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The Effects of Different Salting Methods on Extract Loss from Rainbow Trout

Mustafa Ünlüsayin*, Ruhan Erdilal, Bahar Gümüş and Hayri Gülyavuz

Faculty of Fisheries, Akdeniz University, Dumlupınar Bulvarı, 07059 Kampus - Antalya, Turkey
*Corresponding author: munlusayin@akdeniz.edu.tr

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ABSTRACT

In this study, the effects of three different salting methods on extract loss of rainbow trout was investigated. A total of 120 rainbow trouts were divided into 4 equal groups. Fish of group A served as untreated control. In groups B and C, brine with 8 and 20% (w/w) sodium chloride was used for a period of 6 h and 45 min, respectively. In the third method (group D), dry salting was run by inserting a salt layer with fish layer for a period of 12 h. The contents of moisture and total proteins between the control and treated groups were significantly different ($P<0.05$). Molecular weights of protein bands detected in salted trouts were 125, 110, 104, 88, 66, 63, 52, 41, 34, 32 kDa. The protein bands of 125, 110, 104 and 63 kDa density were not seen in samples taken from control rainbow trout. According to total proteins analysis results, salting fish with brine having 20% NaCl was suited as salting method, as the amount of total protein contents of extract loss in this method was found low. In contrary, the total protein content in extract was higher for dry salting method.

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INTRODUCTION

Salting is a popular procedure for preserving fish. Salting methods are simple and involve salt crystals or brine. There are three types of salting of fish: dry salting, wet salting and a combination of the two methods. Length of salting period as well as salt concentration depends on the expected final product (Bellagha *et al*., 2007).

Sodium chloride diffuses to the outside from muscles due to difference in osmotic pressure between the brine and fish muscle. This process does not continue indefinitely: sodium and chlorine ions form a water-binding complex with protein which itself exerts an osmotic pressure and eventually equilibrium is reached (Horner, 1997). In salted fish, where the salt concentration reaches ≈20%, high ionic strength causes contraction of the myofibrils and dehydration of proteins. Also, pH of the medium and the type of salts used for salting can influence the degree of protein denaturation (Martínez-Alvarez and Gómez-Guillén, 2005).

Ünlüsayın *et al*. (2001) and Gökoğlu *et al*. (2004) found that mean moisture, protein, ash and fat contents of raw rainbow trout were 73-75, 16-20, 1-2 and 3-4%, respectively, while Ünlüsayın *et al*. (2001) found that the total proteins were 31761.38 ± 771.08 µg/ml. Protein profiling revealed protein of 12.5, 35.5, 39, 46 and 54 kDa size (Ünlüsayın *et al*., 2001) and 32, 35, 35.6, 37.9, 39.5, 42.7, 50, 51, 61, 63, 86, 96, 102, 107, 117, 135 kDa size (Michalczyk and Surowka, 2007).

Salting fish is likely to remain in good demand by those who value tradition and taste but it has also gained acceptance in innovative products that provide convenience. The aim of the present study was to determine the extract loss of rainbow trout (*Oncorhynchus mykiss*) with different salting methods.

MATERIALS AND METHODS

Experimental fish

A total of 120 rainbow trouts (*Oncorhynchus mykiss*) were used for this investigation. The fish were purchased from a local fish farm in Antalya (Turkey). Mean total body mass and total length of fish was 274.07 ± 30.03g and 28.73 ± 1.13 cm, respectively. Fish were then transported to the laboratory in polystyrene boxes with crushed ice. These fish were divided into four groups (A, B, C and D), with 30 fish in each group.

Salting

The viscera of the fish were removed and the leftovers such as blood, mucus and tissue pieces were washed with large amount of water. Fish of group A served as untreated control. Those of group B were salted with brine having 8% NaCl (w/w) for 6 h, while fish of

group C were salted using brine with 20% NaCl for 45 min. Fish of group D were subjected to dry salting, which was done by inserting a salt layer over fish layer for a period of 12 h. The fish were then removed from the brine and dry salted rainbow trout were taken out and access of salt was removed before analysis. Salting processes were done with a fish to brine ratio of 1:2. Brine salting and dry salting were conducted in plastic containers at 4 ± 1ºC.

Analytical procedures

All muscle tissues from trouts of all groups were analyzed for moisture, dry matter, proteins, fat, ash and NaCl contents. Moisture contents in fish meat were determined according to AOAC (2002a). Crude protein content (Nx6.25) was calculated using the Kjeldahl method (AOAC, 2002b). Lipid (fat) content was determined according to Soxhlet method (AOAC, 2002c). Crude ash (Inorganic matter in meat) was determined according to Official Methods of Analysis (AOAC, 2002d). Sodium chloride was determined by volumetric method (AOAC, 1995).

After removing water from extract, crude ash in extract was calculated (AOAC, 2002d) and organic matter in extract was calculated by using the following formula:
Organic matter in extract (%) = 100 – crude ash in extract

Protein extraction

For protein extraction, 1.5g of minced muscle tissues of rainbow trout were homogenised at 4°C for 1 min in 9.5 ml physiological saline by a mechanical homogenizer (Heidolph, Slient Crusher M model, Heidolph Instruments GmbH & Co KG, Germany). Samples were stirred constantly for 20 min at 2°C and centrifuged at 5000 rpm for 25 minutes at 4°C in an Elektromag (4808p, İkitelli OSB, İstanbul, Turkey). Protein concentration was determined in the supernatant by kit method (Lowry *et al*., 1951). Optical density was measured at 650 nm in Chebios UV/ spectrophotometer (Optimum-One, Chebios s.r.l., Roma, Italy). The rest of the supernatant was freeze-dried and kept at -18°C for further analysis through SDS-PAGE.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

For discontinuous polyacrylamide gel electrophoresis, dilution of a 30% stock solution of acrylamide was prepared, where the total amount (T) of acrylamide+bis was 2% for the stacking gel and 5.1% for resolving gel. Freeze dried protein samples were reconstituted in appropriate amount of Laemmli (1970) sample buffer to achieve the protein concentration of 13 µg/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 (3h) 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 wide range marked protein markers from 6.5 to 205 kDA range including: Aprotinin, bovine lung (6.5 kDa), α-Lactalbumin, bovine milk (14.2 kDa), Trypsin inhibitor, soybean (20 kDa), Trypsinogen, bovine pancreas (24 kDa), Carbonic anhydrase, bovine erythrocytes (29 kDa), Glyceraldehyde-3-phosphate dehydrogenase, rabbit muscle (36 kDa), Ovalbumin, chicken egg (45 kDa), Glutamic dehydrogenase, bovine liver (55 kDa), Albumin, bovine serum (66 kDa), Phosphorylase B, rabbit muscle (97 kDa), β-Galactosidase, *E. coli* (116 kDa), Myosin, rabbit muscle (205 kDa) from Sigma (Cat. No: M. S8445). 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 washing of the same solution without Coomassie Brillant Blue R-250. Photographs of the gels were taken in 7% acetic acid while they were still wet.

Statistical analysis

Statistical analyses were performed using "SPSS 10.0 for Windows software" (SPSS Inc, Chicago, IL). Differences in the means between groups were analyzed by one-way analysis of variance. Duncan's multiple range tests was applied for multiple means comparisons, where necessary.

RESULTS AND DISCUSSION

The changes in moisture contents of fresh and brine salted rainbow trout are shown in Table 1. Total protein values of fresh, brine salted and dry salted rainbow trout and extracts are given in Table 2.

The average moisture content of fresh rainbow trout was 76.59 ± 0.44% in this study (Table 1). The moisture content of fresh rainbow trout has been recorded by Gökoğlu *et al*. (2004) as 73.38 ± 0.015% and by Ünlüsayın *et al*. (2001) as 74.86 ± 0.55%, which are comparable to our findings. It has been reported that protein, ash, fat and moisture contents of rainbow trout change according to nutrition, living area, fish size, catching season, seasonal and sexual variations as well as environmental conditions (Ünlüsayın *et al*., 2001; Gökoğlu *et al*., 2004).

The moisture contents of fresh and brine salted rainbow trout (8 and 20%) did not vary. However, in dry salted rainbow trout, moisture contents were low ($P<0.05$) compared to other groups (Table 1). Dry salting produced considerable loss of constituent water due to heavy uptake of salt (Martínez-Alvarez and Gómez-Guillén, 2006). Protein contents (dry basis) of three salting methods were significantly lower compared to control ($P<0.05$; Table 1). A part of the fat in muscle was transferred to extract. There was a correlation between fat transfer rate from muscle to extract and salt concentration in muscle. The transfer rate was increased with high salt concentration. Amount of inorganic substances in brine and dry salted rainbow trout were higher compared to fresh rainbow trout. The highest ash was in dry salted rainbow trout (14.69 ± 2.26%; Table 1). Difference in ash content of fresh, 20% brine salted and dry salted rainbow trout was significant ($P<0.05$; Table 1). The pH was 6.47 ± 0.03 for the control group and there were significant differences between brine and dry salted groups ($P<0.05$; Table 1). NaCl contents between 8% and 20% brine salted rainbow trout groups showed non significant differences, but there were significant differences between brine and dry salted rainbow trout groups ($P<0.05$; Table 1). Organic matter in extract of dry salted group was higher (2.10 ± 0.61%)

Table1: Chemical composition of rainbow trout samples and extracts

Parameters	Fresh	Brine salted 8 % (w/w)	Brine salted 20 % (w/w)	Dry salted
Moisture in muscle (%)	76.59 ± 0.44[a]	75.02 ± 1.01[a]	75.06 ± 1.58[a]	69.77 ± 4.91[b]
Dry matter (%)	23.41 ± 0.44[b]	24.98 ± 1.00[b]	24.94 ± 1.58[b]	30.22 ± 4.91[a]
Protein in muscle (%)	76.53 ± 1.00[a]	73.31 ± 0.22[b]	72.47 ± 2.10[b]	71.29 ± 0.91[b]
Fat in muscle (%)	13.75 ± 7.55	12.11 ± 4.17	12.87 ± 2.28	11.46 ± 2.04
Ash in muscle (%)	6.58 ± 0.49[c]	9.39 ± 1.21[bc]	11.15 ± 1.29[b]	14.69 ± 2.76[a]
pH	6.47 ± 0.03[bc]	6.52 ± 0.04[b]	6.88 ± 0.05[a]	6.38 ± 0.09[c]
NaCl (%)	—	5.30 ± 0.51[b]	5.94 ± 0.26[b]	11.99 ± 2.83[a]
Solid matter in extract (%)	—	7.03 ± 0.0[c]	21.02 ± 0.54[a]	8.62 ± 0.03[b]
Organic matter in extract (%)	—	2.86 ± 0.19[a]	2.98 ± 0.13[a]	2.10 ± 0.61[b]
Fat in extract	—	1.07 ± 0.15[b]	1.21 ± 0.05[ab]	1.32 ± 0.07[a]

Values are shown as mean ± standard deviation of triplicate measurements; Different superscripts in the same row indicate significant differences between groups (P<0.05).

compared to both brine salted groups. Fat in extract of 8% brine salted and dry salted rainbow trout groups showed significant difference (P<0.05; Table 1).

After salting, total protein values of the 8% brine salted rainbow trout were decreased to 12651.45 ± 0.39 µg/ml compared to 13184.38 ± 4.04 µg/ml in control group (Table 2). The reason of this reduction seems to be presence of water soluble medium-molecular- weight protein and amino acids such as alanine, glycine, leucine, valine, and glutamic acid, all of which might have been affected during the salting process. However, in 20% brine salted and dry salted rainbow trout, total protein values were higher than in 8% brine salted fish (Table 2). These changes seem to be related with salt concentration. This is explained by the large uptake of salt (NaCl) by the muscle, resulting in competition with muscle protein for water molecules, and denaturation and aggregation of these proteins by a process of "salting out" (Ünlüsayın *et al.,* 2001; Sannaveerappa *et al*., 2004; Martínez-Alvarez and Gómez-Guillén, 2006).

Table 2: Total protein contents in meat of fresh, brine salted and dry salted rainbow trout

Source	Salting methods	Total proteins (µg/ml)
Flesh	Fresh	13184.38 ± 4.04[a]
	Brine (8%) salted (w/w)	12651.45 ± 0.39[d]
	Brine (20%)salted (w/w)	12795.46 ± 0.44[c]
	Dry salted	13066.31 ± 0.20[b]
Extract	Brine (8%) salted (w/w)	250.45 ± 0.56[f]
	Brine (20%)salted (w/w)	128.31 ± 0.34[g]
	Dry salted	1507.32 ± 0.53[e]

Values are shown as mean ± standard deviation of triplicate measurements; Different superscripts within a column indicate significant differences between groups (P<0.05).

It is known that the soluble muscle tissue proteins of fish flesh can be analyzed by electrophoresis (Ünlüsayın *et al.,* 2001). Electrophoretic studies have shown a decrease in the number of bands during wet and dry salting at a slow rate up to 9 h, after which high-molecular-weight proteins decreased faster than medium-molecular-weight proteins in both the 24 h wet- and dry-salted samples. The medium-molecular-weight proteins seem to be more stable (Sannaveerappa *et al*., 2004).

In the present study, protein band patterns were determined by using SDS-PAGE analysis with known mass standards and 10 bands of different molecular weights for rainbow trout were detected (Fig. 1). Different bands were visualized on gels in fish belonging to different salted methods. Also, the density of these bands differed among samples, i.e. for fresh rainbow trout bands had molecular weights as 88, 66, 52, 41, 34, 32 kDa, for 8% brine salted rainbow trout these were 110, 104, 88, 66, 52, 41, 34, 32 kDa and for 20% brine salted rainbow trout bands were of 125, 110, 104, 88, 66, 63, 52, 41, 34, 32 kDa. For dry salted rainbow trout, 9 bands of 110, 104, 88, 66, 63, 52, 41, 34, 32 kDa were seen.

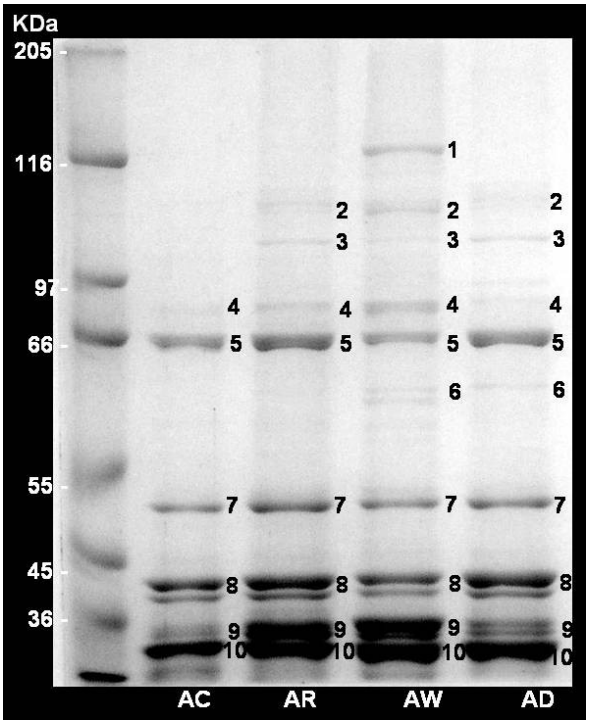

Fig. 1: SDS-PAGE of muscle tissue proteins of fresh, brine salted and dry salted Rainbow Trout. Lines: AC; Fresh. AR; Brine salted 8% (w/w). AW; brine salted 20% (w/w). AD; Dry salted.

The density of 125, 110, 104 and 63 kDa protein bands could not be seen for fresh rainbow trout. In this respect, most of the protein banding patterns of different

salted samples of rainbow trout resembled each other. Especially, 66 kDa protein bands of all rainbow trout samples may be albumin. Martínez-Alvarez and Gómez-Guillén (2005) found a notable loss of soluble muscle proteins by osmosis in salting at pH 6.5; in particular, myosin (MHC) and especially actin, as well as other minority proteins. Similar to earlier reports and as expected, myosin bands (205 kDa) were not visualized on gels from rainbow trout samples of any group (Fig. 1).

Conclusions

In conclusion, we do not recommend salting process with higher than 20% salt concentrations as a preliminary operation for rainbow trout smoking process. The total protein content in extract was lowest for brine salting with 20% NaCl compare to other two groups. Our results may imply that the increase in salt concentration was effective in respect of the extract loss of rainbow trout. Processors may note that some proteins could be denatured during salting process. The determination of protein loss of rainbow trout is of much importance for human consumption in terms of processed sea foods. Thus, protein loss from rainbow trout can be decreased during salting processes by using brine will 20% sodium chloride.

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