Study of different concentration of sodium alginate as a coating film on the shelf- life of frozen dressed kilka (Clupeonella cultriventiris)

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Abstract: The aim of the present study was to investigate the impact of sodium alginate coating on quality and shelf life of frozen dressed kilka fish. Sodium alginate edible coating was prepared in four different concentrations, including 0.25, 0.75, 1.25, 1.75 %. Then dressed kilka were coated for 1h, packed in polyethylene dishes with cellophane blanket and stored at -18 °C. Percentage of moisture and protein, Peroxide value and total microbial count were performed within 0, 1, 2, 3 and 4 months (sample size: 120 packs of 250 g). SPSS Statistical Software, One Way ANOVA test, Turkey test with the 5% significant level (significance level of 5%) have been used as for data analysis. Significant reduction was found in moisture value with increase of Sodiume Alginate concentration during the study ($P<0.05$). There were no significant differences in protein content ($P>0.05$). Results showed that there was significant difference between peroxide and total microbial count samples ($P<0.05$). Use of sodium alginate due to decrease peroxide production and slowing microbial growth speed, can increase shelf life of kilka in storage of freezing up to 4 months.
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Keywords: Kilka, edible coatings, sodium alginate, shelf life

1. Introduction

Fish meat is an important source of protein supply and other nutrients which developing countries have heavily relied on its export (Mahboob *et al*., 2008). Kilka (*Clupeonella cultriventiris*) is one of the economically valuable species of Clupeidae living in Caspian Sea that has naturally a significant status among other foodstuffs for vitamins, essential oils and various types of minerals. Evidently, less than 10% of the fish catchment is directly use by human being and the remaining section has been powdered for consumption(Moradi, 2001).

Freezing is a common preservation method to control or decrease biochemical alterations that occur during meat storage. It, however, doesn't completely inhibit chemical reactions, that lead to quality deterioration of fish. Preservatives such as phosphates are often applied to improve of shelf life of seafood's products. There are numerous studies that focused on natural ingredients to enhance shelf life and avoid of synthetic preservative application (Williams *et al*., 1978). An edible film is generally defined as a thin layer that formed as a coating between food materials. This film can be applied by immersion, spraying and or panning (Chapman *et al*., 1997; Regalado *et al*., 2006).

Edible films and coatings, as well as biodegradable materials offer alternative packaging systems, which may replace some synthetic packaging materials or reduce their application by partially replacement (Regalado *et al*., 2006).

Previous studies show that coatings have some advantages on edible supplies such as reduction of oil based polymeric films, decrease of solid materials and their consumption value, harmlessness for the consumer and selective permeability (Kester & Fennema, 1986; Draget *et al*., 1998; Crapo *et al*., 1999; Cutter, 2006).

Edible films and coatings are intended to maintain the quality and shelf life of food products by controlling the transfer of moisture, oxygen, carbon dioxide, lipids, aromas, flavors, and food additives (Sothornvit & Krotcha, 2005). Polysaccarides and proteins could be used to coat of fish fillets and suppress of quality changes during frozen storage (Sathivel, 2005).

Alginate is a hydrophilic colloidal carbohydrate extracted from various species of brown seaweeds (*Phaeophyceae*). It is molecularly a member of unbranched binary compolymers, residues of D-β-mannuronic acid and L-α-guluronic acid, they are widely varying composition and sequential structure (Draget et al., 1998; King, 1983). Ability to react with polyvalent metal kations to produce strong gels is the most useful and unique property of alginates (Grant *et al*., 1973). this gel is used for in food processing industry to produce restructured foods such as meat products, onion rings, pimento olive fillings, crabsticks, and cocktail berries(Moe et al., 1995), and in the biotechnology industry for producing beads for immobilization of cells or

enzymes (Rhim, 2004; Pavlath *et al*., 1999; Williams *et al*., 1978).

According to basic nutrition alginate films and nutrition coatings researches (Maftoonazad *et al*., 2008), Alginate nutrition films have anti –microbus factors that could enhance shelf-life quality of sliced peach and melon (Maftoonazad *et al*., 2008; Pearson, 1997). Besides, alginate and gelan based coatings is to improve barrier, texture and edible properties of fresh-cut papaya (Tapia *et al*., 2008). Kilka is abundantly available and caught in Caspian Sea. However, there are few efforts on their preservation using edible coating. Accordingly, the present research has been assigned to investigate the effect of sodium alginate coating on shelf life of kilka. Besides, antimicrobial and chemical properties of coated kilka have been considered during frozen storage.

2. Materials and Methods

This study was conducted in the National Research Fish Processing Center, Anzali, Guilan province, Iran. Sodium alginate (Product No: 650.0489) have been diluted to different concentrations of 0.25%, 0.75%, 1.25%, 1.75% (**w**/w) according to Maftoonazad method (Maftoonazad *et al*., 2008). Sodium alginate powder was broken up to 50, 150, 250, and 350 g and diluted in separate tanks containing 20 L tab water.

35 kg fresh kilka were provided from quay of Anzali and then carefully gutted and dressed by hand. The fish were divided to 5 separate treatments including treatment 1 (control, uncoated fish), treatment 2 (coated with 0.25 % sodium alginate), treatment 3 (coated with 0.75 % sodium alginate), treatment 4 (coated with 1.25 % sodium alginate), treatment 5 (coated with 1.75 % sodium alginate). The treated kilka were immersed in nominative concentration of sodium alginate solution for 1h in 3.5 °C and kept in baskets for 1 min to dissenting surplus of solution from fish surfaces. These samples were then packed in polyethylene dishes with cellophane blanket and frozen at -18˚C for 4 month. The control group of fish were just packed in similar polyethylene dishes and stored in accord with treated fish.

Sampling was carried out in 4 phases, numbered as 1 to 4. Phase 1 performed one month after storage and remaining phases were done each one month after each other (2, 3 and 4 month). Moisture were measured by (A.O.A.C, 2000) standard method, protein by method of digestion, titration and distillation by Kajedal (Pearson, 1997), amount of peroxide and total microbial count were determined for each sampling. Lee method was used to determine amount of peroxide (Pearson, 1997) according to the following formula:

Peroxide value (meq/1000g) = (A-B) ×M×1000/W

Where: A = Titration value of sample

B = Titration value of blank (ml of thiosuphate)

M = Molarity of sodium thiosuphate

W = Weight of sample (g)

Total microbial count has also done according to standard ISIRI no. 2394-1 (ISIRI no 2394-1., 2000).

Data were statistically analyzed using one–way ANOVA and the mean values were compared using Tukeys' HSD. A p–value less than 0.05 were considered as significant. All statistical analyses have been done using SPSS software 16.0.

3. Results

Moisture

Study of treatment effect showed significant difference among average moisture of different treatments of 0, 1, 2, 3, and 4 months ($p<0/05$). Study of time effect showed significant difference among different times 1, 2, 3, 4 and 5 treatments ($p<0.05$). Both treatment and time averages have been compared in table 1and showed in figure 1.

Table 1: one way variance analysis results turkey test of treatment and time effects moisture of data

Treatment					Time in
5	4	3	2	1	(Month)
74/00±0/14^{b,A}	74/15±0/03^{ab,A}	74/26±0/05^{a,A}	74/30±0/07^{a,A}	74/11±0/09^{ab,A}	0
73/70±0/04^{c,B}	73/78±0/09^{bc,B}	74/10±0/06^{a,A}	74/17±0/06^{a,A}	73/90±0/08^{b,A}	1
73/24±0/04^{c,C}	73/47±0/02^{b,C}	73/87±0/06^{a,B}	73/96±0/09^{a,B}	73/56±0/09^{b,B}	2
72/85±0/05^{b,D}	72/86±0/08^{b,D}	73/54±0/06^{a,C}	73/65±0/05^{a,C}	73/00±0/08^{b,C}	3
72/50±0/06^{c,E}	72/57±0/02^{bc,E}	73/20±0/07^{a,D}	73/39±0/01^{a,D}	72/75±0/17^{b,C}	4

(a-c)) are used to compare treatments average that have significant difference with different letters, ($p<0.05$)

(A-E) are used to compare times average that have significant difference with different letters, ($p<0.05$).

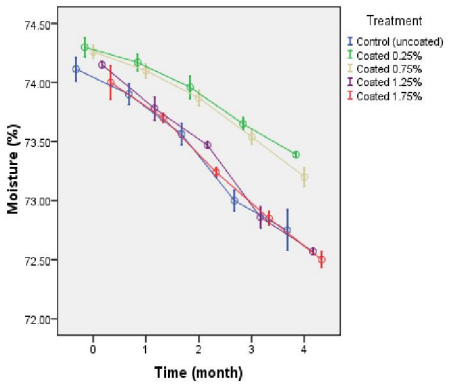

Figure 1: effect of time and treatment on moisture data of uncoated and coated samples with sodium alginate

Protein

There were no significant differences of protein between different treatments (p>0/05). Also study of time effect showed no significant difference among different times (p>0.05). Both treatment and time averages have been compared in table 2 and showed in figure 2.

Table 2: one way variance analysis results turkey test of treatment and time effects protein of data

Treatment					Time in
5	4	3	2	1	(Month)
17/68 ± 0/02	17/70 ± 0/05	17/68 ± 0/07	17/62 ± 0/02	17/65 ± 0/04	0
17/67 ± 0/08	17/63 ± 0/11	17/65 ± 0/05	17/60 ± 0/11	17/58 ± 0/07	1
17/68 ± 0/10	17/65 ± 0/05	17/60 ± 0/00	17/57 ± 0/03	17/53 ± 0/05	2
17/64 ± 0/05	17/61 ± 0/07	17/57 ± 0/06	17/59 ± 0/07	17/55 ± 0/08	3
17/60 ± 0/05	17/61 ± 0/05	17/55 ± 0/10	17/57 ± 0/08	17/50 ± 0/04	4

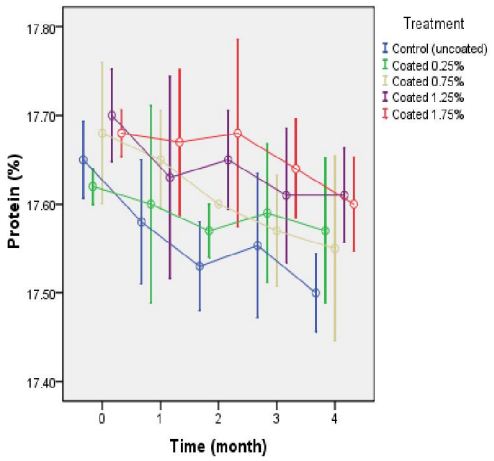

Figure 2: effect of treatment and time on protein data of uncoated and coated samples with sodium alginate

Peroxide

Study of treatment effect showed significant difference among average peroxide of different treatments of 1, 2, 3, and 4 months (p<0/05). Study of time effect showed significant difference among different times 1, 2, 3, 4 and 5 treatments (p<0.05). Both treatment and time averages have been compared in table 3 and showed in figure 3.

Total microbial count

There were significant differences of total microbial counts between treatments during 4 months (p<0/05). Study of time effect showed significant difference among different times of 1, 2 and 3 treatments (p<0.05). Both treatment and time averages have been compared in table 4 and showed in figure 4.

Table 3: one way variance analysis results tukey test of treatment and time effects peroxide of data (meq/kg)

Treatment					Time in
5	4	3	2	1	(Month)
$0/82 \pm 0/04^{C}$	$0/91 \pm 0/01^{E}$	$0/85 \pm 0/06^{E}$	$0/90 \pm 0/05^{E}$	$0/90 \pm 0/02^{D}$	0
$0/95 \pm 0/05^{b,C}$	$1/07 \pm 0/07^{ab,D}$	$0/98 \pm 0/02^{b,D}$	$1/13 \pm 0/03^{a,D}$	$1/00 \pm 0/05^{b,D}$	1
$1/35 \pm 0/07^{b,B}$	$1/37 \pm 0/03^{b,C}$	$1/52 \pm 0/05^{a,C}$	$1/41 \pm 0/03^{ab,C}$	$1/45 \pm 0/06^{ab,C}$	2
$1/57 \pm 0/08^{c,A}$	$1/69 \pm 0/03^{c,B}$	$2/08 \pm 0/08^{ab,B}$	$2/13 \pm 0/01^{a,B}$	$2/00 \pm 0/05^{b,B}$	3
$1/66 \pm 0/05^{d,A}$	$2/04 \pm 0/06^{c,A}$	$2/45 \pm 0/00^{ab,A}$	$2/36 \pm 0/11^{b,A}$	$2/63 \pm 0/06^{a,A}$	4

(a-d)) are used to compare treatments average that have significant difference with different letters, (p<0.05)

(A-E) are used to compare times average that have significant difference with different letters, (p<0.05).

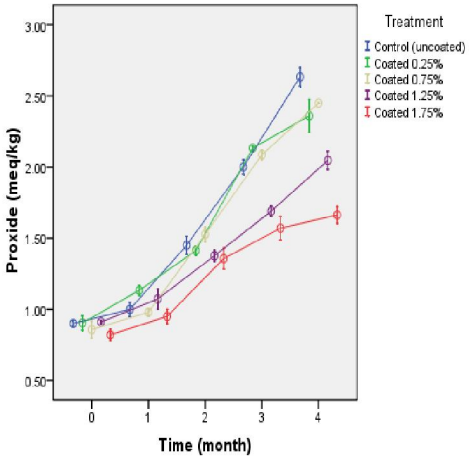

Figure 3: effect of treatment and time on peroxide data of uncoated and coated samples with sodium alginate

Table 4: one way variance analysis results tukey test of treatment and time effects Total microbial count of data (log CFU/g)

Treatment					Time in
5	4	3	2	1	(Month)
2/56 ± 0/72	2/61 ± 0/65	$2/63 \pm 0/68^{B}$	$2/56 \pm 0/72^{B}$	$2/31 \pm 1/19^{B}$	0
2/64 ± 0/67	2/66 ± 0/65	$2/97 \pm 0/15^{AB}$	$2/67 \pm 0/66^{B}$	$2/46 \pm 1/04^{B}$	1
2/98 ± 0/13	2/75 ± 0/66	$2/64 \pm 0/73^{B}$	$2/94 \pm 0/31^{B}$	$2/87 \pm 0/37^{AB}$	2
2/81 ± 0/71	2/91 ± 0/55	$3/46 \pm 0/40^{AB}$	$4/01 \pm 0/38^{AB}$	$3/80 \pm 0/56^{AB}$	3
$3/08 \pm 0/28^{b}$	$3/11 \pm 0/28^{b}$	$4/07 \pm 0/07^{ab,A}$	$4/89 \pm 0/71^{a,A}$	$5/08 \pm 0/68^{a,A}$	4

a-b) are used to compare treatments average that have significant difference with different letters, (p<0.05)

A-B) are used to compare times average that have significant difference with different letters, (p<0.05)

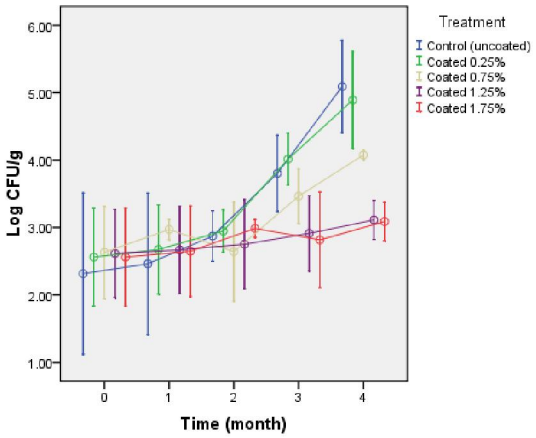

Figure 4: effect of treatment and time on Total microbial count data of uncoated and coated samples with sodium alginate

Discussion

We also significant differences in moisture content in all treatments at months 0, 1, 2, 3 and 4 of storage time, as moisture content was reduced significantly. The studies have demonstrated that linked coatings and alginate films with $CaCl_2$ cations increase water resistance qualities, but applied alginate coatings increase steam permeability and reduce the moisture consequently, ((Rhim et al., 2006; Rhim, 2004). Increased resistance of coated samples rather than uncoated samples Its also found that the content of sodium alginate in coating has a significant effect on moisture content, as treatments No. 2 and 3 (with 0.25 and 0.75 % sodium alginate) had higher moisture content with comparison of control samples, while treatments No. 4 and 5 (with 1.25 and 1.75 % sodium alginate) had lower higher moisture content with comparison of control samples.

There is due to increased lipid to improve water resistance of alginate, and glean coatings (Rojas-Grau et al., 2007). Tapia et al. (2008) observed that gelan coatings resist better against steam than that of alginate.

They proposed that applied higher density rates of alginate (2%) than gelan (0.5%) may be the cause of water resistant reduction of against coated kulak when the moisture is reduced. According to the studies of Maftoonazad et al. (2008) on peach, shelf-life increased with applying the sodium alginate and methyl cellulose. They observed that moisture reduction of the coated fruits with sodium alginate has been more than that of coated fruits with methyl cellulose. Lazarus (1976) studied coating effectiveness of alginate calcium and protective plastic cover to control reduced body weight of sheep. He observed that nutritional film of calcium alginate increased weight of the hot body up to 270g. In fact it is a moisture extractor rather than moisture barrier.

According to the obtained results, protein content of different treatments at months 0, 1, 2, 3 and 4, did not remarkably changed in 0 to 4 months, but coated samples have generally had more proteins than that of testifiers. As alginate is a poly anion and easily reacts to cations and poly cations produce a structure named Egg box, when a network of resembling cavities is formed, the network prohibits heavier proteins than 100KD to pass (Mortazavi, 2006). Thus, sodium alginate coating prevents water contained proteins to erupt.

Preservers can prevent growth of ice crystals and migration of water molecules of protein, thus, they preserve natural form of protein during freeze process (Yoon & Lee, 1990).

Obtained results demonstrated that peroxide average of samples has had significant increase during 4 months and maximized in treatment 1 (uncoated kilka). Generally, performed processing of lipid oxidation and peroxide increased during storage. The coated samples reduced their peroxide by increase of sodium alginate concentration. It is, therefore, concluded that sodium alginate solution effectively prevents peroxide increase. When hydro peroxide content of fish muscles is reduced the compositions compound faster than break up, then peroxide content of fish muscles is increased based on one molecular mechanism (Vidya Sager Peddy & Sriker, 1996). The previous studies showed that peroxide content of Sardine at -18°C increased in 6 month fridge but it was decreased after 6 month due to decomposition of oxidation product (Pacheco-Aguilar *et al*., 2000).

The present study demonstrated significant differences of total microbial count between treatments throughout 4 month. Treatment 1 (uncoated kilka) contained the most bacteria count, while the coated exhibited bacterial count reduction by increasing of sodium alginate concentration, so that the treatment 5 had the least bacteria. It is could be related to anti microbial property of sodium alginate. The recommended shelf-life of frozen stored fish in the present study was 4 months, if the acceptability limit of 107 CFU/g is applied (Duan et al., 2010).

Cutter and Siragusa (1996) observed a lack of effectiveness to control the growth of *Brochothrix thermosphacta* when nisin was applied on beef carcass surface for a long period of storage. In order to improve the efficiency of this bacteriocin, these authors immobilized the antimicrobial peptide in an alginate gel. This gel allowed a controlled release of nisin on meat, which resulted in a 3 log CFU/cm^2 reduction of *B. thermosphacta* on meat carcass

surfaces as compared with beef covered whit nisin solution. Apparently, bactericidal activity of nisin was able to control the growth of *B. thermosphacta* in ground beef when entrapped in alginate gel (Moe *et al*., 1995). The efficiency of numerous films formulation based on calcium- alginate or agar containing nisin to inhibit the antibiotic resistant *Salmonella typhimurium* on poultry drumstick skin was demonstrated by Natrajan and Sheldon (Natrajan & Sheldon, 2000).

Alginate has gel properties which its gel porosity is affected by rate of gluronic acid and mannuronic acid of alginate molecule. Both of the properties produce a semi permeable layer to effectively trap microorganisms (Mir Nezami Ziabari, 2002).

Oms-Oliu et al (Oms-Oliu *et al*., 2008) investigated the polysaccharide-based edible coating of fresh-cut melon and found out the highest growth rate of psychrophilic aerobic microorganisms during 15 days at 4°C on gellan-coated, reaching counts of 8 log CFU/g at the end of storage. On the contrary, its counts in both uncoated and alginate- or pectin-coated samples exceeded 7 log CFU/g after the period of experiment.

It is concluded from the present research that application of sodium alginate due to slowing effect of microbial growth and decrease of peroxide production can increase shelf life of kilka. Finally, 1.25% concentration of sodium alginate has been chosen as the most effective one and it is recommended that lipid based materials must be used in the compound of sodium alginate nutritional film to prevent moisture reduction of kilka when it is preserved in freezer.

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