Chemical, microbiological and sensory evaluation of gutted kilka coated with whey protein based edible film incorporated with sodium alginate during frozen storage

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Abstract

The effects of whey protein (12%) and sodium alginate (0.5%) on chemical, microbial and sensory changes and shelf life in kilka during frozen storage are investigated for up to 6 months. Total bacteria count and *Staphylococcus* bacteria counts(2 / 51 and 1 / 44 log CFU/g) in coated samples showed decrease in comparison with control samples(3.21- 2.28 log CFU/g). Moisture of coated samples had significant increase than control treatment ($p<0.05$). protein, lipid, ash and calorie were higher in test samples as compared with the control samples. Free fatty acids, TBRS, peroxide value, TVN and pH in coated samples showed significant decrease in comparison with control ($p<0.05$). Sensory evaluation of coated samples showed significant difference in comparison with control ($p<0.05$). It is suggested that whey protein edible coating incorporated with sodium alginate can enhance quality and increase shelf life of kilka fish in storage of freezing up to 6 months.

Keywords: Edible film, Whey protein, Sodium alginate, Kilka , Shelf life

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Introduction

Clupeids, also known as kilka, including anchovy (*Clupeonella engrauliformis*), common kilka (*C. cultriventris*), and bigeye kilka (*C. grimmi*), are one of the most abundant fish in the Caspian Sea (Fazli et al, 2007). Freezing is a common method using for control and decreasing biochemical changes in fish during storage.

Advances in food processing and food packaging play a primary role in producing safe food in the world. Packaging maintains the benefits of food processing after the process, enabling foods to travel safely for long distances from their point of origin and still be wholesome at the time of consumption. However, packaging technology must balance food protection with other issues, including energy and material costs, heightened social and environmental consciousness, strict regulations on pollutants and disposal of municipal solid waste. Nowadays, packaging methods including aluminum foil and the active packaging techniques such as atmospheric changes for aquatic products is used in the world. Development of an edible coating to inhibit the growth of pathogenic bacteria in food products is an active research area in the food science field. protein based edible film is the most interesting research object in recent development, although edible films can be prepared using protein(whey protein, soy bean and chitosan) polysaccharide and lipid materials (Ahvenainen, 2003; Bourtoom, 2009). Because edible film is natural product, and due to the biodegradable characteristic of these films. These films were biodegradable and invisible by the naked eye. Edible films have adhesion properties to the food and they are antibacterial and antioxidant nutrients that can penetrate into nutrients. (Chapman et al, 1997).

Edible films have Good mechanical strength and are good oxygen and lipid barriers, but like other protein-based films are poor barriers to moisture due to their hydrophilic nature (McHugh and Krochta 1994). Edible films are good alternative for preservatives in order to inhibit bacterial growth in the products (Manish et al, 2004). This method of packaging without using preservative can reduce toxicity and allergic reactions for consumers (Shariatifar, 2007). The functional properties, relative abundance and low cost of whey proteins make them suitable for production of transparent, flexible, colorless and odorless edible films (Sedaght, 1996). Sodium alginate powder was used in this project for coating kilka. Alginate is used in the food industry. Alginates are extracted from brown seaweed and are available in sodium, ammonium, calcium and potassium derivatives. In the presence of calcium and an acid some alginates can form resilient gels. Alginate is present in the cell walls of brown algae as the calcium, magnesium and sodium salts of Alginic acid.

A few studies are available on whey protein edible coating applied to seafood in Iran (Motalebi et al, 2010), but there is not any research published on whey protein and sodium alginate together. The aim of this investigation was to investigate the effect of whey protein incorporated with sodium alginate , on

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moisture content, microbial characteristic and enhancing shelf life of coated kilka during frozen storage.

Materials and methods

Sampling

Features of fresh fish were monitored according to national standards (Iranian National Standard NO.5623, 2002b). Kilka fish were washed with chlorinated water for processing. The amount of 180 kg of common kilka was used. Fresh fish was provided from quay of Bandar Anzali, in Guilan Province.

Coated samples processing

Fish were carefully gutted and dressed by hand, and then they were washed again with chlorinated water. The Gutted kilka were placed in concentration of 12% whey protein incorporated with (0.5%) sodium alginate (Merk). Each 500 g immediately packed in polyethylene dishes with cellophane blanket and stored at -18°C for 6 months.

Control samples processing

Fish were carefully gutted and dressed by hand, and then they were washed again with chlorinated water. Each 500 g immediately packed in polyethylene dishes with cellophane blanket and stored at -18°C for 6 months.

Experimentes

Experimental and control samples were processed in three replications. Quality of coated and control samples was tested with microbiological, chemical and sensory assessment.

Total microbial count, was assessed on 48 package (Andrews et al., 2003), count of *Staphylococcus* (Bennett and Lancette 2001), *E. coli* forms (Feng et al., 2002) and *Pseudomonas* (Holt et al., 1994) were done in microbiology laboratory of National Fish Processing Research Center. Sampling was carried out in 9 phases. Phase 1 (before processing), phase 2 (gutted kilka), phase 3(after coating and before cold storage), phase 4(one day after processing), phase 5-9(after each month up to month 6).

Chemical analysis on samples packed with edible films and controls (42 packs), including calorie measurement according (Iranian National Standard NO. 8867, 2006); Moisture (Iranian National Standard 5625, 2002c); protein (Iranian National Standard NO.924, 1995); lipid (Iranian National Standard NO. 742, 2002a); free fatty acid (Iranian National Standard NO. 493, 2004); Thiobarbituric acid (Iranian National Standard NO. 10494, 2004); ash(Iranian National Standard NO.744, 2002); Total volatile nitrogen (TVN) (Iranian National Standard NO. 5625, 2002c); Peroxide (Iranian National Standard NO. 493, 2004); pH (Iranian National Standard NO. 1028, 2007). These tests were done in 7 phases; phase 1 (before processing) and phase 2-7(monthly). Each measurement was repeated three times.

Sensory analyses were done, on edible film coated pack and control (14 packs) including color, odor, flavor, texture and total acceptability were carried out by home evaluators (ISO 2006- 2008, 1988). In this test, 30 evaluators compared the quality of samples and scored 1-4 point. Less point in each index indicates higher quality of samples.

Statistical analysis

The results of microbial tests were analyzed by SPSS Software and Two Way

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Variance Analysis, and results of the sensory tests were analyzed by Ranking and Freedman Test.

Results

Results of bacterial analysis before processing are shown in Table 1.Mean of total bacterial count and *Staphylococcus* bacterial contamination were 2 / 51 and 1 / 44 log CFU/g in coated samples prior and after six months cold storage and 3.21-2.28 log CFU/g in Control group. Coated samples did not show any *Escherichia coli* and *Pseudomonas* bacteria (Table 2). Based on Kolmogorof-Smirnof test, distribution of bacterial analysis was normal. There was significant differences in coated and uncoated samples in bacterial counts during storage period($p<0.05$). In control samples moisture, peroxide, TVN, pH, free fatty acids, thiobarbitoric acid content were respectively 59.43%, 3.75 meq/ kgoil, 16.28 mg/100 g, 6.91, 9.21 g / 100 and 0 .15 mg/kg. In coated samples, moisture, peroxide, TVN, pH, free fatty acid, thiobarbitoric acids content were respectively 73.62%, 0.06 meq/kgoil, 9.88 mg/100 g , 5.37, 1.07 g/ 100 and 0 .004 mg/kg tissue (Table 3).

Distribution of chemical analysis was normal based on Kolmogorof-Smirnof test. Based on two way ANOVA, there were significant changes in moisture, TVN, pH and peroxide from the first day to sixth month in control samples($p<0.05$) (Table 4).

Changes in free fatty acid from the first day of cold storage to fifth month was significant ($p<0.05$) and it was insignificant in sixth month ($p>0.05$) (Table 4), pH was significant between first day and sixth month($p<0.05$) (Table 3). TBA was significant between first day and sixth month ($p<0.05$) and it was significant between first, 2^{d}, 3^{rd}, 4^{th}, and 5^{th} months ($p<0.05$) (Table 4). There were no significant difference in chemical analysis of coated samples ($p>0.05$).

Protein, lipid, ash and calorie of coated sample were higher than control (Table 4).

Sensory analysis showed significant difference between coated and control samples ($p<0.05$) (Table 5).

According to the statistical analysis of chemical assessment on Friedman test, coated samples had better quality than control samples. Coated samples retained their quality until the end of cold storage while based on moisture content and sensory assessment, control samples kept their quality up to 3 months.

Table 1: Bacterial counts of uncoated samples(Log CFU/g) in two forms of gutted and before processing of kilka

Bacteria	Total count	*Staphylococcus*	*Pseudomonas*	*Coli* form	*E. coli*
Sampling before processing	4.49±0.12	2.69±0.23	Less than 10 /g	Less than 10 /g	Less than 10 /g
Gutted kilka	3.81±0.11	2.95±0.15	Less than 10 /g	Less than 10 /g	Less than 10 /g

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Table2: Bacterial counts of coated and control samples, 6 month in cold storage (Log CFU/g)

Time / Index	*Pseudomonas*		*Coliform*		*Staphylococcus*		Total count	
Samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples
Before cold storage	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.93±0.15^{a}	2.38±0.30a	3.81±0.11^{a}	3.17±0.34a
One day after cold storage	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.85 ± 0.12^{b}	2.14±0.26b	3.76 ± 0.21^{b}	0.22b± 3.04
month 1	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.57±0.42^{c}	1.87±0.32c	3.46±0.11^{c}	0.19c ± 2.90
month 2	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.32±0.32^{d}	1.54±0.25d	3.32±0.25^{d}	2.69 ±0.21d
month 3	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.17±0.17^{e}	1.21±0.20e	3.20±0.32^{e}	2.35±0.25e
month 4	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	2.00± 0.18^{f}	1.00±0.18f	2.95±0.14^{f}	2.00±0.11f
month 5	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	1.72±0.22^{g}	Less than 10 g/g	2.77± 0.12^{g}	1.45±0.16g
month 6	Less than 10 /g	Less than 10 /g	Less than 10 /g	Less than 10 /g	1.69± 0.28^{h}	Less than 10 /gg	2.47± 0.24^{h}	1.12±0.19h

Dissimilar letters indicate significant differences between different times of cold storage ($p<0.05$)
Similar letters indicate insignificant differences between different times of cold storage ($p>0.05$)

Table 3: Chemical analysis of 6 month cold storage in coated and control samples

Index / Time	Peroxide value (meq/ kgoil)		TBA (mg/kg)		pH		TVN (mg/100 g)		Moisture (%)		Free fatty acids (g/100)	
Samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples	Control samples	Test samples
One day after cold storage	0.2 ± 0.01a	0.15 ± 0.05a	0.024±0.03 a	0.001± 0.001a	6.3± 0.10 a	6.2±0.10a	10.0± 0.36 a	9.8± 0.30a	72.2 ± 0.35a	73.38±0.07a	4.10±0.25 a	0.7± 0.05a
month 1	1.7 ±0/10 b	0.15 ± 0.05a	0.07±0.01 b	0.001± 0.001a	6.4± 0.20 b	6.2±0.15a	12.2± 0.30 b	9.8± 0.35a	67.35 ± 0.25b	73.38±0.04a	6.83± 0.32*	0.7± 0.15a
month 2	3.2 ± 0.10 c	0.15 ± 0.05a	0.10 ± 0.03 c	0.002± 0.001a	6.7± 0.10 c	6.3±0.05a	14.6± 0.30 c	9.8± 1.04a	63.20±0.10c	73.38±0.07a	8.34 ± 0.25 c	0.7± 0.15a
month 3	4.5±0 0.10 d	0.15 ± 0.06a	0.14±0.01d	0.001± 0.002a	7.0± 0.15 d	6.3±0.15a	16.2± 0.30 d	9.8± 0.30a	58.90±1.61d	73.28±0.04a	9.52 ± 0.28 d	0.8± 0.10a
month 4	6.0±0.25 e	0.20 ± 0.05a	0.17 ± 0.01e	0.001± 0.002a	7.2± 0.15 e	6.4±0.10a	18.5± 0.30 e	10.0± 0.30a	54.15±0.16e	73.27±0.04a	10.96 ± 0.46 e	0.8± 0.20a
month 5	5.6 ± 0.60 f	0.20 ± 0.08a	0.25 ± 0.03 f	0.002± 0.003a	7.3± 0.26 f	6.5±0.26a	20.8± 0.50 f	10.0± 0.36a	50.43±0.14f	73.70±0.05a	12.37 ± 1.33 f	0.8± 0.15a
month 6	5.1 ±0/30 g	0.15 ± 0.08a	0.32 ± 0.02 g	0.002± 0.003a	7.5± 0.15 g	6.5±0.15a	21.7± 0.25 g	10.0± 0.15a	46.16±0.17g	73.36±0.02a	12.38± 0.10 f	0.9± 0.25a

Dissimilar letters indicate significant differences between different times of cold storage ($p<0.05$)
Similar letters indicate insignificant differences between different times of cold storage ($p>0.05$)

Table 4: Nutritional value analysis of coated and control samples

Index / Sample	Calorei (kcal/kg)	Ash(%)	Carbohydrate (%)	Lipid(%)	Protein(%)
Coated samples	134.63	2.88 ±0.20	0.25 ±0.17	4.62 ± 0.25	18.95 ± 0.45
Control samples	117.28	2.87 ± 0.35	4.59 ± 0.76	18.91 ± 0.45	73.63 ± 0.65

Table 5: Analysis of sensory assessment in coated and control samples and fresh kilka during cold storage in coated samples

Index / Treatment	Taste	Texture	Odor	color	Overall acceptable
Coated sample	34 a	32 a	45 a	40 a	31 a
Control (freeze) sample	80 c	63 c	92 c	80 c	85 c
Fresh kilka	66 b	85 b	68 b	60 b	64 b
– coated sample) LSD (control sample	46> 19.6	31> 19.6	47> 19.6	40> 19.6	54> 19.6
– coated sample) LSD (fresh kilka	32> 19.6	53> 19.6	23> 19.6	20> 19.6	33> 19.6
- control sample) LSD (fresh kilka	26> 19.6	22> 19.6	24> 19.6	20> 19.6	21> 19.6

Dissimilar letters indicate significant differences between different times of cold storage ($p<0.05$)
Similar letters indicate insignificant differences between different times of cold storage ($p>0.05$)
LSD: Less Significant differences

Discussion

Total bacterial count and *Staphylococcus* bacteria(1.44-2.51 log CFU/g) were lower in coated samples in comparison with control (2.28-3.21 log CFU/g). Based on our experiment there was a decrease in bacterial count of treatments before cold storage in comparison with control. During cold storage was observed more decrease in total bacterial counts and *Staphilococcus* bacteria count of coated samples in comparison with control. These results are in agreement with Krochta, 1996; Cagri,2002, 2003; Fujki ,2009.

Probiotic microorganism of whey protein produce organic acids such as lactic and acetic, nicin bacteriocine , hydrogen peroxide, ethanol, asetaldehide, NH_3, diacethyle, decrease in redox potential and

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pH(Cappuccino and Sherman, 1999; Adams and Mous, 2002). These organic acids have antibacterial properties and inhibit *Staphylococcus* ،*Coli*form ، *Escherichia coli* and *Pseudomonas bacteria* growth (Novac et al, 2003; Zinoviadou et al, 2007). Because of the sodium alginate ability to eliminate food poisoning micro- organisms, there was reduction in bacterial count of coated samples in comparison with control.

Sodium alginate have ability to produce 100 µ capsules with 17 nm pores but the real cause of this property in freeze and fresh samples is unknown yet. In addition gel formation of sodium alginate is due to Glucronic acid/Monoroiec acid. These properties cause a semi-permeable layer on product that can reduce microorganism infiltration into coated samples (Martin, 1994; Fujki, 2009).

Bactericidal property of sodium alginate and whey protein decrease microorganism amount in coated samples in comparison with control samples, before cold storage. After cold storage, low temperature and sodium alginate decreased the total bacterial count and *Staphylococcus* bacteria in comparison with control samples (Adams and Mous, 1999).

Moisture of coated samples (73.62%) increased significantly than control samples(59.43%). Gel formation of sodium alginate prevents moisture decrease of coated samples. Our results are in accordance with Sanker (1995), Hiroshi (2001), Amberdekar (2007), Moieni (2009) and Anker and Hermansson (2010).

In addition, chelating of calcium ions and reducing the link of protein by producing ion bridge , increases single and multi- layer water storage in myofibril, and prevent dehydration of tissue in sodium alginate coated samples (Chapman et al, 1997). Combinational coatings due to their high protein solubility of whey protein, water absorption and enhancement of protein size, retain moisture content of tissue and prevent flavor loss and chemical reactions in coated sample (Marsh and Bugusu, 2007).

Due to space in fish fillet of control samples and changes of temperature in cold storage, kilka lost moisture content (Safari, 1990) that causes about 3.5% weight loss (Kochakian, 2002). Air flow in cold storage and formation of ice crystals are also responsible for weight loss (Janson and Nikelson, 1998). Ice formation decreases moisture content and increases lipid oxidation, causing low quality in texture and color of uncoated samples (Janson and Nikelson, 1994).

In the covered samples compared with the control samples, changes of TVN during the time storage were not significant. This factor in coated samples (9.88 mg/100g) in comparison with control samples(16.28 mg/100g) showed decrease during cold storage. These results are in accordance with Manish (2006) and Morrissey (2009). Edible coating films, prevent moisture loss, form free fatty acids, denature protein and prevent protease activity(Salmani et al, 2002; Nassiri and Danesh, 2007) .

In the covered samples (5.37) compared with the control samples(6.91), changes of pH during the time storage were not significant.

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These results are in accordance with Dies (2006). 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(Tall, 1995).

Ash in coated samples (2.88%) was higher than control (2.87%) and fresh kilka (2.87%). Our results were in accordance with Marsh (2007). Increase of ash in coated samples is due to sodium ions in sodium alginate, and presence of sodium, calcium and sulphated amino acid in whey protein (Fatemi, 1990).

Protein of coated samples (18.96%) were higher than uncoated samples (18.04%) and fresh kilka(18.91%). Present results were according to Zeng (1997) and Ahmed (2006). Sodium alginate films content D & B Manoronic acid and alpha L glucronic acid, whey protein content alpha lacatlbomin and beta lacto glubulin (Renken and Kill, 1993).

Lipid in coated samples (4.62%) were higher than control (4.03%) and fresh kilka (4.59%). Present results were according to Zeng (1997). This increase was due to fatty acids in sodium alginate and phospholipid, lipoprotein and glicirids of milk (Coels, 2003).

Due to added value of edible films, Calorie of coated samples (134.63 kcal/kg) was higher than control (108.43 kcal/kg) and fresh kilka (117.28 kcal/kg). Based on our experiment, protein, lipid and carbohydrate of coated samples were higher than control. Increase in calorie of coated samples was due to conversion ratio of these components (Martin, 1994).

In the covered samples compared with the control samples, changes of free fatty acids during the time storage were not significant. Free fatty acids in coated samples (1.07 g/100) was lower than control samples (9.21 g/100). The concentration of free fatty acids in control increased from the first month up to month 5. But according to Aubourg (1995), amount of fatty acid remained constant in end of cold storage. Because edible films have good mechanical strength and are good oxygen and lipid barriers, there is decrease in fatty acid of coated samples. (Rezaie et al, 2006). Lipid oxidation and hydrolysis is caused by lipolitic enzymes in fish. Lipase in tissue and lipolitic enzyme from *Staphilococcus*, hydrolysis fat and produce free fatty acid (Jairus et al, 1996; Cappuccino and Sherman, 1999). Accumulation of fatty acid in muscle and combination with protein release bad smell. In addition, due to denatured proteins fatty acids cause tissue changes and reduce quality.

In present study, in the covered samples compared with the control samples, changes of TBRS and peroxide value during the time storage were not significant. TBA and peroxide value in control (0.15 mg/kg and 3.75 meq/ kgoil) were higher than coated samples (0.004 mg/kg and 0.06 meq/ kgoil).

Peroxide value in control was increased from the first month to month 5 then it was decreased in month 6. TBA was increased during cold storage. These results were in agreement with Tall (1995). Peroxide begins to disintegrate, leading to production of aldehydes, cetones and TBA (Moieni et al., 2009). But in coated samples due to the

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properties of edible films increase of TBA and peroxide is prevented (Amberdekar, 2007). TBA is a widely used indicator for the assessment of lipid oxidation (Nishimoto et al, 1985). It causes changes in sensory properties (Coles et al, 2003). Lower TBA in coated samples is due to decrease in oxidation.

Coated samples have more brilliance and soft texture than control, our results are similar to Hegenbart (2006) Rokwer (2006), Bigelow (2007) and Trout (2009) findings. Lateral Chain of alginate react with molecules of and forms a sticky layer on the product .In addition alpha lactalbomin and beta lacto glubilin in whey protein form large particles and finally increase viscosity of coated samples (Marsh and Bugusu, 2007).

Sodium alginate prevents myofibril denaturation and rancidity of frozen fish. Separation of calcium from phosphate due to sodium alginate films, controls flavor of frozen fish. Diacetyll and lactose in whey protein make good taste in products (Hiroshi and Yukinori 2001; Crapo et al, 1999).

Coated samples had more brilliance color than control, our results are according to Ben (1999) and Shah (1999). Color transparency in treatments and control is due to hydrophilic molecules in sodium alginate structure and shiny gel formation on fish surface. Sodium alginate keeps food color and freshness leading to increase food shelf life. Color of freeze coated samples remained constant (Rockwer et al, 2006).

There was significant difference in odor factor between coated samples and control. Our results are similar to Silva (1993). Progress of oxidation and presence of compounds such as TBRS in fish meat causes some changes in its sensory specifications such as smell (Aubourg et al, 1995; Shah, 1999).

According to the results of the experiments, a meaningful difference in the index of overall acceptable among the covered samples compared with the control sample were observed. No statistically significant differences were observed in the results of chemical experiments of the covered samples and presence of the meaningful difference at the results of the chemical experiments of the control sample. 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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