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Alkali-assisted extraction of proteins from meat and bone residues of harp seal (*Phoca groenlandica*)

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Proteins from mechanically separated seal meat (MSSM) or bone residues thereof were extracted at pH 10.5 over a 1-h period at 20°C and subsequently precipitated at pH 4.5–5.5. Washing of the resultant precipitate with acetone removed a large portion of haem pigments and effectively improved the colour of the product. The recovery of proteins from MSSM ranged from 56.92 to 63.88% and that for bone residues was 12.02–13.07%, depending on the extraction temperature employed. Precipitates obtained at pH 4.5 contained 85.88% moisture, 12.43% proteins, 0.31% minerals and 1.08% lipids. The moisture content of the acetone-washed product was decreased to 8–15% with a lipid content of 1.10%, on a dry-weight basis. Protein efficiency ratio (PER) values for extracted proteins from MSSM (3.13) and seal bones (3.09) were higher than those for beef (2.8) and pork (2.5) and similar to that for cod (3.1). Tryptophan was the limiting amino acid in the protein isolates. Among the free amino acids, taurine was present at 22.9 mg%. This amino acid is important for functional regulation of the eyes, heart, muscles and central nervous system. Alkali-extracted proteins of seal meat had excellent fat adsorption, emulsifying capacity and emulsion stability. The nitrogen solubility index (NSI) value of the products was similar to those obtained for base-extracted proteins from chicken bone residues. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The population of harp seal (*Phoca groenlandica*) in the Atlantic regions of Canada is currently estimated at 3.1–4.6 million. Seals feed on a variety of fish species and consume up to 5 million metric tons of capelin, crustaceans, herring, salmonids and cod. Federal regulations allow the harvest of up to 250 000 seals annually; however, only 50 000–70 000 animals were caught until 1995. This provided approximately 1.2–2.1 million kg of meat each year (Synowiecki & Shahidi, 1991).

Seal meat is a rich source of nutritionally valuable proteins (Shahidi *et al.*, 1990). However, full utilization of the meat is limited due to its dark colour and intense flavour. Seal bone residues from mechanical deboning of seal carcasses are also a rich source of protein (about 25%). A simple base-extraction process was employed to recover protein residues from deboning of chicken carcasses which were subsequently used as an additive in luncheon meats without alteration of their quality (Jelen *et al.*, 1982). Caldironi & Ockerman (1982) also substituted alkali-extracted proteins from beef bones in beef sausages at levels of up to 15% and obtained very acceptable products. Lysinoalanine and other toxic amino acid derivatives were not detected under mild base-extraction conditions proposed by Lawrence & Jelen (1982). The present study employed a base-extraction process to recover proteins from mechanically separated seal meat (MSSM) and bone residues thereof in order to achieve full utilization of carcass components of harp seal.

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MATERIALS AND METHODS

Harp seals (*Phoca groenlandica*), age from 1 to 4 years, were caught in the coastal areas of Newfoundland during April and May, bled and skinned, blubber fat removed and eviscerated. The carcasses were subjected to mechanical deboning using a Poss deboner (Model PDE500, Poss Limited, Toronto, Ontario, Canada) and components were kept frozen in vacuum-packed

polyethylene pouches at −20°C until used. Base-extracted proteins from MSSM or seal bone residues were prepared according to the methods developed by Jelen *et al.* (1979) and McCurdy *et al.* (1986) for the recovery of proteins from beef and chicken bones, respectively. The main steps in the preparation of alkali-extracted proteins included extraction of proteins at pH 10.5 over a 1-h period, separation of unextracted connective tissues and precipitation of proteins at pH 4.5–5.5. Approximately 150 g of MSSM or seal bone residues were mixed with 600 ml of distilled water to which 4 M NaOH solution was added to reach pH 10.5. Extraction was carried out at 20 or 80°C for 1 h. The unextracted residues were separated by centrifugation at 2000 *g* for 15 min. Alkali-extracted proteins were precipitated with 4 M HCl solution at pH 4.5–6.0 and centrifuged at 2000 *g* over a 15-min period. The precipitate was then washed three times with 100 ml of acetone, filtered under suction using Whatman No. 1 filter paper and dried.

Moisture, crude protein (N×6.25) and ash contents in the products were determined according to AOAC (1990). Total lipids were extracted using a chloroform–methanol–water mixture (Bligh & Dyer, 1959). The amino acid composition of proteins was determined after digestion of samples in 6 M HCl at 110°C (Blackburn, 1968) using a Beckman 121 MB amino acid analyser (Beckman Instruments Inc., Palo Alto, CA, USA). Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, using performic acid oxidation prior to their digestion in 6 M HCl (Blackburn, 1968). Analysis of tryptophan was carried out by hydrolysis of the sample under vacuum in 3 M mercaptoethanesulphonic acid at 110°C (Penke *et al.*, 1974).

Essential amino acid index (EAA) (FAO/WHO/UNU Report, 1985) and protein efficiency ratio (PER) values were then calculated by consideration of the content of 10 designated amino acids from the equations developed by Lee *et al.* (1978) as described elsewhere (Shahidi & Synowiecki, 1993).

For determination of free amino acids, 1 g of sample was dissolved in ice-cold 6% perchloric acid (1:2, w/v) and then mixed using a Polytron homogenizer (Brinkmann Kinematica AG, Littau-Switzerland, Model PT3000). After a 30-min incubation period, samples were centrifuged at 3000 *g* for 10 min at 5°C. The pH of the extract was adjusted to 7.0 using a 33% (w/v) KOH solution. The precipitated potassium perchlorate was removed by centrifugation at 3000 *g* for 10 min. The supernatant was acidified with 10 M HCl to pH 2.2 and diluted with 0.3 M lithium citrate buffer, pH 2.2 (2:1, v/v). Free amino acids were analysed using a Beckman 121MB amino acid analyser using a Benson D-X 8.25 resin and a single column, according to the three-buffer lithium method, as described in the Beckman 121MB-TB-0.17 application notes.

The tristimulus Hunter colour parameters *L* (lightness, 100 = white, 0 = black), *a* (red, +; green, −) and *b* (yellow, +; blue, −) of the samples were measured using a Colormet colorimeter (Instrumar Engineering Ltd, St. John's, Newfoundland, Canada). The unit was standardized with a B-143 white calibration tile having a Hunter *L* value of 94.5 ± 0.2, an *a* value of −1.0 ± 0.1 and a *b* value of 0.0 ± 0.2.

The solubility of base-extracted proteins was determined using 0.2 g of sample suspended in 20 ml of distilled water. Adjustment of the pH values of the mixtures was achieved using 0.1 M solutions of NaOH or HCl. After standing for 10 min at room temperature, the mixtures were then centrifuged at 12 000 *g* for 15 min. The supernatants were diluted to 50 ml with water and 20 ml aliquots were used for Kjeldahl analysis (AOAC, 1990). The solubility was expressed as a percentage of soluble proteins at a given pH.

The nitrogen solubility index (NSI) in NaCl solutions was assayed at pH 6.1 according to Ozimek *et al.* (1986). The content of soluble nitrogen compounds was determined using the Kjeldahl procedure (AOAC, 1990).

Moisture and fat adsorption, emulsifying capacity, emulsion stability, whippability and foam stability were determined as described elsewhere (Shahidi *et al.*, 1995). Analysis of variance and Tukey's studentized range tests (Snedecor & Cochran, 1980) were used to determine differences in mean values of replicates of each measurement. Significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

The amount of base used for extraction of proteins from MSSM was 9.6 g NaOH/kg meat. The presence of large amounts (5.3%) of haemoproteins in seal meat is responsible for the dark colour of lyophilized precipitates. Hence, washing of the precipitated proteins with acetone was effective in removing some of the pigment residues and markedly improved the colour of the product (Table 1).

The highest loss of protein from MSSM in the extraction process was due to unextracted connective tissues (12.9–14.2%) and the presence of some unprecipitated proteins in the supernatant (Table 2). An increase in the extraction temperature from 20 to 80°C improved the recovery of proteins from MSSM and seal bone residues from 56.92 to 63.88% and from 12.02 to 13.07%, respectively. However, at higher temperatures, formation of lysinoalanine, lanthionine, ornithinoalanine and other amino acid derivatives might occur. Lawrence & Jelen (1982) reported the formation of lysinoalanine at less than 200 ppm after 4 h of base extraction (pH = 10.7) of chicken bone residues at 50°C. However, no lysinoalanine was detected in this study in any of the samples which were heated for 1 h.

The yield of proteins from seal bone residues (12.0%) was similar to the 9–16% recovery reported by McCurdy *et al.* (1986) in a pilot-scale processing of residues from mechanically separated chicken meat. Higher protein recovery (56.92%) from MSSM was perhaps due to its lower content of connective tissues. The best yield of proteins was achieved at a precipitation pH of 4.5–5.5, corresponding to the isoelectric range of

Table 1. Hunter colour parameters of lyophilized, base-extracted seal muscle proteins precipitated under different pH conditions[1]

Hunter value	pH of protein precipitation			
	4.5[2]	4.5	5.5	6.0
L	20.5 ± 0.1[a]	52.2 ± 0.1[b]	51.0 ± 0.2[c]	50.1 ± 0.2[d]
a	7.4 ± 0.1[a]	10.2 + 0.1[b]	10.8 ± 0.2[c]	12.1 ± 0.1[d]
b	8.2 ± 0.2[a]	16.6 ± 0.0[b]	18.6 + 0.1[c]	20.1 + 0.0[d]
Hue	48.0 ± 0.5[a]	58.4 + 0.1[b]	59.9 + 0.2[c]	58.8 + 0.3[d]
Chroma	11.1 + 0.1[a]	19.5 ± 0.1[b]	21.5 + 0.1[c]	23.5 ± 0.2[d]

[1]Results are mean values of four colour measurements ± standard deviation. Values in each row with the same superscript are not significantly different ($P > 0.05$) from one another.
[2]Sample without acetone decolorization.

Table 2. Nitrogen balance and recovery yield from mechanically separated seal meat (MSSM)[1]

Specification	pH of protein precipitation		
	4.5	5.5	6.0
MSSM	100	100	100
Unextracted residues	12.92 ± 0.20[a]	13.71 ± 0.50[b]	14.19 ± 0.28[b]
Supernatant after protein precipitation	27.92 ± 0.09[a]	27.63 + 0.05[b]	29.34 + 0.13[c]
Recovery yield of precipitated proteins	56.92 ± 0.10[a]	56.85 ± 0.06[b]	55.21 ± 0.11[b]

[1]Results are mean values of three separate batch operations ± standard deviation.Values in each row with the same superscript are not significantly ($P > 0.05$) different from one another.

most muscle proteins (Table 2). Increased loss of unprecipitated proteins was noticed at a pH near 6.0. The precipitate obtained at pH 4.5 and centrifuged at 2000*g* contained 85.28% moisture, 12.4% crude proteins, 0.31% minerals and 1.08% lipids. Washing of this product with acetone decreased its moisture content to 8–15% and lipids, on a dry basis, from 7.64 to 1.10% (Table 3).

There were relatively small differences in the amino acid composition of base-extracted seal muscle proteins and proteins from MSSM (Table 4). Base-extracted proteins contained slightly less proline, glycine and hydroxyproline, which are considered characteristic amino acids of collagen. An increased amount of these amino acids in proteins extracted at 80°C shows a better solubilization of collagen from unextracted residues at a higher temperature (Table 2). Bone residues contained 23.6–25.0% proteins and, although most of these are collagenous matter, the amino acid composition of alkali-extracted proteins from seal bones was similar to that of MSSM (Table 4). Proteins in seal bone residues contained slightly higher amounts of glycine, leucine and hydroxyproline, and less isoleucine, methionine and tryptophan, than those present in the alkali-extracted seal muscle proteins. The low content of tryptophan in alkali-extracted proteins from MSSM (0.53%) and seal bone residues (0.50%) is responsible for the low essential amino acid indices (EAA) of 79.8 and 76.8, respectively, of the products. However, the protein efficiency ratio (PER) values of alkali-extracted proteins from MSSM (3.13) and seal bone residues (3.09) were higher than those of beef (2.87) and pork (2.5) and similar to that of cod (3.1).

Total content of free amino acids in MSSM and base-extracted seal muscle proteins was 0.15 and 0.19%, respectively. The base-extracted proteins contained more aspartic acid, cystine and glutamic acid than MSSM (Table 5). About 55% of free amino acids consisted of taurine, alanine, arginine, aspartic acid, glutamine and glutamic acid. Free amino acid fractions of base-extracted proteins also contained 29.3% of sweet and 23.7% of bitter taste compounds (Table 5). Taurine

Table 3. Proximate composition of proteins precipitated under different pH conditions from base-extracted seal meat (at pH = 10.5, 1 h at 20°C)[1]

pH	Moisture	Protein[2]	Ash[2]	Lipids[2]
4.5[3]	85.88 ± 0.30	88.0 ± 0.42[a]	2.18 ± 0.25[a]	7.64 ± 0.10[a]
4.5	86.47 ± 1.05	96.0 ± 0.21[b]	1.20 ± 0.04[b]	1.10 ± 0.12[b]
5.5	84.01 ± 0.73	95.9 ± 0.01[c]	1.14 ± 0.03[b]	1.12 ± 0.02[b]
6.0	86.45 ± 0.52	95.6 ± 0.09[d]	1.24 ± 0.05[b]	1.32 ± 0.06[b]

[1]Results are average values of three or four replicates ± standard deviation. Values in each column with the same superscript are not significantly different ($P > 0.05$).
[2]Values are percentage on a dry-weight basis.
[3]Sample without acetone decolorization. The original wet precipitate contained: 12.4 ± 0.06% protein, 0.31 ± 0.03% ash and 1.08 ± 0.10% lipids.

Table 4. Amino acid composition (g/100 g proteins) of MSSM and base-extracted proteins by NaOH solution (pH 10.5) at 20 or 80°C from MSSM and seal bone residues[1]

Amino acid	MSSM	Base-extracted MSSM		Base-extracted seal bone residues	
		20°C	80°C	20°C	80°C
Alanine	5.88 ± 0.03	6.03 ± 0.05	6.06 ± 0.05	5.90 ± 0.01	5.91 ± 0.30
Arginine	6.21 ± 0.05	6.32 ± 0.58	5.80 ± 0.08	5.52 ± 0.01	5.32 ± 0.12
Aspartic acid	8.23 ± 0.15	8.26 ± 0.02	8.30 ± 0.09	8.51 ± 0.09	8.44 ± 0.16
Cysteine	0.87 ± 0.01	1.41 ± 0.01	1.27 ± 0.01	1.34 ± 0.01	1.17 ± 0.06
Glutamic acid	11.5 ± 0.03	11.6 ± 0.03	11.6 ± 0.27	11.6 ± 0.13	12.1 ± 0.02
Glycine	4.47 ± 0.05	4.19 ± 0.05	4.70 ± 0.04	4.57 ± 0.05	5.33 ± 0.25
Histidine	5.01 ± 0.09	5.05 ± 0.05	5.08 ± 0.05	5.61 ± 0.04	5.78 ± 0.88
Hydroxylysine	0.10 ± 0.01	—	—	—	—
Hydroxyproline	0.55 ± 0.01	0.04 ± 0.01	0.43 ± 0.06	0.22 ± 0.05	0.73 ± 0.03
Isoleucine	4.58 ± 0.04	5.25 ± 0.13	5.29 ± 0.09	4.76 ± 0.06	4.68 ± 0.02
Leucine	7.44 ± 0.03	7.91 ± 0.03	7.88 ± 0.19	8.55 ± 0.20	8.84 ± 0.08
Lysine	8.72 ± 0.06	9.97 ± 0.10	9.55 ± 0.09	9.87 ± 0.04	10.30 ± 1.72
Methionine	1.64 ± 0.07	2.04 ± 0.02	2.08 ± 0.01	1.60 ± 0.01	1.60 ± 0.08
Phenylalanine	4.57 ± 0.05	4.60 ± 0.03	4.64 ± 0.06	4.74 ± 0.05	4.77 ± 0.05
Proline	3.89 ± 0.02	3.56 ± 0.10	3.83 ± 0.04	3.85 ± 0.04	4.21 ± 0.35
Serine	3.98 ± 0.02	3.98 ± 0.08	3.83 ± 0.02	4.02 ± 0.10	3.98 ± 0.05
Threonine	4.53 ± 0.06	4.28 ± 0.04	4.12 ± 0.05	4.07 ± 0.03	4.05 ± 0.03
Tryptophan	1.20 ± 0.01	0.53 ± 0.01	0.53 ± 0.01	0.50 ± 0.01	0.48 ± 0.04
Tyrosine	2.85 ± 0.01	3.10 ± 0.03	2.98 ± 0.06	2.79 ± 0.01	2.63 ± 0.03
Valine	5.80 ± 0.07	6.00 ± 0.07	6.07 ± 0.03	6.08 ± 0.03	6.05 ± 0.04

[1]Results are mean values of three replicates ± standard deviation.

Table 5. Content of free amino acids (mg/100 g sample) in MSSM and base-extracted seal muscle proteins[1]

Amino acid	MSSM	Base-extracted proteins
Alanine	41.4 ± 0.34	20.3 ± 1.37
Arginine	17.2 ± 0.31	12.0 ± 0.43
Aspartic acid	9.02 ± 1.23	13.8 ± 0.78
Asparagine	3.13 ± 0.00	—
Cystine	1.05 ± 0.14	4.61 ± 0.37
Glutamic acid	20.3 ± 0.64	25.3 ± 0.22
Glutamine	32.7 ± 0.00	13.5 ± 0.13
Glycine	14.0 ± 0.21	7.86 ± 0.08
Histidine	9.05 ± 0.31	7.92 ± 0.10
Hydroxyproline	2.98 ± 0.12	—
Isoleucine	8.93 ± 0.18	4.18 ± 0.05
Leucine	20.8 ± 0.35	9.40 ± 0.21
Lysine	18.8 ± 0.45	9.75 ± 0.06
Methionine	9.16 ± 0.15	3.80 ± 0.11
Ornithine	2.15 ± 0.13	—
Phenylalanine	10.2 ± 0.19	6.10 ± 0.08
Proline	9.60 ± 0.20	3.62 ± 0.11
Serine	17.1 ± 0.32	9.13 ± 0.12
Taurine	59.3 ± 3.43	22.9 ± 0.31
Threonine	12.9 ± 0.23	6.17 ± 0.09
Tryptophan	1.75 ± 0.05	—
Tyrosine	9.86 ± 0.17	6.50 ± 0.04
Valine	15.4 ± 0.20	6.95 ± 0.12

[1]Results are mean values of three replicates ± standard deviation.

Table 6. Selected functional characteristics of base-extracted (pH = 10.5) seal muscle proteins precipitated at pH = 4.5[1]

Functionality	Functionality (%) for proteins	
	Lyophilized	Washed with acetone
Fat adsorption	346.5 ± 3.5[a]	275.0 ± 0.5[b]
Moisture adsorption	8.3 ± 1.8[a]	14.5 ± 2.1[b]
Emulsifying capacity	64.3 ± 3.8[a]	95.4 ± 0.3[b]
Emulsion stability	93.3 ± 1.7[a]	92.7 ± 0.5[b]
Whippability	25.0 ± 1.8[a]	13.5 ± 2.6[b]
Foam stability		
20 min	64.6 ± 3.6[a]	67.2 ± 2.7[a]
30 min	44.7 ± 1.9[a]	50.1 ± 1.8[b]
60 min	33.9 ± 2.3[a]	36.4 ± 1.9[a]
120 min	10.4 ± 0.8[a]	18.2 ± 1.4[b]

[1]Results are mean values of three measurements ± standard deviation. Values in each row with the same superscript are not significantly different ($P > 0.05$) from one another.

(2-aminoethanesulphonic acid) was present at levels of 59.3 and 22.9 mg%, respectively, in MSSM and base-extracted proteins. Taurine is an important metabolite of the sulphur-containing amino acids and its acceptance as an essential growth factor followed recognition of its role in bile acid synthesis and in the prevention of certain pathological problems. Taurine is pervasive in most mammals and its accumulation is necessary for functional regulation of the eyes, heart, muscles, brain and central nervous system (Trautwein & Hayes, 1990). The low content of histidine (7.9 mg%) in the products may also be important as its bacterial decay leads to the production of histamine, which may cause scrombosis or histamine poisoning. Base-extracted proteins contained 30.5 mg% of anserine and 209 mg% of carnosine, compared with corresponding amounts of 70.0 and 311 mg% in MSSM. These peptides contain β-alanine bound to histidine or 1- (or 3-) methylhistidine. The physiological role of carnosine and anserine is not clear; however, they may be involved in the revitalization of exhausted muscles or may serve as part of the buffer system in the muscle.

Table 7. Nitrogen solubility index (NSI) of base-extracted seal meat and chicken bone protein residues (% of total nitrogen)[1]

NaCl concentration (M)	Proteins from MSSM (lyophilized)	MSSM (acetone washed)	Chicken bones[2]
0.0	7.22 ± 0.25	5.10 ± 0.20	11.1 ± 0.0
0.3	10.8 ± 0.14	7.36 ± 0.18	13.2 ± 0.7
0.5	12.0 ± 0.08	9.78 ± 0.15	12.8 ± 1.2
1.0	13.6 ± 0.10	12.0 ± 0.21	13.2 ± 0.4
2.0	12.3 ± 0.12	9.92 ± 0.12	12.7 ± 0.0

[1]Results are mean values of three determinations ± standard deviation.
[2]According to Ozimek *et al.* (1986).

Selected functionalities of lyophilized and acetone-washed, base-extracted proteins are presented in Table 6. Both products had excellent fat adsorption, emulsifying capacity and emulsion stability properties. The solubility of base-extracted proteins, determined in the pH range of 3.1–8.5, attained a minimum of 7.2% for the lyophilized product and 5.1% for that washed with acetone at pH 6.1. The nitrogen solubility index (NSI) value assayed at an NaCl concentration of up to 2.0 M was similar to that reported by Ozimek *et al.* (1986) for base-extracted proteins from chicken bone residues (Table 7). The washing of the product with acetone had a minor effect on its NSI values.

In conclusion, the present study has indicated that the nutritional quality of alkali-extracted seal proteins is retained when the extraction is carried out at room temperature. Tryptophan was the limiting amino acid in products prepared from bone residues or MSSM. The base-extracted proteins thus prepared may be used as an ingredient in muscle food formulations and in products containing high levels of tryptophan.

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