www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 75-79 (2010) DOI: 10.4194/trjfas.2010.0111

The Effects of Salt-Boiling on Protein Loss of *Penaeus semisulcatus*

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Received 22 October 2008
Accepted 11 October 2009

Abstract

The aim of this study was to investigate the protein losses of *Penaeus semisulcatus* when different brine solutions (10% or 12% NaCl) were used with whole or only the abdomen flesh and the boiling times were varied. The moisture and protein content (dry basis) of fresh and salt-boil shrimp were significantly different ($P<0.05$) with the total protein content of shrimp decreasing. The SDS-PAGE bands for 62 kDa and 33 kDa protein could not be seen for fresh shrimp muscle tissue. The results indicate that the best method for salt-boiling shrimp was with whole shrimp boiled for 8 min at 10% NaCl concentration. Increased salt concentration and boiling time increased protein loss.

Keywords: salt-boiled shrimp, shrimp, *Penaeus semisulcatus,* proteins loss, SDS-PAGE.

Tuzlama-Haşlamanın *Penaeus semisulcatus*'un Protein Kayıpları Üzerine Etkileri

Özet

Bu çalışmanın amacı bütün veya sadece abdomen etleriyle farklı tuz solüsyonları (%10 veya %12 NaCl) kullanıldığında ve haşlama süreleri değiştiğinde *Penaeus semisulcatus*'un protein kayıplarını araştırmaktır. Taze ve tuzlanmış-haşlanmış karideslerin su ve protein içerikleri (kuru madde) ile karidesin toplam protein içeriğinde meydana gelen azalma önemli derecede farklıdır ($P<0,05$). Taze karides kasında 62 kDa ve 33 kDa SDS-PAGE bantları görülememiştir. Sonuçlar, tuzlanan-haşlanan karidesler için en iyi metodun %10 NaCl konsantrasyonunda 8 dakikalık bütün haldeki karides haşlama olduğunu göstermiştir. Tuz konsantrasyonu ve haşlama süresindeki artış protein kayıplarını arttırmıştır.

Anahtar Kelimeler: tuzlama-haşlama karides, karides, *Penaeus semisulcatus*, proteinlerin kaybı, SDS-PAGE.

Introduction

Salting of seafood is a traditional method used around the world to preserve and increase their shelf life. Salting methods including for seafood are simple and involve dry salt crystals or a brine, sometimes both. The length of salting as well as the salt concentration depends on the required final product (Bellagha *et al*., 2007).

The NaCl diffuses in to seafood muscle due to the osmotic pressure (Horner, 1997). In salted seafood, where the salt concentration reaches about 20%, high ionic strength causes contraction of the myofibrils and dehydration of protein. Also, the pH of the final product and the type of salts used can influence the degree of protein denaturation (Martínez-Alvarez and Gómez-Guillén, 2005).

Martinez *et al*. (2001) investigated the changes during ice storage in the soluble protein fractions that were extracted from shrimp muscle in water after low-salt and high-salt treatments. From SDS-PAGE, they found degradation of the myosin heavy chain at all salt concentrations and an increase in the number and intensity of bands of molecular weight about 100 kDa.

Different species of shrimp such as *Penaeus semisulcatus*, *Penaeus japonicus*, *Penaeus kerathurus*, *Metapenaeus stebbingi*, *Metapenaeus monoceros*, *Parapenaeus longirostris*, *Trachypenaeus curvirostris* and *Ariteus antennatus* (Gökoğlu and Kaya, 2005; Kumlu, 2001) are harvested in Turkey. The total amount of shrimp caught in Turkey was 6,339 tons in 2005 and 960 tons of this amount were from the Mediterranean Sea (Turkstat, 2005). Shrimp

are generally consumed as in Turkey fresh, salt-boiled, canned, smoked and frozen products (Bayızıt *et al*., 2003).

The aims of the salt-boiled process for shrimp are to reduce the load of microorganisms to an acceptable level and to improve the flavour of shrimp. A suitable salt-boiled process is important to get a suitable salt concentration to minimize loss of muscle protein and maintain the red colour.

The aim of this study was to determine the effects of the salt-boiled process on protein content of shrimp to avoid nutrient loss.

Material and Methods

Raw Material

Shrimps were caught in December 2006 in the Mediterranean Sea by a commercial fishing trawler. Shrimp were put in boxes including ice just after being caught. Totally, four treatments of 30 shrimp each were used for 4 different treatments (120 shrimp). Mean total body mass and mantle length of individuals were 31.4±3.2 g and 3.66±0.35 cm respectively. Shrimp were then transported to our laboratory in polystyrene boxes including ice immediately.

Salt-boiling Processes

The salt-boiling processes (salt concentration, boiling time, ratio of shrimp to brine, boiling steps etc.) were chosen based on the literature (Niamnuy *et al*., 2007; Cadun *et al*., 2005; Prachayawarakorn, 2002) and seafood industrial practices of the Antalya (Turkey) region as follows.

Method I: Raw whole-shrimp were put into boiling salt solution containing 10% NaCl. After 8 min, shrimp were removed from the salt solution and the cephalothorax, abdominal segments and telson of the shrimp were removed.

Method II: This method of salt-boiling was performed in two steps. Firstly, raw whole-shrimp were put into boiling water. After 5 min, shrimp were removed from the water and shelled as explained above. Some of the abdominal flesh was used for analysis; the other flesh was used for the second step. For the second step, the abdominal flesh was put into a boiling salt solution containing 12% NaCl. After 1 min, the flesh of the shrimp was removed from the salt solution.

Both salt-boiling processes were done with a shrimp to brine ratio of 1:2. The analyses were repeated in triplicate for each boiling condition. All samples were stored at 4±1ºC in a refrigerator before the analysis performed.

Analytical Procedures

The chemical contents of shrimp meat and extract were determined according to the Official Methods of Analysis (AOAC, 2002). Moisture content was determined according to the Official Method 950.46 (2002a). Crude protein content (Nx6.25) was calculated using the Kjeldahl method 928.08 (2002b). Lipid (fat) content was determined according to the Soxhlet method 960.39 (2002c). Crude ash (inorganic matter) was determined according to method 920.153 (2002d). Sodium chloride was determined according to the volumetric method 935.47 (AOAC, 1995). After the water in the extract was removed by evaporating, crude ash in the extract was calculated (AOAC, 2002d) and the organic matter in the dry extract was calculated using the following equation:

Organic Matter = 100 – Crude Ash in the Extract in the Extract (%)

Protein Extraction

Minced muscle tissue 1.5 g were homogenised at 4°C for 1 min in 9.5 ml physiological saline (0.9% NaCl) using a mechanical homogenizer (Heidolph, Silent Crusher M model, Heidolph Instruments GmbH & Co KG, Stuttgart, Germany), setting 6. Samples were stirred constantly for 20 min at 2°C and then centrifuged at 3000xg for 25 minutes at 4°C in an Elektromag (4808p, İkitelli OSB, İstanbul, Turkey). Protein concentration was in the supernatant determined by the colorimetric method of Lowry *et al*. (1951) using a Total Protein Kit (Protein determination without protein precipitation procedure, Sigma-Aldrich Chemie GmbH, München, Germany, Code TP0300 and L3540). Optical density was measured at 650 nm in a Chebios UV/VIS spectrophotometer (Optimum-One, Chebios s.r.l., Rome, Italy). The rest of the supernatant was kept at -18°C for further analysis.

SDS-Page

Discontinuous gel for PAGE was prepared using a dilution of a 30% stock solution of acrylamide where the total amount (T) of both acrylamide and bis acrylamide is 2% for the stacking gel and 5.1% for the resolving gel. Freeze-dried protein samples were reconstituted in an appropriate amount of Laemmli (1970) sample buffer to achieve a protein concentration of 13 µg/µl and loaded in each well of the gels. Electrophoresis (Mini-Protean II/Bio-Rad loboratories in Hercules, California, USA) was carried out at 35 mA for one slab until the tracking dye reached the bottom of the gel (3 h) in a chamber with cooling to approximately 10°C. The molecular weight of each protein band could then be calculated according to the standard curve using purified wide range of marker proteins including aprotinin from bovine lung (6.5 kDa), α-lactalbumin from bovine milk (14.2 kDa), trypsin inhibitor from soybean (20

kDa), trypsinogen from bovine pancreas (24 kDa), carbonic anhydrase from bovine erythrocytes (29 kDa), glyceraldehyde–3-phosphate dehydrogenase from rabbit muscle (36 kDa), ovalbumin from chicken egg (45 kDa), glutamic dehydrogenase from bovine liver (55 kDa), albumin from bovine serum (66 kDa), phosphorylase b from rabbit muscle (97 kDa), β-galactosidase from *E. coli* (116 kDa), myosin from rabbit muscle (205 kDa) (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.

Statistical Analysis

Statistical analyses were performed using "SPSS 10.0 for Window software" (SPSS Inc, Chicago, IL, USA). 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 and Discussion

Water losses of shrimp for each salt-boiling process were determined; the loss in salt-boiling shrimp with method I was higher than Step 1 of method II ($P<0.05$) but after Step 2 of method II, water losses for both methods were the same. The average moisture content of fresh shrimp was 75.48±1.72% in this study (Table 1). The moisture content of fresh shrimp was similar to previous reports ranging from 71 to 80% (Balogun and Akegbejo-Samsons, 1992; Diler and Ataş, 2003; FAO, 2001).

The difference between fresh and salt-boiling shrimp's crude protein content (dry basis) was significant ($P<0.05$). After the salt-boiled process, the ratio of crude protein decreased owing to the muscle protein lost into the extract. The lowest protein content in muscle was observed at Step 1 of method II protein content of muscle (% dry basis) increased from 77.42% in step 1 of method II to 81.33% in Step 2 of method II (Table 1). The reason of this may be from the lower moisture content of muscle in Step 2: Because when boiling shrimp in salt solution in Step 2, the salt went in the muscle and was released the water by muscle. Therefore, protein content also increased with the increment of dry matter from the calculation.

The average lipid content of fresh shrimp was 1.79±0.12%. The lipid content of fresh shrimp ranged from 0.79 to 1.11% and from 1.51 to 2.04% according to Balogun and Akegbejo-Samsons (1992) and Yerlikaya and Gökoğlu (2005), respectively. There was no change in the lipid contents of salt-boiled shrimp's muscle. However, lipid contents of the extract increased on Step 2 of Method II ($P<0.05$). This showed that the crude lipid was lost in the extract during the salt-boiled process (Table 1).

The organic matter in the extract contained protein, lipid and carbohydrate. Organic matters were found to be 5.47% for the first method and 8.65% for the second method (Table 1 and Figure 1). From the results, it was seen that more organic matter (only protein and carbohydrate, not lipid) was lost into the extract using the second method than the first method.

The difference between fresh and salt-boiled shrimp's crude ash contents was found to be significant ($P<0.05$) (Table 1) as expected.

It has been reported that protein, ash, lipid and moisture contents of shrimp change according to nutrition, living area, size, catching season, seasonal and sexual variations as well as environmental conditions (Wen *et al*., 2001; Gökoğlu and Yerlikaya, 2003; Rosa and Nunes, 2004; Huang *et al*., 2005; Yerlikaya and Gökoğlu, 2005).

Total protein values of fresh and salt-boiled shrimp are given in Table 2. The amounts of protein in the extracts were quite low so the Lowry method was used. After the salt-boiled process, total protein values for shrimp for the second method decreased from 13522.45±0.13 µg/ml to 4213.81±0.13 µg/ml, presumably due to extraction of water soluble proteins during boiling (Ünlüsayın *et al*., 2001). It was found

Table 1. Chemical composition of shrimp samples

Part of Shrimp	Analysis	Fresh	Method I	Method II	
				Step 1	Step 2
Muscle	Moisture (%)	75.48±1.72^{a}	69.26±1.71^{c}	72.28±0.21^{b}	70.12±0.18^{bc}
	Dry Matter (%)	24.52±1.78^{c}	30.74±1.71^{a}	27.72±0.21^{b}	29.88±0.19^{ab}
	Protein (%) (in dry)	83.81±0.76^{a}	79.15±0.35^{c}	77.42±0.07^{d}	81.33±0.65^{b}
	Lipid (%) (in dry)	1.79±0.12^{a}	2.28±0.40^{a}	2.30±0.20^{a}	2.34±0.23^{a}
	Ash (%) (in dry)	7.63±0.13^{b}	7.22±0.18^{b}	5.58±0.05^{c}	9.40±0.79^{a}
	pH	6.69±0.03^{b}	6.83±0.18^{ab}	6.97±0.07^{a}	6.82±0.07^{ab}
	NaCl (%)	—	1.27±0.03^{b}	0.62±0.03^{c}	1.71±0.03^{a}
Extract	Solid Matter (%)	—	10.87±0.11^{b}	1.28±0.43^{c}	15.59±1.56^{a}
	Inorganic Matter (%)	—	5.40±1.28^{a}	—	6.94±4.04^{a}
	Lipid (%)	—	0.50±0.10^{b}	0.42±0.14^{b}	1.21±0.05^{a}

Values are shown as mean ± standard deviation of triplicate measurements for three independent and parallel experiments.
Different superscript letters in the same row indicate significant differences between groups ($P<0.05$).

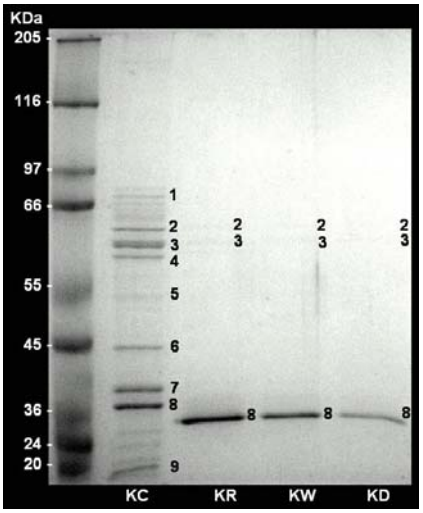

Figure 1. SDS-PAGE of muscle tissue proteins of shrimp samples.
Lines: KC: Fresh shrimp, KR: Flesh using Method I. KW: Flesh at step 1 of method II, KD: Flesh at step 2 of method II.
Trypsin inhibitor, soybean (20 kDa), trypsinogen, bovine pancreas (24 kDa), glyceraldehyde-3-phosphate dehydrogenase, 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).

Table 2. The total protein quantities of shrimp samples (µg/ml)

Sample	Type of process		Total protein (µg/ml)
Muscle	Fresh		13522.45±0.13[a]
	Method I		4598.44±0.32[c]
	Method II	Step 1	4213.81±0.13[d]
		Step 2	5030.56±0.34[d]
Extract	Method I		1823.07±0.15[f]
	Method II	Step 1	1685.30±0.18[g]
		Step 2	223.22±0.24[i]
		Total	1908.52±0.21[e]

Values are shown as mean ± standard deviation of triplicate measurements for three independent and parallel experiments. Different superscript letters within a column indicate significant differences between groups (P<0.05).

that the values of total protein in the extracts were 1823.07 and 1908.52 µg/ml for the first method and second method, respectively. This result indicates that the protein loss of Method II was higher than that of Method I. These changes were related to the concentration of salt solution, boiling times and whether whole or only the abdominal flesh. Protein losses have been 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; Horner, 1997; Yapar, 1999; Ünlüsayın *et al*., 2001; Sannaveerappa *et al*., 2004; Martínez-Alvarez and Gómez-Guillén, 2006).

Peptide bands patterns were determined by using SDS-PAGE with known mass standards; 9 bands (69, 63, 61, 59, 54, 45, 39, 37 and 19 kDa) for fresh shrimp were detected (Fig. 1). However, just 3 of them (63, 61 and 37 kDa) were found after salt boiling. In general, the number of bands decreases during salting processes owing to protein denaturation (Martínez-Alvarez and Gómez-Guillén, 2006).

The protein banding patterns of salt-boiled shrimp resembled each other. Especially 45 kDa protein band of fresh shrimp was believed to correspond to ovalbumin. Martinez *et al.* (2001) investigated changes in the soluble protein fractions that were extracted from shrimp muscle in water, low-salt and high-salt solutions during ice storage. According to their results, degradation of the myosin heavy chain occurred at all salt concentrations and one band of about 67 kDa disappeared during storage. Similar to earlier reports and as expected, most of the bands after the salt-boiled process and differently from the previous study myosin heavy chain for any sample disappeared on the gels (Figure 1).

In conclusion, method I for the salt-boiling process of shrimp was found to be the best because of the minimum loss of protein in shrimp muscle. Hence, it was concluded that the first method was better than the second method for salt-boiled of shrimp. Although protein content changed depending on the concentration of salt solution, boiling times and whether whole pieces or only the abdomen flesh, lipid content did not change. The first method had the lowest concentration of salt, the shortest boiling time and permitted a presentation as a whole shrimp.

Processors should note that some proteins could denature during the salt-boiling process. The most required quality of salt-boiled shrimp is minimum loss; because dietary protein has played a significant role in improving human health beyond their well-recognized nutritional value. Consequently, we do not recommend salt-boiled process with higher than 10% salt concentration as a preliminary operation for

shrimp processing. This method can be used at home and by food processing industry.

Acknowledgements

This work was supported by the Scientific Research Projects Commission of Akdeniz University (AÜBAP–2006.01.0121.002). The authors thank Dr.Numan Hoda for critically reviewing the manuscript. Also, the authors thank Olgaç Güven and Dr.Ali Günlü for helping on this study.

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