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The Influence of Different Salting Processes on Protein Loss of Cuttlefish (*Sepia officinalis*)*

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Özet: *Sübye (*Sepia officinalis*)'nin protein kayıpları üzerine farklı tuzlama yöntemlerinin etkileri*. Bu çalışmanın amacı; farklı tuzlama metotlarıyla tuzlanan sübye (*Sepia officinalis* L., 1758)'nin protein kayıplarını araştırmaktır. %8 ve %20 konsantrasyonlarında NaCl içeren iki farklı tuz solüsyonu çalışılmıştır. Üçüncü yöntem olan kuru tuzlama yöntemi ise balık yüzeyinin tamamı tuz ile kaplanarak yapılmıştır. Taze ve %8'lik solüsyonla tuzlanan sübyelerin nem içeriğindeki değişimler önemsizdi (P>0,05). %8 ve %20'lik konsantrasyonlarla tuzlanan sübyelerin protein içeriğindeki (kuru ağırlık üzerinden) değişimler önemsiz (P>0,05) olarak belirlenmiştir. Tuzlamadan sonra tüm gruplarda sübyelerin toplam protein içeriği azalmıştır (P<0,05). Taze ve kuru tuzlanmış örneklerde kas doku proteinlerinin SDS-PAGE jel elektroforezinde 205 kDa ve 35 kDa yoğunluğundaki protein bantları görülmemiştir. Sonuçlara göre sübye için en iyi tuzlama metodu %8'lik konsantrasyonla tuzlanmış grup olarak belirlenmiştir. Tuz konsantrasyonundaki artış protein kayıplarını artırmıştır.

Anahtar Kelimeler: Tuzlama, *Sepia*, Protein, SDS-PAGE, Kimyasal kompozisyon

Abstract: The aim of this study was to investigate protein loss of cuttlefish (*Sepia officinalis* L., 1758) under different salting methods. Two different brine solutions containing 8% and 20% NaCl were investigated. In the third treatment, dry salting was run by covering a salt layer with fish layer. Unsalted fish were used as control. The changes in moisture contents of fresh and 8% brined cuttlefish were not significant (P>0.05). Protein contents (dry basis) of brine salted cuttlefish (8% and 20%) were not significantly different. After salting, total protein contents of cuttlefish were decreased in brined and dry salted samples (P<0.05). The density of 205 kDa and 35 kDa protein bands couldn't be seen for fresh and dry salted cuttlefish in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of muscle tissue proteins. The results indicate that best salting method for cuttlefish flesh was brine salting with a concentration of 8%. Increased salt concentration increased in protein loss.

Key Words: Salting, *Sepia*, Protein, SDS-PAGE, Chemical composition

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Introduction

Salting of fish was long worldwide traditions as means to preserve and increase the shelf life. In addition, it was a preliminary application in some smoking, drying and marinating processes (Ismail and Wootton, 1992) that were been mostly empirically developed and was remained unchanged for millennia. Salt inhibits the growth of spoilage-causing microorganisms by drawing water out of tissue through osmosis. This process was not continued 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 was reached (Horner, 1992). Length of salting period as well as salt concentration depends on the expected final product (Bellagha *et al*., 2007). Salt uptake depends on many factors including species, muscle type, fish size, fillet thickness, weight, composition (lipid content and distribution), physiological state, salting method, and brine concentration, duration of salting step, and fish-to-salt ratio, ambient temperature, freezing and thawing (Ismail and Wootton, 1992; Jittinandana *et al*., 2002; Wang *et al*., 2000).

Byun *et al*., (2000) were studied on squid (*Todarodes pacificus*) salted with brine solutions containing 5, 10, and 20% (wt/wt) sodium chloride. The results were showed that the combination of low salt concentration (10%) and gamma radiation was effective in processing salted and fermented squid and extending its shelf life compared to control (20% of salt) without adding any food additives. Sepia sp. was holded in 2.5% brining for 2-3 minutes than sun-dried. Protein ratio of Sepia sp. was found as 67.5% (Burt, 1988).

In salted fish, where the salt concentration reaches ≈20%, high ionic strength was caused contraction of the myofibrils and dehydration of protein in process. Also, the pH of the medium and the type of salts were used for salting can influence the degree of protein denaturation (Martínez-Alvarez and Gómez-Guillén, 2005).

Lipid was reported to be a limiting factor for salt and water diffusion during the salting and drying steps as a consequence of its hydrophobicity (Jason, 1965).

Cephalopods were commercially important group in fisheries and had gained increasing attention as an alternative to the traditional marine harvest and would be gain much

larger importance in the future to supply mankind with marine living resources (Piatowski *et al.*, 2001). Total cephalopod landings were increased steadily since 1950s (FAO, 2005).

The aim of the preset study was to determine the effects of salting on cuttlefish flesh as a preprocessing before smoked and also the protein loss of species with different salting methods. Determination of the best salting method as a preliminary application for processing was crucial to avoid nutrient loss.

Materials and Methods

Common cuttlefish (*Sepia officinalis* L., 1758) was used for this investigation. Cuttlefish were gillnetted with local fisherman from Boğazkent, (Serik-Antalya, Turkey) in April-2007. Mean total body mass and mantle length of individuals were 98.6±17.7 g and 9.2±0.5 cm, respectively. Samples were then transported to laboratory in polystyrene boxes in crushed ice during approximately 60 min. Totally, for treatments of 30 common cuttlefish each were used for 4 different treatments.

The viscera, head and arms of the cuttlefishes were removed and mantles were washed with water. Salt, also known as rock salt, is a crystalline mineral that is composed primarily of sodium chloride (NaCl) and that used for salting process was purchased from local market (Antalya). The salt processes (salt concentration, ratio of cuttlefish to brine) were chosen based on seafood industrial practices of the firms (Antalya-Turkey) as follows. Brine salting and dry salting processes were conducted in plastic containers at 4±1ºC. The concentrations of salt in brine solutions were 8% (R) and 20% (W) (w/w), and curing processes using these brine concentrations with a fish to brine ratio of 1:1 were carried out for a period of 6 h and 45 min respectively. Dry salting (D) was run for 12 h and salting process was carried by covering the surface of fish. Thereby squid was inserted into a salt layer. After the curing process salted mantles of the cuttlefishes were then used for the analysis. Unsalted fish were used as control. pH was measured in the dorsal muscle with a digital electronic pH meter with a glass electrode (WTW Mark, 320, Germany).

The chemical contents of common cuttlefish flesh and extract were determined according to Official Methods of Analysis. Moisture contents were in fish flesh determined according to method 950.46 (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 method 920.153 (AOAC, 2002d). Sodium chloride was determined by volumetric method (AOAC, 1995). Solid matter in extract was determined according to Gravimetric method 952.08 (AOAC, 2002a). After the water in extract was removed, crude ash in extract was calculated according to method Official Methods of Analysis (AOAC, 2002d) and the organic matter in extract was calculated by using the following formula:

Organic matter in extract (%) = 100 – Crude ash in extract.

A total of 1.5 g minced muscle tissue of cuttlefish were homogenised at 4°C for 1 min in 9.5 ml physiological saline (0.9% NaCl) with a mechanical homogenizer (Heidolph, Slient Crusher M model, Heidolph Instruments GmbH & Co KG, Germany), dialled to 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 in the supernatant by kit method (Lowry *et al.*, 1951), (Protein Determination without Protein Precipitation Procedure; Sigma, Code TP0300 and L3540). Optical density was measured at 650 nm in Chebios UV/ spectrophotometer (Optimum-One, Chebios s.r.l., Roma, Italy). The remainder of the supernatant was freeze-dried and kept at -18°C for further analysis.

Discontinuous PAGE was prepared dilution of a 30% stock solution of acrylamide 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 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 % comassie 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 comassie blue R-250. Picture of them were taken in 7% acetic acid while they were still wet.

Statistical analyses were performed using “SPSS 10.0 for Windows software” (SPSS Inc, Chicago, IL). Differences in the means between groups were analysed by one-way ANOVA. Two-tailed *P* values were used, and statistical significance was set to $P < 0.05$.

Results and Discussion

There was no change in moisture content of cuttlefish salted with 8% salt concentration. However, water losses in both cuttlefish salted with 20% brine and dry salt were determined; these losses were higher in dry salted cuttlefish than brine salted. The average moisture content of fresh cuttlefish was 77.40±0.25% in this study (Table 1). The moisture contents of

fresh cuttlefish reported by Özoğul *et al.*, (2008) were 81.02±0.18% in spring, 78.02±0.21% in autumn, and 79.51 ± 0.28% in winter. Özoğul (2012) also reported mantle of cuttlefish caught from the Gulf of Mersin had high protein (22.20% for female and 22.15% for male) and low fat contents (0.86% for female and 1.01% for male). Zlatanos *et al.*, (2006) reported moisture content of 81.2±2.0%. Changes in protein content of cuttlefish salted with 8% and 20% salt were similar and after the salting process, the crude protein content decreased than the fresh samples, owing to the muscle protein lost into the extract. Loss of protein for dry salted samples is more than brine salted samples (Table 1). The lipid content of species was found to be very low and species was all considered as lean. The results indicate that this cephalopod species is excellent protein sources and has low fat content. These results are supported by the findings of other researchers (Özoğul *et al.*, 2008; Zlatanos *et al.*, 2006). Because of the low lipid content of cuttlefish meat, changes in lipid content after salting were insignificant (P>0.05).

Table 1. Chemical composition of cuttlefish samples

Part of cuttlefish	Analysis	OC	OR	OW	OD
Flesh	Moisture (%)	77.40±0.25^{a}	76.36±0.42^{a}	73.04±1.02^{b}	67.62±1.47^{c}
	Dry Matter (%)	22.60±0.25^{c}	23.64±0.42^{c}	26.96±1.02^{b}	32.38±1.47^{a}
	Protein (%)	84.81±2.49^{a}	73.90±2.42^{b}	72.81±6.23^{b}	54.92±2.07^{c}
	Lipid (%)	5.65±1.94^{a}	4.66±1.25^{a}	5.71±1.77^{a}	3.86±0.43^{a}
	Ash (%)	7.19±2.13^{b}	18.52±2.20^{a}	16.48±2.21^{a}	19.03±1.11^{a}
	pH	6.75±0.13^{a}	6.44±0.09^{b}	6.60±0.07^{ab}	6.45±0.20^{b}
	NaCl (%)	–	14.56±1.19^{b}	15.15±0.63^{b}	17.40±0.66^{a}
Extract	Solid Matter (%)	–	6.66±1.24^{b}	16.75±0.24^{a}	8.25±2.08^{b}
	Inorganic Matter (%)	–	12.01±2.79^{b}	20.67±4.58^{a}	19.28±1.13^{a}
	Lipid (%)	–	1.02±0.07^{a}	1.01±0.05^{a}	0.98±0.06^{a}

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).
OC: Fresh cuttlefish.; OR: Brine salted 8% (w/w) cuttlefish meat; OW; Brine salted 20% (w/w) cuttlefish meat OD; Dry salted cuttlefish meat.

Total protein values of fresh cuttlefish meat and extracts of brine salted and dry salted cuttlefish were given on Table 2. Protein band patterns were determined by using SDS-PAGE analysis with known mass standards; we have detected 9 bands for cuttlefish (Figure 1). Different bands were visualized on gels belonging to different salted methods. Also, the density of these bands are differed among samples, i.e. bands in 118, 65, 38 kDa are denser for brine salted (8%) and (20%) cuttlefishes than the control and dry salted samples. For brine salted (with 8% brine solution) cuttlefish 32 kDa protein band is apparently denser than the samples salted by the other methods.

It is known that the soluble muscle tissue protein of fish flesh can be analyzed by electrophoresis (Ünlüsayın *et al.*, 2001). That was found about electrophoretic studies, the number of bands decreases during salting process (Martínez-Alvarez and Gómez-Guillén, 2006). Electrophoretic studies showed 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 protein seems to be more stable (Sannaveerappa *et al.*, 2004).

Table 2. Total protein quantities of fresh, brine salted and dry salted cuttlefish samples

Parameter	µg/ml
OC	6592.29±0.30^{d}
OR	12039.31±0.36^{b}
OW	12675.49±0.31^{a}
OD	10501.45±0.26^{c}
ORe	233.07±0.16^{g}
OWe	243.74±1.10^{f}
ODe	905.10±0.30^{e}

Values are shown as mean ± standard deviation of triplicate measurements.
Different superscript letters within a column indicate significant differences between groups (P<0.05).
OC; Fresh cuttlefish, OR; Brine salted 8% (w/w) cuttlefish meat, ORe; Brine salted 8% (w/w) cuttlefish extract, OW; Brine salted 20% (w/w) cuttlefish meat, OWe; Brine salted 20% (w/w) cuttlefish extract, OD; Dry salted cuttlefish meat, ODe; Dry salted cuttlefish

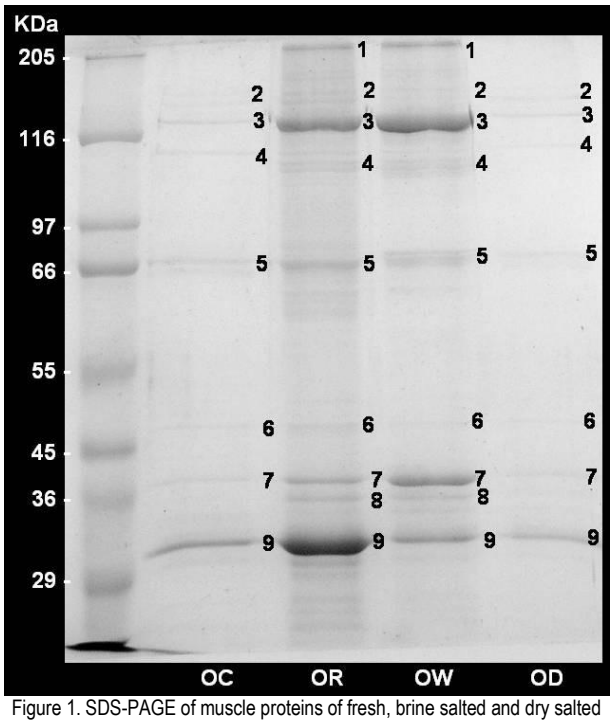

Figure 1. SDS-PAGE of muscle proteins of fresh, brine salted and dry salted cuttlefish samples.
OC; Fresh cuttlefish., OR; Brine salted 8% (w/w) cuttlefish meat OW; Brine salted 20% (w/w) cuttlefish meat, OD; Dry salted cuttlefish meat

Electrophoretic profiles of proteins released into various cuttlefish samples were shown in Figure 1. The density of 205 kDa and 36 kDa protein bands couldn't be seen for both fresh and dry salted cuttlefish. All the other protein bands (150, 118, 106, 67, 48, 38 and 31) can be seen for all brine methods. In this respect, protein banding patterns of different salted samples of cuttlefish resembled each other.

Conclusion

Salting process with 8% brine seems to be the best for cuttlefish samples because of the minimum loss of organic material. For both brining with 20% solution and dry salting, organic material losses have been determined. Although protein content changed depending on the concentration of salt solution lipid content did not change. Our results may imply that the increase in salt concentration was effective in respect of the extract loss of common cuttlefish. Low lipid content of cuttlefish meat causes more water loss and salt diffusion at salt concentrations. Consequently we don't recommend salting with high salt concentrations as a preliminary operation for processing for cephalopod species.

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