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Effects of whey protein edible coating on bacterial, chemical and sensory characteristics of frozen common Kilka

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Abstract

The objective of current study was to investigate the effects of whey protein coating on the quality of common kilka during frozen storage period. For this study, common kilka was coated with 12% whey protein concentration. Also, non- coated kilka was used as a control sample. Coated and non-coated samples were then stored at -18 ^{o}C for six months. Bacterial, chemical and sensory properties of the samples were determined in each month. Results showed that total bacterial counts and S*taphylococcus* bacteria count were lower in the test samples compared to the control samples. *Coliform, Escherichia coli* and *Pseudomonas* bacterial contamination were negative till the end of the storage period in the covered samples. Chemical factors consisting of humidity, protein, lipid, ash and calorie were higher in the test samples compared to the control samples. Peroxide value, free fatty acids, thiobarbitoric acid, TVB-N and pH were lower in the test samples. Sensory tests had a better quality in the test samples compared to the control samples. No statistically significant decreases were observed in chemical factors in the covered samples compared to the control samples ($p<.05$). In sensory specifications, there was a significant difference between the covered samples and the control samples ($p<.05$). According to the results of experiments and statistical analysis, the covered samples had a favorable quality till the end of the storage period but the control samples had lost their quality.

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

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Introduction

Kilka fish belong to the genus *Clupeonella*, family Clupeidae. These fish are composed of three species consisting of *Clupeonalla delicatula* ، *C. engrauliformis* and *C. grimmi* (Naderi and Abdoli, 2004). They can be processed into salted , smoked, pickled conserved, dried and frozen fish. But in Iran, kilka products in the market are canned, packagedin frozen shape and/or fresh. The annual catch has increased from 19610 tons in 2004) to over 25483 tons in 2009. Around 10–12% 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 (Iranian fisheries, 2008). Consumption of fresh kilka fish dropped during the years 2004-2009 (from 6.00 to 2.20%). Consumption of canned kilka also dropped for 5.2% to about 0.8% during the same period, whereas consumption of the frozen fish rose from 1.00% to 6.20% during these years (Seifzadeh, 2010). 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(Moeini, 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).

Kilkahaving a valuable protein and digestible fats, rich vitamins and minerals has attained an important position among animal products. In an overall view, the value of each food product such as kilka depends on nutritional specifications and its acceptability in the societies; therefore, accurate processing of this fish and preparation of favorable variety products have special importance for the market (Martin, 1994).

The final step of the food production chain is packaging. Packaging occupies the middle ground of production, distribution and consumption. Many small and large companies in all over the world are very active in packaging field. The competition of these companies with each other has led to great increase in the quality and quantity of packaging forms. In most fields, the producers as well as the consumers are not well aware of the high value of proper modern packaging techniques. Currently, numerous packaging and preservation methods such as non – biological decomposable synthetic chemicals are used for food preservation. Recently, new packaging materials such as edible films that are biologically decomposable have been presented to the market (Shariatifar, 2007).

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

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period of cold or frozen storage(Ahmed,2006). 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 (Ahvenian, 2003). 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 films made of whey protein have been used for packaging of salmon, hot-dog, sausage, cracker and frozen fish-fillet (Stuchell and Krockta, 1995). Packaging of fish with edible film was originally used in Iran by Mirhashemi Rostami (2000). He used coconut oil for packaging of *Huso huso* fish in his project. But no research hasnot been carried out using whey protein films for packaging of fish in this country. In other countries, films consisting of whey proteins have been used for food packaging by Piyachonkwan (1995), Stuchell (1995), Krockta (1996), Shah (1999), Crapo (1999), Cagri (2002, 2003) and Min (2006 , 2007).The objective of current study was to investigate the effects of whey protein coating on the quality of common kilka during frozen storage.

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 national standards of Iran (INS[1], 2002b) were recorded. The fish were chilled down to 0 ºC 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.

Before processing fish were washed with chlorinated water. The fish were first washed. Then the heads were cut, and viscera were taken out. The cleaned fish were washed again.

Experimental design (Treatments)

Two treatments were used for this research. The treatments included coated Kilka and non coated Kilka.

The cleaned fish immersed in whey protein at a concentration of 12% in no time. After this stage, the fish was packaged in disposable dishes and covered by cellophane.

Control samples processing

For preparation of the control sample, Kilka fish was washed at first. Then the fish heads were cut, and viscera were taken out. The fish were washed again after cleaning and

[1] Iranian National Standard

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were packaged in disposable dishes with cellophane covers in 500 gr in weight.

The prepared samples (coating and control) were kept at -18 ^{o}C in cold-storage for a period of six months. Quality of these samples were studied similar to the test samples on the determined times by microbial, chemical and sensory tests. These samples were processed three times. Each step of the tests was repeated three times.

Their for experimental and control samples (48 packages) consisted of total bacterial counts (Andrews & Hammak, 2003 & Maturin, 2001) for *Staphylococcus* (Bennet and Lancette, 2001 & Holt et al ., 1994*), Coliform* (Feng et al, 2002*), Escherichia coli* (Feng *et al*., 2002) and *Psedoumonas* (Hasegawa, 1987). Microbial tests were carried out during nine steps. The first microbial tests on fish samples were conducted immediately after receiving the raw fish samples. The second test was conducted after cleaning. The third stage was conducted one day after processing and then the following tests were conducted on , the second step after cleaning the Kilka, the third step, one day after processing and the remaining steps, once a month after processing at determined times for a period of six months. In each step, these tests were repeated three times.

The chemical tests for the packaged samples with edible film and control (42 packages) consist of measurement of calorie (Iranian National Standard No 8867, 2005, protein (Iranian National Standard No 924, 1994) humidity (Iranian National Standard No 5625, 2002), lipid (Iranian National Standard No 742, 2002), ash (Iranian National Standard No 744, 2001), peroxide value (Iranian National Standard No 493, 2003), TVN (Iranian National Standard No 5625, 2002), TBA (Iranian National Standard No 10494, 2006), FFA (Iranian National Standard No 493, 2003) and pH (Iranian National Standard No 1028, 2007). These tests were conducted in eight steps which included the raw fish samples, one day after processing and other steps once a month from the first month after processing up to the sixth month, on determined 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, odor, color and taste with the method of Ranking and through execution of 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.

The results of microbial and chemical 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 samples are shown in the Tables 1 and 2. As indicated no *Coliform, Escherichia coli* and *Pseudomonas* bacterial contamination was observed in the whey protein covered samples during storage time. The mean total bacterial counts and *Staphylococcus* bacteria count in processed samples by whey protein were 2.47 and 1.61

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log CFU/g and in control samples 3.13 and 2.18 log CFU/g from 1 day until six months after processing, respectively.

There was a meaningful difference in the microbial results in the control samples and covered samples from the first day up to the sixth month ($p<.05$). Analysis of results obtained using Two – Way ANOVA at 95 % confidence level indicate significant differences in total bacterial counts and *Staphylococcus* bacteria count both in the covered samples as well as in the control samples ($p>.05$).

The chemical and proximate analysis results of the samples are shown in the Tables 3 and 4. The mean of moisture, peroxide value, TVB-N, pH, free fatty acids, thiobarbitouric acid , protein, fat, ash and calorie in the covered samples were 73.91%, 0/13meq/kgoil, 9.84mg/100g, 6.25, 1.15gr/100, 0/006 mg/kg, 19.00%, 4.25%, 2.10% and 120.73kcal/kg and in control samples 59.43%, 3.75 meq/kgoil, 16.22 mg/100g, 6.71, 9.21gr/100, 0/15mg/kg, 18.2%, 4.00%, 1.80% and 108.8 kcal/kg, respectively.

Variations of free fatty acids were meaningful from one day after keeping up to five months ($p<.05$) and at the sixth months. It was not meaningful in control samples.

Variations of pH and TBA showed significant differences between the first day with the sixth month after processing in control samples ($p<.05$).

Analysis of results obtained using Two – Way ANOVA at 95% confidence level indicate no significant differences in moisture, free fatty acids, thiobarbitouric acid, TVB-N and peroxide valuable in the covered samples ($p>.05$) compared with the control ones ($p<.05$).

According to Kolmogrof-Smirnof Test, distribution of microbial and chemical data was normal.

The sensory results of the samples are shown in the Table 5. Based on Freedman test in sensory properties, there was a meaningful difference between covered samples and control sample ($p<.05$).

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

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

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

Bacteria Samples	Total bacterial counts	*Staphylococcus* bacteria
Whole Kilka	4.49±0.12	2.39±0.23
Cleaned Kilka fish	3.81±0.11	2.51±0.15

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Table 2: Microbial factors results in covered and control samples during storage period (log CFU/g)

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

The different letters in the same column within the different storage time indicate significant differences ($p<.05$).

Values are mean + standard deviation.

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Table 3: Chemical factors results in covered and control samples during storage period

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

The different letters in the same column within the different storage time indicate significant differences ($p<.05$). Values are mean + standard deviation.

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Table 4: Proximate analysis results in covered and control samples during storage period

Experiments / Time	Moisture%		Protein%		Fat%		Ash%		Calorie kcal/kg	
Samples	Control	Coated	control	Coated	Control	Coated	Control	Coated	control	Coated
First day	72.2±0.35 [a]	73.93±0.04	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
First month	67.35±0.25 [b]	73.93±0.14	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
Second month	63.20±0.10 [c]	73.93±0.05	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
Third month	58.90±1.61 [d]	73.91±0.04	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
Forth month	54.15±0.16 [e]	73.91±0.04	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
Fifth month	50.43±0.14 [f]	73.90±0.04	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73
Sixth month	46.16±0.17 [g]	73.90±0.06	18.2±0.65	19.00±0.07	4.00±0.36	4.25±0.03	1.80±0.15	2.10±0.25	108.8	120.73

The different letters in the same column within the different storage time indicate significant differences ($p<.05$). Values are mean + standard deviation.

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Table 5: Sensory analysis and statistical analysis results in covered and control samples

Properties / Samples	Color	Odor	Tissue	Taste	Total acceptance
Covered samples	75 [a]	70 [a]	79 [a]	79 [a]	79 [a]
Control samples	110 [b]	106 [b]	96 [b]	104 [b]	112 [b]
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

The different letters in the same column within the different storage time indicate significant differences (p<.05). Values are mean + standard deviation.

Discussion

The total bacterial counts and Staphylococcus bacteria in the covered samples have been 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 & Moss, 2002; Nasar *et al*., 2008 & Seifzadeh, 2002). These compounds have anti-microbial specifications and are able to prevent the growth of *Staphylococcus, Psedoumonas, Coliform* and *Escherchia coli* (Zinoviadou *et al*., 2009 & Cagri *et al*., 2002 & 2003). 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 & Krochta, 1995). Absorption of water by protein and connection and linking of protein chains to each other increase on the size of protein that may cause to increase the viscosity, humidity and texture at the covered samples by this film, compared with the control sample (Sanker & Raghunath, 1995). In the control samples, because of presence of empty space between fish filets and also temperature rise and fall of the cold-storage, the Kilka in the packages has lost the humidity and will be dried and naturally it leads to decrease in weight about 3.5% after three months (Kochakian, 2002). This status may appear because of production of ice crystals at the products, too. Freezing is considered a basic action of dehydration that causes to exit the frozen humidity in vapor status from the food products. Air circulation at the cold- storage may intensify the humidity exit, too. This status may quicken the ruin of proteins and oxidation of lipids, and causes to decrease the quality of the taste and color change in Kilika without cover (Deman, 1999 & Safari, 1990 & Fatemi, 1990).

Amount of TVB-N in Kilka without cover during the storage period at the cold-storage showed an increase. This may be due

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to the effect of decrease of humidity and production of free fatty acids on the denaturizing of the Protein. These factors decreased in covered samples compared with the control sample. We can relate its reason to the specification of whey protein that cause to prevention of the proteolyses, decrease of humidity and production of free fatty acids on denaturizing of protein and naturally increase in TVB-N(Morrissey et al., 2009; Piyachomkwan and Penner, 1995; Salmani et al., 2002).

Amount of ash showed an increase in the covered samples compared with control sample, That is because of presence of sodium, potassium, calcium, manganese and phosphate ions, and also presence of sulfur holder amino acids in the compound of whey protein and this point that these amino acids are considered as mineral elements of food products (Lyndon, 1999).

Amount of protein showed an increase in covered samples compared with the control sample. This may be due to the effect of some protein compounds such as α-lactalbomine and β-lactoglobine of this film (Shahidi and Botta, 1990).

Concentration of lipid showed an increase in the covered samples compared with control sample. That is because of presence of glysirids 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 hydrolyze are influenced by the action of lipolytic enzymes (Deis,2006 and 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 analyzed bacteria, have been able to be active at low water activity and may cause hydrolyze of lipids and production of non-saturated fatty acids through the lipolyze process(Cappuccino and Sherman, 1999; Min et al., 2005, 2007). Releasing of fatty acids with high numbers of carbons by lipase enzyme may not provide a specific bad taste but through passing the time, effects of accumulation of free fatty acids in muscles of fish because of their combination with protein of muscle may cause unfavorable taste and tissue damages (Crapo et al., 1999). In addition to fatty acids, because of protein denaturation, it provided some tissue changes and decrease of quality. In the control samples, concentration of these acids has been increased from the first month up to the fifth month. But the nearly constant concentration of these acids at the last processes of keeping has probably been because of 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 the free fatty acids has been measured less in the samples covered by whey protein, compared with the control sample. This may be due to the prevention from losing the water from the surface and inside the body by the fine holes present at the body surface, prevention from the oxygen contact with the fish tissue and combination with non-saturated fatty acids and oxidation, and lack of absorption of light by the fish body surface (Lyndon, 1999; Ahvenainen, 2003).

Freezing caused the fish tissue denaturizing and moisture loses leads to the weight loss which may be due to the oxygen penetration

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into the fish tissue and oxidation increasing of free fatty acids and peroxide value. But, peroxide, will start to induce decomposition, leading to the aldehydes, ketone and seton production, subsequently, in peroxide value reduction to over time (Krochta et al., 1996).

First products of lipid oxidation are hydro-peroxide and they are broken to secondary products such as aldeydes that can react with proteins. This compound react with pigments and other molecules present at the fish, too. Therefore it leads to loss the color and producing bad smell (Rezaei *et al*., 2003).

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 (Shah, 1999; Aubourg et al., 1995).

Amount of peroxide and TBA was measured lower in the covered samples compared with control sample. 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 film, prevention of oxygen absorption, prevention from humidity decrease, decrease of oxidation and production of secondary products of oxidation such as aldeydes and specifications of these compounds (Tall and Harris, 1995; Gigirey and Desousa, 1999).

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

The covered samples have better taste and smell compared with the control sample that is due to the effect of di-acetyl and lactose in the compound of this film. Proteins of α-lactalbomine and β-lactoglobine present at whey protein cause production and accumulation of big solution and masses. This process may lead to production of small sedimentations. These accumulations and connection of them with water, have increased the amount of water and consequently cause to increase the viscosity and development of the taste of the product (Bigelow and Lee, 2007; Chapman et al., 1997).

Amount of calorie has shown an increase in the samples covered by edible film compared with the control samples. It is because of increase of 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 more than the control sample and with consideration to the conversion coefficient of each gram of protein, carbohydrate and lipid to energy. Amount of calorie of these samples has shown an increase (Marsh and Bugusu, 2007).

Through passing of time, lipid oxidation products such as hydro-peroxides have been analyzed and some compounds such as aldeyds and others have been produced in the control sample. These compounds have alkali specification and cause an increase in the pH

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of the product. Therefore in the covered samples, changes of pH during the time storage were not significant (Anker and Hermansoon, 2010).

Similar results were obtained by Rokwer, Biglelow, Fujki, Trout and Manish.

According to the results of the experiments, a meaningful difference in the index of total acceptance 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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