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The Investigation of Extract Loss of Horse Mackerel (*Trachurus trachurus* L., 1758) with Different Salting Methods*

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Özet: ***Farklı tuzlama metotları ile tuzlanan istavrit balığı (***Trachurus trachurus ***L., 1758)'nın ekstrakt kayıplarının belirlenmesi.*** Bu araştırmanın amacı farklı tuzlama metodlarıyla tuzlanan istavrit balığının ekstrakt kayıplarının belirlenmesi içindir. Bu çalışmada, %8 ve %20 NaCl konsantrasyonu içeren iki farklı tuzlu-su çözeltisi ve kuru tuzlama metodu kullanılmıştır. %8 ve %20'lik tuzlu-su çözeltisi ile tuzlanan örneklerde nem ve protein içeriğindeki değişimlerin önemsiz (p>0,05) olduğu belirlenmiştir. SDS-PAGE jel elektroforezinde, kontrol grubunda 205, 116, 86 ve 29 kDa yoğunluğundaki protein bantları görülmemiştir. İstavrit için en uygun tuzlama metodu %20'lik tuzlu-su çözeltisi ile tuzlama olarak belirlenmiştir. Bizim sonuçlarımız tuz konsantrasyonundaki artışın istavritin ekstrat kayıpları üzerinde etkili olduğunu belirtmektedir.

Anahtar Kelimeler: Tuzlama, ekstrakt, protein, *Trachurus trachurus*, SDS-PAGE.

Abstract: The aim of this study was to investigate the extract loss of horse mackerel in different salting methods. In this study, two different brine solutions containing 8% and 20% NaCl and dry salt were used. The changes in moisture and protein content were not determined significantly different (p>0.05). The density of 205 116 86 and 29 kDa protein bands could not be seen for fresh horse mackerel in SDS-PAGE of muscle tissue proteins. The brine salted (20%) horse mackerel was observed to be appropriate for salting. Our results indicate that the increase in salt concentration was effective in respect of the extract loss of horse mackerel.

Key Words: Salting, extract, protein, *Trachurus trachurus*, SDS-PAGE.

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Introduction

Salting is a method based on long tradition and still a favourite for many festive dishes, ranging from Scandinavia to South European countries (Borgstrom 1965). Salting methods are simple and involve salt crystals or brine. Sodium chloride diffuses to the outside due to the osmotic pressure between the brine and fish muscle solution. This process does not continue indefinitely: sodium and chlorine ions form a water-binding complex with protein which itself exert an osmotic pressure, eventually, balancing that due to the surrounding brine, and equilibrium is reached (Horner 1997). Tambo et al (1992) studied the change in myofibrillar protein of cured horse mackerel meat induced by dehydration. They found myosin heavy chain in the cured meat together with rise in temperature applied to dehydration, myofibrillar Ca-ATPase was rapidly inactivated and no change occurred during dehydration of such cured meat at 20 ºC or 40 ºC.

Horse mackerel (*Trachurus trachurus*) have been made to utilize them as smoked, canned, chilled, frozen fish product (Simeonidou et al 1997, Aubourg 2001). The preservative effect of salt has been recognised according to a decrease in water activity, less availability to microbial attack, and enhancement of functional properties, leading to an increase of the shelf-life time (Aubourg and Ugliano 2002). In addition, it is a preliminary operation in some smoking, drying and marinating processes (Ismail and Wootton, 1992). Total amount of horse mackerel caught in Turkey was 22.991 tons in 2007 and the 624 tons from Mediterranean Sea (Turkish Statistical Institute 2007). Saltfish is likely to remain in good demand by those that value tradition and taste but it has also gained acceptance in innovative products that provide convenience as added value.

The aim of this study was to determine the extract loss of horse mackerel with different salting methods. Determination of the best salting method in three different groups as a preliminary operation for processing is crucial to avoid nutrient loss.

Materials and Methods

Horse mackerel *(Trachurus trachurus)* was obtained from a local fisherman in Mediterranean Sea (Boğazkent-Serik-Antalya,Turkey) and then transported to our laboratory in polystyrene boxes in crushed ice. 30 fish samples for each method were used. The mean weight of individual samples was determined as 41.6±10.5 g and the mean total length was 17.8±1.3 cm.

After gutted and washed, the fish were divided into four lots; first group was fresh fish (control) and others were placed with keeping 8%, 20% salt solutions (fish/brine solution ratio: 1:1) and dry salting. Brine salting and dry salting was conducted in plastic containers at 4±1 ºC. Brine solution was consisting of 8% and 20% (w/v) sodium chloride (NaCl)

concentration, for a period of 6 h for 8%, 45 min for 20% concentrations. Dry salting process was performed by adding 1 layer salt and 1 layer fish in every 12 h. The fish were then removed from the brine and dry salted horse mackerel were taken out and access of salt eliminated and began to analysis.

The chemical contents of horse mackerel meat and extract were determined according to Association of Official Methods of Analysis. Moisture content was determined according to AOAC (2002a). Crude protein content (Nx6.25) was calculated using the Kjeldahl method AOAC (2002b). Lipid content was determined according to Soxhlet method described in AOAC (2002c). Crude ash was determined according to method AOAC (2002d). Concentration of sodium chloride was determined by using Volumetric method was described by AOAC (1995). After the water in extract was removed, crude ash in extract was calculated according to method AOAC (2002d) and the organic matter in extract was calculated to the following equation:

Organic matter in extract (%) = 100–Crude Ash in Extract

1.5 g of minced muscle tissue of horse mackerel were homogenised at 4 °C for 1 min in 9.5 ml physiological saline (0.9% NaCl) by a mechanical homogenizer (Heidolph, Slient Crusher M model, Heidolph Instruments GmbH & Co KG, Germany), setting 6. Samples were stirred constantly for 20 min at 2 °C then centrifuged at 5000 rpm for 25 minutes at 4 °C in an Elektromag (4808p, İkitelli OSB, İstanbul, Turkey). Protein concentration was determined by the colorimetric method of Lowry et al (1951) using by Total Protein Kit (Protein Determination without Protein Precipitation Procedure. Sigma, Code TP0300 and L3540). Optical density was measured at 650 nm by using 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.

Discontinuous PAGE was prepared by using dilution of a 30% stock solution of acrylamide where the total amount (T) of acrylamide+bis is 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 microgram/microliter 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 marker proteins 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 washes 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.

All measurements were performed in triplicate and the values expressed as the mean±SD. Statistical analyses were performed using SPSS 9.0 for Windows. Analysis of variance (ANOVA) was used and statistical significance was set at $p< 0.05$.

Results and discussion

The change of moisture contents for 8% and 20% brine horse mackerel were observed to be insignificant ($p>0.05$). The average moisture content of fresh horse mackerel was higher (78.91±0.19%) (Table 1). The moisture content of fresh horse mackerel was detected on several researches by Silva et al (2006) as 75.34±0.09% and Tzikas et al (2007) as 78.2%. The chemical composition of fish muscle varies greatly from one species and one individual to another depending on age, sex, environment and season (Turan et al 2006). The variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning (Bandarra et al 2001, Tzikas et al 2007). For this reason it is difficult to compare our findings with those of other researchers. However, on dry salted horse mackerel, these changes were determined to be significant different ($p<0.05$) (Table 1). Dry salting produced considerable loss of constituent water due to heavy uptake of salt (Martínez-Alvarez and Gómez-Guillén 2006). Changes in protein content (dry basis) of 8% and 20% brine salted horse mackerel samples were observed to be insignificant ($p>0.05$). But fat content in muscle of same samples were found significant ($p<0.05$). A part of fat in muscle was transferred in extract. There were correlation between transfer rate from fat in muscle to extract and salt concentration in muscle. The transfer rate was increased with high salt concentration. Changes of ash content of dry salted horse mackerel were found significant ($p<0.05$) (Table 1).

Total micro protein values of fresh, brine salted and dry salted horse mackerel were given on Table 2. After salting, total micro protein values of the brine salted horse mackerel (20%) were decreased 9336.58±0.33 µg/ml. The reason of this reduction is due to presence of water soluble-medium-molecular- weight proteins and amino acids such as alanine, glycine, leucine, valine, and glutamic acid, all of which might be affected during the salting process. However, on brine salted (8%) samples 10219.79±0.28 µg/ml and dry salted samples 10195.40±0.26 µg/ml increased values were observed (Table 2). These changes were 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” (Voskresensky 1965, Ünlüsayın et al 2001, Martínez-Alvarez and Gómez-Guillén 2006) resulting in hydration capacity (Horner 1997).

Table1. Chemical composition of horse mackerel samples.

Part of horse mackerel	Analysis	Fresh	Brine salted 8% (w/v)	Brine salted 20% (w/v)	Dry salted
Flesh	Moisture (%)	78.91±0.19^{a}	76.14±0.19^{b}	76.70±1.43^{b}	74.31±0.05^{c}
	Dry Matter(%)	21.09±0.19^{c}	23.87±0.19^{b}	23.30±1.43^{b}	25.69±0.05^{a}
	Protein (%)	82.23±1.14^{a}	76.81±0.51^{ab}	80.24±5.61^{ab}	76.07±0.45^{b}
	Lipid (%)	4.99±0.50^{a}	2.97±0.25^{ab}	1.67±0.27^{b}	2.79±1.87^{ab}
	Ash (%)	7.12±0.28^{c}	10.55±1.03^{b}	11.03±0.83^{b}	17.91±1.30^{a}
	pH	6.58±0.33^{a}	6.42±0.13^{a}	6.88±0.40^{a}	6.57±0.05^{a}
	NaCl (%)	–	7.75±0.16^{b}	6.77±0.31^{b}	13.36±1.82^{a}
Extract	Solid Matter(%)	–	8.00±0.22^{c}	23.13±1.40^{a}	15.76±0.51^{b}
	Inorganic Matter (%)	–	6.67±1.81^{a}	4.31±0.40^{a}	4.93±2.42^{a}
	Lipid (%)	–	1.28±0.04^{a}	0.71±0.08^{b}	0.57±0.03^{c}

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

Table 2. The total micro protein quantities of fresh, brine salted and dry salted horse mackerel (µg/ml).

Type of salting methods	Total micro protein quantity (µg/ml) of horse mackerel
Fresh horse mackerel	9937.24±0.63c
Brine salted 8% (w/v) horse mackerel	10219.79±0.28a
Brine salted 20% (w/v) horse mackerel	9336.58±0.33d
Dry salted horse mackerel	10195.40±0.26b
Brine salted 8% (w/v) extract	313.36±0.42e
Brine salted 20% (w/v) extract	128.18±0.17f
Dry salted extract	73.03±0.07g

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

Protein band patterns were determined by using SDS-PAGE analysis with known mass standards; 9 bands for horse mackerel were detected (Figure 1). Different bands were visualized on gels belonging to different salted methods. Also, the density of these bands were differed among samples, i.e. for fresh horse mackerel 97, 66, 55, 45, 36, 29 kDa, for brine salted (8%) horse mackerel 116, 97, 86, 66, 55, 45, 36, 29 kDa and for brine salted (20%) horse mackerel 116, 97, 86, 66, 55, 45, 36, 29 kDa bands and for dry salted horse mackerel 205, 116, 97, 86, 66, 55, 45, 36, 29 kDa were dense.

Soluble muscle tissue protein of fish flesh can be analyzed by electrophoresis (Ünlüsayın et al 2001). Electrophoretic studies showed the differences in composition and pH of brines used prior to dry salting of Atlantic cod (*Gadus morhua*) slightly affected the composition of muscle as regards the major constituents and functional quality of the muscle protein (Martínez-Alvarez and Gómez-Guillén 2006).

Figure 1 shows the electrophoretic profiles of proteins released into various horse mackerel samples, and of the resulting fraction of muscle protein. The density of 205, 116, 86 kDa and 29 kDa protein bands couldn't be seen for fresh horse mackerel. Another protein bands were detected (97, 66, 55, 45, 36, 29 kDa) for different salted samples. Generally, myofibrillar and sarcoplasmic proteins denaturated by processing or by ice storage can be fully dissolved from fish muscle by the use of a chaotropic agent such as urea or an anionic detergent such as SDS and be further analysed by IEF or by SDS-PAGE (Bandarra et al 2001). In this respect, protein banding patterns of different salted samples of horse mackerel resembled each other earlier reports and as expected, myosin bands were not visualized on gels belonging to different horse mackerel samples (Figure 1).

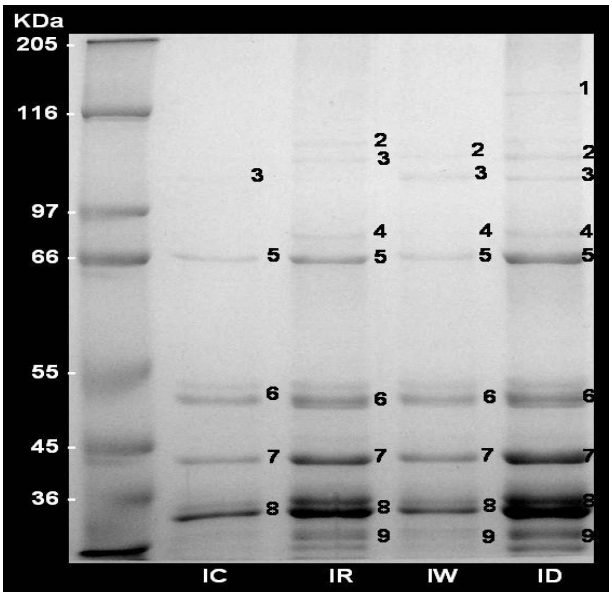

Figure 1. SDS-PAGE of muscle tissue proteins of fresh, brine salted and dry salted horse mackerel. Lines: IC: Fresh horse mackerel, IR: Brine salted 8% (w/v) horse mackerel, IW: Brine salted 20% (w/v) horse mackerel, ID: Dry salted horse mackerel.

In conclusion, brine salted (20%) horse mackerel was showed better results for salting. Our research results may imply that the change in salt concentration was effective in respect of the extract loss of horse mackerel. Seafood industry relating the chemical composition of fish, needing to know the nature of the raw material before the techniques of chilling, freezing, smoking or canning can be correctly applied. In addition, processors should note that during salting protein denaturation may occur. Our data also supports investigation of extract loss of horse mackerel is very important for consumption in processed sea foods.

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