Full Length Research Paper

Evaluation of fat quality in packaged common kilka fish soaked in whey protein compared with sodium alginate

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Whey protein and sodium alginate (12% and 0.5% concentrations, respectively) were used for fish packaging at time 0. The covered samples were kept at -18 °C for a period of six months. Humidity, peroxide value, free fatty acids, thiobarbitoric acid, TVN and pH were higher in the samples covered with whey protein compared with other samples. But, fat content was lower in these samples. No statistically significant decreases were observed in chemical factors in the covered samples compared with the control samples (p<0.05). The control samples lost their quality during the storage period. Fish soaked with sodium alginate had better quality compared with the other samples.

Key words: Chemical factors. Whey protein, Sodium alginate, Fat quality, Kilka fish.

INTRODUCTION

Kilka fish belong to the genus Clupeonella, the order Clupeiformes, the phylum cleopiform, and the family Clupeidae. These fish are composed of three species consisting of Clupeonella delicatula, Clupeonalla 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.

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 the Guilan province. Around 9– 13% 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 the Mazandaran Province, of which 5–12 % is used for human consumption and the remaining 88–95% for animal feeds. Consumption of fresh Kilka fish dropped during the years 2004-2009 (from 6% to around 2.2%). Consumption of canned Kilka fish also dropped for 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 (fisheries studies and statistical group, 2007). The frozen fish packs, due to their longer storage time as well as wider countrywide distribution, had much higher sales rate 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. 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 (Moeini, 2009). 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 (Ahvenian,2003). Use of edible films for packaging of kilka fish seems to be an ideal method for proper long storage fish preservation. 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 (Seifzadeh, 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 coating are invisible to the naked eye (Dies, 2006). Sodium alginate are derived from brown seaweeds. These films are transparent. 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 (Martin,1994).

Edible film whey protein has been used for packaging of Salmon, Hot-dog, sausage, cracker and frozen fish-fillet (Coles, 2003). But no research has not been carried out using whey protein and sodium alginate films for packaging of fish in Iran. In other countries, films consisting of whey proteins have been used for food packaging by Crapo (1999), Krockta (1996), Shah (1999), Stuchell (1995), Morrissey (2009) and Piyachonkw (1995). These results indicate sensory quality deterioration prevention, lipid oxidation decrease and shelf life extension in cold storage and films consisting of sodium alginate have been used for food packaging by Zeng(1997), Rokwer (2006), Biglelow (2009), Trout (2009) and Manish(2004). 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.

MATERIAL 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 national standards of Iran were recorded. The fish were chilled down to 0 °C under a cover of ice at a fish: ice ratio of 2:1 in insulated tubs and then 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

Before processing fish were washed with chlorinated water. Then the heads were 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 and 0.5% concentration of sodium alginate with at time=0, separately. Following this, the fish were packaged in disposable dishes and covered with cellophane (500 gr). These samples were kept of -18 °C for six months. Three replicates were used in this study. Chemical tests were carried out to test the quality of samples after storage. These samples were treated in three replicates.

CHEMICAL ANALYSIS

The chemical tests for the packaged samples with edible film and control (35 packages) included measurement of humidity and TVN (INS, No.5625, 2002), lipid (INS, No. 742, 2002), peroxide value and FFA (INS, No. 493, 2003), TBA (INS, No. 10494, 2006) and pH (INS, No. 10282007). 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 month, at specific times. Each step of the tests was repeated three times.

STATISTICAL ANALYSIS

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

RESULTS

The mean peroxide value, TVN, pH, free fatty acids and thiobarbitouric acid in the control samples were 3.75 meq/kg, 16.22 mg/100g, 6.71, 9.21 gr/100 and 0/15 mg/kg, respectively. The mean of moisture, peroxide value, TVN, pH, free fatty acids, thiobarbitouric acid and fat in the fish samples covered with whey protein were 73.91%, 0/13 meq/kg, 9.84 mg/100g, 6.31, 1.15 gr/100, 0/006 mg/kg and 4.25%, respectively.

The mean moisture, peroxide value, TVN, pH, free fatty acids, thiobarbitouric acid and fat in the fish samples covered with sodium alginate were 73.34%, 0.06 meq/kg, 9.88 mg/100g, 6.25, 0.77 gr/100, 0/001 mg/kg and 4.62%, respectively.

The mean moisture, peroxide value, TVN, pH, free fatty acids, thiobarbitouric acid and fat in the control samples were 59.43%, 3.75 meq/kg, 16.22 mg/100g, 6.71, 9.21 gr/100, 0/15 mg/kg and 3.99%, respectively.

According to the test results and statistical analysis, better general qualities were observed in the covered samples compared with the control ones. Variations of free fatty acids were meaningful from one day after storage up to five months (p<0.05) and at the sixth months. It was not meaningful in the control samples.

Variations of pH and TBA showed significant differences between the first day with the sixth month after processing in the control samples (p<0.05).

According to the test results and statistical analysis, better general qualities were observed in the covered samples compared with the control ones. Based on the statistical test, the covered samples had preserved their quality up to the end of storage period whereas the control samples had lost their quality after
Table 1: Chemical factor results in covered samples by whey protein during storage period

<table>
<thead>
<tr>
<th>Experiments Time</th>
<th>Moisture%</th>
<th>PV value meq/kg oil</th>
<th>FFA gr/100</th>
<th>TBA mg/kg</th>
<th>pH</th>
<th>TVN mg/100gr</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day</td>
<td>73.93±0.04</td>
<td>0.08±0.10</td>
<td>1.1±0.15</td>
<td>0.006±0.001</td>
<td>6.2±0.20</td>
<td>9.8±0.35</td>
</tr>
<tr>
<td>First month</td>
<td>73.93±0.14</td>
<td>0.08±0.20</td>
<td>1.1±0.25</td>
<td>0.006±0.002</td>
<td>6.2±0.10</td>
<td>9.8±0.20</td>
</tr>
<tr>
<td>Second month</td>
<td>73.93±0.05</td>
<td>0.08±0.20</td>
<td>1.1±0.35</td>
<td>0.006±0.002</td>
<td>6.2±0.20</td>
<td>9.8±0.25</td>
</tr>
<tr>
<td>Third month</td>
<td>73.91±0.04</td>
<td>0.08±0.15</td>
<td>1.1±0.02</td>
<td>0.007±0.02</td>
<td>6.3±0.20</td>
<td>9.8±0.55</td>
</tr>
<tr>
<td>Forth month</td>
<td>73.91±0.04</td>
<td>0.09±0.26</td>
<td>1.2±0.05</td>
<td>0.007±0.001</td>
<td>6.3±0.20</td>
<td>9.9±0.41</td>
</tr>
<tr>
<td>Fifth month</td>
<td>73.90±0.04</td>
<td>0.09±0.15</td>
<td>1.2±0.15</td>
<td>0.007±0.11</td>
<td>6.4±0.10</td>
<td>9.9±0.30</td>
</tr>
<tr>
<td>Sixth month</td>
<td>73.90±0.06</td>
<td>0.1±0.25</td>
<td>1.3±0.35</td>
<td>0.008±0.025</td>
<td>6.6±0.15</td>
<td>9.9±0.30</td>
</tr>
</tbody>
</table>

Fat content: 4.25±0.03%

Table 2: Chemical factor results in covered samples by sodium alginate during storage period

<table>
<thead>
<tr>
<th>Experiments Time</th>
<th>Moisture%</th>
<th>PV value meq/kg oil</th>
<th>FFA gr/100</th>
<th>TBA mg/kg</th>
<th>pH</th>
<th>TVN mg/100gr</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day</td>
<td>73.63±0.30</td>
<td>0.05±0.15</td>
<td>1±0.20</td>
<td>0.004±0.001</td>
<td>6.2±0.15</td>
<td>9.8±0.20</td>
</tr>
<tr>
<td>First month</td>
<td>73.63±0.10</td>
<td>0.05±0.15</td>
<td>1±0.17</td>
<td>0.004±0.002</td>
<td>6.2±0.15</td>
<td>9.8±0.30</td>
</tr>
<tr>
<td>Second month</td>
<td>73.63±0.06</td>
<td>0.05±0.25</td>
<td>1.1±0.37</td>
<td>0.004±0.03</td>
<td>6.2±0.20</td>
<td>9.8±0.40</td>
</tr>
<tr>
<td>Third month</td>
<td>73.62±0.07</td>
<td>0.06±0.10</td>
<td>1.1±0.30</td>
<td>0.005±0.02</td>
<td>6.3±0.10</td>
<td>9.8±0.30</td>
</tr>
<tr>
<td>Forth month</td>
<td>73.62±0.04</td>
<td>0.06±0.30</td>
<td>1.1±0.26</td>
<td>0.005±0.002</td>
<td>6.3±0.15</td>
<td>10±0.30</td>
</tr>
<tr>
<td>Fifth month</td>
<td>73.61±0.05</td>
<td>0.06±0.40</td>
<td>1.1±0.25</td>
<td>0.006±0.04</td>
<td>6.3±0.15</td>
<td>10±0.30</td>
</tr>
<tr>
<td>Sixth month</td>
<td>73.61±0.09</td>
<td>0.7±0.30</td>
<td>1.2±0.20</td>
<td>0.006±0.47</td>
<td>6.3±0.15</td>
<td>10±0.23</td>
</tr>
</tbody>
</table>

Fat content: 4.62±0.25%

three months.

The fish samples soaked with sodium alginate had better quality compared with the ones covered with whey protein.

DISCUSSION

Humidity was higher in fish samples soaked with whey protein and sodium alginate solution (73.91%) and sodium alginate (73.34%) compared with the control samples (59.43%). Similar results were obtained by Rokwer, Stuchell, Kochakian and Moeini.

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 (Anker, 2010). Absorption of water by protein and adhesion and linking of protein chains to each other increases the size of protein that may cause to increase the viscosity, humidity and texture of the covered samples by this film, compared with the control sample (Stuchell, 1995).

In samples covered by sodium alginate, formation of gel by sodium alginate helped to prevent of decrease in moisture level. Sodium alginate is calcium ions chelating agent. It formation a calcium ion bridge. Therefore, Na-Alg had the effects of increasing the monolayer and multilayer water around myofibril (Mf), decreasing water activity, and also had inhibitory effects on Mf dehydration. The inhibitory effects were markedly correlated with the state of the water. These results suggested that Na-Alg inhibits dehydration-induced denaturizing of myofibril by stabilizing water around Mf. Mf has an inhibitory effect of dehydration degeneration, and the state of the water suppression was found between having a relationship. Therefore humidity and tissue quality kept at the covered samples by this film, compared with the control sample (Hiroshi and Yukinori, 2001).

In the control samples, because of presence of 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 decrease in weight of about 3.5% after three months. This condition may also appear due to the production of ice crystals in the product (Auburg, 1995). 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 causes to decrease the quality of taste and color change in Kilka without cover (Safari, 1990).

TVN content in the Kilka covered by sodium alginate (9.88 mg/100 g) and one samples by whey protein (9.84 mg/100g) compared with the control samples (16.22 mg/100g) during the cold storage period showed a
increase. This factor in whey protein covered Kilka (9.84 mg/100g) compared with covered samples by sodium alginate (9.88 mg/100g) during the cold storage period showed decrease, too. Similar results were obtained by Manish and Morrissey. This may be due to the effect of decrease in humidity and production of free fatty acids on the denaturizing 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 denaturizing of protein and naturally increases TVN (Piyachomkwan, 1995).

The lipid content showed an increase in the whey protein covered samples (4.25%) compared with the control sample (3.99%). Similar results were obtained by Krockta. That is because of the presence of glysirids of milk lipid, phospho-lipids and lipo-proteins in whey protein (Shah, 1999).

The lipid content showed an increase in the sodium alginate covered samples (4.52%) compared with the control sample (3.99%). Similar results were obtained by Manish. This may be retarded to the presence of free fatty acids consisting of arashidonic acid, eicozapentanoic acid and docozahexanoic acid in sodium alginate (Safari, 1990).

Fat variations were variable in the control samples compared with the samples covered with whey protein and sodium alginate. This can be due to a decrease in moisture, freezing effect during cold storage, penetration of oxygen and fat oxidation (Gigirey, 1999).

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 (Hegenbart, 2006). Releasing of 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 (Zeng, 1997). 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 decrease of raw materials and increase of oxidation in free fatty acids (Rezaei, 2003).

Based on the conducted experiments, the amount of the free fatty acids measured in the samples covered by whey protein (1.15 g/100) and sodium alginate (0.77 g/100) was lower than the control sample (9.21 g/100). 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 (Safari, 1990).

A suitable index for determination of progress in fat oxidation and producing 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 (Rezaei, 2003).

Peroxide value and TBA contents were lower in the whey protein covered samples (0.13 meq/kg/100g and 0.006 mg/kg) and sodium alginate in covering (0.06 meq/kg/100g and 0.001 mg/kg) compared with the control sample (3.75 meq/kg/100g and 0.15 mg/kg). Similar results were obtained by Shah. We can say that it is because of prevention of decrease of water in fish tissue while freezing, decrease of water activity and free radicals production, covering specifications of edible films, prevention of oxygen absorption, prevention of humidity decrease, decrease of oxidation and production of secondary products of oxidation such as aldehydes and

<table>
<thead>
<tr>
<th>Experiments Time</th>
<th>Moisture%</th>
<th>PV value meq/kgoil</th>
<th>FFA Gr/100</th>
<th>TBA mg/kg</th>
<th>pH</th>
<th>TVN mg/100gr</th>
<th>Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day</td>
<td>72.2±0.35a</td>
<td>0.20±0.01a</td>
<td>4.10±0.25a</td>
<td>0.03±0.024a</td>
<td>6.2±0.10a</td>
<td>9.8±0.36a</td>
<td>4.54±0.36a</td>
</tr>
<tr>
<td>First month</td>
<td>67.35±0.25b</td>
<td>1.70±0.10b</td>
<td>6.83±0.32b</td>
<td>0.07±0.1a</td>
<td>6.2±0.20a</td>
<td>12±0.30a</td>
<td>4.45±0.28a</td>
</tr>
<tr>
<td>Second month</td>
<td>63.20±0.10c</td>
<td>3.20±0.10c</td>
<td>8.34±0.25c</td>
<td>0.10±0.03ab</td>
<td>6.4±0.10a</td>
<td>14.6±0.30c</td>
<td>4.26±0.43c</td>
</tr>
<tr>
<td>Third month</td>
<td>58.90±1.61d</td>
<td>4.50±0.10d</td>
<td>9.52±0.28d</td>
<td>0.14±0.01ab</td>
<td>6.7±0.15b</td>
<td>16.2±0.30d</td>
<td>3.98±0.14d</td>
</tr>
<tr>
<td>Forth month</td>
<td>54.15±0.16e</td>
<td>6.00±0.25e</td>
<td>10.96±0.46e</td>
<td>0.17±0.01ab</td>
<td>7±0.15bc</td>
<td>18.5±0.30e</td>
<td>3.74±0.29e</td>
</tr>
<tr>
<td>Fifth month</td>
<td>50.43±0.14f</td>
<td>5.60±0.6f</td>
<td>12.37±1.31f</td>
<td>0.25±0.03abc</td>
<td>7.2±0.26bc</td>
<td>20.8±0.50f</td>
<td>3.58±0.18f</td>
</tr>
<tr>
<td>Sixth month</td>
<td>46.16±0.17g</td>
<td>5.10±0.30g</td>
<td>12.38±0.10g</td>
<td>0.32±0.02bc</td>
<td>7.3±0.15c</td>
<td>21.7±0.25g</td>
<td>3.39±0.39g</td>
</tr>
</tbody>
</table>
specifications of these compounds (Marsh, 2007; Sanker, 1995).

In the present research, amounts of measured TBA showed an increasing trend up to the end of cold-storage in the control Kilka. This is 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 (Shahidi, 1994). Peroxide will start to induce decomposition, leading to the aldehydes, ketone and seton production. Subsequently, the peroxide value was reduced to over time (Silva, 1993).

The pH rate was measured lower in the samples covered with whey protein (6.31) and sodium alginate (6.25) compared with the control sample (6.71). Similar results were obtained by Piyachomkwan and Trout. Through passing of time, lipid oxidation products such as hydro-peroxides have been analyzed and some compounds such as aldehyds 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 covered samples, changes of pH during the time storage were not significant (Crapo, 1999). The amounts of chemical factors consisting of peroxide value, free fatty acids, thiobarbituric acid and pH in the samples covered with whey protein were higher compared with the samples covered with sodium alginate. This may be due to the production of free fatty acids by lactic acid bacteria in these samples and their oxidation (Trout, 2009).

The covered samples by sodium alginate had better quality compared with the covered samples by whey protein. This can be due to the lower rates of chemical factors in these samples. No statistically significant differences were observed in the results of chemical experiments of the covered samples. But, meaningful difference was noted in the results of the chemical experiments in the control sample. Therefore, 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 after three months.

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