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PAPER

Effects of cooking methods on electrophoretic patterns of rainbow trout

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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 with microwaved sample, while the lowest rate occurred with deep-fried sample.

Introduction

Fish is rarely eaten raw but is usually cooked in different ways before consumption. During cooking, chemical and physical reactions take place that improve or impair the food nutritional value (Finot, 1997; Bognar, 1998). Moreover, this effect is also dependent on the type of cooking (Gall *et al*., 1983). In general during fish heating (cooking and sterilization), sarcoplasmic and myofibrillar proteins are denaturated and coagulated. In heat, denaturation of flesh proteins, H-bonds, which are involved in the secondary and tertiary structures of proteins, are broken, resulting in an unfolding of the native configuration. The extent of these changes depends on the temperature and time and affects the yields and final quality of the fishery product. According to March (1984) and Deman (1999) cooking of fish causes solubilization of proteins and hence leads to loss of proteins from the final product.

SDS-PAGE (Sodium Dodecyl Sulphate-Polycarylamide Gel Electrophoresis) has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Bechtel 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*, WALBAUM 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 on 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

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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).

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

	Raw	Microwave-cooked	Baked	Grilled	Deep-fried
Moisture	69.24±0.59^{a}	57.74±1.04^{c}	61.91±0.20^{b}	60.39±0.60^{b}	38.77±0.87^{d}
Protein	22.46±0.22^{d}	31.38±0.91^{b}	28.77±0.10^{c}	29.60±0.61^{c}	40.09±0.61^{a}
Lipid	6.27±0.13^{d}	10.52±0.12^{b}	7.71±0.14^{dc}	8.05±0.70^{c}	19.03±1.42^{a}
Ash	1.25±0.02^{d}	2.37±0.02^{b}	2.00±0.00^{c}	1.99±0.07^{c}	2.68±0.01^{a}

$^{a-d}$within the row values with different letters are significantly different ($P<0.05$). Data are shown as mean±SD.

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 non-appearance 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.

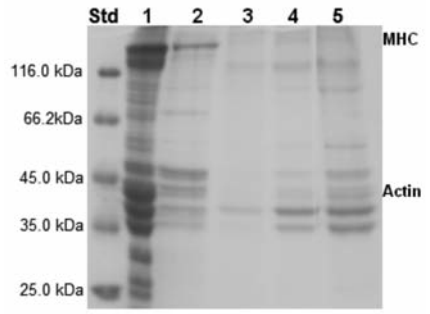

Figure 1. Electrophoretic pattern of rainbow trout subjected to different cooking methods (std, standard; 1, raw; 2, deep-frying; 3, microwave; 4, grilling; 5, baking).

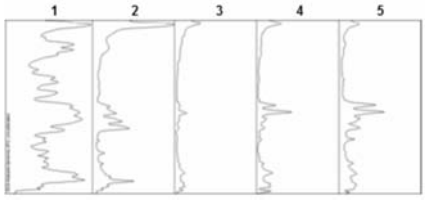

Figure 2. Densitometric analyses of protein bands of raw and cooked samples of rainbow trout (1, raw; 2, deep-frying; 3, microwave; 4, grilling; 5, baking).

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.

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