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Water dispersions of myofibrillar proteins from capelin (*Mallotus villosus*)

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Mechanically deboned whole capelin was subjected to washing with water, 0·5% sodium chloride, and 0·5% sodium bicarbonate. The washing process yielded myofibrillar proteins which, upon homogenization in cold water and heat treatment at 70°C for 15 min followed by centrifugation, produced a low-viscosity dispersion. The proteins in the dispersion were stable to heating at 100°C and remained soluble in solution over a wide range of pH and in the presence of some salts. The amino acid composition of the dispersion was similar to that of the original mince. The product was nearly bland in taste as most of its flavour-active free amino acids were removed by the washing process.

INTRODUCTION

Capelin (*Mallotus villosus*) is an important pelagic species and perhaps the most important of all forage fish found abundantly in cold waters of the Arctic and sub-Arctic in both the Atlantic and Pacific regions. In North America, capelin is present from Hudson Bay to Nova Scotia, especially around Newfoundland and Labrador. Its total global annual landing is about 200 000 metric tonnes, Canada contributing 20 000–40 000 metric tonnes to this amount (Andrews, 1988).

While female roe capelin has found a lucrative market in Japan, male and spent capelin are generally dumped or reduced to meal or silage (Eide et al., 1982). Recently, fish sauce, dried products and hydrolyzed protein preparations have been produced from capelin in Newfoundland. Nonetheless, there is a general desire to better utilize capelin for novel foods for human consumption (Whittle & Hardy, 1990).

Production of a thermostable, low viscosity water dispersion of myofibrillar proteins from Atlantic mackerel (*Scomber scombrus*) was recently reported from our laboratories (Venugopal & Shahidi, 1994). The protein dispersion so produced did not precipitate upon heating of the acidified solutions at 100°C or upon centrifugation. In this paper we report on an aqueous extraction process for production of a thermostable protein dispersion from muscle tissues of capelin.

MATERIALS AND METHODS

Capelin (*Mallotus villosus*) was obtained from a local fish processor. Whole capelin was subjected to mechanical deboning using a Baader 694 deboning machine with a drum orifice size of 3 mm. The process yielded 13·7 kg deboned meat from 16·0 kg starting material.

Mechanically deboned capelin meat (MDCM) (500 g) was suspended in 1000 ml cold water and stirred gently over a 30 min period. The suspension was then filtered through three layers of cheesecloth to remove the wash water. The residue was then suspended in 1000 ml of a 0·5% (w/v) solution of sodium chloride and then in a 0·5% (w/v) sodium bicarbonate solution. After a 30 min standing, the residue was collected as before and the resultant meat was further washed with water to remove any residual bicarbonate. The resultant washed MDCM was used for further studies.

The washed meat was suspended in cold water at proportions ranging from 10 to 50% (w/v) and was homogenized in a Waring blendor. The homogenate was held in a water bath at 70°C for 15 min and then cooled to room temperature and centrifuged at 2675 $\times g$ for 20 min using an ICE CENTRA MP4 centrifuge (International Equipment Co., Needham Heights, MA, USA). The supernatant was collected and its protein content determined using the Kjeldahl procedure (AOAC, 1990).

Thermal stability

Aliquots (20 ml) of the dispersion were transferred to screw-capped glass vials. They were then held in a boiling water bath for 15 min. After cooling to room temperature, the vials were centrifuged at 2675 $\times g$ for 15 min. The content of protein in the supernatant was then determined.

Stability to pH variation and salts

The pH of the dispersion was adjusted between 3·5 and 10·0 by addition of a 2M HCl or NaOH solution. The samples were then heated in a boiling water bath for 15 min, cooled to room temperature and centrifuged. The content of protein in the supernatant was then determined.

In order to examine the effect of salts, known volumes of a 1M NaCl, KCl or $CaCl_2$ were added to the dispersion to obtain 0–50 mM salt solutions. After thorough mixing, the samples were heat-treated in a boiling water bath for 15 min, cooled and centrifuged. The percentage of protein remaining in the supernatant was then determined.

In another set of experiments, the influence of sodium tripolyphosphate (STPP) on the solubility characteristics of dispersed proteins was tested. The concentration of STPP in the dispersion was 25 mM. After mixing, the samples were heat-processed, cooled and centrifuged as described above.

Viscosity measurements

The viscosity of dispersions was measured using a Brookfield synchro-lectric viscometer model LVT (D. W. Brookfield Ltd, Cooksville, ON, Canada) calibrated using a Brookfield standard with a viscosity of 98·2 cp. During measurements, the dispersion (200 ml) was held in a water bath at the desired temperature. The viscosity was measured routinely at 60 rpm using spindle No. 2 or 3. The values were recorded after 1 min of rotation of spindle in the dispersion. Viscosity values were obtained from the conversion factor provided by the manufacturer (Tung, 1978). The apparent viscosity (Rao, 1977) values were expressed in Pascal seconds (Pa.s).

Total and free amino acid compositions

The unwashed as well as the washed dispersions of MDCM were frozen at –60°C and lyophilized. The resultant powders were digested at 110°C in 6M HCl for 24 h (Blackburn, 1968). The amino acid composition of the resultant hydrolyzate was determined using a Beckman 121 MB amino acid analyser. Tryptophan was determined after a 24 h hydrolysis of the protein at 100°C in the presence of 3M mercaptoethane sulphonic acid (Penke *et al.*, 1974). Methionine and cysteine were subjected to performic acid oxidation prior to hydrolysis in 6M HCl (Blackburn, 1968).

The content of free amino acids in the unwashed and washed dispersed MDCM was determined by homogenizing (Polytron homogenizer, Speed 4) 10 g of the sample with 20 ml ice cold 6% (w/v) perchloric acid. After 30 min 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 recovered after centrifugation for 10 min at 3000 ×*g*. The supernatant was acidified with 10M HCl to pH 2·2, diluted at a ratio of 1 : 1·5 (v/v) with a 1·0% (w/v) lithium citrate buffer, pH 2·2. The content of free amino acids was determined using a Beckman 121 MB amino acid analyser.

RESULTS AND DISCUSSION

The washing process partially removed soluble components of the meat and gave a lighter coloured and nearly odourless preparation. The proximate composition of unwashed and washed MDCM is given in Table 1. The washed meat had a higher moisture and a lower lipid and protein content as compared with its unwashed counterpart. A similar observation was made by Lee *et al.* (1991) for bicarbonate-washed sardine proteins. The washing process employed a procedure similar to that generally used for preparation of surimi from fatty fish species (Venugopal & Shahidi, 1995). This treatment involved exposure of proteins to the moderately high pH of the bicarbonate solution and enhanced the unfolding of protein chains, thus influencing their solubility and gelling characteristics (Tanford, 1968; Schmidt, 1981; Howell *et al.*, 1991). The thermal and centrifugal stabilities of the dispersed proteins in aqueous solution are shown in Table 2. The protein in the dispersion was stable to heating for up to 30 min at 100°C followed by centrifugation at 2675 ×*g* for 15 min. Over 88% of the original proteins remained soluble in the dispersion. Heating of aqueous dispersions containing 10–50% (w/v) of washed MDCM at 70°C followed by centrifugation allowed over 92% of the proteins to remain in water.

The influence of addition of NaCl, KCl, or $CaCl_2$ with or without STPP, in combination with heat, on the stability of capelin dispersions is shown in Fig. 1.

Table 1. Proximate composition of unwashed and washed mechanically deboned capelin meat (MDCM)[a] (mean ± SD, *n* = 3)

Constituent (%)	Unwashed MDCM	Washed MDCM
Moisture	83·98 ± 0·20	93·19 ± 0·10
Protein	12·70 ± 0·31	4·51 ± 0·15
Lipid	1·98 ± 0·01	1·13 ± 0·04

[a]MDCM was washed with water, 0·5% NaCl and 0·5% $NaHCO_3$.

Table 2. Effect of heating time at 100°C on protein solubility of washed MDCM[a] (mean ± SD, *n* = 3)

Heating time (min)	Protein content of solution (g %)	Protein remaining in solution (%)
0	2·27 ± 0·00	100
10	2·10 ± 0·05	92·5 ± 2·2
20	2·00 ± 0·05	88·0 ± 2·2
25	2·00 ± 0·05	88·0 ± 2·2
30	2·00 ± 0·04	88·0 ± 1·8

[a]Dispersions were heated at 100°C, cooled under cold water and then centrifuged at 2675 ×*g* for 15 min. pH of original dispersion = 7·10.

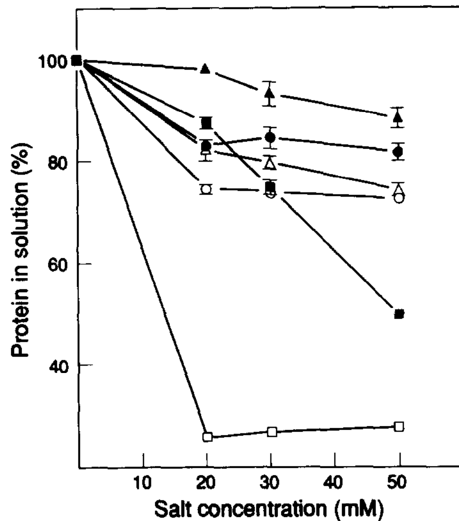

Fig. 1. Effect of presence of salts: (○) NaCl; (△) KCl; and (□) $CaCl_2$, on the solubility of proteins from washed mechanically deboned capelin meat. Full symbols represent corresponding data in the presence of 25 mM sodium tripolyphosphate. Each point represents mean value of triplicate determinations. Standard deviations from means for proteins in solution did not exceed 2·2% as shown by error bars.

While proteins remained fairly soluble in 50 mM NaCl or KCl solution, presence of $CaCl_2$ resulted in precipitation of 72–74% of proteins. STPP, together with the above salts, generally enhanced the solubility of proteins. These results are in concurrence with those of Hermansson (1986) who showed partial separation of water from protein gels in the presence of 5% salt.

The apparent viscosity of the dispersion depended on its content of protein (Fig. 2). At a washed meat to water ratio of 1 : 1 (w/v), the dispersion (2·27% protein, w/v) was too viscous to be effectively homogenized. However, upon heating of the dispersion to 70 or 100°C for 15 min, and subsequent cooling to room temperature, the apparent viscosity of the solutions was drastically reduced. While the viscosity of dispersions heated to 70°C showed some concentration dependence, the viscosity of the protein solution heated to 100°C remained constant at about 0·15 Pa.s.

The influence of pH on the solubility of capelin proteins was further examined. Results shown in Fig. 3 show that, while proteins remained totally soluble at pH 7·0–7·5, a minimum solubility was reached when pH was lowered to 5·5 which is close to the isoelectric point of myofibrillar proteins of fish. However, under most pH conditions over 85% of proteins remained soluble. The viscosity of the dispersions remained low (< 0·20 Pa.s) under all conditions, except when precipitation occurred.

Table 3 shows the total amino acid profile of the unwashed and washed dispersions of MDCM proteins. The extraction process did not have any marked effect on the amino acid profile of the samples. The largest change (approximately 7·6–8·3%) was noted for glycine and proline contents, respectively, presumably due to the concentration of connective tissues in the washed MDCM. Meanwhile, the total content of free amino acids in the washed MDCM was reduced by about 92% (Table 4).

In summary, the present study demonstrates that a thermostable, low-viscosity dispersion of myofibrillar proteins of capelin could be prepared. Potential areas of application of this preparation in production of functional protein powders and in formulation of extruded products is being examined.

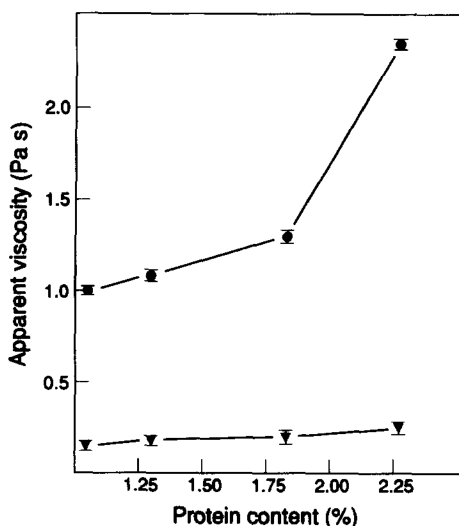

Fig. 2. Effect of protein concentration with or without prior heat treatment (100°C) and centrifugation (2675 ×g) on the viscosity of dispersions from washed mechanically deboned capelin meat. (●) Without heating; and (▼) with heating. Each point represents mean value of triplicate determinations. Standard deviations did not exceed 0·03 Pa.s units as shown by error bars.

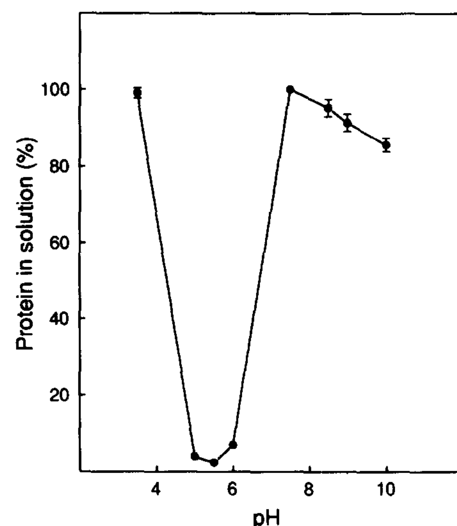

Fig. 3. Effect of pH variation followed by heating (100°C) and centrifugation on the solubility of proteins from washed mechanically deboned capelin meat. Each point represents mean value of triplicate determinations. Standard deviations from means for proteins in solution did not exceed 2·2% as shown by error bars.

Table 3. Amino acid composition of unwashed and washed aqueous dispersions of mechanically deboned capelin meat (MDCM) in g/100g protein (mean ± SD, n = 3)

Amino acid	Unwashed MDCM	Washed MDCM
Alanine	6·08 ± 0·06	6·11 ± 0·08
Arginine	6·33 ± 0·12	6·53 ± 0·20
Aspartic acid	10·88 ± 0·10	10·87 ± 0·10
Cysteine	0·93 ± 0·02	0·90 ± 0·03
Glutamic acid	14·86 ± 0·12	14·64 ± 0·16
Glycine	5·33 ± 0·12	5·74 ± 0·25
Histidine	2·35 ± 0·05	2·18 ± 0·03
Isoleucine	4·72 ± 0·18	4·88 ± 0·10
Leucine	9·19 ± 0·16	9·25 ± 0·20
Lysine	9·54 ± 0·08	9·47 ± 0·05
Methionine	3·08 ± 0·10	3·11 ± 0·09
Phenylalanine	4·42 ± 0·10	4·2 ± 0·15
Proline	3·63 ± 0·05	3·93 ± 0·05
Serine	4·41 ± 0·10	4·26 ± 0·18
Threonine	4·83 ± 0·12	4·95 ± 0·10
Tryptophan	1·08 ± 0·10	0·99 ± 0·10
Tyrosine	4·00 ± 0·08	4·11 ± 0·05
Valine	5·72 ± 0·10	5·78 ± 0·10

Table 4. Free amino acid content of unwashed and washed dispersions of mechanically deboned capelin meat (MDCM) in mg/100g protein (mean ± SD, n = 3)

Amino acid	Unwashed MDCM	Washed MDCM
Alanine	44·8 ± 0·6	3·3 ± 0·2
Anserine	66·1 ± 1·5	Trace
Arginine	11·7 ± 0·3	3·5 ± 0·1
Aspartic acid	22·7 ± 1·0	2·3 ± 0·1
Cysteine	2·3 ± 0·1	Trace
Glutamic acid	43·6 ± 1·3	2·8 ± 0·3
Glycine	22·1 ± 0·6	1·4 ± 0·1
Histidine	10·0 ± 0·3	0·8 ± 0·1
Isoleucine	19·5 ± 0·5	1·6 ± 0·2
Leucine	37·4 ± 0·8	3·0 ± 0·1
Lysine	39·3 ± 0·9	10·2 ± 0·1
Methionine	14·0 ± 0·6	1·5 ± 0·2
Ornithine	3·5 ± 0·6	1·5 ± 0·3
Phenylalanine	17·7 ± 0·5	2·4 ± 0·3
Proline	22·6 ± 0·2	1·3 ± 0·2
Serine	26·9 ± 0·8	2·7 ± 0·5
Taurine	129·6 ± 2·3	4·1 ± 0·6
Threonine	22·3 ± 0·1	1·6 ± 0·1
Tryptophan	3·1 ± 0·3	0·2 ± 0·1
Tyrosine	16·6 ± 0·2	2·1 ± 0·1
Valine	31·1 ± 0·5	2·3 ± 0·3
Total	606·9	48·6

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