SDS-PAGE CHANGES OF RAW, SMOKED AND MARINATED RAINBOW TROUT (*Oncorhynchus mykiss*) DURING FROZEN STORAGE

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ABSTRACT

This study aimed to determine effects on protein of frozen storage time (-20 °C) on properties of raw, marinated and smoked rainbow trout (*Oncorhynchus mykiss*). Therefore, the fish were smoked and marinated. All filleted fish samples including also raw were vacuum packed and subjected to 9 months of frozen storage and analyzed at intervals of a month. Changes in muscle proteins of raw, smoked and marinated trout samples (*Oncorhynchus mykiss*) have been examined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE and densitometric analysis revealed that intensity and the number of protein bands weren't changed until the ninth month of raw and marinating process while the bands of some protein pattern denaturated when trout muscle was subjected to the 9th month of smoking process. Myosin heavy chain (MHC) band with molecular weights of 205 kDa and actin band with molecular weights of 45 kDa appeared in the during 9 months of raw, smoking and marinating process. Similarly, other main myofibrillar proteins; tropomyosin (37.7 kDa), troponin-T (32.9 kDa), myosin light chain 1 (27.2 kDa), and myosin light chain 2 (20.4 kDa) also didn't show any differences during 9 months storage. Subsequently, there were significant monthly changes in the protein percentages caused by smoking and marinating processes excluding raw ($P<0.01$).

KEYWWORDS:

Trout, Protein, Actin, Myosin, SDS-PAGE, Smoking, Marinating

1. INTRODUCTION

Fishery products have an important place among processed foods both from health and taste aspects, and they differ from other foods of animal origin in that they are quite sensitive to processing methods and storage and therefore they easily lose their quality[1]. Fish muscle is composed of myofibrillar proteins, sarcoplasmic proteins, storma proteins, connective tissue, nucleotides, polypeptides and non-protein nitrogen compounds. The major alteration in fish tissue occurs largely in the myofibrillar proteins, the main muscle proteins, comprising myosin and actin[2]. Freezing is one the best methods for fish preservation. But, measurement of sensory, chemical and physical changes have shown deterioration of fish quality continues to some extent during frozen storage[3].

Changes in heat, pH and salt concentrations and reducing agents denature proteins in fishery products like all other food proteins and destruction secondary and tertiary structures[4]. It was reported that about 90% of fish proteins are generally denatured between 60-65 ^{o}C, while the remaining 10% (tropomyosin) can remain stable without being denatured for a long time until 100 ^{o}C[5,6]. Denaturing temperature varies depending on fish species, protein type and the ambient temperature[7].

It is known that most of protein bands disappear due to hot smoking and denaturing in zander, trout and eel [8]. Smoking and marinating are the oldest traditional fish preservation methods. There are many studies conducted on changes in physical, chemical, sensorial and microbiological quality of smoked and marinated fish products[9-13]. However, there are only a limited number of studies investigating protein changes through electrophoretic methods.

In this study, the changes in protein profiles of rainbow trout preserved with raw, marinating and smoking methods are observed with SDS-PAGE during the frozen storage period, and protein quality losses were determined.

2. MATERIAL and METHODS

2.1.Preparation of samples

Rainbow trout samples (*Oncorhynchus mykiss*) were obtained fresh from local rainbow trout farm in Adana (Turkey), stored on ice and transferred to the laboratory. The mean length and weight of fishes were measured as 26.20±0.42 cm and 234.98±11.62 g,

respectively. Subsequently, pretreatments (washing, evisceration, deheading and filleting) were carried out and two fillets were obtained from each carcass. Fillet samples were randomly divided into three groups: raw, smoking and marinating (9 fillets for each groups). Then, smoking and marinating processes were performed.

2.2. Smoking process

Trout fillets were kept in 20% of 1:1 (w/v) brine solution at 4 °C for 1 hour. Afterwards, samples were washed again and dried. For pre-heating, fishes were kept at 90 °C for about 1 hour, smoked at 80 °C and cooled.

2.3. Marinating process

Trout fillets were submerged in marinating solution. As marinating solution, 3% (v/v) acetic acid and 10% (w/v) NaCl were used. Fillets were submerged in this solution at 1:1.5 (w/v) ratio and kept for 48 h and at the end of this period, they were taken out of the solution and filtered for 15 min.

2.4. Raw samples

For control, filleted fish samples were vacuum packed without any treatment. Similarly, vacuumed samples were kept at -20 °C for 9 months.

2.5. Packing

Smoked, marinated and raw samples were vacuum packed with Reepack (Mod RV 50) trademark vacuum machine. These samples were stored at -20 °C for 9 months.

2.6.Protein extraction and analysis

Crude protein content was calculated by converting the nitrogen content according to Kjeldahl's methods[14]. Raw, marinated and smoked samples for electrophoresis were prepared by homogenizing 1 gr minced samples. From each one of raw, marinated and smoked samples, 1 g of sample was taken and homogenized within 100 ml cold (~ 5 °C) distilled deionized water with an Ultratorax for 30 s. Raw and marinated samples were diluted at 1:1 (w/v) in buffer prepared for raw samples (4% (w/v) SDS, 0.125 M tris, pH 6.8, 20% (v/v) glycerol, 10% (v/v) β-mercaptoethanol), while the smoked samples were diluted at 1:3 (w/v) ratio with the buffer used for cooked samples (0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 Mm EDTA, 0.01% sodium azide)[15,16]. Monthly prepared samples were kept at

-20 °C, and homogenized using a Polytron at room temperature for 1 min after nine months. The samples were then centrifuged for 20 min at room temperature and the supernatants were collected. Proteins of raw, marinated and smoked samples were estimated as described by previously using bovine serum albumin as the standard[17].

2.7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

All raw, marinated and smoked homogenates were then boiled in a water bath (100°C) for 3 min and loaded in each gel lane. Unstained SDS-PAGE molecular weight standard, MW range 14.4-116.0 (Fermentas, SM0431) was diluted 1:2 with loading dye.

SDS-PAGE analysis was carried out according to the method of Laemmli[18]. This was done with a SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA, USA) using a 3% (w/v) acrylamide stacking gel and 10% (w/v) acrylamide resolving gel[19].

After electrophoresis, the gels were stained for 1 h with Coomassie brillant blue R250 dye (0.2% w/v Coomassie brillant blue, 50% (v/v) methanol, 10% (v/v) glacial acetic acid). Afterwards, removal of the residual stain on the surface of gels was done with a destaining solution. Finally, gels preserved in acetic acid (5% v/v methanol, 7% v/v glacial acetic acid) were photographed on a white illuminated desk in a dark room. Densitometric analysis of protein bands were performed using Image Program, Version 1.4.

3.RESULTS

In the present study, protein patterns of monthly frozen stored rainbow trout samples (*Oncorhynchusmykiss*) were determined by using SDS-PAGE analysis by known standards (β-galactosidase 116.0, Bovine serum albumin 66.2, Ovalbumin 45.0, Lactate dehydrogenase 35.0, REase Bsp981 25.0, β-lactoglobulin 18.4, and Lysozyme 14.4). The SDS-PAGE pattern of total proteins from raw, marinated and smoked samples in *Figure 1, Figure 3* and *Figure 5*, respectively. The pattern reveals multiple bands in the molecular weight range from 116.0 kDa to 14.4 kDa. The band appearing at over 116.0 kDa is myosin (205 kDa) heavy chain and at 45 kDa is actin components. The protein profiles by SDS-PAGE help us to understand the process of aggregation during frozen storage.

The protein profile assessed by SDS-PAGE showed no difference in proteins pattern during 9 months in raw rainbow trout proteins frozen-storage at -20 °C. Similarly, there was no significant change in actin and myosin bands, either (*Figure 1, Figure 2*).

Muscle protein patterns didn't showed a different profile during frozen-storage marinated trout samples for 9 months (*Figure 3, Figure 4*).

When the changes in proteins were investigated for the monthly stored (-20 °C) and smoked rainbow trout for 9 months, it was determined that intensity of protein bands started to decrease in 9^{th} months. In addition, there wasn't also evident decrease in the intensity of actin and myosin bands during 9 months (*Figure 5, Figure 6*).

The protein quantities (%) of monthly frozen stored rainbow trout samples obtained in this study were subject to variance analysis (one-way ANOVA) using SPSS 9.0 Windows software, and the mean values of important variance sources were compared with Duncan's multiple range test at p=0.01 significance level and the results are given in *Table 1*.

4.DISCUSSION

The statistical analysis of the protein changes demonstrated a significant difference in the results of the protein (%) analysis of the monthly frozen stored trout samples with marinating, smoking methods excluding raw (*Table 1*). The crude protein content of rainbow trout ranged between 18.34 (raw sample) to 16.29% for marinated samples. This decrease could be attributed to proteolytic enzymes. Besides, salting treatment decreased the protein rate of the marinated fish product as a result of protein being dissolved in the brine and other nitrogenous substance including free amino acids[20].

No significant decrease was detected in raw and marinated rainbow trout protein bands stored at -20 °C until 9 months. Similarly, there was no significant change in actin and myosin bands, either. But, in the smoked trout samples, when the intensity of myosin and protein bands of over 116 kDa molecular weight didn't change. However, it was determined that intensity of same protein bands started to decrease in 9^{th} months. Similarly, Bauchart *et al*. [21] reported the occurrence of low molecular weight peptides (<5 kDa) in post mortem rainbow trout muscle, during ice storage, and evaluated their stability during cooking. They showed that muscle of trout was poor in peptides and their concentration was almost unaffected by the seven days of ice storage and vacuum cooking for 5 min at 70 °C.

In smoking, protein band intensities were observed to not decrease from the 1st to 8th months, but some protein bands completely disappeared in 9th months. In addition, there was an evident decrease in the intensity of actin and myosin bands in the later months. Hultmann et al.[22] studied in effects of storage period on different properties related to proteins and enzymes in cold-smoked salmon. They determined that the intensity of the myosin heavy chain band was reduced with further storage of smoked samples as a result of SDS-PAGE analysis. Our results in accordance with the results of Hultmann et al. [22]. In another study, Verrez-Bagnis et al.[23] observed the post mortem evolution of protein patterns in sea bass muscle by SDS-PAGE and two-dimensional electrophoresis (2-DE) after 0, 2, 4, and 6 days cold storage. They indicated very few changes in protein patterns and revealed the gradual disappearance of a protein band of 16 kDa immediately after fish death. Mackie et al. [24] identificated species of smoked and gravad fish products by SDS-PAGE, urea isoelectric focusing and native isoelectric focusing. They found that the exception of *S.alpinus*, the profiles of the smoked and gravad samples changed to varying extents from those of the corresponding raw reference profiles as a result of the processing.

There was a significant difference in protein quantity of samples by months storing at -20 °C ($P<0.01$). The crude protein content rainbow trout ranged between 18.34 (raw sample) to 26.10 % for smoked samples. This increase could be attributed to the applied salting and heat treatments. The protein content of the smoked fish products increased with salting and heat treatment because of decreasing water amount[25].

Keyvan *et al*.[26] investigated the effect of duration of frozen storage on chemical analysis, lipid damage and extractability of myofibrillar proteins of Kutum (*Rutilus frisi kutum*). They showed that the major alternation in fish tissue occurs largely in the myofibrillar proteins.

Unlusayin et al.[8] studied chemical changes in rainbow trout, pike perch and eel after hot smoking. They defined 18 protein bands in rainbow trout, 12 bands in pike perch, 14 bands in eel, with the disapparing of the most bands after smoking and storage on SDS-PAGE.

Michalczyk et al.[27] investigated the type and scope of changes in rainbow trout (*Oncorhynchus mykiss*) gravad proteins during processing and vacuum storage at +3°C and -30 °C. As a result of SDS-PAGE analysis, they determined that when gravads were cold stored for 8 weeks intensity of 274 kDa, 160 kDa bands and the C-protein increased compared

to the fresh gravad. They showed that lowering of relative intensity of bands representing actin and myosin heavy chain. On the other hand, they stated that lowering actin and MHC bands could be due to their degradation caused by cathepsins.

The results of the polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that protein band intensities started to decrease in the 2nd month in marinated and smoked samples excluding raw samples. Some protein bands were relatively reduced in the later weeks and even some bands completely disappeared. However, SDS-PAGE analyses revealed that intensity of the myosin heavy chain, actin, tropomyosin, troponin-T, myosin light chain 1, and myosin light chain 2 bounds was not reduced until 9th months.

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REFERENECES

[1] Poulter, R.G., Ledward, D.A., Godber, S., Hall, G., Rowlands, B. (1985) .Heat stability of fish muscle proteins. J Food Technol, 20: 203-217.

[2] Badii, F., Howell. N.K. (2001) A comparision of biochemical changes in cod (*Gadus morha*) and haddock (*Melnogrammus aeglefinus*) fillets during frozen storage. J Sci Food Agr, 82: 87-97.

[3] Celik, M., Diller, A., Kucukgulmez, A.A. (2005). Comparision of the proximate composition and fatty acid profiles of Zander (*Sander lucioperca*) from two different region and climatic condition. J Agr Food Chem, 92: 637-641.

[4] Ledword, D.A. (1979). Protein-polysaccharides interactions In, Blansshard, M.V. and Mitchell, J.R. (Eds): Polysaccharidesin in Food pp. 56-78, Butterwolih and Co. Ltd Sydney.

[5] Opstvedt, J., Miller, R., Hardy, R.W., Spinelli, J. (1984). Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairneri*). J Agr Food Chem, 32: 929–935.

[6] Murueta, J.H.C., Toro, M.A.N., Carreno, F.G. (2007). Concentratesof fish protein from by catch species produced by various drying processes. Food Chem, 100: 705–711.

[7] Opstvedt, J. (1988). Influence of drying and smoking on protein quality. Fish Smoking and Drying, In**,** Burt J R (Ed): Elsevier Applied Science Publishers *LTD*. pp. 41-53, London and New York.

[8] Ünlüsayın, M., Kaleli, S., Gulyavuz, H. (2001). The determination of flesh productivity and protein compotents of some fish species after hot smoking. J Sci Food Agr, 81: 661-664.

[9] Kolodziejska, I., Niecikowska, C., Januszewska, E., Sikorski, Z.E. (2002). The microbial and sensory quality of mackerel hot smoked in mild conditions. Lebensm-Wiss Technol 35: 87-92.

[10] Kılınc, B., Caklı, S. (2004). Chemical, microbiological and sensory changes in thawed frozen fillets of sardine (*Sardina pilchardus*) during marination. Food Chem, 88(2): 275-280.

[11] Basti, A.A., Misaghi, A., Salehi, T.Z., Kamkar, A. (2006). Bacterial pathogens in fresh, smoked and salted Iranian fish. Food Control, 17(3):183-188.

[12] Sallam, K.I., Ahmed, A.M., Elgazzar, M.M., Eldaly, E.A. (2007). Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4 °C. Food Chem, 102(4):1061-1070.

[13] Yanar, Y., Küçükgülmez, A., Ersoy, B., Çelik, M. (2007). Cookıng effects on fatty acid composition of cultured sea bass (*Dıcentrarchus Labrax*) fillets. J Muscle Foods, 18(1): 88–94.

[14] AOAC, (1990). Official methods of analysis, 15th ed. Association of the Offical Analtical Chemists, 15th edn. AOAC, Arlington, Virginia, 405 p.

[15] Xiong, S., Xiong, Y.L., Blanchard, S.P., Wang, B., Tidwell, J.H. (2002). Evalution of tenderness in prawns (*Machrobrachium rosenbergii*) marinated in various salt and acid solitions. Int J Food Sci Tech, 37: 291-296.

[16] An, H., Marshall, M.R., Otwell, W.S., Wei, C.I. (1988). Electrophoretic identification of raw and cooked shrimp using various protein extraction system. J Food Sci, 53(2): 313-318.

[17] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J Biol Chem, 193: 265-271.

[18] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

[19] Srinivasan, S., Xiong, Y.L., Blanchard, S.P., Tidwell, J.H. (1997). Physicochemical changesin prawns (*Macrobrachium rosenbergii*) subjected to multiple freze-thaw cycles. J Food Sci, 62: 123-127.

[20] Clucas, I.J., Ward, A.R. (1996). Post-Harvest Fisheries Development: A guide to Handling, Preservation, Processing and Quality. Chathman Maritime, Kent ME44TB, United Kingdom

[21] Bauchart, C., Chambon, C., Mirand, P.P., Savary-Auzeloux, I., Remond, D., Morzel, M. (2007). Peptides in rainbow trout (*Oncorhynchus mykiss*) muscle subjected to ice storage and cooking. Food Chem, 100(4): 1566-1572.

[22] Hultmann, L., Røra, A.M.B., Steinsland, I., Skara, T., Rustad, T., (2004). Proteolytic activity and properties of proteins in smoked salmon (Salmo salar)- effects of smoking temperature. Food Chem, 85(3), 377-387.

[23] Verrez-Bagnis,V., Ladrat, C., Morzel, M., Noel, J., Fleurence, J., (2001). Protein changes in post mortem sea bass (*Dicentrarchus labrax*) muscle monitored by one-and two dimensional gel electrophoresis. Electrophoresis, 22: 1539-1544.

[24] Mackie, I., Craig, A., Etienne, M., Jérôme, M., Fleurence, J., Jessen, F., Smelt, A., Kruijt, A., Yman, I.M., Ferm, M., Martinez, I., Pérez-Martín, R., Piñeiro, C., Rehbein, H., (2000). Species identification of smoked and gravad fish products by sodium dodecylsulphate polyacrylamide gel electrophoresis, urea isoelectric focusing and native isoelectric focusing: a collaborative study. Food Chem, 71(1): 1-7.

[25] Kolsarıcı, N., Özkaya, Ö. (1998). Effect of smoking methods on shelf-life of rainbow trout (*Salmo gairdneri*), Turk J Vet Anim Sci, 22: 273-284.

[26] Keyvan, A., Moini, S., Ghaemi, N., Haghdoost, A.A., Jalili, S., Pourkabir, M. (2008). Effect of frozen storage time on the lipid deterioration and protein denaturation during Caspian Sea white fish (*Rutilus frisi kutum*). J Fish Aquat Sci, 3(6): 404-409.

[27] Michalczyk, M., Surowka, K. (2007). Changes in protein fractions of rainbow trout (*Oncorhynchus mykiss*) gravads during production and storage. Food Chem, 104(4): 1006–1013.

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FIGURES

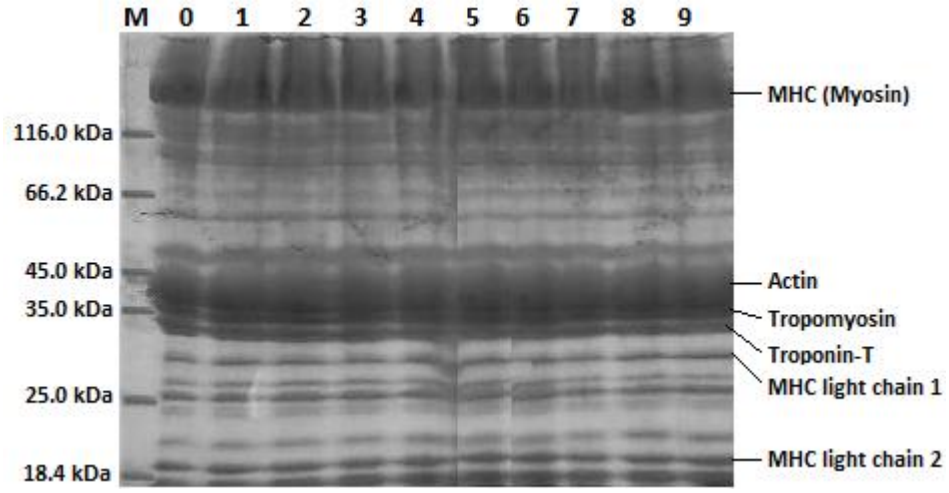


Figure 1. The comparison of total proteins of monthly frozen stored (-20 °C) raw rainbow trout (*Oncorhynchus mykiss*) samples in SDS-PAGE (M: marker, 0: control, 1-9: 1-9 months respectively)

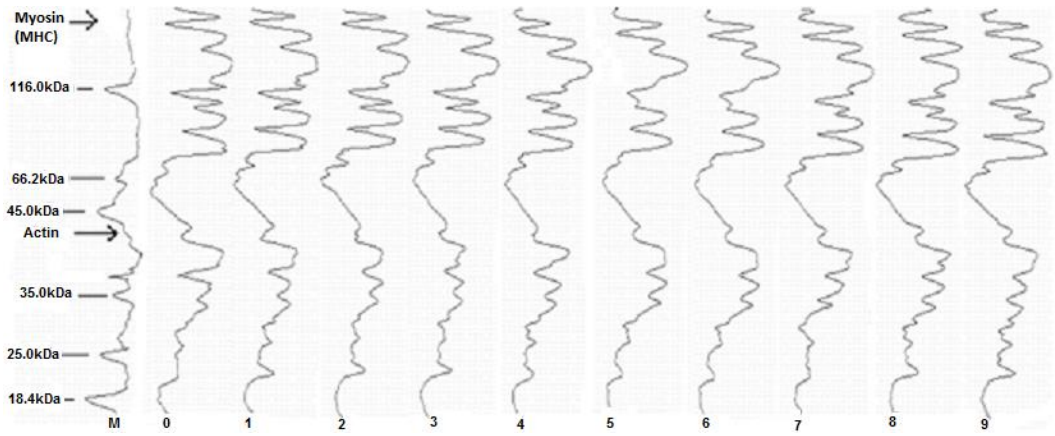


Figure 2. The densitometric analysis of protein bands of monthly frozen stored (-20 °C) raw rainbow trout (*Oncorhynchus mykiss*) (M: marker, 0: control, 1-9: 1-9 months respectively)

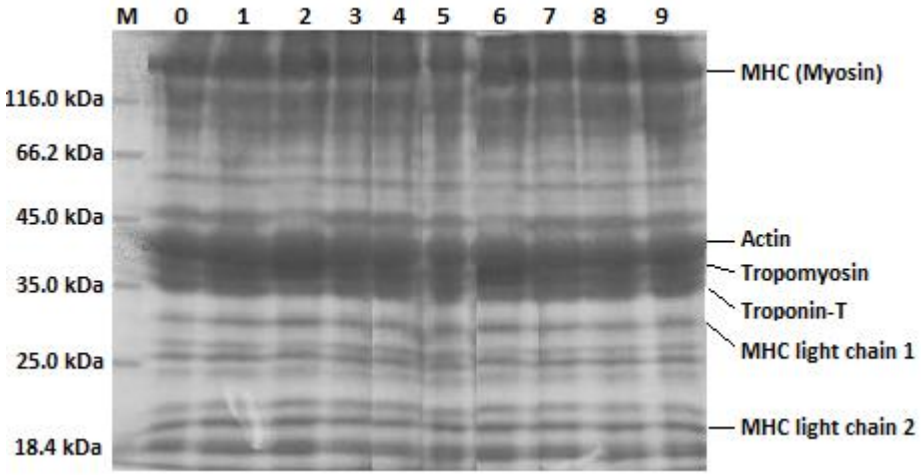


Figure 3. The comparison of total proteins of monthly frozen stored (-20 °C) marinated rainbow trout (*Oncorhynchus mykiss*) samples in SDS-PAGE (M: marker, 0: control, 1-9: 1-9 months respectively)

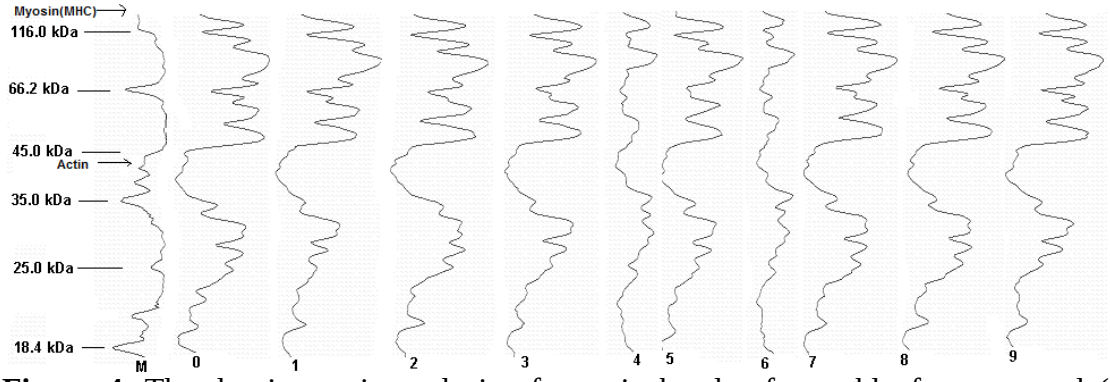


Figure 4. The densitometric analysis of protein bands of monthly frozen stored (-20 °C) marinated rainbow trout (*Oncorhynchus mykiss*) (M: marker, 0: control, 1-9: 1-9 months respectively)

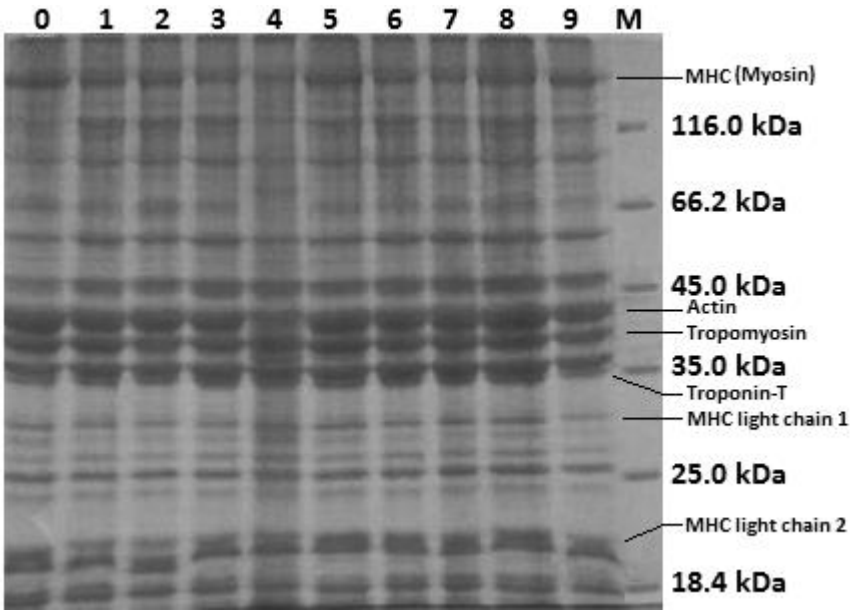


Figure 5. The comparison of total proteins of monthly frozen stored (-20 °C) smoked rainbow trout (*Oncorhynchus mykiss*) samples in SDS-PAGE (M: marker, 0: control, 1-9: 1-9 months respectively)

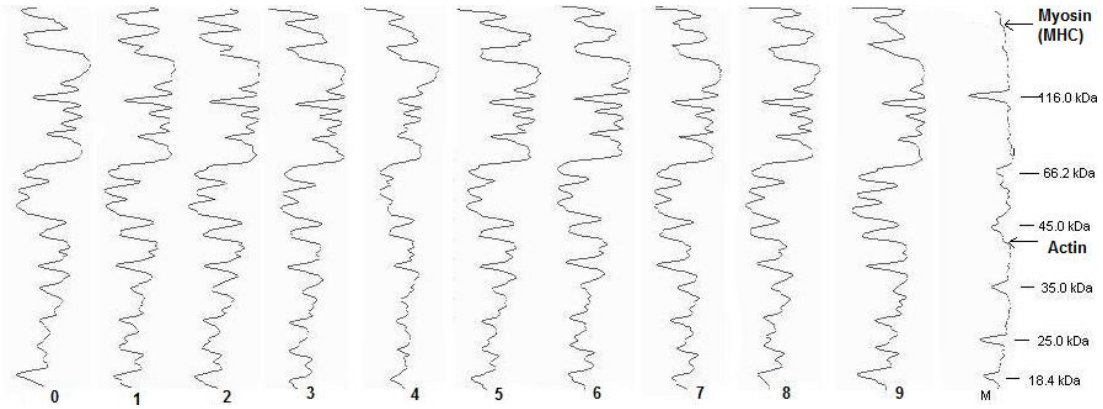


Figure 6. The densitometric analysis of protein bands of monthly frozen stored (-20 °C) smoked rainbow trout (Oncorhynchus mykiss) (M: marker, 0: control, 1-9: 1-9 months respectively)

TABLE

Table 1. Protein content (%) of frozen stored (-20 °C) rainbow trout (*Oncorhynchus mykiss*)

Months	**Protein (%)**		
	Raw	**Marinated**	**Smoked**
1	18.34±0.14^{a}	17.63±0.05^{a}	24.15±0.32^{cd}
2	18.63±0.31^{a}	17.65±0.09^{a}	24.27±0.14^{bcd}
3	18.47±0.28^{a}	16.99±0.02^{bc}	24.10±0.42^{cd}
4	18.59±0.31^{a}	16.85±0. 10^{cde}	25.05±0.46^{ab}
5	18.75±0.23^{a}	16.89±0.07^{bcd}	25.61±0.14^{a}
6	18.52±0.32^{a}	17.33±0.11^{abc}	25.34±0.31^{a}
7	18.49±0.31^{a}	16.73±0.02^{cde}	25.06±0.02^{ab}
8	18.65±0.23^{a}	16.47±0.35^{de}	25.57±0.09^{a}
9	18.04±0.15^{a}	17.07±0.06^{bc}	24.83±0.14^{abc}
10	17.89±0.45^{a}	16.40±0.15^{e}	23.82±0.06^{d}

The variation between values shown with different letters in the same column is significant ($P<0.01$)