensory tests. These samples were processed three times. Each step of the tests was repeated three times and the samples were treated in three replicates.

The results of microbial tests were analyzed by SPSS Software and Two Way Variance Analysis, and results of the sensory tests were analyzed by Ranking and Freedman Test.

## Results

The bacterial counts of the fresh samples are shown in table 1. As it was indicated, no *Coliform*, *Escherichia coli* and *Pseudomonas* bacterial contamination was observed in these samples. Total bacterial counts in the Kilka samples after processing were lower than the samples before processing, while *Staphylococcus* bacterial counts in Kilka samples after processing were higher than the samples before processing.

The mean of proximate analysis in covered and control samples are shown in table 2. Humidity, protein, fat, ash and calorie content in the covered samples were higher as compared with the control samples. Analysis of results obtained using Two-Way ANOVA at 95% confidence level indicated no significant differences in humidity in covered samples as compared with uncovered samples during storage ( $p>0.05$ ).

Bacterial counts were lower in the covered samples compared with the control samples (Table 4). The mean total bacterial counts and *Staphylococcus* bacteria count in samples processed by whey protein were 2.47 and 1.61 log CFU/g and 3.13 and 2.18 logCFU/g in control samples from 1 day until six months after processing, respectively. No *Coliform*, *Escherichia coli* and *Pseudomonas* bacterial contamination was observed in the whey protein covered and control samples (prior and during storage period).

Chemical factors were lower in the covered samples compared with the control samples (Table 3). The mean

peroxide value, TVN, pH, free fatty acids and thiobarbitouric acid in the covered samples were 0/13meq/kgoil, 9.84mg/100g, 6.25, 1.15gr/100 and 0/006 mg/kg, respectively.

The mean peroxide value, TVN, pH, free fatty acids and thiobarbitouric acid in the control samples were 3.75 meq/kgoil, 16.22 mg/100g, 6.71, 9.21gr/100 and 0/15mg/kg, respectively. According to Kolmogrov-Smirnov Test, distribution of microbial and chemical data was normal. Sensory analysis in the control and covered samples are shown in table 6. Based on the Freedman test significant differences ( $P<0.05$ ) were observed between covered samples and the control sample regarding odor, color, tissue, texture and overall acceptability. The covered samples had better quality. Analysis of results obtained using Two-Way ANOVA at 95 % confidence level indicated no significant differences in free fatty acids, thiobarbitouric acid, TVN and peroxide values in the covered samples ( $P>0.05$ ) compared with the control ones ( $P<0.05$ ). Variations of free fatty acids were significant from one day after

keeping up to five months ( $P<0.05$ ) and at the sixth month in control samples, but it wasn't meaningful in covered samples. Variations of pH and TBA showed significant differences between the first day and the sixth months after processing in control samples ( $P<0.05$ ).

According to the results of the chemical, bacterial and sensory tests and statistical analysis, better general quality was observed in the covered samples compared with the control sample.

Statistical analysis of results obtained from bacterial analysis in the control and covered samples are shown in table 4. Analysis of results obtained using Two-Way ANOVA at 95 % confidence level indicated significant differences in total bacterial counts and *Staphylococcus* bacteria count both in the covered samples as well as in the control samples from the first day up to the sixth month ( $P>0.05$ ).

Based on the statistical tests, the covered samples had preserved their quality up to the end of the storage period while the control samples had lost their quality after three months.

**Table 1: Microbial analysis results in fresh Kilka fish (log CFU/g) (N= 10 )**

Samples	Bacteria Total bacterial counts	<i>Staphylococcus</i> bacteria
Whole Kilka (before processing)	4.49±0.12	2.39±0.23
Cleaned Kilka fish	3.81±0.11	2.51±0.15

**Table 2: Proximate analysis in covered and control samples (N= 35 pack from each treatment)**

	Moisture%	Protein %	Fat%	Ash%	Calorie (kcal/kg)
Covered samples	73.91±0.04	19.00±0.07	4.25±0.03	2.10±0.25	120.73
Control samples	58.91±0.13	18.2±0.65	4.00±0.36	1.80±0.15	108.8

**Table 3: Chemical factor results in covered samples during storage (35 packs from each treatment)**

Experiments	PV value		FFA		TBA		pH		TVN	
	meq/kgoil		gr/100		mg/kg				mg/100gr	
Samples	control	Covered	control	Covered	Control	Covered	control	Covered	control	Covered
First day	0.20±0.01 <sup>a</sup>	0.08±0.10	4.10±0.25 <sup>a</sup>	1.1±0.15	0.03±0.024 <sup>a</sup>	0.006±0.001	6.2±0.10 <sup>a</sup>	6.2±0.20	9.8±0.36 <sup>a</sup>	9.8±0.35
First month	1.7±0.10 <sup>b</sup>	0.08±0.20	6.83±0.32 <sup>b</sup>	1.1±0.25	0.07±0.1 <sup>a</sup>	0.006±0.002	6.2±0.20 <sup>a</sup>	6.2±0.10	12±0.30 <sup>b</sup>	9.8±0.20
Second month	3.20±0.10 <sup>c</sup>	0.08±0.20	8.34±0.25 <sup>c</sup>	1.1±0.35	0.10±0.03 <sup>ab</sup>	0.006±0.002	6.4±0.10 <sup>a</sup>	6.2±0.20	14.6±0.30 <sup>c</sup>	9.8±0.25
Third month	4.50±0.10 <sup>d</sup>	0.08±0.15	9.52±0.28 <sup>d</sup>	1.1±0.02	0.14±0.01 <sup>ab</sup>	0.007±0.02	6.7±0.15 <sup>b</sup>	6.3±0.20	16.2±0.30 <sup>d</sup>	9.8±0.55
Forth month	6.00±0.25 <sup>e</sup>	0.09±0.26	10.96±0.46 <sup>e</sup>	1.2±0.05	0.17±0.01 <sup>ab</sup>	0.007±0.001	7±0.15 <sup>bc</sup>	6.3±0.20	18.5±0.30 <sup>e</sup>	9.9±0.41
Fifth month	5.60±0.6 <sup>f</sup>	0.09±0.15	12.37±1.31 <sup>f</sup>	1.2±0.15	0.25±0.03 <sup>abc</sup>	0.007±0.11	7.2±0.26 <sup>bc</sup>	6.3±0.10	20.8±0.50 <sup>f</sup>	9.9±0.30
Sixth month	5.10±0.30 <sup>g</sup>	0.1±0.25	12.38±0.10 <sup>f</sup>	1.3±0.35	0.32±0.02 <sup>bc</sup>	0.008±0.025	7.3±0.15 <sup>c</sup>	6.3±0.15	21.7±0.25 <sup>g</sup>	9.9±0.30

The different letters in the same column within the different storage time indicate significant differences ( $P < 0.05$ ). Values are mean + standard deviation.

**Table 4: Statistical and bacterial analysis results in control and covered samples (logCFU/g) (N= 35 pack from each treatment)**

Bacteria Samples	Total bacterial counts		<i>Staphylococcus</i> bacteria	
	control	Covered	control	Covered
First day	3.67±0.21 <sup>a</sup>	3.43±0.32 <sup>a</sup>	2.85±0.12 <sup>a</sup>	2.25±0.12 <sup>a</sup>
First month	3.46±0.11 <sup>b</sup>	3.34±0.22 <sup>b</sup>	2.57±0.42 <sup>b</sup>	2.1±0.22 <sup>b</sup>
Second month	3.32±0.25 <sup>c</sup>	3.25±0.42 <sup>c</sup>	2.32±0.32 <sup>c</sup>	1.84±0.31 <sup>c</sup>
Third month	3.20±0.32 <sup>d</sup>	3.07±0.18 <sup>d</sup>	2.17±0.17 <sup>d</sup>	1.59±0.18 <sup>d</sup>
Forth month	2.95±0.14 <sup>e</sup>	2.69±0.14 <sup>e</sup>	2.00±0.18 <sup>e</sup>	1.35±0.11 <sup>e</sup>
Fifth month	2.77±0.12 <sup>f</sup>	2.00±0.11 <sup>f</sup>	1.72±0.22 <sup>f</sup>	1.20±0.15 <sup>e</sup>
Sixth month	2.47±0.24 <sup>g</sup>	1.45±0.17 <sup>f</sup>	1.69±0.28 <sup>g</sup>	1.00±0.13 <sup>e</sup>

The different letters in the same column within the different storage time indicate significant differences ( $P < 0.05$ ). Values are mean  $\pm$  standard deviation

**Table 5: Sensory analysis results in covered samples and control samples (N= 14 pack from each treatment)**

Properties	Color	Odor	Tissue	Taste	Overall acceptable
Covered samples	75 <sup>a</sup>	70 <sup>a</sup>	79 <sup>a</sup>	79 <sup>a</sup>	79 <sup>a</sup>
Control samples	110 <sup>b</sup>	106 <sup>b</sup>	96 <sup>b</sup>	104 <sup>b</sup>	112 <sup>b</sup>
Score	110 – 75	106 – 76	99 – 69	104 – 64	112 – 68
LSD	35>19.6	36>19.6	23>19.6	40>19.6	44>19.6

Different letters in the same column within the different treatment indicate significant differences ( $P < 0.05$ )

## Discussion

The total bacterial counts and *Staphylococcus* bacteria in the covered samples had decreased. This may be due to the effect of lactic acid, acetic acid, probiotic bacteria and production of ethanol and nisin. Pro-biotic micro-organisms of whey protein are able to produce organic acids such as lactic acid, acetic acid, nisin bacteriocin, hydrogen peroxide, ethanol, acetaldehyde, ammoniac, di-acetyl, Eh and pH reduction (Adams and Moss, 2002; Seifzadeh, 2002; Nasar et al., 2008). These compounds have

anti-microbial specifications and are able to prevent the growth of *Staphylococcus*, *Pseudomonas*, *Coliform* and *Escherchia coli* (Cagri et al., 2002 and 2003; Zinoviadou et al., 2009). Naturally, the whey proteins have a globule structure and high solubility and emulsification. This film has protein, lactose and minerals and is able to increase the ability of connection to water in Kilka (Stuchell and Krochta, 1995). Absorption of water by protein and adhesion and linking of protein chains to each other increases the size of protein

which may cause an increase in the viscosity, humidity and texture of the covered samples by this film, compared with the control sample (Sanker and Raghunath, 1995). In the control samples, because of the empty space between fish fillets and also rise and fall in temperature of the cold-storage, the Kilka in the packages lost their humidity and got dried, which naturally led to a 3.5% decrease in weight after three months (Kochakian, 2002). This condition may also appear due to the production of ice crystals in the product. Freezing is considered a basic action of dehydration that causes the release of frozen humidity in vapor state from food products. Air circulation in the cold-storage may also intensify the release of humidity. This condition may quicken the degradation of proteins and oxidation of lipids, and cause a decrease in the quality of taste and color change in Kilka without cover (Fatemi, 1990; Safari, 1990; Deman, 1999).

There was a decrease in the amount of TVN in covered Kilka compared with Kilka without cover during the storage period in cold-storage. This may be due to the effect of the decrease in humidity and production of free fatty acids on the denaturing of protein. These factors decreased in covered samples compared with the control sample. We can relate this to the specification of whey protein that causes the prevention of proteolyses, decrease of humidity and production of free fatty acids on denaturing of protein and naturally increases TVN (Piyachomkwan and Penner, 1995; Salmani et al., 2002; Morrissey et al., 2009).

Ash content showed an increase in the covered samples compared with control samples. That is because of the

presence of sodium, potassium, calcium, manganese and phosphate ions, and the presence of sulfur holder amino acids in the whey protein and due to the fact that these amino acids are considered as mineral elements of food products (Lyndon, 1999). Protein content showed an increase in covered samples compared with the control sample. This may be due to the effect of some protein compounds such as  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin of this film (Shahidi and Botta, 1990). Concentration of lipid showed an increase in the covered samples compared with the control sample. That is because of the presence of glycerides of milk lipid, phospho-lipids and lipo-proteins in whey protein. Oxidation of the lipid arising from the reaction of lipid with oxygen and its hydrolysis are influenced by the action of lipolytic enzymes (Deis, 2006; Min et al., 2006). The enzyme of lipase of the tissue, the excreted lipolytic enzyme from the *Staphylococcus* bacteria and those enzymes that are released from the dead and decomposed bacteria, have been able to be active at low water activity and may cause hydrolysis of lipids and production of non-saturated fatty acids through the lipolyze process (Cappuccino and Sherman, 1999; Min et al., 2005, 2007). Releasing fatty acids with high numbers of carbons by lipase enzyme may not provide a specific bad taste but with the passage of time, effects of accumulation of free fatty acids in muscles of fish may cause unfavorable taste and tissue damage because of their combination with protein of muscle (Crapo et al., 1999). In addition to fatty acids, protein denaturation caused some tissue changes and lowering of quality. In the control samples, concentration of these acids increased from the first month up to the fifth month.



But the nearly constant concentration of these acids at the end of the processes of keeping has probably been because of the decrease of raw materials and increase of oxidation in free fatty acids (Silva and Ammerman, 1993; Rezaei et al., 2002).

Based on the conducted experiments, the amount of free fatty acids measured in the samples covered by whey protein was lower than the control sample. This may be due to the prevention of water loss from the surface and from inside the body by the fine holes present on the body surface, prevention of 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 (Lyndon, 1999; Ahvenainen, 2003). Freezing caused fish tissue denaturizing and moisture loss leading to weight loss which may be due to the oxygen penetration into the fish tissue and an increase in free fatty acids and peroxide value caused by oxidation. But, peroxide will start to induce decomposition, leading to aldehyde, ketone and seton production, and subsequently to peroxide value reduction over time ( Krochta et al., 1996). The first products of lipid oxidation are hydro-peroxide and they are broken into secondary products such as aldehydes that can react with proteins. These compounds react with pigments and other molecules present in the fish, too. Therefore it leads to loss in color and production of bad smell (Rezaei et al., 2003).

A suitable index for determining progress in fat oxidation and production of carbonyl compounds is measurement of TBA. Presence of such compounds in fish meat causes some changes in its sensory specifications such as taste and smell (Aubourg et al., 1995; Shah, 1999). The amount of peroxide value and TBA was

lower in the covered samples compared with the control sample. We can say that it is because of preventing the decrease of water in fish tissue while freezing, decrease of water activity and free radicals production, covering specifications of edible film, preventing oxygen absorption, preventing humidity decrease, decrease of oxidation and production of secondary products of oxidation such as aldehydes and specifications of these compounds (Tall and Harris, 1995; Gigirey and Desousa, 1999).

In the present research, amounts of measured TBA showed an increasing trend up to the end of cold-storage in the control Kilka. This may be due to the effect of freezing on decrease in humidity, decrease in the water activity factor, increase in oxidation at low water activity, production of free radicals, production of free fatty acids and being ready for oxidation (Coles and McDowell, 2003; Hegenbart, 2006).

The covered samples had better taste and smell compared to the control sample and this is due to the effect of diacetyl and lactose in the composition of this film. Proteins of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin present in whey protein cause production and accumulation of big solution and masses. This process may lead to production of small sedimentations. These accumulations and their linkage with water, has increased the amount of water consequently causing an increase in viscosity and development of the tissue of the product. Samples covered with whey protein had better color during the storage period compared with control samples. This can be due to retarded oxidation (Chapman et al., 1997; Bigelow and Lee, 2007). Calorie content showed an increase in the samples covered by edible film compared with the control samples. It is

because of increase in the added value raised from the edible covers. Based on the conducted experiments, the amount of protein, lipid and carbohydrates in the samples covered by this film is higher than the control sample and with consideration to the conversion coefficient of each gram of protein, carbohydrate and lipid to energy. Calorie content in these samples has also shown an increase (Marsh and Bugusu, 2007; Hassan Zati, 2010; Motalebi, 2010). Through the passing of time, lipid oxidation products such as hydro-peroxides are analyzed and some compounds such as aldehydes and others are produced in the control sample. These compounds have alkali specification and cause an increase in the pH of the product. Therefore in the covered samples, changes of pH during the time storage were not significant (Anker and Hermanson, 2010). Similar results were obtained by Rokwer, Bigelow, Fujki, Trout and Manish.

According to the results of the experiments, meaningful differences in the index of covered samples in comparison with the control sample were observed. No statistically significant differences were observed in the results of chemical indexes of the covered samples in comparison to those of the control samples. The covered samples up to the end of storage period at cold-room had a favorite quality but the control samples had lost their quality.

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