Effects of cooking methods on electrophoretic patterns of rainbow trout

Makbule Baylan, 1 Bahri D. Ozcan, 2 Aygül Kucukgulmez, 3 Yasemen Yanar 1
1 Faculty of Fisheries, Cukurova University, Adana, Turkey
2 Faculty of Arts and Sciences, Osmaniye Korkut Ata University, Osmaniye, Turkey

Abstract

The aim of this study was to determine the effects of different cooking methods on the electrophoretic patterns of rainbow trout (Oncorhynchus mykiss) fillets using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Raw rainbow trout were deep-fried, microwaved, grilled, and baked and then monitored for changes in the electrophoretic pattern. All cooking methods resulted in significant moisture loss when compared to the raw sample (P<0.05). Water losses, occurring during cooking resulted in a higher protein content in all of the cooked fish, with regard to raw fish. Deep fried fish had higher lipid content than raw or other cooked fish. The electrophoretic pattern of samples showed a considerable number of protein bands. The bands did not disappear completely, but changed remarkably after cooking. Considering myosin and actin bands, the highest rate of bands disappearance was observed among which frying, grilling, baking and microwaving are most common. There have been many notable electrophoretic studies dealing with the tissue changes of various fish species after freezing (Owusu-Ansah and Hultin, 1986; LeBlanc and LeBlanc, 1989; Ragnarsson and Regenstein, 1989; Türköz et al., 2000), smoking (Ünlüsayın et al., 2001), microwaving (Yowell and Flurkey, 1986), and irradiation, ice and chilled storage (Al-Kahtani et al., 1998; Aubourg et al., 2005; Silva et al., 2006). However, no research related to the effects of cooking methods on the electrophoretic patterns of rainbow trout has been encountered yet.

The purpose of this study, therefore, was to determine the effects of various cooking such as frying, baking, grilling, and microwave cooking on the electrophoretic patterns of rainbow trout (Oncorhynchus mykiss) fillets using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and methods

Preparation of samples

Rainbow trout (Oncorhynchus mykiss, WAL-BAUM 1792), (60 individuals; average weight and length: 170.18±4.31 g and 29.75±0.36 cm, respectively) was obtained from a local rainbow trout farm in Adana (Turkey), stored in ice in an insulated box and transferred to the laboratory. The head, scales and viscera were removed from each fish, and two fillets were obtained from each carcass. Fillet samples were randomly divided into five groups (24 fillets each): the first group for analyzing in raw, and the other four groups for analyzing after grilling, baking, deep frying and microwave cooking, respectively. Grilling: samples were grilled with an electrically operated grill at 180°C 20 min. Baking: the oven having been preheated at 180°C for 20 min, samples were put into and held at this temperature for 10 min on each side. Frying: samples were deep-fried at 180°C for 4 min (2 min for each side) in a conventional frying pan with 500 ml sunflower oil per 250 g fillet. After frying, excess fat was removed by gently pressing the fillets with filter paper. Microwave cooking processes were carried out in a microwave oven at 2450 MHz for 10 min. Cooking was done in the conventional cooking ways benefited by common people in their houses when preparing fish for a meal, but no salt or additional ingredients were added. Sunflower oil, which is the most common oil in Turkish cuisine, was used in deep-frying. Raw and cooked samples were homogenized with a blender.

Proximate analysis

The samples were homogenized and subjected to moisture and ash analyses using AOAC (1995) methods. Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl’s method (AOAC, 1995). Lipid content was determined by the method of Bligh and Dyer (1959). The data with respect to proximate composition were subjected to analyses of variance (one-way ANOVA) at the 5% level, using Duncan’s multiple range test (Duncan, 1955).

SDS-PAGE

Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine proteolytic changes in raw and cooked rainbow trout fillets using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify the different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Rechelt and Parrish, 1983). In Turkey, fish is generally marketed as fresh, chilled or frozen and it is consumed primarily in traditional ways, among which frying, grilling, baking and microwaving are most common. There have been many notable electrophoretic studies dealing with the tissue changes of various fish species after freezing (Owusu-Ansah and Hultin, 1986; LeBlanc and LeBlanc, 1989; Ragnarsson and Regenstein, 1989; Türköz et al., 2000), smoking (Ünlüsayın et al., 2001), microwaving (Yowell and Flurkey, 1986), and irradiation, ice and chilled storage (Al-Kahtani et al., 1998; Aubourg et al., 2005; Silva et al., 2006). However, no research related to the effects of cooking methods on the electrophoretic patterns of rainbow trout has been encountered yet.

The purpose of this study, therefore, was to determine the effects of various cooking such as frying, baking, grilling, and microwave cooking on the electrophoretic patterns of rainbow trout (Oncorhynchus mykiss) fillets using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and methods

Preparation of samples

Rainbow trout (Oncorhynchus mykiss, WAL-BAUM 1792), (60 individuals; average weight and length: 170.18±4.31 g and 29.75±0.36 cm, respectively) was obtained from a local rainbow trout farm in Adana (Turkey), stored in ice in an insulated box and transferred to the laboratory. The head, scales and viscera were removed from each fish, and two fillets were obtained from each carcass. Fillet samples were randomly divided into five groups (24 fillets each): the first group for analyzing in raw, and the other four groups for analyzing after grilling, baking, deep frying and microwave cooking, respectively. Grilling: samples were grilled with an electrically operated grill at 180°C 20 min. Baking: the oven having been preheated at 180°C for 20 min, samples were put into and held at this temperature for 10 min on each side. Frying: samples were deep-fried at 180°C for 4 min (2 min for each side) in a conventional frying pan with 500 ml sunflower oil per 250 g fillet. After frying, excess fat was removed by gently pressing the fillets with filter paper. Microwave cooking processes were carried out in a microwave oven at 2450 MHz for 10 min. Cooking was done in the conventional cooking ways benefited by common people in their houses when preparing fish for a meal, but no salt or additional ingredients were added. Sunflower oil, which is the most common oil in Turkish cuisine, was used in deep-frying. Raw and cooked samples were homogenized with a blender.

Proximate analysis

The samples were homogenized and subjected to moisture and ash analyses using AOAC (1995) methods. Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl’s method (AOAC, 1995). Lipid content was determined by the method of Bligh and Dyer (1959). The data with respect to proximate composition were subjected to analyses of variance (one-way ANOVA) at the 5% level, using Duncan’s multiple range test (Duncan, 1955).

SDS-PAGE

Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine proteolytic changes in raw and cooked rainbow trout fillets using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify the different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Rechelt and Parrish, 1983). In Turkey, fish is generally marketed as fresh, chilled or frozen and it is consumed primarily in traditional ways, among which frying, grilling, baking and microwaving are most common. There have been many notable electrophoretic studies dealing with the tissue changes of various fish species after freezing (Owusu-Ansah and Hultin, 1986; LeBlanc and LeBlanc, 1989; Ragnarsson and Regenstein, 1989; Türköz et al., 2000), smoking (Ünlüsayın et al., 2001), microwaving (Yowell and Flurkey, 1986), and irradiation, ice and chilled storage (Al-Kahtani et al., 1998; Aubourg et al., 2005; Silva et al., 2006). However, no research related to the effects of cooking methods on the electrophoretic patterns of rainbow trout has been encountered yet.

The purpose of this study, therefore, was to determine the effects of various cooking such as frying, baking, grilling, and microwave cooking on the electrophoretic patterns of rainbow trout (Oncorhynchus mykiss) fillets using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
cooked samples (Laemmli, 1970). This was done with a SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA, USA) using a 3% acrylamide stacking gel and a 10% acrylamide resolving gel (Srinivasan et al., 1997). Raw samples for electrophoresis were prepared by homogenizing 1 g minced raw muscle in 100 mL cold (~5°C) distilled deionized water with an Ultratorax for 30 s. The homogenate was diluted 1:1 in the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol and 10% β-mercaptoethanol, yielding a sample protein concentration of approximately 1 mg mL-1, assuming a 20% protein content in raw muscle tissue (Xiong et al., 2002). The cooked samples were chopped, mixed in a baker with 1% SDS (contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% (w/v) sodium azide) at a ratio of 1:3 (w/v), and homogenized using a Polytron at room temperature for 1 min. The samples were then centrifuged for 20 min at room temperature (An et al., 1988), the supernatants were collected, and protein contents determined by Lowry method (Lowry et al., 1951). All raw and cooked samples 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. After the electrophoresis, the gel was stained for 1 hour with Coomassie blue R 250 dye in methanol-acetic acid-water solution (4:1:5, by volume) and destained in the same solution without dye.

**Image analysis**

The gels were scanned and the images analysed with the Image program, version 1.40. Molecular weights of protein bands were calculated and densitometric analysis were performed.

**Results and discussion**

Data on moisture, protein, lipid and ash contents of the raw and cooked rainbow trout are presented in Table 1. There was a significant total moisture loss in all the cooking methods when compared to the uncooked sample (P<0.05), with the deep-fried having the greatest loss and microwave-cooked showing the second largest loss. The mean moisture of the baked and grilled samples did not differ significantly from each other, but did differ from the other cooking methods and raw sample. Water losses, occurring during cooking resulted in a higher protein content in all of the cooked fish, with regard to raw fish. There was an apparent net increase in protein levels of cooked rainbow trout compared to the raw ones. Frying of fish, which is commonly practiced, resulted in higher amount of protein. This is in accordance with the findings of Gall et al., (1983) where deep fried fish fillet had significantly higher protein than raw fillet. Total lipids in raw and baked samples did not differ significantly. Deep fried fish had higher lipid content than raw or other cooked fish, mainly due to the absorption of fat by the fish. Similar findings have been reported in Mai et al., (1978).

Changes in rainbow trout caused by cooking methods were followed by SDS-PAGE. Figure 1 shows the effect of cooking methods on rainbow trout electrophoretic pattern. The electrophoretic pattern of samples showed a considerable number of protein bands and thus, all the major proteins generally present in fish. Through electrophoretic analysis, it was seen that the bands, particularly myosin and actin, did not disappear completely, but changed remarkably after cooking. It can be thought that, if some cleavage of MHC and actin into smaller polypeptide chains occurs, the nonappearance and/or decreasing of these bands in our gel would indicate that these are smaller than 5 kDa. It was reported that protein bands with molecular weights lower than 5 kDa are not separated in the SDS-PAGE (Silva et al., 2006). In this study, while density of the myosin bands were decreased 34, 60, 57 and 52%, the actin bands were decreased 41, 63, 59 and 48% in deep-fried, microwaved, grilled and baked respectively (Figure 2).

The comparison of the effect of the different cooking methods in the MHC/A ratio showed 1.040, 1.153, 1.130, 1.086 and 0.963 in the raw, deep-frying, microwave, grilling and baking respectively. These results show that, actin density was decreased in raw, deep-fried, microwaved and grilled samples towards to MHC, but it was increased in the baked sample. There have been many studies of qualitative changes in protein of various fish species using SDS-PAGE, but these seem to be focused on or dealt with only frozen samples. In these studies, some losses were reported on actin and myosin bands (LeBlanc and LeBlanc, 1989; Türköz et al., 2000). However, there is not enough study regarding the effects of cooking methods on muscle proteins of any fish in the literature yet. Among these few studies, Ünlüsayın et al. (2001) reported remarkable losses on the bands of pike perch, rainbow trout and eel after smoking.

**Table 1. Proximate composition of raw and cooked rainbow trout in percentage.**

|            | Raw   | Microwave-cooked | Baked | Grilled | Deep-fried |
|------------|-------|------------------|-------|---------|------------|
| Moisture   | 69.2±4.59a | 57.7±4.10b      | 61.9±2.00b | 60.39±0.60b | 38.77±0.87a |
| Protein    | 22.46±0.22a | 31.38±0.91b      | 28.77±1.01a | 29.0±0.61c  | 40.09±0.61a |
| Lipid      | 6.27±0.13a | 10.52±0.12b      | 7.71±0.14c  | 8.05±0.70c  | 19.03±1.42a |
| Ash        | 1.35±0.02a | 2.37±0.02b       | 2.00±0.00a  | 1.99±0.07c  | 2.68±0.04b  |

*within the row values with different letters are significantly different (P<0.05). Data are shown as means±SD.

**Conclusions**

Taking into account the above findings and the results of the present study, it is clear that remarkable changes affect the quality the proteins in the different cooking methods. Although deep-fried fish had a higher level of lipid than raw and other cooked rainbow trout,
considering of actin and myosin bands, lowest rate of band disappearance was observed with deep-fried samples.

References

Al-Kahtani, H.A., Abu-Tarboush, H.M., Atia, M., Bajar, A.S., Ahmed, M.A., El-Mojaddidi, M.A., 1998. Amino acid and protein changes in tilapia and Spanish mackerel after irradiation and storage. Radiat. Phys. Chem. 51:107-114.

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

AOAC, 1995. Official Methods of Analysis, 16th Ed. Association of Official Analytical Chemists, Washington, DC, USA.

Aubourg, S.P., Piñeiro, C., Gallardo, J.M., Velazquez, J.B., 2005. Biochemical changes and quality loss during chilled storage of farmed turbot (Psetta maxima). Food Chem. 90:445-452.

Bechtel, P.J., Parrish, F.C.J., 1983. Effects of postmortem storage and temperature on muscle protein degradation: analysis by SDS gel electrophoresis. J. Food Sci. 48:294-297.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys. 37:911-917.

Bognár, A., 1998. Comparative study of frying to other cooking techniques influence on the nutritive value. Grasas Aceites 49:250-260.

Deman, J.M., 1999. Principles of Food Chemistry. 3rd ed., Aspen Publ., Inc., Gaithersburg, MD, USA.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Finot, P.A., 1997. Effects of Processing and Storage on the Nutritional Value of Food Proteins. In: S. Damodaran and A. Paraf (eds.) Food Proteins and their Applications. Marcel Dekker, Inc., New York, NY, USA, pp 551-557.

Gall, K.L., Otwell, W.S., Koburger, J.A., Appledorf, H., 1983. Effects of four cooking methods on proximate, mineral and fatty acid composition of fish fillets. J. Food Sci. 48:1068-1074.

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

LeBlanc, E., LeBlanc, R.J., 1989. Separation of cod (Gadus morhua) fillet proteins by electrophoresis and HPLC after various frozen storage treatments. J. Food Sci. 54:827-834.

Lowry, O.H., Rosebrough, N.T., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Mai, J., Shimp, J., Wehrauch, J., Kinsella, J.E., 1978. Lipids of fish fillets: Changes following cooking by different methods. J. Food Sci. 48:1068-1074.

March, B.E., 1984. Effect of processing on the nutritive value of foods. In: R.J.R. Miloslave (ed.) Handbook of Nutritive Value of Processed Food: Food for Human Use. CRC Press Inc., Boca Raton, FL, USA, pp 363-381.

Owusu-Ansah, Y.J., Hultin, H.O., 1986. Chemical and physical changes in red hake fillets during frozen storage. J. Food Sci. 51:1402-1406.

Ragnarsson, K., Regenstein, J.M., 1989. Changes in electrophoretic patterns of gadoid and non-gadoid fish muscle during frozen storage. J. Food Sci. 54:819-823.

Silva, H.A., Mendes, R., Nunes, M.L., Empis, J., 2006. Protein changes after irradiation and ice storage of horse mackerel (Trachurus trachurus). Eur. Food Res. Technol. 224:83-90.

Srinivasan, S., Xiong, Y., Blanchard, S.P., Ticidwell, J.H., 1997. Physicochemical changes in prawns (Macrobrachium rosenbergii) subjected to multiple freeze-thaw cycles. J. Food Sci. 62:123-127.

Türköz, Y., Arslan, A., Gönulalan, Z., Ileri, T., 2000. Electrophoretic identification of fish species using various extraction systems, and investigation of the effect of frozen storage and cooking on fish muscle proteins. Firat University Journals of Health Sciences 14:31-38.

Ünlüsayin, M., Kaleli, S., Gülyavuz, H., 2001. The determination of flesh productivity and protein components of some fish species after hot smoking. J. Sci. Food Agr. 81:661-664.

Xiong, S., Xiong, Y., Blanchard, P., Wang, B., Ticidwell, J., 2002. Evaluation of tenderness in prawns (Macrobrachium rosenbergii) marinated in various salt and acid solutions. Int. J. Food Sci. Tech. 37:291-296.

Yowell, K., Flurkey, W.H., 1986. Effect of freezing and microwave heating on proteins from codfish fillets: Analysis by SDS Polycrylamide gel electrophoresis. J. Food Sci. 51:508-509.