Solubilization and Thermostability of Water Dispersions of Muscle Structural Proteins of Atlantic Herring (*Clupea harengus*)

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Fillets of Atlantic herring (*Clupea harengus*) were cut into small pieces and washed in cold water at a ratio of 1:3 (w/v), then in a 0.5% (w/v) solution of sodium bicarbonate, and finally in cold water again. The washed meat was homogenized in ice-cold water at 12.5–20.0% (w/v). The apparent viscosity of the homogenate was dependent upon protein concentration. Heating of the homogenate up to 45 °C decreased the apparent viscosity, while at higher temperatures proteins were precipitated. Addition of acetic acid to lower the pH of the dispersion to 3.5 decreased the apparent viscosity without causing protein precipitation. The proteins in the acidified dispersion were highly stable to combinations of heat (100 °C for 30 min), centrifugation (up to 13500*g*) and presence of NaCl or $CaCl_2$ (20 mM each). However, increasing the pH above 4.0 resulted in the precipitation of proteins. The amino acid composition of the acidified, heated dispersion was comparable to that of the unwashed herring meat. The treatment, however, caused cross-linking of proteins as suggested by the sulfhydryl group content of the dispersion.

Keywords: *Herring, water dispersion, thermostability, solubilization, low viscosity, acetic acid, extruded products, protein extender*

INTRODUCTION

The world catch of herring and other pelagic fish including sardine and anchovies, among others, is above 20 million metric tons (Flick *et al.*, 1990). Because of the high lipid content and problems associated with rancidity development and flavor reversion, the fresh fish has limited consumer acceptance. Therefore, there is a need for exploring the potential use of pelagic fatty fish species such as herring for human consumption since their proteins possess excellent amino acid scores and digestibility characteristics (Sikorski *et al.*, 1990).

Fish proteins may be used to fortify the nutritive value of cereal-based foods, for preparing powders and hydrolysates as dietetic and hypoallergenic agents, and also as animal feed. However, fish myosin and actomyosin are generally regarded as being sparingly soluble in water or low-salt solutions at neutral pH values. This may be due to the tendency of myosin molecules to interact with one another under physiological conditions and in the presence of several low molecular weight compounds and enzymes that remain adhered to these proteins in their native state (Suzuki, 1981; Nakagawa *et al.*, 1989). A significant portion of the adhering compounds is removed upon washing, which enhances the gelation of proteins, and hence the resultant washed fish meat can be used for development of various texturized products (Suzuki, 1981; Venugopal and Shahidi, 1994a,b). In addition, fish proteins are sensitive to denaturation, myosin from cold water species being more prone to denaturation than that from tropical species (Howell *et al.*, 1991). The present paper reports the development of a process to prepare a free-flowing (low-viscosity), thermostable, and shelf-stable myofibrillar protein dispersion of mackerel in water. It is expected that such a preparation would facilitate the use of the fish muscle as a fortifying agent in product development.

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MATERIALS AND METHODS

Materials. Fresh Atlantic herring (*Clupea harengus*) was skinned and filleted, and the dark meat was manually removed. The dark meat-free fillets were comminuted into small pieces (0.1–0.2 g each), 500 g of which was washed with cold water (<10 °C) at a meat to water ratio of 1:3 (w/v). The meat was then suspended in fresh cold water at a meat to water ratio of 1:3 (w/v) and held overnight in a cold room (2–4 °C). The slurry was passed through a stainless steel sieve (mesh size, 100) to drain off the water. The meat was then resuspended in the same volume of cold water containing 0.5% (w/v) sodium bicarbonate. After standing for 2 h at 2–4 °C, the meat was recovered by passing through the sieve (see above). The treated meat was given a third wash by suspending it in cold water at a meat to water ratio of 1:3 (w/v) and holding it for 1 h. The washed material was resuspended in cold water at varying proportions ranging from 12.5–20% (w/v) (total volume, 200 mL) and was homogenized in a Waring blender for 1 min. Glacial acetic acid (1 mL) was then added dropwise to the homogenate to lower the pH to 3.5 as determined by pH meter. For heat treatment, samples were incubated in a water bath at 50 or 100 °C for 0–30 min and then cooled under a stream of cold running tap water. For all experiments reported, protein extractability of the washed meat is defined as percentage of the ratio of the weight difference of washed meat and the residue to the initial amount of washed meat.

Viscosity Measurements. The viscosity of the homogenate containing 0.94–1.54% protein (w/v) dispersions was measured using a Brookfield synchrolectric viscometer Model LVT (Cooksville, ON) calibrated using a Brookfield standard having a viscosity of 98.2 cP. During the measurements, 200 mL of the dispersion in a 250-mL beaker was held in water bath temperatures ranging from 5 to 45 °C. The viscosity was measured routinely at a speed of 60 rpm using spindle No. 2 or 3. The values were recorded after 1 min of rotation of the spindle in the dispersion. Variations in the viscosity of the same sample measured by the two spindles were always within 10% of each other; in such cases, the average values were used. The viscosity values were obtained using a conversion factor provided by the manufacturer (Tung, 1978) and were expressed in Pascal seconds (Pa·s).

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Viscosity was measured before acidification, immediately after the addition of glacial acetic acid (1 mL), and also after heating (at 50 or 100 °C over a period of 0–30 min) and cooling of the dispersions (200 mL) to room temperature.

Thermal and Centrifugal Stability. Aliquots (15 mL) of the acidified protein dispersion (containing 0.94% protein) were transferred into screw-capped vials and were held in boiling water for 0–30 min. After heat treatment, samples were cooled under running tap water and were centrifuged at 5000*g* for 15 min in a Sorvall RC-2 refrigerated centrifuge. The supernatant was passed through glass wool to remove emulsified oil. The protein content of the supernatant was then determined, as described in a later section. The amount of water separated, if any, was also noted and was expressed as percentage (v/v) of total volume of dispersion centrifuged.

For determination of centrifugal stability of samples, the heat-treated (in boiling water bath for 15 min) and cooled dispersion (15 mL) was centrifuged for 10 min at 5000–13500*g*. The supernatant was passed through glass wool to remove the emulsion layer and the protein content of the supernatant was determined.

Stability to pH Change. The pH of the acetic acid-treated dispersion was increased from 3.5–7.5 by the addition of a 2 N NaOH solution. The sample was then held in a boiling water bath for 15 min and cooled under a stream of cold water. The treated sample was centrifuged at 5000*g* for 15 min, as before, and the protein content of the supernatant was determined.

Effect of Salt on Stability. Solutions of NaCl (1 M) and $CaCl_2$ (1 M) were added to 10 mL of the dispersion to obtain a final concentration of 0–100 mM of each salt. The mixture was heat treated at 100 °C for 15 min in a water bath, cooled, and subsequently centrifuged at 5000*g* for 15 min, and the protein content of the supernatant was then determined.

Effect of Storage on Color Stability. The final acidified and heated (100 °C, 10 min) dispersion (50 mL) was aerobically sealed in 16 × 20 cm polyethylene pouches and stored at 2 or 25 °C. During the course of storage, tristimulus Hunter color parameters *L* (light/darkness, 100 for white and 0 for black), *a* (red, +; green, −) and *b* (yellow, +; blue, −) of the pouch surface were measured using a Colormet colorimeter (Instrumar Engineering Ltd., St. John's, NF). A white color plate with specifications $L = 94.5$, $a = -1.0$, and $b = 0.0$ was used for calibration.

Pepsin Digestibility. Activated pepsinogen (0.5 or 1.0 mL of a 1 mg/mL solution; Sigma, 3550 units of activity/mg of protein) was added to aliquots (20 mL) of the diluted dispersion (containing 63 mg % of protein). The mixture was incubated at 50 °C. At 5-min intervals (0–30 min), aliquots (2 mL) of the mixture were pipetted into 2 mL of 10% (w/v) trichloroacetic acid. After 18 h at room temperature, the samples were filtered through a Whatman No.1 filter paper, and the amount of tyrosine in the filtrate was determined.

Proximate Composition. Protein content (N × 6.25) of comminuted unwashed and washed meat as well as the acidified dispersion was calculated on the acidified basis of total nitrogen determined by the Kjeldahl method (AOAC, 1990). In experiments on stability to pH and salts, the procedure of Lowry *et al.* (1951) was used for determination of protein content, using bovine serum albumin as a standard. For tyrosine quantification, L-tyrosine was used as a standard. Moisture content of samples was determined by drying in a forced-air convection oven at 105 °C (AOAC, 1990). Lipids were quantified after their extraction with a chloroform/methanol/water mixture, as described by Bligh and Dyer (1959).

Amino Acid Composition. The unwashed herring meat as well as the acidified dispersion (protein content of 1.54%) was frozen at −80 °C and lyophilized. The resultant powders were digested at 110 °C in 6 N HCl containing 0.05% phenol for 24 h (Blackburn, 1968). The amino acid composition of the hydrolysates was determined using a Beckman 121 MB amino acid analyzer. Tryptophan was estimated by 24-h hydrolysis of the protein at 110 °C in the presence of 3 N mercaptoethanesulfonic acid under vacuum (Penke *et al.*, 1974). Methionine and cysteine were subjected to performic acid oxidation prior to hydrolysis in 6 N HCl (Blackburn, 1968). The content of free amino acids was determined by homogenizing (Polytron homogenizer, speed 4) 10 g of the sample with 20 mL of ice-cold 6% (w/v) perchloric acid. After 30 min of incubation at 0 °C, samples were centrifuged at 3000*g* for 10 min at 5 °C. The procedure was repeated twice, and the pH of the combined supernatants was adjusted to 7.0 using a 33% (w/v) KOH solution. Perchlorate precipitates were removed after centrifugation for 10 min at 3000*g*. The supernatant was acidified with 10 N HCl to pH 2.2 and diluted at 2:3 (v/v) with a 1.0% (w/v) lithium citrate buffer, pH 2.2. The extract was then analyzed on a Beckman 121 MB amino acid analyzer.

Table 1. Proximate Composition (Weight Percent) of Whole and Washed Herring Meat[a]

component	whole	washed
moisture	72.4 ± 0.1	86.7 ± 2.3
crude protein (N × 6.25)	17.4 ± 1.2	8.2 ± 0.2
lipid	8.7 ± 1.2	4.1 ± 0.1

[a] Results are mean values of four replicates ± standard deviation. Values in each row are significantly ($p < 0.05$) different from one another.

Sulfhydryl Group Measurements. Free and total sulfhydryl contents of prepared herring meat and the dispersion were determined by using a spectrophotometric method and employing a 0.016 M solution of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). Details of this procedure have previously been described by Synowiecki and Shahidi (1991).

Electrophoresis. SDS–PAGE in discontinuous buffer system was performed according to the method described by Laemmli (1970) using Pharmacia gel electrophoresis apparatus GE-4 with Pharmacia electrophoresis power supply ECPS 2000/300. Stacking gels were 3.5% (w/v) acrylamide with 0.093% (w/v) bis-(acrylamide), and resolving gels were 5 or 10% acrylamide containing 0.133 or 0.266% (w/v) bis(acrylamide), respectively. The low molecular weight standard (Sigma Chemical Co., St. Louis, Mo) included proteins of the following molecular masses (kDa): 14.2 (α-lactoalbumin), 20.1 (soybean trypsin inhibitor), 24 (trypsinogen), 29 (carbonic anhydrase), 36 (glyceraldehyde-3-phosphate dehydrogenase), 45 (egg albumin), and 66 (bovine albumin). Cross-linked bovine albumin was utilized as the high molecular weight standard which included monomer, dimer, trimer, and tetramer of molecular masses 66, 132, 198, and 264, kDa, respectively. One hundred micrograms of protein from the original unwashed herring meat and the heat-treated (100 °C, 15 min) dispersions treated with 1% sodium dodecyl sulfate (SDS) per gel was applied, and electrophoresis was performed at a constant current of 5 mA per gel. Gels were stained with Coomassie blue G 250 (Sigma).

Statistical Analysis. Analysis of variance and Tukey's Studentized range tests (Snedcor and Cochran, 1980) were used to determine differences in mean values based on data from replicates of each measurement. Significance was determined at 95% level of probability.

RESULTS AND DISCUSSION

The washing process partially removed pigments and other soluble components of the meat and gave a colorless and odorless preparation. The proximate compositions of unwashed and washed meat are given in Table 1. The washed meat had a higher moisture and a lower lipid content as compared with its unwashed counterpart. Despite the leaching out of soluble components, the mass of the washed meat increased by approximately 20% (w/w), possibly due to increased hydration of the myofibrillar proteins.

The washing process employed a procedure similar to that generally used for preparation of surimi from fatty fish species (Venugopal and Shahidi, 1994a) and consisted of sequential washing of the comminuted meat with cold water, a bicarbonate solution, and water. The treatment, which involved exposure of proteins to moderately high pH (bicarbonate wash), followed by readjustment of pH to neutrality, has been shown to improve the gelling ability of protein molecules (Schmidt, 1981). Thus, heating of unacidified dispersions caused coagulation of proteins and

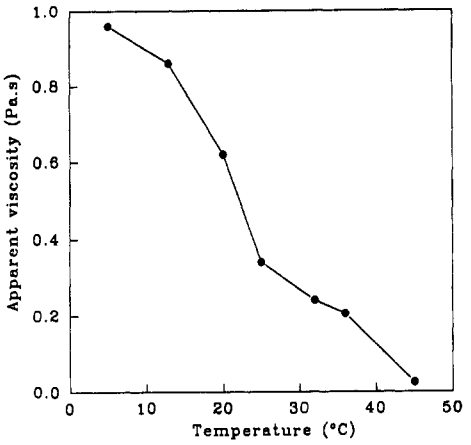

Figure 1. Influence of temperature on the apparent viscosity of 12.5% washed herring meat dispersion [protein content, 0.94% (w/v)]. The values are significantly ($p < 0.05$) different from one another.

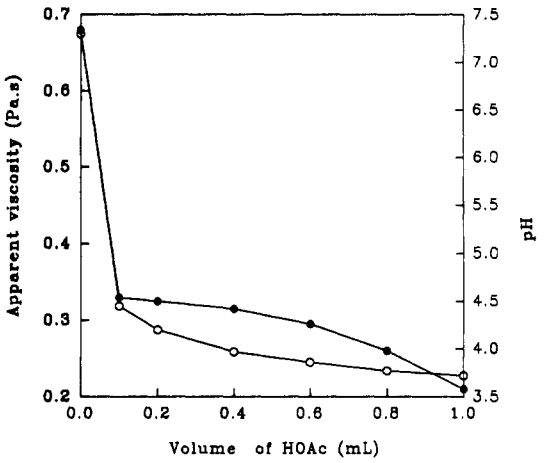

Figure 2. Influence of acetic acid concentration on pH and apparent viscosity of washed herring meat dispersion [200 mL, protein content, 0.94% (w/v)]: (●) pH; (○) apparent viscosity. Apparent viscosity values are significantly ($p < 0.05$) different from one another at HOAc addition of <0.4 mL, and pH values are not significantly ($p > 0.05$) different from one another upon addition of 0.1–0.6 mL of HOAc.

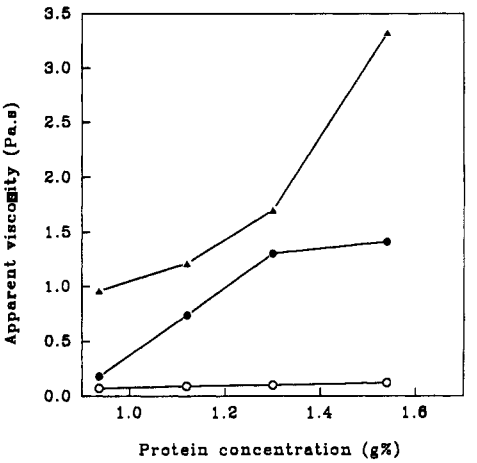

Figure 3. Effect of protein concentration on the apparent viscosity of washed herring meat dispersion [500 mL, 0.94% (w/v) protein content] with addition of HOAc and heat. Symbols for the addition of HOAc: (▲) no addition; (●) 1 mL; (○) 1 mL followed by heating at 50 °C. The values for different protein concentrations are significantly ($p < 0.05$) different from one another.

separation of water. This effect could be through enhanced unfolding of proteins, making them less compact, more solvated, and flexible (Tanford, 1968), increasing the hydrophilicity of the proteins through sodium salt formation of carboxyl groups (Dickinson and Stainsby, 1982) and by exposure of buried sulfhydryl groups available for aggregation (Schmidt, 1981). Gelation has been suggested to impart thermostability to proteins (Lilliford, 1986; Howell *et al.*, 1991). Gelation of myosin molecules depends upon heating conditions (Sharp and Offer, 1992) and involves irreversible aggregation of myosin heads through formation of disulfide bonds and helix–coil transition of the tail part of the molecules resulting in a three-dimensional network (Niwa, 1992; Stone and Stanley, 1992). This is also supported by the low content of sulfhydryl groups in the acidified, heated protein dispersion as compared with unwashed herring meat. The present results are also in agreement with those of Synowiecki and Shahidi (1991) and Jiang *et al.* (1989) on the heat-induced changes in seal and milk fish proteins, respectively, and lend further support to the findings of Wu *et al.* (1991), who reported enhanced solubility of washed fish muscle proteins in water.

The dispersions obtained by homogenization of the washed meat in water were highly viscous, similar to that observed for other proteins (Tung, 1978; Schmidt, 1981); their apparent viscosity depended upon temperature and protein concentration. Figure 1 illustrates the influence of temperature on apparent viscosity of a 12.5% (w/v) herring meat dispersion [protein content, 0.94% (w/v)]. The apparent viscosity decreased with increasing temperature and reached a minimum at 45 °C. However, at temperatures of 50 °C and above, the proteins were precipitated. Holding of the original dispersion at 0 °C for up to 4 days did not change its viscosity. In slurries, intermolecular protein–protein interactions dominate and are responsible for the observed viscosity behavior (Shen, 1982). It is likely that these interactions, which are influenced by temperature or low pH, determine the stability of proteins. Lowering of pH can induce conformational changes in protein molecules, influencing their α-helix content (Fink *et al.*, 1990) which leads to the gelation of fish myosin (Fretheim *et al.*, 1985) and shark myofibrillar proteins (Venugopal *et al.*, 1994). The heat-induced changes of the unacidified dispersions are comparable to the modori phenomenon of surimi gel, in which the homogeneous dispersion of a protein network might undergo intense shrinking at high temperatures. This results in the liberation of water, causing heterogeneity in the dispersion (Niwa, 1992). Similar observations were made for soy proteins (Hermansson, 1986).

The influence of addition of varying amounts of acetic acid (HOAc) on the pH and apparent viscosity of the homogenate [200-mL dispersion, 0.94% (w/v) protein content] is shown in Figure 2. The initial pH of the dispersion was 7.3 and reached a value of 3.7 upon addition of 1.0 mL of glacial acetic acid to it. The lowering of pH with varying amounts of HOAc corresponded with a decrease in apparent viscosity of the dispersion.

The apparent viscosity of the dispersion depended on its protein content (Figure 3). At a meat content of 20.5% [protein concentration of 1.54% (w/v)], the washed preparations were too viscous to be effectively homogenized. Addition of 1 mL of glacial acetic acid to the dispersions [200 mL, 0.94% (w/v) protein content] instantly reduced their apparent viscosity. Further loss of apparent viscosity was observed when the acidified dispersions were subjected to heat treatment at 100 °C followed by cooling at room temperature.

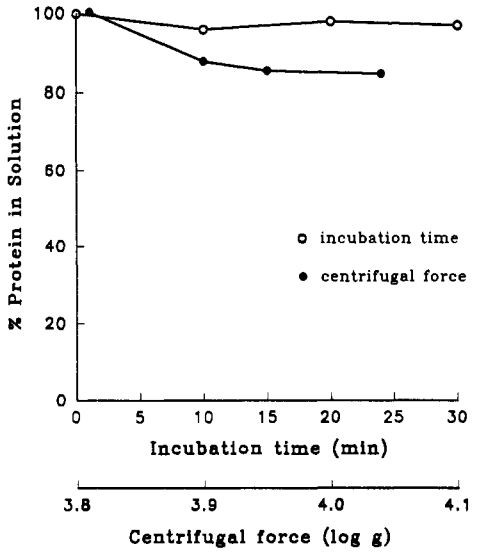

Figure 4. Thermal and centrifugal stability of acetic acid-treated herring meat dispersion. The dispersion [protein content, 0.94% (w/v)] was incubated at 100 °C for 0–30 min, cooled, and centrifuged at 5000*g* for 15 min. For centrifugal stability, the dispersion was incubated at 100 °C for 15 min, cooled, and centrifuged for 10 min at different *g* values. The protein content of supernatants was then determined.

The thermal and centrifugal stabilities of the acetic acid-treated dispersion (protein content of 0.94%) are shown in Figure 4. The protein in the dispersion was stable to heating for up to 30 min at 100 °C followed by centrifugation at 5000*g* for 15 min. The heat-treated (100 °C for 15 min) and cooled dispersion was also stable to centrifugation at up to 13500*g* for 10 min. When acidified dispersions at higher initial protein levels [(0.96–1.58% (w/v)] were subjected to heating (100 °C for 15 min), cooling, and centrifugation at 5000*g* for 15 min, more than 96% of the proteins remained soluble. Thus, proteins in the washed meat could be almost completely extracted into the solution. The centrifugation step also helped the removal of a lipid-containing emulsion layer (about 2 g/50 mL of dispersion) that was formed on top of the slurry.

Table 2 depicts the effect of heat treatment (100 °C for 15 min) on the stability of both unacidified and acidified dispersions. Unlike the acidified sample, the unacidified homogenate showed heavy precipitation of proteins. The heated and cooled acidified dispersion had low apparent viscosity, while that of the unacidified preparation could not be measured due to protein separation. Centrifugation of samples devoid of acid at 5000*g* for 15 min showed more than 95% separation of water; however, no water was separated from their acidified counterparts.

The influence of addition of NaCl or $CaCl_2$, in combination with heat, on the stability of the dispersion is shown in Figure 5. It was noted that while proteins were stable

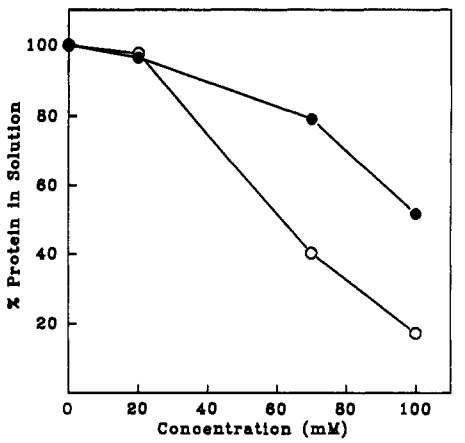

Figure 5. Effect of NaCl (●) and $CaCl_2$ (○) concentrations and heating (100 °C for 15 min) on stability of washed acidified herring meat dispersion [protein content, 0.94% (w/v)]. The values, except for 20 mM concentration, are significantly ($p < 0.05$) different from one another.

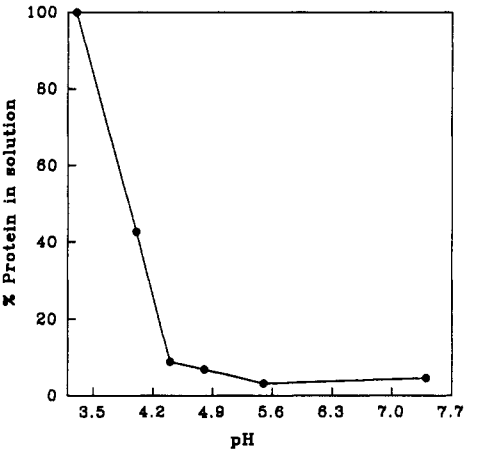

Figure 6. Effect of increasing pH followed by heating (100 °C for 15 min) on the soluble protein content of acidified washed herring meat dispersion [protein content, 0.94% (w/v)]. The values are significantly ($p < 0.05$) different from one another at pH <5.6.

to 20 mM salt solution, precipitation occurred at higher salt concentrations. Addition of a $CaCl_2$ solution caused 20–40% more precipitation of proteins in the dispersion than that caused by NaCl. At a 100 mM NaCl, approximately 50% of proteins were still in solution. However, increasing the pH followed by heating greatly affected the stability of proteins in the dispersion. Thus, most of the proteins were precipitated at a pH >4.0 (Figure 6).

The prepared dispersion was susceptible to digestion by pepsin as shown by a significant increase in its tyrosine content when it was incubated in the presence of pepsin

Table 2. Effect of Heating on Apparent Viscosity and Water Separation of Washed Herring Meat Dispersion[a]

protein concn (wt %)	apparent viscosity (Pa·s) initial[b]	HOAc (0.5 %) and heat[c]	no HOAc but heat[d]	water separation (%) HOAc (0.5 %)	No HOAc
0.94	0.96 ± 0.10	0.01 ± 0.02	ND[b]	nil	95
1.12	1.23 ± 0.10	0.02 ± 0.02	ND	nil	96
1.30	1.54 ± 0.05	0.04 ± 0.02	ND	nil	96
1.54	3.38 ± 0.14	0.07 ± 0.02	ND	nil	96

[a] The dispersions (200 mL) were heat treated (100 °C for 15 min) and cooled, and then apparent viscosity was measured. Acetic acid was added before heat treatment. Water separation was measured after centrifugation of the samples at 5000*g* for 15 min. Initial viscosity in the absence of acetic acid at 2–4 °C was measured after heating. Values reported are mean values of triplicate determinations ± standard deviation. [b] Values in this column were significantly ($p < 0.05$) different from one another. [c] Only the last number in this column was significantly ($p < 0.05$) different from others. [d] Protein precipitation occurred.

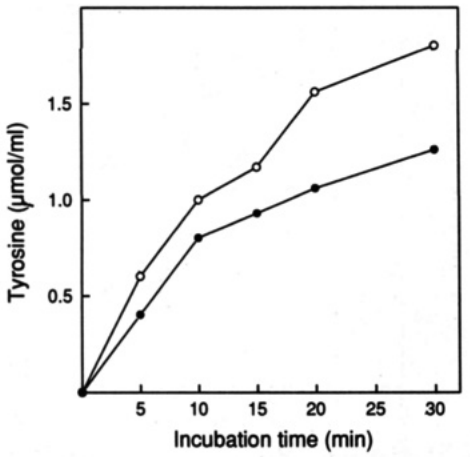

Figure 7. Pepsin digestibility of acidified and heated herring meat dispersion. (fdt) 0.5 mL of enzyme; (cop) 1.0 mL of enzyme. Conditions of enzyme treatment are given under Materials and Methods. Values are significantly ($p < 0.05$) different from one another.

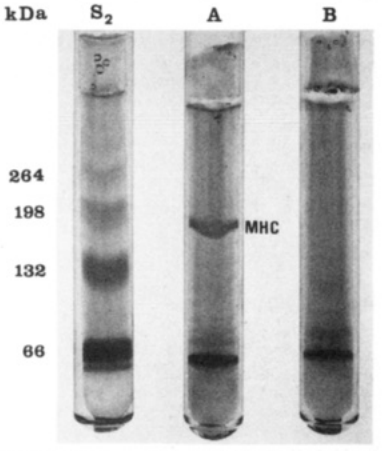

Figure 8. SDS–PAGE profiles of (A) the whole herring meat and (B) the dispersion on a 5% polyacrylamide gel. The profile of standards, S_2, is given: 66, 132, 198, and 264 kDa. MHC denotes myosin heavy chain.

(Figure 7). Heating (100 °C for 15 min) of the dispersion (protein content of 0.94%) alone resulted in the release of only 0.10 and 0.24 μmol of tyrosine/mL when 1 and 2% glacial acetic acid, respectively, was used without addition of any enzyme.

SDS–PAGE patterns of the original meat and the prepared dispersion were different. Thus, the myosin heavy chains (MHC) were observed only in unwashed native proteins on a 5% gel, while they were absent in the dispersion (Figure 8). On a 10% gel MHC was located on the top of gel of native proteins and myosin light chain (MLC) corresponded to a 25-kDa standard (Figure 9). The observation of the absence of the band corresponding to MHC in the dispersion is in concurrence with that of Kamat *et al.* (1992), who demonstrated a decrease in MHC content of surimi gels during incubation at temperatures ranging from 4–50 °C. This was attributed to non-disulfide covalent cross-linking or proteolysis in the case of Alaska pollock or croaker surimi (Kamat *et al.*, 1992). The present results are also in agreement with a recent report on solubilization of myofibrillar proteins of Atlantic mackerel (Venugopal and Shahidi, 1994b). Nevertheless, the molecular mechanisms of reduction of viscosity and stabilization of washed herring muscle proteins by combination of low pH and heat require further studies.

Table 3 shows the total amino acids profile of the dispersion and that of the unwashed meat. The extraction

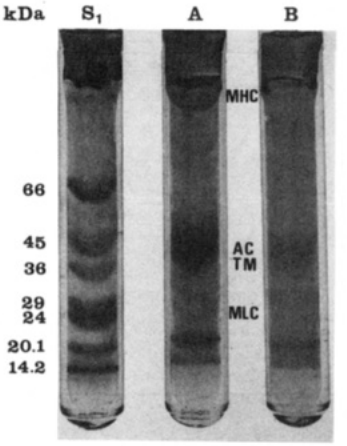

Figure 9. SDS–PAGE profiles of (A) the whole herring meat and (B) the dispersion on a 10% polyacrylamide gel. The profile of standards, S_1, is given: 14.2, 20.1, 24, 29, 36, 45, and 66 kDa. MHC denotes myosin heavy chain; MLC, myosin light chain; TM, tropomyosin; and AC, actin.

Table 3. Amino Acid Composition of Original Herring Meat and the Dispersion[a]

	total amino acid (mg/g)		free amino acid (μg/g)	
amino acid	original meat	dispersion	original meat	dispersion
aspartic acid	99.2	108.0	230	39
hydroxyproline	1.1	2.9		
threonine	45.4	50.0	340	23
serine	38.3	39.0	50	14
cysteine	17.3	4.8		
glutamic acid	135.0	142.0	100	34
proline	34.7	38.0	50	14
glycine	44.8	44.0	107	13
alanine	59.7	59.0	760	46
valine	60.0	55.5	350	32
isoleucine	42.9	47.7	85	23
leucine	80.3	81.0	280	11
tryosine	35.7	37.5	46	40
pheylalanine	40.0	44.0	90	54
lysine	89.8	94.5	710	26
histidine	27.1	21.7	160	8
arginine	59.3	65.3	100	9
tryptophan	12.1	12.4	50	
methionine	24.1	25.0	340	54

[a] Values given are averages of two determinations. Corresponding results for total amino acids, in each case, for the original herring meat and dispersion were significantly ($p > 0.05$) different from one another, except for serine, glycine, alanine, and leucine. For free amino acids, the corresponding results for the original meat and dispersion in all cases were significantly ($p < 0.05$) different from one another.

process did not have any marked effect on the amino acid profile of the meat, but free amino acid content of the dispersion was generally reduced by 60%. Meanwhile, the content of free and total sulfhydryl groups of the unwashed meat and the acidified and heated dispersion was altered (Table 4). The untreated meat had a high content of free sulfhydryl groups as compared to the dispersion. While disulfide bonds were present in the dispersion, they were absent in the original meat.

Solubility of large molecules such as the myofibrillar proteins may be defined as the percentage of protein that goes into solution or exists as a colloidal dispersion under specified conditions without precipitating by moderate centrifugal forces (Skaara and Regenstein, 1991). The present preparation of fish myofibrillar proteins in water had remarkable centrifugal and thermal stability. Thus, proteins in acidified dispersion were stable to heating at 100 °C, centrifugation, and presence of salt up to a certain concentration.

Table 4. Content of Sulfhydryl and Disulfide Groups in Original Herring Meat and Heated Dispersion[a]

protein source	sulfhydryl groups: initial sample	sulfhydryl groups: after S–S ond reduction	disulfide bonds
original meat	44.22 ± 1.06	44.49 ± 0.65	0.20 ± 0.19
dispersion	3.59 ± 0.12	14.02 ± 0.85	5.01 ± 0.35

[a] The values are expressed as μmol/g protein and are mean values of three determinations ± standard deviation. Results for sulfhydryl groups for the original meat before and after S–S bond reduction were not significantly ($p > 0.05$) different, but for the dispersion were significantly ($p < 0.05$) different from each other.

Table 5. Effect of Storage on Color Characteristics of Acidified and Heated Dispersion[a]

storage period	stored at 2 °C: *L*	*a*	*b*	stored at 25 °C: *L*	*a*	*b*
1 week	73.5 ± 1.5	−1.1 ± 0.2	9.3 ± 0.5	73.4 ± 0.5	−0.4 ± 0.2	15.4 ± 0.5
3 weeks	75.2 ± 1.6	0.9 ± 0.5	10.5 ± 0.9	72.1 ± 0.9	0.8 ± 0.2	19.2 ± 0.4

[a] Results are mean values of six determinations ± standard deviation. Initial Hunter *L*, *a*, *b* values of the dispersion were 74.7 ± 0.8, −1.9 ● 0.3, and 9.1 ± 0.9, respectively. Storage of the dispersion at 2 °C resulted in a significant ($p > 0.05$ change in the Hunter *a* value. For 25 °C-stored sample, the Hunter *a* value changed to the same extent as that at 2 °C, but the *b* value changed drastically ($p < 0.05$).

Storage of the preparations at ambient temperatures under aerobic conditions led to their slight yellowing as determined by Hunter color values. Thus, the *b* values indicative of yellowing increased during storage of the dispersions at ambient temperatures. Refrigerated samples did not undergo any color change (Table 5).

While preparation of dispersions containing more than 20% washed meat was difficult, due to high viscosities, addition of acetic acid to a 30% mixture of washed meat prior to homogenization circumvented the problem. The mixtures may be incubated at 2–4 °C for 18 h followed by homogenization without encountering problems related to the thickness of the slurry. The preparation had a protein content of 2.5% after heat treatment and cooling.

Interest in stable proteins is increasing not only to understand their structure–function relationships but also to explore their potential areas of application (Kristjansson and Kinsella, 1991; Nosoh and Sekiguchi, 1991; Doi, 1993). Advantages of the present preparations are their high thermostability, low viscosity, and presumed resistance against microbial spoilage due to the presence of acetic acid. Potential areas of application of fish protein dispersions for product development are being examined.

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