Solubility and Viscosity of Herring (*Clupea harengus*) Proteins as Affected by Freezing and Frozen Storage

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ABSTRACT: The aim of this work was to evaluate the effects of freezing and frozen storage at –24 °C on the quality of Icelandic herring fillets, focusing on protein solubility and viscosity at pH 2.7 and 11 used for pH-aided protein isolation. The evaluation of quality was based on chemical analyses, protein degradation measurements, and changes in protein solubility and viscosity at pH 2.7 and 11 after up to 6-mo frozen storage of the herring fillets. Lipid oxidation measured as TBARS values increased significantly during the frozen storage ($P < 0.05$). Protein solubility at pH 2.7 decreased during frozen storage for 6 mo, where the solubility was about 10% lower after 6-mo frozen storage compared to the beginning ($P < 0.05$). At pH 11, the solubility became approximately 15% lower after 6-mo frozen storage compared to initial solubility ($P < 0.05$). Viscosity, measured at pH 2.7, increased after 3 mo of frozen storage ($P < 0.05$). At pH 11, the viscosity increased significantly after 1-wk frozen storage, compared to fresh herring fillets, but did not increase significantly with further storage ($P < 0.05$). Changes found in solubility and viscosity indicated protein degradation due to freezing and frozen storage. SDS-PAGE analysis did not reveal any protein cross-linking or aggregation formation, either with frozen storage or due to exposure to low pH.

Keywords: frozen storage, herring, rancidity, solubility, viscosity

Introduction

Currently, the most widely used proteins in the food industry originate from soybeans or milk where sale has increased considerably in the recent years (Jansen and Krijger 2003). At the same time, many fish species are underutilized for human consumption and protein rich by-products are used for production of animal feed or low-value products. Herring (*Clupea harengus*) is one of these species (FAO 1997). In Iceland, the proportion of the herring catch used for food production has been widely variable (Statice 2006). The market situation for herring products, along with the availability of the species from one fishing season to another, is the reason for this variability. Price for protein intended for human consumption or as feed differs greatly (NMFS 2006). It is therefore of interest to develop new methods to use herring as food, expand its usability in new products, and thereby reinforce the herring market. One of the problems with the development of new and improved food products from herring is the variability in the chemical composition of the raw material and the different fat composition depending on seasons. Frozen storage inhibits microbial spoilage, and can be used to overcome this variability, but other changes do occur including modification of muscle proteins that affects their structural and functional properties (Connell 1960; Mackie 1993; Badii and Howell 2001). What causes the changes during frozen storage is not exactly clear but products from lipid oxidation may be involved. Lipids in herring are vulnerable to oxidation that rapidly gives rise to rancidity that makes the effect of the oxidation reaction particularly important during frozen storage (Undeland and Lingnert 1999). Using a pH-aided isolation technology may be a new possibility to improve the functionality, storability, odor, and taste of proteins extracted from fish (Hultin and Kelleher 2000a, 2000b). This technology opens up possibilities to produce functional- and food-grade proteins from fatty fish such as herring, which has been difficult with traditional production methods because of difficulties in removing the lipids (Shimizu and others 1992). The method is based on the following steps: (1) proteins made soluble at low pH (2.7) or at high pH (11); (2) high-speed centrifugation used to remove fat and impurities; and finally (3) precipitation of the proteins at pH 5.5. The aim of this work was to evaluate the effects of freezing and frozen storage on the stability of lipids, protein solubility, and viscosity of whole muscle Icelandic herring proteins for protein isolation.

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Materials and Methods

Herring catch and herring fillets preparation

The herring was caught southeast of Iceland in September 2001. The fish was kept in refrigerated seawater (RSW; temperature 0 to 3 °C) aboard the fishing vessel until landing about 24 h postcatch. After landing, the herring was size-graded, beheaded, filleted, and skinned in machines, iced, and sent to the Icelandic Fisheries Laboratories (IFL), Reykjavík. Approximately 60 pieces of fillets were packed in bags of 1 kg and frozen at –24 °C. A part of the fillets was kept on ice until analyzed. Samples were taken before freezing and after 7 d, 1, 3, and 6 mo of frozen storage at –24 °C.

Sample preparation before viscosity and protein solubility measurements

Frozen herring fillets were thawed overnight at 0 °C. The fillets were minced twice in a Kenwood food processor and washed twice

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with cold water (4 °C), 1 part herring and 3 parts water. The resulting slurry was gently stirred for about 10 min at each wash and then drained through a layer of cheesecloth. Protein solubility and viscosity were measured in homogenized solution that included 1 part washed herring muscle and 9 parts cold water. The solution was homogenized for 45 s in a Ultra Turrax T25 (20500 rpm) and pH adjusted to desired value between 2 and 12 with 1 M NaOH or 1 M HCl. Viscosity was measured directly in the sample after adjusting to desired pH value but before measuring protein solubility the sample was centrifuged for 30 min at 8000 rpm in Sorvall RC-5B refrigerated superspeed centrifuge with GS-3 rotor at approximately 4 °C. Soluble proteins were measured in the middle supernatant layer.

Proximate analysis of herring fillets and mince

Chemical composition was measured in unfrozen herring fillets. Furthermore, protein quantity was measured in washed herring mince at each sampling point. The nitrogen content was determined by the Kjeldahl method in a Kjeltec Auto sampler 1035/30 system (N ∗ 6.25). Water content in muscle was measured as weight loss after 4 h at 103 ± 1 °C. The fat content was determined using Soxhlet according to Method BA 38 and application note Tecator number AN 301 (AOCS 1997). Salt content was determined by an AOAC method number 937.09 for measurement of salt in fish (AOAC 2000).

Thiobarbithuric acid reactive substances (TBARS)

TBARS were determined by a slightly modified steam distillation method (Tarladgis and others 1960), where the sample size was reduced to 5 g and antioxidants (5 mL of 0.5% propyl gallate and 0.5% ethylene diamine tetraacetic acid in water) were added to the sample during blending. Malondialdehyd-bis-(diethyl acetate) was used as a standard.

Protein solubility

Solubility was tested according to Stefansson and Hultin (1994) with some modifications. Soluble protein was determined by the Biuret method (Layne 1957; Torten and Whitaker 1964) by combining 1 part sample, 4 parts Biuret reagent, and 1 part 10% deoxycholic acid. The deoxycholic acid was added to eliminate lipids from the test solution that could interfere with the measurement. Bovine serum albumin (BSA) was used as a standard. The protein content in the initial homogenate before solubilization was determined by the Kjeldahl method as stated earlier.

Viscosity in herring homogenate

The viscosity was measured after protein solubilization/precipitation at different pH values from 2 to 12 according to Undeland and others (2003). The homogenates were placed into a 400-mL Pyrex beaker on ice. Viscosity was measured at 4 to 6 °C using Bohlin visco 88 BV with nr C30 spindle (30 mm in diameter) at 1000 rpm. Each sample was measured twice and the results were presented as mPa·s.

Electrophoresis

Samples for electrophoresis were taken from the homogenate. Sampling was made from fresh herring and after 1-wk, 1-, and 6-mo frozen storage. SDS-PAGE electrophoresis were performed according to the discontinuous electrophoresis method of Laemmli (1970) using 4% stacking gel and 15% acrylamide gel. The molecular weight markers used were from Bio-Rad (nr 161-0304 and 161-0303).

Measurements of pH

The pH was measured with Orion pH meter (model 290A, Orion, Boston, Mass., U.S.A.).

Statistical analysis

Mean values were compared with a 1-way analysis of variance (ANOVA) using NCSS (version 2000; NCSS Statistical Software, Utah, U.S.A.) with respect to statistical significance (significance level $P < 0.05$).

Results and Discussion

Chemical composition

Chemical composition of raw material, skinned herring fillets was 68% water, 18.1% protein, 12.4% lipids, 0.2% salt, and 1.4% ashes.

Lipid oxidation

Lipids in herring are susceptible to oxidation caused by the unsaturated nature of the lipids and the fact that herring is a dark-fleshed fish which contains a large number of both enzymatic and nonenzymatic prooxidants (Chaijan and others 2006). During frozen storage, the TBARS increased significantly through the storage time ($P < 0.05$) (Figure 1), which is in accordance with what other researches have discovered (Undeland and Lingnert 1999; Tokur and others 2006). The increase in TBARS indicated the formation of secondary oxidation products (Frankel 1998). Two days passed from catch until the herring fillets were frozen. During that time, the lipids had already started to oxidize explaining why the TBARS were around 0.75 mg mal/kg before freezing, emphasizing the importance of good raw material handling before freezing (Figure 1).

Changes in protein solubility during frozen storage

The solubility of proteins in fresh herring muscle homogenate changed with pH where it was highest at low and high pH values and lowest near the isoelectric point at pH 5 to 6 (Figure 2). The shape of the protein solubility curve with pH is well known, both for proteins in general and fish proteins such as myofibrillar proteins from cod (Spinelli and others 1972) and Atlantic croaker (Kristinsson and Liang 2006). At the pH of unadjusted washed herring muscle homogenate (pH approximately 7), using fresh muscle, approximately 16% of the proteins was soluble but approximately 90% was soluble at pH < 3.5 and pH > 11. The level of sarcoplasmic proteins usually found in fish muscle tissue is approximately 25% to 30% (FAO 1995). It was therefore apparent that by washing the homogenate with water the amount of sarcoplasmic proteins can be decreased. Protein isolation is performed at low pH (2.7) or at high pH (11) and it is therefore of interest to describe how the protein solubility at those pH values is affected by frozen storage of the raw material. Solubility of herring proteins extracted at both pH 2.7 and pH 11 decreased with frozen storage up to 6 mo (Table 1). The protein solubility at pH 2.7 changed significantly ($P < 0.05$) compared to fresh muscle during 6-mo frozen storage and was 80% compared to 90% in fresh muscle. Protein solubility at pH 11 of the washed herring muscle changed significantly during 6-mo frozen storage and had diminished from 87% protein solubility in fresh muscle to 72% of proteins soluble (Table 1). Decrease in protein solubility after frozen storage has been shown by other researchers (Undeland and others 2003). The decreased solubility indicated that protein denaturation had occurred due to the freezing and frozen storage. The increased TBARS showed that aldehyde had been generated

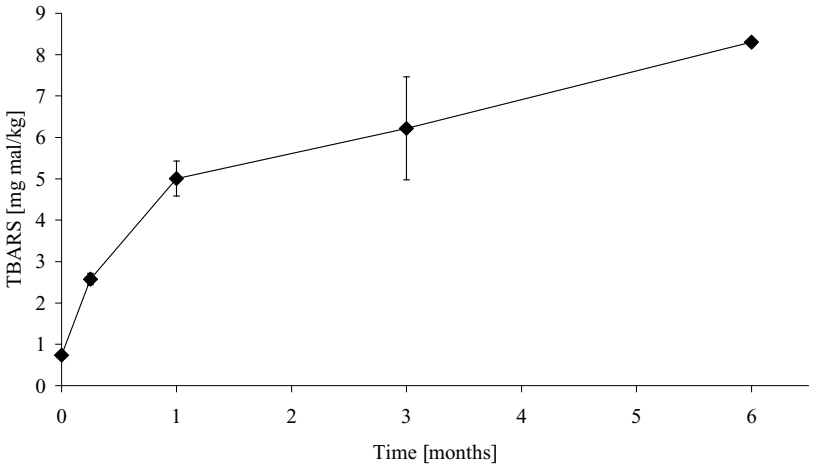

Figure 1—TBARS value of skinless herring fillets after up to 6 mo frozen storage at –24 °C. The herring was caught in September 2001. Each value is an average of 2 measurements with standard deviation. Significant difference between all values except between 1 and 3 mo frozen storage ($P < 0.05$).

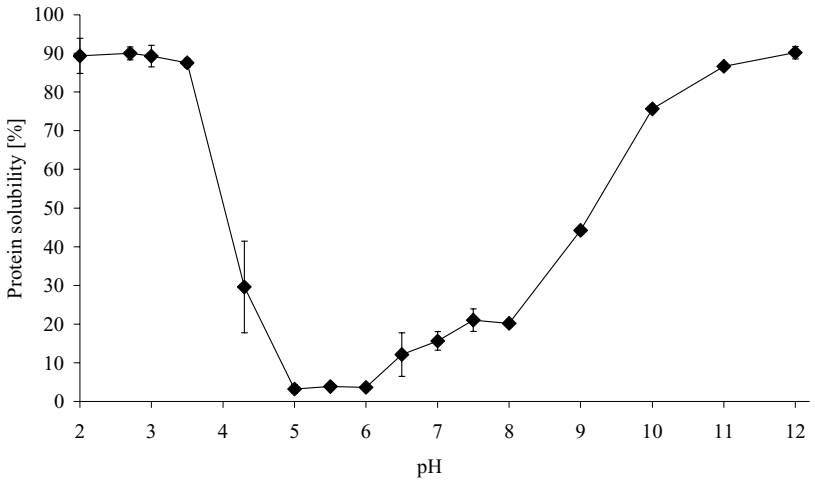

Figure 2—Protein solubility at 4 to 6 °C of washed herring muscle homogenate at selected pH values between 2 and 12. Each value is an average of 2 measurements with standard deviation. The herring was caught in September 2001 and was measured within 48 h post-catch.

due to lipid oxidation. Aldehyde could participate in protein degradation (Buttkus 1967; Tironi and others 2002).

Viscosity of herring muscle homogenate affected by frozen storage

Viscosity of unfrozen herring protein homogenate changed with pH in a similar way as the protein solubility; viscosity was the lowest around the isoelectric point and increased as the pH was lowered or increased (Figure 3A). When the pH of a homogenate from fresh, washed herring muscle was acidified, the viscosity increased sharply below pH 4.5, reached a maximum at pH 3, and decreased sharply when the pH was decreased below this value. The same pattern was observed when the pH was increased in homogenate from unfrozen herring muscle; the viscosity increased sharply above pH 7.5, reached a maximum at pH 10, and then decreased again when the pH was increased further (Figure 3A). This peak pattern changed at pH 11 when the raw material had been frozen. After 1-wk frozen storage of the raw material, the peak had diminished and after 6-mo frozen storage the peak had disappeared and the viscosity increased even more at pH 12 instead of declining as in the fresh muscle. This change was not seen at the low pH (Figure 3A). Viscosity increased at pH 2.7 significantly after 3-mo frozen storage compared to fresh muscle ($P < 0.05$). Freezing and frozen storage for 1 wk increased viscosity significantly at pH 11. Further frozen storage did not change the viscosity significantly (Figure 3B). Viscosity at pH 7 did not change significantly ($P < 0.05$).

The difference in viscosity with pH of herring muscle homogenate and the effects of frozen storage have been observed by other researchers (Undeland and others 2003). However, the change found on the viscosity peaks by frozen storage (Figure 3A) is not consistent with what Undeland and others (2003) observed in white muscle homogenate from New England herring. In that study, the viscosity peak disappeared in the low pH range but not in the high range by frozen storage at –18 °C for 18 d opposite to what was found here. Based on the findings by Kristinsson and Hultin (2003) that the myosin molecule is more affected by low pH then high pH, it was expected that the viscosity curve would change with frozen storage in the same manner as found by Undeland and others (2003). It should though be kept in mind that in the present study the whole muscle from herring fillets was used, not just the

Table 1—Protein solubility of washed herring muscle homogenate at pH 2.7, pH 7, and pH 11 after different time in frozen storage at –24 °C

Months	pH 2.7	pH 7	pH 11
0	$90.0^{a,b} \pm 3.0$	$15.6^{a} \pm 2.4$	$86.6^{a} \pm 0.5$
0.25	$93.4^{a} \pm 1.0$	$25.5^{b} \pm 3.1$	$85.5^{a} \pm 1.7$
1	$89.4^{a,} \pm 3.7$	$31.8^{b} \pm 2.7$	$90.2^{b} \pm 3.4$
3	$84.7^{b,c} \pm 2.3$	$30.0^{b} \pm 9.2$	$85.2^{a} \pm 1.9$
6	$80.9^{c} \pm 4.6$	$24.2^{b} \pm 2.7$	$71.6^{c} \pm 1.7$

[a]Values are means ± SD of 6 determinations.
Means with the same letter in each column are not significantly different ($P < 0.05$).

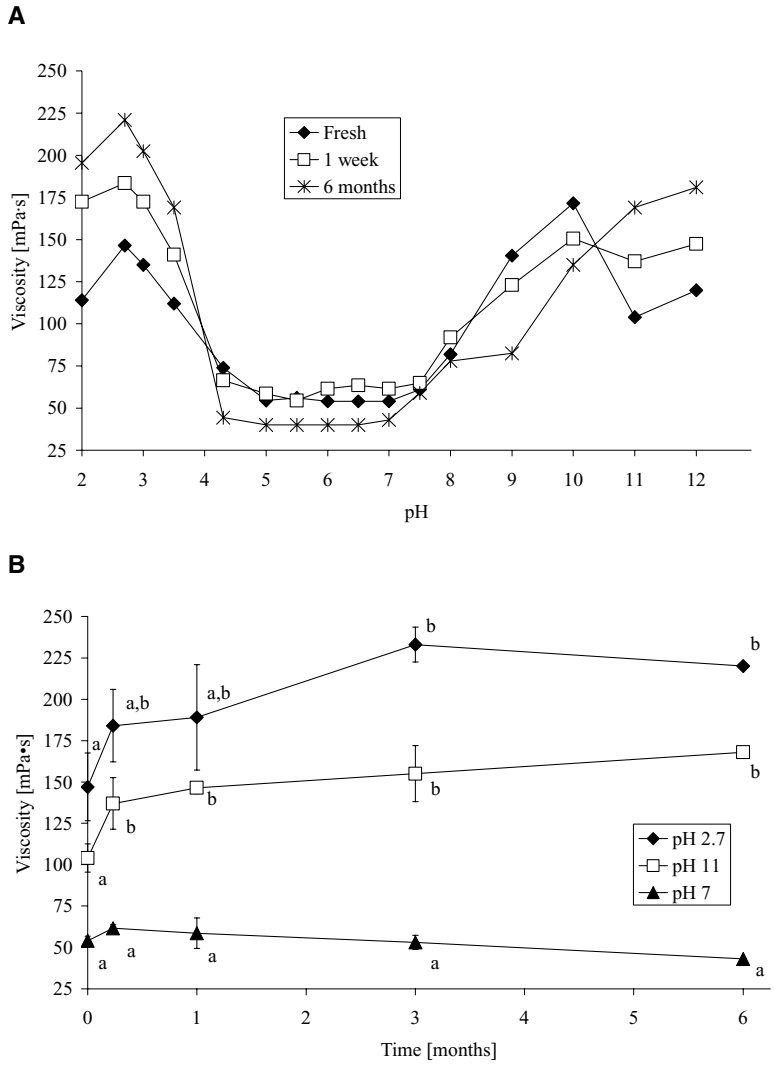

Figure 3—(A) Viscosity of washed herring muscle homogenate at different pH—fresh and after up to 6 mo at –24 °C. Each value is an average of 2 measurements. (B) Viscosity of washed herring muscle homogenate at pH 2.7, 7, and 11 after up to 6 mo frozen storage at –24 °C. Each value is an average of 2 measurements with standard deviation. Means with the same letter in each line are not significantly different ($P < 0.05$).

white muscle. Lipid content was therefore higher or 12.4% compared to 2.4% in white muscle. Kristinsson and Hultin (2003) reported that alkali treatment of cod myosin and washed cod muscle significantly improved their emulsifying properties compared with an acid treatment. The high lipid content of the homogenate might therefore be causing an emulsion at high pH causing different viscosity behavior than when using herring muscle homogenate with low lipid content.

Electrophoresis

No changes were found in SDS electrograms for herring proteins homogenate by freezing or by frozen storage for up to 6 mo (Figure 4), neither in bands identified as myosin (approximately 200 kDa) or actin (approximately 45 kDa) (see black arrows in Figure 4). Furthermore, no changes were found in the SDS profile when proteins were exposed to low pH, after washing of the homogenate or in the washing water (results not shown). However, it is known that freezing and thawing of herring causes liberation of proteolytic enzymes from the muscle tissue that can participate in proteolysis (Stefánsson and others 2000). The thawing of the herring fillets overnight at 0 °C did not cause detectable protein degradation (Figure 4).

Conclusions

This study showed that protein solubility and viscosity of homogenates of Icelandic herring fillets at pH 2.7 and pH 11 changed significantly during frozen storage of the raw material at –24 °C. The decreased solubility and increased viscosity

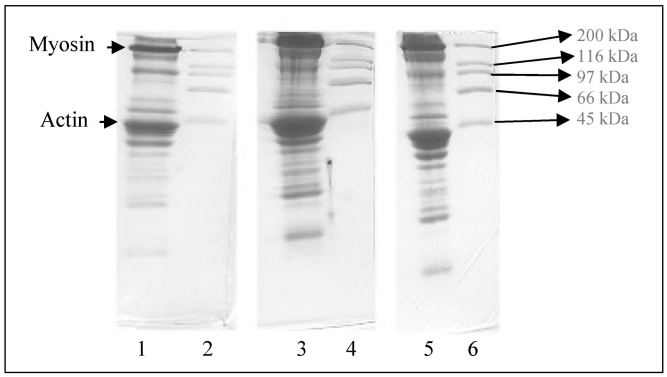

Figure 4—SDS-PAGE of homogenates made from fresh herring (lane 1), herring frozen for 1 wk (lane 3) and 6 mo (lane 5) at –24 °C along with high range (45 to 200 kDa) molecular weight SDS-PAGE standards (lanes 2, 4, and 6)

indicated that frozen storage of the herring raw material affected the structural and functional properties of the muscle proteins. The increased TBARS showed that the lipids of the raw material oxidized which might be the cause for the negative changes during frozen storage.

Since the protein solubility changed significantly during frozen storage it is recommended that frozen storage of the raw material (frozen Icelandic herring fillets) should not exceed 3 mo before being used as a raw material for protein isolation.

Acknowledgments

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