Effect of whey protein coating on physico-chemical properties of gutted Kilka during frozen storage

Hasanzati Rostami A.[1]* ; Motallebi A. A.[2] ; Khanipour A. A.[3] ; Soltani M.[4] ; Khanedan N.[1]

Received: April 2009 Accepted: July 2009

Abstract

The aim of this paper was to study the effects of whey protein coating on chemical and physical properties of gutted Kilka during frozen storage. Kilka was coated by dipping in whey protein solution with different concentrations of 3, 7, 10 and 13%, for 1h. Then, after being packed in polyethylene dishes, they were covered in cellophane blanket and stored in -18°C. There were 125 testing and control packages, each of them weight 250 grams. Heme iron, peroxide value, protein, lipid and pH have measured after 0, 1, 2, 3 and 4 months storage in freezer. Results of heme iron, peroxide value and lipid of control treatments have shown significant difference among experimental treatments ($p<0.05$). Protein and pH value of control treatments did not show significant difference with other treatments ($p>0.05$). Our results showed that coating process with different whey protein concentration leads to decrease in peroxide value and increases the iron content. 13% whey protein concentration was provided the most satisfactory results.

Keywords: Edible films and coatings, Whey protein, Kilka Fish, Heme iron, Lipid oxidation

1 -Science and Research Branch, Islamic Azad University, P.O.BOX: 14515-755 Tehran, Iran.
2 -Iranian Fisheries Research Organization, P.O.BOX: 14155-6116 Tehran, Iran.
3-National Fish Processing Research Center, P.O.BOX: 43145-1655 Bandar Anzali, Iran.
4-Faculty of Veterinary Medicine, University of Tehran, P.O.BOX: 14155-6453 Tehran, Iran.
* Corresponding author's email: arostami306@gmail.com

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

Introduction

Whey, the byproduct of cheese-making, is produced in large quantities and its annual production increases continuously. Much of this whey is not utilized, and it creates serious pollution and waste disposal problems (Ozdemir and Floros, 2001). Their potential as an agent to form edible films has generated interest in the last decade, because of their favorable functional properties and industrial surplus (Gounga et al., 2007). The formation of edible films and coatings from whey proteins can increase the utilization of whey, improve the nutritional value of foods and prolong shelf life (Ozdemir and Floros, 2008). Investigations for their ability to produce edible films and especially properties of milk protein based edible films have been extensively reviewed by McHugh and Krochta (1994) and Chen (1995). Milk protein based edible films are excellent oxygen, lipid, and aroma barriers; however, due to their hydrophilic nature, they have poor moisture barrier properties (Chen, 1995; Krochta and DeMulder-Johnston, 1997; Miller and Krochta, 1997; Chick and Ustunol, 1998; Sydim and Sarikus, 2006). Fish is one of the most important sources of protein and other nutrients in many parts of the world and some countries rely highly on fish export for their income (Mahboob et al., 2008; Motalebi et al., 2010). Fish meat may decompose more easily than other meat products (Panchavarnam, et al., 2003); it must be consumed immediately after fishing or must be stored under suitable conditions to reach consumers without losing its nutritional value (Kilincceker et al., 2009). Deterioration of fish meat mostly occurs in the fat-containing portions. The proportion of unsaturated fatty acids in fish fat is approximately 80% (Kilincceker et al., 2009).These fatty acids are affected by the environmental oxygen that oxidizes and spoils the fish meat. As a result, the taste becomes bitter, and later on, color changes occur. All these alter the perceptions and satisfaction of the consumers. Longer shelf life and better quality can be made possible by using different processing techniques such as freezing and appropriate combinations of these techniques (Kilincceker et al., 2009). Cold storage and freezing are the normally employed methods for fish preservation, but they do not completely inhibit the quality deterioration of fish (Jeon et al., 2002; Duan et al., 2010). Several studies (Kulp and Loewe, 1990; Williams and Mittal, 1999; Cutter, 2006) have shown that edible coatings made of protein, polysaccharide, and oil-containing materials help to prolong the shelf life and preserve the attributes of edible quality (Kilincceker et al., 2009). Edible coatings are important for sensitive foods, such as seafood (Kilincceker et al., 2009). A number of coating materials have been tested in attempt to maintain quality and prolong shelf life of meat products (Piayachomkwan and Penner, 1995; Stuchell and Krotcha et al., 1995; Crapo et al., 1999; Shah et al., 1999; Sathivel, 2005; Kilinccerker et al., 2009; Motalebi et al., 2010). In Iran Mirhashemi Rostami has used Coconut oil for coating on *Acipenser Persicus*, and whey protein has not been used for fish coating in Iran, so far (Motalebi et al., 2010). There are two

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

types of dietary iron based on different mechanisms of absorption: nonheme and heme. Nonheme iron, found in plant and animal products, has a low bioavailability ranging from 2 to 20%, and is influenced greatly by a variety of enhancing and inhibiting components in the diet. Heme iron, on the other hand, is found only in meat, fish, and poultry (MFP), has a much higher bioavailability ranging from 15 to 35%, and is not affected by other dietary constituents (Clark et al., 1997). The objective of present study was to evaluate the effects of coating made with whey protein on Physic-Chemical properties of Kilka during frozen storage.

Materials and methods

Fresh fish were caught from quay of Bandaranzali, in Guilan. Approximately 50 kg fresh fish were transferred to the Laboratory in the National Fish Processing Research Center (Bandaranzali, Iran), in 2009. Whey protein concentrate (WPC) (Kheizaran Food Industries Company) was used to prepare edible coating according to Perez-Gago et al. (1999).

Different concentrations of whey protein were chosen as recommended by previous studies (Mor and Shoemaker, 1999; Franssen et al., 2004; Perez-Gago et al., 2006), including 3%, 7%, 10% and 13% (w/w), by breaking up 600, 1400, 2000 and 2600 g Whey protein concentrate in separate tanks with 20 liter water. The quantity of used Kilka fish for preparation of experimental and control treatment has spotted 125 packages, each weighted 250 g (3 times repetition for each test). Pate and tail of fish were cut off, and then gutted. Then 25 kilos of Kilka fish were dipped in whey protein solution with nominative concentration for 1h. After bringing out, samples were kept in baskets about 1 min to dissent surplus of solution from fish surfaces. 7 kilos of fish were not coated and were spotted as control samples. After packaging all samples in polyethylene dishes with cellophane blanket, they were stored at -18°C, for 4 months.

The following treatments were carried out in the present study: treatment 1 (uncoated Kilka fish), treatment 2 (coated fish with 3% WPC), treatment 3 (coated with 7% WPC), treatment 4 (coated with 10% WPC) and treatment 5 (coated with 13% WPC). Then 5 samples were taken as follows: one day after storage followed by sampling at the beginning of each month, for 4 months.

Lee method was used for determining amount of peroxide (Pearson, 1997). Soksule and kjeldahl methods were used to determine the amount of lipid and protein, respectively (Parvane, 1998), and A.O.A.C was used to determine pH (A.O.A.C, 2000). Content of heme iron was determined by Hornsey method with total pigment analyzes (Clark et al., 1997).

Samples (10 ± 0.1 g) of Kilka were accurately weighed into 50-mL centrifuge tubes. To this was added about half of an acidified acetone solution containing 40 ml of acetone, 9 ml of water and 1 ml of HCl. Each sample was homogenized for 15 s with a mixer (Moulinex M.R.) and the remaining acidified acetone solution was added. The samples were mixed thoroughly; the tubes were capped tightly and allowed to stand in the dark for at least 1 h before being centrifuged at 2200

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

g for 10 min. The supernatant was then filtered (GF/A filter paper, Whatman, Maidstone, England) and the absorbance was measured at 640 nm against a reagent blank with a spectrophotomer (Cecilce 1010). The absorbance was multiplied by the factor 6800 and then divided by the sample weight to give the concentration of total pigments in the meat as µg hemeatin/g meat. The iron content was calculated with the factor of 0.0882 µg iron/µg hemeatin (Clark et al., 1997).

To analyze statistical results, One-way ANOVA was applied and the mean comparison was done through Tukey test at reliability level of 5%. Data analysis was done in SPSS software (release 16.0).

Results

One-way ANOVA showed that there is significant difference between peroxide value for different treatments (control, coated %3, %7, %10 and %13) during phases 0, 1, 2, 3 and 4 ($p<0.05$) (Fig.1). There was significant difference in level 5 between mean of peroxide in different times in treatments ($p<0.05$).

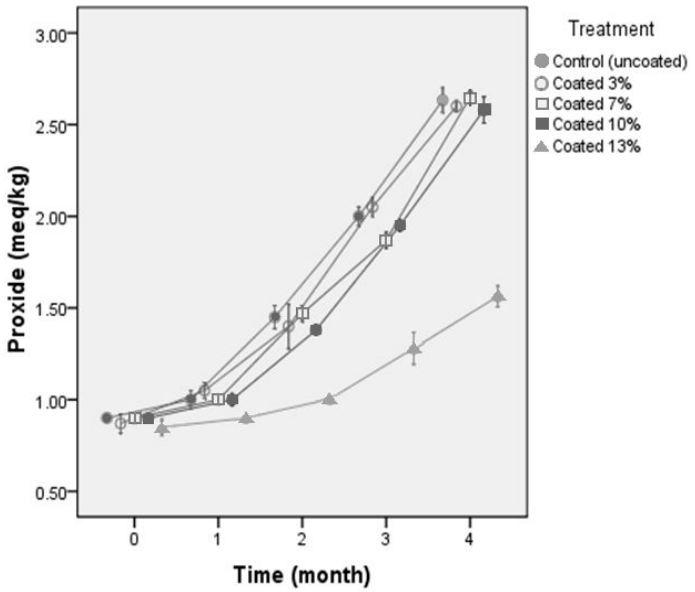

Figure 1: Effect of whey protein concentration and time on peroxide value in coated and uncoated samples.

Peroxide value increased from 0.9 meq/kg to 2.63, 2.60, 2.65, 2.58 and 1.65, respectively for treatments 1, 2, 3, 4 and 5, during 4 months. The minimum value of peroxide observed in samples coated with whey protein % 13 concentrations (treatment 5).

Lipid content of samples was shown in Fig. 2. As indicated, lipid content in all samples decreased with increasing the storage time. Also, significant difference was observed in coated sample as compared to the non-coated samples. Maximum value of lipid (6.87%) was observed in treatment 5, after 4 month storage.

Results showed that there was significant difference between heme iron

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

content among different treatments (control, coated %3, %7, %10 and %13) during 0, 1, 2, 3 and 4 months of experiment ($p<0.05$) (Fig. 3). One-way ANOVA results showed that there was significant difference in level 5 between mean of heme iron in different times in treatments 1 to 5 ($p<0.05$).

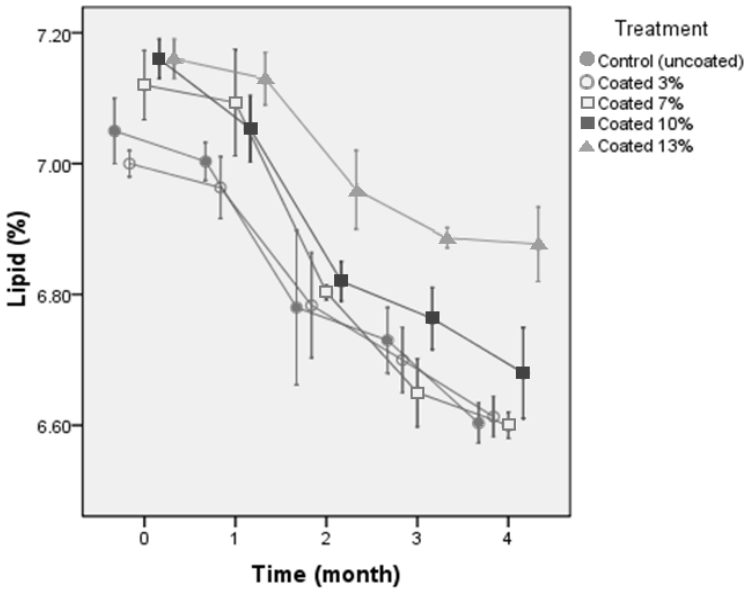

Figure 2: Effect of whey protein concentration and time on lipid between coated and uncoated samples

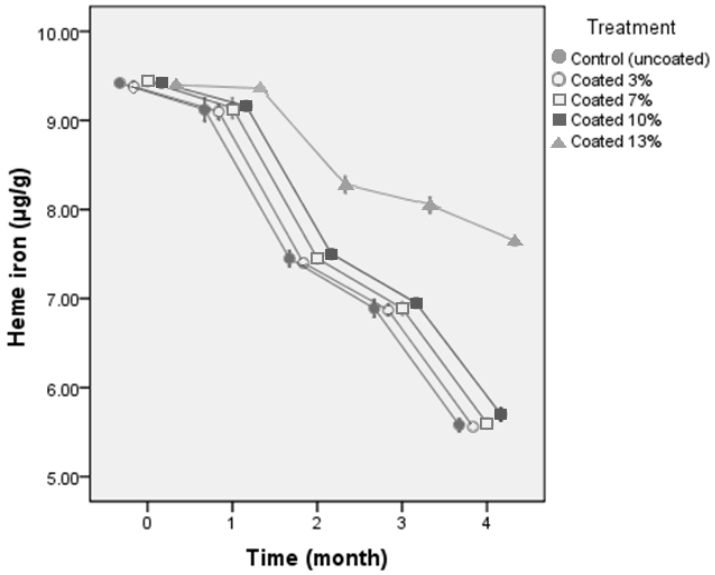

Figure 3: Effect of whey protein concentration and time on Heme iron between coated and uncoated samples

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

In treatment 1-5, heme iron reduced from 9.42, 9.38, 9.44, 9.42 and 9.4 mg/g meat, respectively, to 5.58, 5.56, 5.60, 5.70 and 7, 65 mg/g meat after 4 month. Maximum value of heme iron was observed in samples coated with whey protein % 13 concentrations (treatment 5).

Protein content of all samples was presented in Fig. 4, ranging from 17.65 – 17.33 g/ 100 g of fish muscle. As indicated no significant (P>0.05) differences was observed between the samples. pH value of the coated and non-coated samples was shown in Fig. 5.

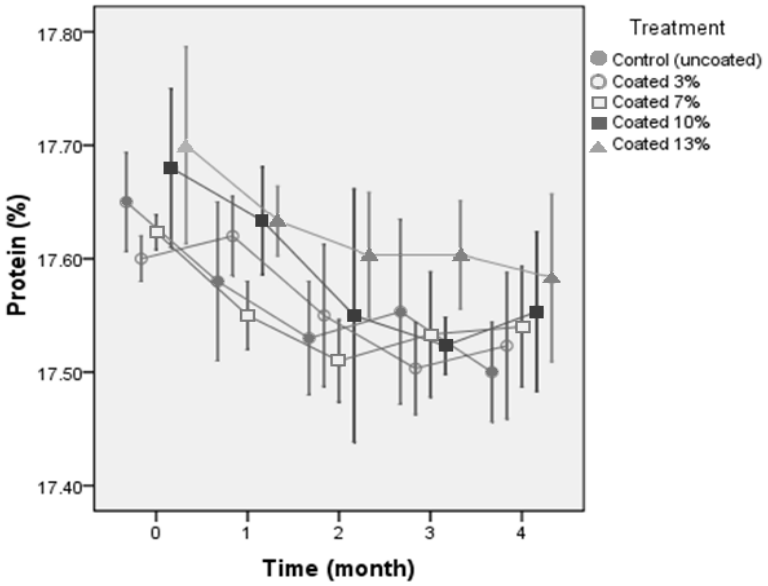

Figure 4: Effect of whey protein concentration and time on protein between coated and uncoated samples.

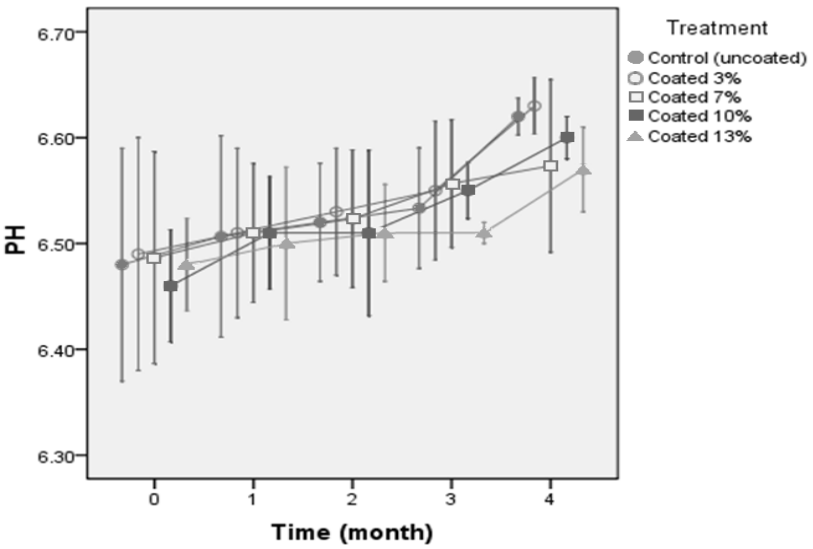

Figure 5: Effect of whey protein concentration and time on pH between coated and uncoated samples

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

The pH value was observed between 6.48 – 6.60. This factor showed no significant (P>0.05) difference among treatments.

Discussion

In oil and fatty foods, the peroxide value must be less than 5 meq/kg fatty materials (Pearson, 1997). Peroxide value of 10 and higher is considered as unusable (Parvane, 1998). Lipid oxidation is one of the most important factors responsible for quality deterioration of fish during frozen storage (Pourashouri et al., 2007). Lipid oxidation is a complex process whereby polyunsaturated fatty acids are degraded via formation of free radicals, causing flavour, texture, colour and nutritional deterioration of foodstuffs (Estévez and Cava, 2004). The peroxide value of all coated samples was lower than uncoated samples during storage, indicating the coating can reduce the lipid oxidation. Peroxide value samples coated with 13% whey protein was significantly (P<0.05) lower than that in other treatments. This difference is more significant in coated samples with 13% concentration whey protein, as lipid percentage is more than other treatments in this level. This might be due to the barrier role of whey protein coating against penetration of oxygen in the fish muscle. Sathivel (2005) demonstrated that salmon fillets coated with chitosan (1 and 2% concentration) and soy protein concentrate retard lipid oxidation during 3 month frozen storage. Stuchell and Krochta (1995) reported that King Salmon coated with whey protein isolates showed delayed lipid oxidation during frozen storage. Jeon et al. (2002) demonstrated that chitosan coated Atlantic cod and herring had reduced lipid oxidation. Whey protein films and coatings have been shown to be excellent oxygen barriers (Franssen et al., 2004). Reducing the peroxide value in coated kilka could be related to the oxygen barrier properties of whey protein coating on the fish muscle. In the present study, during storage, lipid contents varied. It is believed that oxidation of lipids in fatty fish is a major problem during storage and processing, particularly in dark muscles of pelagic species (Tokur and Korkmaz, 2007). In the present study, the edible coating showed significant difference in lipid percentage in Kilka fish treatments (p<0.05).

During storage for 4 month, heme iron value reduced in all samples. Iron is introduced as a valid peroxidant in fatty products. According to the results, it was expected that heme iron amount be significantly different in our samples. The decrease in concentrations of heme iron during 4 month, may be is due to release of iron from the heme iron complex by oxidation and conversion of heme to non-heme iron (Turhan et al., 2004).

In 1995, Piayachomkwon used whey protein on Pacific whiting surimi. Results have shown autolysis had missed in coated surimi samples with 2% whey protein concentration with 80 or 95% protein value. In coated surimi samples with 2% concentration of whey protein with 34% protein content, autolysis has reduced 57% in compare with control samples. In this study no changes were observed in protein content of coated samples, due to low content of protein in whey protein concentrate (9-12%).

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

Sathivel (2005) has shown pH of raw noncoated pink salmon fillets was 6.63 after 3 month frozen storage the pH of raw pink salmon fillets coated with chitosan or protein ranged from 6.4 to 6.7. It has been reported that freezing may cause changes in the pH of the fish muscle. However, this was not observed in this study.

This study indicated that whey protein can be used as edible coating for reducing the lipid oxidation during freezing storage. The best result was found when 13% whey protein concentration was used.

Acknowledgments

Special gratitude to the staff in Iranian Fisheries Research Organization for their technical and beneficial supports during this study. Special thanks to personnel and researchers of this organization and National Fish Processing Research Center.

References

Association of Official Analytical Chemists, 2000. Official methods of analysis. 17th ed., Washington.DC: Association of official analytical chemists.

Chen, H., 1995. Functional properties and applications of edible films made form milk proteins. *Journal of Dairy Science***,** 78, 2563–2583.

Chick, J. and Ustunol, Z., 1998. Mechanical and barrier properties of lactic acid and rennet-precipitated casein-based edible films. *Journal of Food Science*, 63, 1024–1027.

Clark, E. M., Mahoney, A. W. and Carpenter, C. E., 1997. Heme and total iron in ready-to-eat chicken. *Journal of Agricultural Food and Chemistry*, 45, 124-126.

Crapo, C., Himelboom, B.; Pfutzenreutev, R. and Lee, C., 1999. Texture modification processes for giant grenadier fillets. *Journal Aquatic Food Product Technology*, 8, 27-40.

Cutter, C. N., 2006. Opportunities for bio based packaging technologies to improve the quality and safety of fresh and further processed muscle food. *Journal of Meat Science*, 74, 131-142.

Duan, J., Cherian, G. and Zhao, Y., 2010. Quality enhancement in fresh and frozen lingcod (Ophiodon elongates) fillets by employment of fish oil incorporated chitosan coatings. *Food Chemistry*, 119,524-532.

Estévez, M. and Cava R., 2004. Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. *Journal of Meat Science*, 68, 551- 558.

Franssen, L. R., Rumsey, T. R. and Krotcha, J. M., 2004. Whey protein film composition effects on potassium sorbate and Natamycin diffusion. *Journal of Food Science*, 69, 347-351.

Gounga, M. E., Xu, S. Y. and Wang, Z., 2007. Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering*, 83, 521–530.

Jeon, Y .J., Kamil, J. Y. V. A. and Shahidi, F., 2002. Chitosan as edible invisible film for quality preservation

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

of herring and Atlantic cod. *Journal of Agriculture Food Chemistry*, 50, 5167-78.

Kilincceker, O, Dogan, I. S. and Kucuknev, E., 2009. Effect of edible coatings on the quality of frozen fish fillets. *LWT-Food science and Technology*, 42, 868-873.

Krochta, J. M. and DeMulder-Johnston, C., 1997. Edible and biodegradable.

Kulp, K. and Loewe, R., 1990. Batters and breadings in food processing. St. Paul, MN, USA 55121–2097, American Association of Cereal Chemist Inc. 276 p.

Mahboob, S.; Hussain, M. I.; Iqbal, Z. and Nadeem, S., 2008. Estimation of volatile organic compounds in farmed and wild rohu, *Labeo rohita*. *Iranian journal of fisheries sciences*, 8, 65-72.

Mc Hugh, T. H. and Krochta, J. M., 1994. Milk protein based edible films and coatings. *Food Technology*, 97, 97-103.

Miller, K. S. and Krochta, J. M., 1997. Oxygen and aroma barrier properties of edible films: a review. *Trends in Food Science and Technology*,8 , 228-237.

Min, S., Harris, L. J., Han, J. H. and Krochta, J. M. 2005. Listeria monocytogenes inhibition by whey protein films and coatings incorporating lysozyme. *Journal of Food Port*. 68, 2317-2325.

Min, S., Harris, L. J., Krochta, J. M., 2006. Antimicrobial effects of lactoferin, lysozyme and lactoperoxidase system and edible whey protein films incorporating the lactoperoxidase system against *Salmonella enterica* and *Escherichia coli* O157:H7. Journal *of Food Science* 70, 332-338.

Mor, Y., and Sheomaker, C. F., 1999. Compressive properties of whey protein composite gels containing Fractionated Milk fat. *Journal of food science*, 64, 1078-1082.

Motalebi, A. A., Hasanzati Rostami, A., Khanipour, A. A. and Soltani, M. 2010. Impacts of whey protein edible coating on chemical and microbial factors of gutted kilka during frozen storage. *Iranian Journal of Fisheries Sciences,* 9, 255-264.

Ozdemir, M. and Floros, J. D., 2001. Analysis and modeling of potassium sorbate discusion through edible whey protein films. *Journal of Food Engineering* 47, 149-155.

Ozdemir, M. and Floros, J. D., 2008. Optimization of edible whey protein films containing preservatives for mechanical and optical properties. *Journal of Food Engineering*, 84, 116–123.

Panchavarnam, S., Basu, S., Manisha, K., Warrier, S. B. and Venugopal, V., 2003. Preparation and use of fresh water fish, rohu (*Labeo rohita*) protein dispersion in shelf life extension of the fish steaks. Lebens m. Wiss., u-Technology 36, 433-439.

Parvane, V., 1998. [Quality control and food chemical tests.] University of Tehran Press, 325 P. (in Persian)

Pearson, d., 1997. Laboratory techniques in food analysis .4th ed. Butter Worth and Co. Ltd., UK, pp. 316-320.

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]

Perez-Gago, M. B., Nadaud, P. and Krochta, J. M., 1999. Water Vapor permeability, solubility and tensile properties of heat-denaturated versus native whey protein films. *Journal of Food Science* 64, 1034-1038.

Perez-Gago, M.B., Serra, M.; Alonos, M., Mateos, M. and Del Rio, M. A., 2006. Effect of solid content and lipid content of whey protein isolate-Bees Wax Edible Coatings on color change of fresh-cut apples. *Journal of Food Science*, 68, 2186-2191.

Piayachomkwan, K. and Penner, M. H., 1995. Inhabitation of Pacific Whiting Surimi-associated protease bye Whey Protein Concentrate. *Journal of Food Biochemistry*, 18, 345.

Pourashori, P., Shabanpour, B., Daghigh Rohi, J. and Shabani, A., 2007. Inhibitory effect of citric acid on rancidity of frozen catfish (*Silurus glanis*) fillets. *Iranian Journal of Fisheries Sciences*, **7**, 215-228.

Sathivel, S., 2005. Chitosan and protein Coatings affect yield, Moisture loss, and lipid oxidation of apple pink salmon fillets During Frozen storage. *Journal of Food Science*, 70, 445-459.

Seydim, A. C. and Sarikus, G., 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils, *Food Research International*, 39, 639–644.

Shah, A. J., Hansen, B. and Larsen, R. produced by extrusion with addition of whey protein concentrate. *Food Australia*, 51, 104-106.

Stuchell, Y. M. and Krotcha, J. M., 1995. Edible coating on frozen king salmon effect of Whey protein isolate and acetylated monoglycerides on moisture loss and lipid oxidation. *Journal of Food Science* 60, 28-31.

Tokur, B. and Korkmaza, K., 2007. The effects of an iron-catalyzed oxidation system on lipids and proteins of dark muscle fish. *Food Chemistry*, 104, 754-760.

Turhan, S., Ustun, N. S. and Altunkaynak, T. B., 2004. Effect of cooking methods on total and heme iron contents of anchovy (*Engraulis encrasicholus*). *Food Chemistry*, 88, 169-172.

Williams, R. and Mittal, G. S., 1999. Water and fat transfer properties of polysaccharide films on fried pastry mix. *Lebensmittel-Wissenschaft-und-Technologies*, 32, 440–445.

[Downloaded from jifro.ir on 2026-11-23]

[DOR: 20.1001.1.15622916.2010.9.3.6.2]