## BASE EXTRACTION OF PROTEINS FROM SEAL MEAT AND BONE RESIDUES

F. SHAHIDI and J. SYNOWIECKI

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF A1B 3X9 Canada

#### INTRODUCTION

The population of the harp seal (*Phoca groenlandica*) in the Atlantic regions of Canada is currently estimated at 3.1-3.6 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 186,000 seals annually, however only 50,000 to 70,000 animals have been caught in recent years. This provides approximately 1.2-2.1 million kilograms of meat each year (Synowiecki and 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%). The present study reports a facile base-extraction process for the extraction of proteins from seal meat and bone residues in order to allow utilization of the entire seal carcass.

#### MATERIALS & METHODS

Harp seal (*Phoca groenlandica*) from 1 to 4 years of age were caught in the coastal areas of Newfoundland during the month of April, bled and skinned, the blubber fat was removed and the carcasses were eviscerated. The carcasses were subjected to mechanical deboning using a Poss deboner (Model PDE 500, Poss Limited, Toronto, ON) and components kept frozen in vacuum packed polyethylene pouches at -20°C until used.

Approximately 150g of MSSM or seal bone residues were mixed with 600 mL of distilled water, to which 4N NaOH solution was added to reach pH 10.5. Extraction was carried out at 20°C for 1 h. The unextracted residues were separated by centrifugation at 2000xg for 15 min. Alkali extracted proteins were precipitated with 4N HCl solution at pH 4.5 - 6.0 and centrifuged at 2000xg over a 15 min period. The precipitate was then washed 3 times with 100mL of acetone, filtered under suction on Whatman No. 1 filter paper, and evaporated.

Moisture nitrogen (protein content =  $N \times 6.25$ ) and ash contents in products were determined according to AOAC (1990). Total lipids were extracted using a chloroform-methanol-water mixture (Bligh and Dyer, 1959). The amino acid composition of proteins are determined and essential amino acid index (EAA) and protein efficiency ratios (PER) were calculated by consideration of the content of 10 designated amino acids as described elsewhere (Shahidi and Synowiecki, 1993). Tristimulus Hunter L, a, b of samples were measured using a Colormet colorimeter (Instrumar Engineering Ltd., St. John's, NF).

### RESULTS & DISCUSSION

Base extracted proteins from MSSM or seal bone residues were prepared according to method developed by Jelen et al. (1979) and McCurdy et al. (1986) for recovery of proteins from beef bones and mechanically separated chicken meat residues. The main steps in the preparation of alkali extracted proteins involved extraction at pH 1.05 using approximately 9.6g NaOH per kg of meat, separation of unextracted connective tissues, and precipitation of proteins at pH 4.5-5.5. Large amounts (5.3%) of hemoproteins in seal meat are responsible for the dark colour of the liophilized precipitates and hence washing of the precipitated proteins with acetone was found to remove some of the residues and effectively improved the colour of the product (Table 1).

Table 1. Hunter colour parameters of lyophilized based-extracted seal muscle proteins precipitated under different pH conditions. <sup>1</sup>

Hunter value	pH of protein precipitation			
	4.52	4.5	5.5	6.0
L	20.5+0.1	52.2±0.1	51.0±0.2	$50.1 \pm 0.2$
a	$7.4 \pm 0.1$	$10.2 \pm 0.1$	$10.8 \pm 0.2$	$12.1 \pm 0.1$
b	8.2+0.2	16.6±0.0	$18.6 \pm 0.1$	$20.1 \pm 0.0$
Hue	48.0±0.5	58.4±0.1	$59.9 \pm 0.2$	$58.8 \pm 0.3$
Chroma	11.1±0.1	19.5±0.1	$21.5 \pm 0.1$	$23.5 \pm 0.2$

<sup>1</sup>Results are mean values of 4 colour measurements ± standard deviation.

<sup>2</sup>Sample without acetone decolorization.

The highest loss of proteins was due to unextracted connective tissues (12.9 - 14.2%) and presence of some unprecipitated proteins in the supernatant. An increase in the extraction temperature from 20 to 80°C improved the protein recovery of proteins from MSSM and seal bone residues from 56.92 to 63.88% and from 12.02 to 13.07%, respectively. However, formation of lysinoalanine and other amino acid derivatives at higher temperatures may be contemplated. The yield of proteins from seal bone

Table 2. Amino acid composition of base-extracted crude proteins (%) at pH 10.5 and 20°C.

Amino Acid	From MSSM	From Seal bone residues
Alanine	$6.03 \pm 0.05$	$5.90 \pm 0.01$
Arginine	$6.32 \pm 0.50$	5.52 + 0.01
Aspartic acid	$8.26 \pm 0.02$	8.51 + 0.09
Cysteine	$1.41 \pm 0.01$	1.34 + 0.01
Glutamic acid	$11.65 \pm 0.03$	$11.58 \pm 0.13$
Glycine	$4.19 \pm 0.05$	$4.57 \pm 0.05$
Histidine	$5.05 \pm 0.05$	5.61 + 0.04
Hydroxyproline	$0.04 \pm 0.01$	0.22 + 0.05
Isoleucine	$5.25 \pm 0.13$	$4.76 \pm 0.06$
Leucine	$7.91 \pm 0.03$	$8.55 \pm 0.20$
Lysine	$9.97 \pm 0.10$	9.87 + 0.04
Methionine	$2.04 \pm 0.02$	$1.60 \pm 0.01$
Phenylalanine	$4.60 \pm 0.03$	$4.74 \pm 0.05$
Proline	$3.56 \pm 0.10$	$3.85 \pm 0.04$
Serine	$3.98 \pm 0.08$	$4.02 \pm 0.10$
Threonine	$4.28 \pm 0.04$	$4.07 \pm 0.03$
Tryptophan	$0.53 \pm 0.01$	$0.50 \pm 0.01$
Tyrosine	$3.10 \pm 0.03$	$2.79 \pm 0.01$
Valine	$6.00 \pm 0.07$	$6.08 \pm 0.03$

 $^{1}$ Results are mean values of 3 replicates  $\pm$  standard deviation and MSSM refers to mechanically-separated seal meat.

residues (12.02%) is similar to the 9-16% recovery reported by McCurdy et al. (1986) in a pilot-scale processing of mechanically separated chicken meat residues. 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 (Table 2). Increased loss of unprecipitated proteins was noticed at pH near 6.0. The precipitate obtained at pH 4.5 and centrifuged at 2000xg contained 85.28% moisture, 12.43% proteins, 0.31% minerals and 1.08% lipids. Washing of this product with acetone decrease its content of moisture to 8-15% and lipids, on a dry basis, from 7.64 to 1.10%. The amino acid composition of proteins extracted from MSSM and seal bones residues were similar. Proteins from seal bone residues contained slightly higher amounts of glycine, leucine and hydroxyproline, and less isoleucine and methionine than those present in the alkali extracted seal muscle proteins (Table 2). 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 index (EAA) values of 79.77 and 76.77, respectively, for 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 (2.50) and similar to that of cod (3.10).

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