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Determination of Total Serum Protein Levels Fed by Hot Smoked Rainbow Trout (*Oncorhynchus mykiss*) Diets in Rats

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Abstract: The effects on total serum protein levels of rats fed by hot smoked Rainbow trout (*Oncorhynchus mykiss*) were investigated. Four diets containing fresh and hot smoked rainbow trout flesh and vitamin were prepared and commercial pellet food purchased. Four groups of female Wistar rats were fed with the diets for 28 days. Total serum protein and detection of protein bands using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page) were evaluated. The total serum protein level of rat fed by hot smoked rainbow trout flesh+vitamin diet were increased significantly according to the other groups ($p<0.017$, $p<0.002$). Eight protein bands were visualised on rat gels. The molecular weights of protein bands detected were 166, 112, 90, 63, 47, 45, 36 and 14 kDa, respectively.

Key words: Rat, hot smoked rainbow trout, diet, SDS-Page, serum protein levels

INTRODUCTION

Smoking is one of the oldest methods of food preservation and is still widely used in fish processing. In Europe, about 15% of the total quantity of fish for human consumption is offered on the market in the form of either cold-or hot-smoked products. The temperature of the smoke is in the range 12-25°C during cold-smoking and 25-45°C in warm-smoking. In hot-smoking, the process may be carried out in different stages, during which the temperature of the smoke ranges from about 40-100°C and that in the centre of the product may reach up to 85°C. The rate of deposition of different components depends upon the temperature, humidity, flow rate and density of the smoke, the water solubility and volatility of the particular compounds, as well as on the properties of the surface of the fish (Stolyhwo and Sikorski, 2005).

The key role of nitrite and nitrogen oxides in forming N-nitroso compounds by interaction with secondary and tertiary amino compounds has led to the examination worldwide of foods for the presence of N-nitroso compounds, which have been found almost exclusively in those foods containing nitrite or which have become exposed to nitrogen oxides. Among these are cured meats, especially bacon and especially when cooked (Lijinsky, 1999).

The heating of proteins causes denaturation, which is rupture of the secondary and higher structures. Generally speaking, approximately 90% of the protein is denatured about 60 to 65°C, the remaining 10% (tropomyosin) may be held at 100°C for prolonged period of time without being denatured. At higher temperatures protein suffers thermal degradation. Thus at 115°C losses of cysteine/cystine occur both at low (14%) and high moisture content (Opstvedt *et al*., 1984). The amounts of sarcoplasmic proteins and myofibrillar proteins extractable from fish muscle heated to temperatures up to 100°C were measured (Rehbein, 1992). Changing the physical environment easily

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changes the conformational structure of fish proteins. Treatment with high salt concentrations or heat may lead to denaturation, after which the native protein structure has been irreversibly changed (Huss, 1988).

Some papers have contributed to the biological role of selenium in mammalian cells and tissues. While about 30 selenium-containing proteins or subunits in tissues of mammals have been reported (Gao and Wang, 2000; Chen *et al*., 2002) only a few of them, glutathione peroxidases (GSH-Px) family, type-1 iodothyronine 5'-deiodinase and selenoprotein P, have been identified and sequenced in human tissues. Smoked meat products exhibit an increased resistance to oxidative changes and whilst oxidation can lead to the destruction of some vitamins, it would be expected that certain smoke constituents could help protect such oxidizable vitamins in smoked fish products (Burt, 1986).

The objective of this study was to determine to effect of total serum protein levels fed by hot smoked rainbow trout (*Oncorhynchus mykiss*) diets in rats.

MATERIALS AND METHODS

Sample Preparation

Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) was purchased from a local fish farm (Sağdurlar Balıkçılık-Isparta-Turkey). One hundred and ten rainbow trout (200-250 g fish^{-1}) were used in this research. Fish samples were prepared and hot smoked using an AFOS-type mechanical kiln according to a smouldering method previously described (Unlusayin *et al*., 2001). Briefly, the viscera of the fish were removed and the leftovers such as blood, mucus and tissue pieces were washed away with a large amount of water. The fish were kept in 20% (w/w) salt solution (fish/brine solution ratio 1:1) at 16°C for a period of 60 min. The fish were then removed from the brine solution, hung on the kiln, strained and kept at 20°C for about 20 min. Oak sawdust was used for smoking. For the first 45 min a temperature of 30°C was applied. Over the next 180 min the temperature was gradually increased to 50, 60 and finally 70°C. During the final 45 min the temperature was kept at 80°C. The whole smoking process took about 4.5 h. Previously filleted and after minced samples of fresh and smoked rainbow trout flesh were stored for 5 weeks at 4°C constant temperature. Fish samples were used for rat diets.

Diet Preparation and Analyses

Diets and feeding procedure prepared according to the method of Zupthen *et al*. (2001). Four experimental diet groups were planned and 30 g food used per day for each rat. Animals were allowed commercial pellet food (Group B-initial) and fresh rainbow trout flesh (Group K-control) and hot smoked rainbow trout (Group D) and hot smoked rainbow trout flesh+vitamin (ACE plus selenium-Koçak-FARM-Turkey, 30 mg vitamin per day for each rat; contents: 200 mg natural β-carotene, 200 mg vitamin C, 200 mg natural vitamin E, 50 mg Selenhefe and red iron oxide, lecithin, aerosil 200), (Group V).

Moisture content was determined according to AOAC (1996). Lipid (fat) content was determined according to Soxhlet methods described in Keskin (1980). Crude ash (550±1°C) was determined according to TSE 1746 (1974). Crude protein content (N×6.25) was calculated using the Kjeldahl (1983) method.

Biological Assay

Thirty-two female Wistar-Albino rats (3 months age), initial body weight 140±0.4 g (mean±SEM), provided by the Veterinary Faculty of Konya Selçuk University, were used for biological assay. Eight animals were housed per cage and animals were acclimatized to standard animal laboratory conditions (12 h light/dark cycle, temperature 22±1°C and 50±5% humidity). Four

experimental groups of eight animals were randomly assigned to the dietary treatments. The animals were allowed limited (twice a day) food and had free access to demineralised water. The Suleyman Demirel University, Ethic Committee of School Medicine, approved the study.

The seven days of adaptation period and 28 days of experimental period of rats were continuous.

Chemicals

The biuret reagent solution (R2) containing 0.024 mol L^{-1} copper sulfate plus 0.063 mol L^{-1} sodium and potassium tartrate plus 0.03 mol L^{-1} potassium iodate in a 0.02 mol L^{-1} sodium hydroxide solution was prepared by dissolving 6.0 g $CuSO_4 \cdot 5H_2O$, 18.0 g $KNAC_4H_4O_6 \cdot 4H_2O$, 5.0 g KI and 8.0 g NaOH in 1000 mL of water (Zaia *et al*., 1999). A 6.0 g L^{-1} BSA (Sigma Chemical Co.) solution was prepared with distilled water and used as standard in all assays.

Total Protein Analysis

On day 28 the animals were anaesthetised using diethyl ether (Merck) and blood was drawn from the heart. Aspirated blood was kept on ice for 30 min, centrifuged (Hettich EBA 85) and serum was collected. All experiment tubes were marked as blind, test and standard. Into the blind tube; 500 μL physiological saline and 500 μL the biuret reagent solution were added. Ten microliters serum and 500 μL physiological saline and 500 μL biuret reagent solution were put in to test tube. The finally, into the standard tube; 10 μL prepared BSA and 500 μL saline (0.9%) and 500 μL the biuret reagent solution were added. All tubes were rinsed approx. Thirty seconds and acclimatized for 5 min at 20±1°C. Afterwards both of tubes measured on spectrophotometer (Shimadzu UV-1201V) at 540 nm against distilled water. Data has been calculated according to the standard concentration.

SDS Polyacrylamide Gel Electrophoresis (SDS-Page)

Discontinuous SDS-Page was prepared using a stacking gel of 2% acrylamide/bis acrylamide (30%) and a separating gel of 5.1% acrylamide/bis acrylamide (30%). Freeze dried protein samples were reconstituted in appropriate amount of Laemmli (1970) sample buffer to achieve a protein concentration of 3 μL and loaded in each well of the gels. Electrophoresis (Mini-Protean II/Bio-Rad) was carried out at 35 mA one slab until the tracking dye reached the bottom of the gel (3 h) in chamber with cooling to approximately 10°C. The molecular weight of each protein band could then be calculated according to the standard curve of purified marker proteins including α Lactalbumin-bovin milk (14.2 kDa), trypsin inhibitor-soybean (20.1 kDa), carbonic anhydrase-bovine erythrocytes (29 kDa), ovalabumin-egg (45 kDa), albumin-bovine (66 kDa), Phosphorylase b-rabbit muscle (97.4 kDa), β. Galactosidase-*E. coli* (116 kDa), Myosin-rabbit muscle (205 kDa) from Sigma (Cat. No: M. 2789). Following electrophoresis, gels were stained with 0.04 Coomassie Brillant Blue R-250 in 2-propanol: acetic acid: water (25:10:65) overnight at room temperature. Excess stain was removed with several washes of the same solution without Coomassie Brillant Blue R-250. Photographs of the gels were taken when still wet, in 7% acetic acid.

Statistics

Statistical analyses were performed using SPSS 10.0 for windows software (SPSS Inc, Chicago, IL). The Mann-Whitney U-test was done for two-sample comparisons (Zar, 1999). Differences in the means between groups were analysed by one-way ANOVA. Two-tailed p values were used and statistical significance was at $p<0.05$.

RESULTS

The average weights of rats were determined as 143.31±2.245 g (mean±SEM) at the beginning of experiment and at the end of 143.46±4.440 g (mean±SEM). Differences in weight between initial

Table 1: Chemical composition of fresh and hot-smoked rainbow trout samples (on wet weight basis). Values are shown as mean±standard deviation of triplicate measurements

Rainbow trout	Water	Protein	Lipid	Ash	Carbohydrate
	---------- (%) ----------				
Fresh	76.02±0.17[a]	17.24±0.01[b]	3.98±0.46[b]	1.93±0.28[b]	0.83±0.45[b]
Hot smoked	63.15±0.02[b]	23.57±0.28[a]	5.76±0.11[a]	3.92±0.12[a]	3.60±0.21[a]

Different letter(s) within a column indicate significant differences between groups ($p<0.05$). Values are reported as means±SD

Table 2: The comparison of total serum proteins in rats fed by several diet groups values are shown as mean±standard deviation of triplicate measurements

Groups	N	X±SD (g/100 mL)	Median	p*
B	7	4.94±0.62	4.83	p>0.05
K	7	4.86±0.18	4.83	
B	7	4.94±0.62	4.83	p<0.017
V	7	6.29±1.09	6.75	
B	7	4.94±0.62	4.83	p>0.05
D	6	5.25±0.70	5.00	
K	7	4.86±0.18	4.83	p<0.002
V	7	6.29±1.09	6.75	
K	7	4.86±0.18	4.83	p<0.05
D	6	5.25±0.70	5.00	
V	7	6.29±1.09	6.75	p<0.05
D	6	5.25±0.70	5.00	

*: p>0.05 as statistically were not significant in averages. B: Initial, K: Control, V: Hot smoked rainbow trout+vitamin, D: Hot smoked rainbow trout

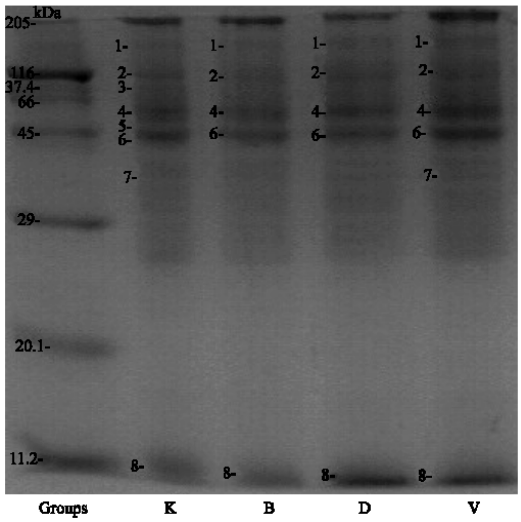

Fig. 1: Separation of total serum proteins of rats using SDS-Page analysis (K: Control, B: Initial, D: Hot smoked rainbow trout, V: Hot smoked rainbow trout+vitamin)

and end of experiment were not significant (p>0.05). During the feeding, all of rats were stayed survival. Table 1 shows the results of chemical composition of fresh and hot-smoked rainbow trout samples. Differences in fat content between fresh and hot smoked rainbow trout were significant (p<0.05).

Table 2 and Figure 1 shows the results of total serum proteins in rats fed by several diet groups.

The total serum protein level of rat of group V, fed by hot smoked rainbow trout flesh+vitamin diet were increased significantly according to the other groups respectively groups BV, groups KV (p<0.017, p<0.002), (Table 2, Fig. 1). Differences in total serum protein levels between group K and B and group D were found insignificant (p>0.05).

The total serum proteins of rats were separated using SDS-Page analysis (Fig. 1). Eight protein bands were visualised on rat gels. The molecular weights of protein bands detected were 166, 112, 90, 63, 47, 45, 36 and 14 kDa, respectively. Number 3 and 5 (90 and 47 kDa) bands were seen only in group K and they were not seen in other groups.

In addition, protein band number 7 (36 kDa) was found in groups K and V. But these bands did not present in groups B and D. Protein band number 4 (63 kDa) was found dense in group V. But this band was reduced in other groups. The number 8 (14 kDa) protein band was very dense containing fish diets groups K, D and V. But this protein band was reduced in group B. Protein band number 6 (45 kDa) was detected dense in groups K and V. However, protein bands number of 1, 2, 4, 6 and 8 (166, 112, 63, 45 and 14 kDa) were found as common proteins in every group.

DISCUSSION

Differences in chemical components between fresh and hot smoked rainbow trout flesh were significant ($p<0.05$). This finding is similar to the results mentioned in a previous study (Unlusayin *et al*., 2001).

Smoked fish constitute a significant part of the human diet, important because of their desirable sensory properties, high nutritional value and abundance, in fatty species, of lipids rich in n-3 fatty acid residues. The wood smoke used in smoking of fish may contain, depending predominantly on the temperature of generation, a large variety of Polycyclic Aromatic Hydrocarbons (PAHs), including the most carcinogenic ones (Stolyhwo and Sikorski, 2005).

According to the Gao and Wang (2000), the selenium-containing protein present in 46-56 kDa band could be probably an isoform of selenoprotein P similar that in rat plasma. Also, selenium in serum may associate with serum proteins non-specifically or weakly, or may be present as small molecules, such as selenoamino acids, selenide, etc., which would be lost from the gel during the separation procedure. We noticed that 47 kDa protein band was found in present study. It may be similar protein band that were described literature.

Normal adult rat serum contains an enzymatic activity that cleaves [^{125}I] rhIGFBP-3 into three proteolytic fragments. This serum activity is lacking in hypoxic rats and restored by rhGH-, but not rhIGFI infusion. Partial characterization of the activity suggests that it is a cation-dependent serine protease. The enzymes responsible for the degradation of IGFBP-3 by human term pregnancy and adult rat serum are different (Rutishauser *et al*., 1993). These findings are not related directly with present study subject but probably our rat serum may be containing similar enzymatic activity in this study. So it could be effect serum protein levels belong to their protein structures.

Some doctors and nutritionists think antioxidant vitamins and minerals may help prevent cancer when included in a healthy, balanced diet. Vitamin A, C and E and the mineral selenium are all anti-oxidants. We do not know for sure whether eating these can help prevent cancer, because this is a very difficult thing to prove. In theory, these antioxidants help to prevent body cells being damaged by oxygen particles called free radicals. The damage can lead to the cells becoming cancerous. As well as being an anti-oxidant, selenium stimulates the immune system. Selenium is an essential trace element to animals and humans. It has been related to a variety of human health problem (Balz, 1999). According to the our study results, the total serum protein levels of rat were reduced in smoked fish diet. But in contradiction the result obtained smoked+vitamin diet was increased. Our results may imply that free radicals, which are present in smoked fish diet, were denatured cell structure and reduced protein levels. These finding is confirmed above theory.

In conclusion, it was defined that protein metabolism became destroy with tissue loss who consumed smoked fish diets. The nourishment by smoked fish diets with containing antioxidant substances was reduced harmful effect of free radicals and there were positive effects on protein metabolism.

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