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# Isolation of mucopolysaccharides from processing discards of seal and beef

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Bone residues after mechanical deboning of carcass meat of harp seal (*Phoca groenlandica*) may be used as an alternative source of mucopolysaccharides (MPS) for medical and cosmetic purposes. The content of MPS in seal processing discards and bovine diaphragm tissues was 0·79 and 0·91%, on a dry weight basis, respectively. However, the highest content of MPS was present in seal and bovine trachea cartilage (10·93 and 10·12%, respectively). Crude MPS from different sources were isolated with a yield of 57·1–72·9%. Optimum isolation involved hydrolysis of tissues with a proteolytic enzyme such as papain at 55°C for 12 h at a tissue to enzyme solution ratio of 1:3 (w/v), separation of enzyme by precipitation with trichloroacetic acid, purification of hydrolysate with 2 M NaOH at 20°C and subsequent dialysis and precipitation of MPS by methanol. The crude MPS obtained from seal processing discards contained 61·64% polysaccharides, 15·73% residual peptides and 17·53% ash.

## INTRODUCTION

Mucopolysaccharides (MPS) are the main components of amorphous substances of connective tissues which surround collagen and elastin fibres. They are composed of uronic acid and sulphated or acetylated hexosamine residues. The MPS in the tissues are usually combined with proteins to form mucoproteins. Among others, hyaluronic acid and chondroitin sulphates are the main components of MPS fraction of connective tissues (Lindahl & Roden, 1972). MPS have a high molecular weight ranging from 30 000–50 000 (chondroitin sulphates) to 3–8 millions Da (hyaluronic acid). They are widely distributed throughout the mammalian body and are components of connective tissues, skin, cartilage, bone joints and eye vitreous humour. MPS-containing tissues are generally present in processing discards of muscle foods. MPS possess good physiological properties and are used as a component of cosmetics for skin care and medicines for curing of joints and eye diseases (Nieduszczycki, 1985).

During mechanical deboning of seal meat the bone residues left behind contained a large amount of connective tissues and cartilage (Shahidi *et al.*, 1990). The purpose of this study was to prepare MPS from seal processing discards and to compare them with those from beef. In addition, developing of an adequate method for isolation of crude MPS from seal bone residues was intended.

## MATERIALS AND METHODS

### Materials

Adult harp seals (*Phoca groenlandica*) were hunted in the coastal areas of Newfoundland during the month of April, were bled, skinned, blubber removed and eviscerated. Carcasses weighing up to 30 kg without head and flippers, were placed inside plastic bags and stored on ice for up to 3 days. Each carcass was then washed with a stream of cold water (+10°C) for about 15 s to remove most of the surface blood. Mechanical separation of meat from carcasses of 15 seals was carried out using a Poss deboner (Model PDE500, Poss Limited, Toronto, ON, Canada). Small portions of seal bone residues were vacuum-packed in polyethylene pouches and were kept frozen at −20°C until use. Fresh samples of bovine skin, diaphragm and trachea cartilage from 10 animals were washed with a stream of cold water (+10°C) for 15 s. They were then cut into small pieces, mixed, vacuum-packed in polyethylene pouches and kept frozen at −20°C up to 4 weeks until use. Before determinations, frozen samples were ground for 30 s with dry ice in a Waring Blender (Model 33 BL73,

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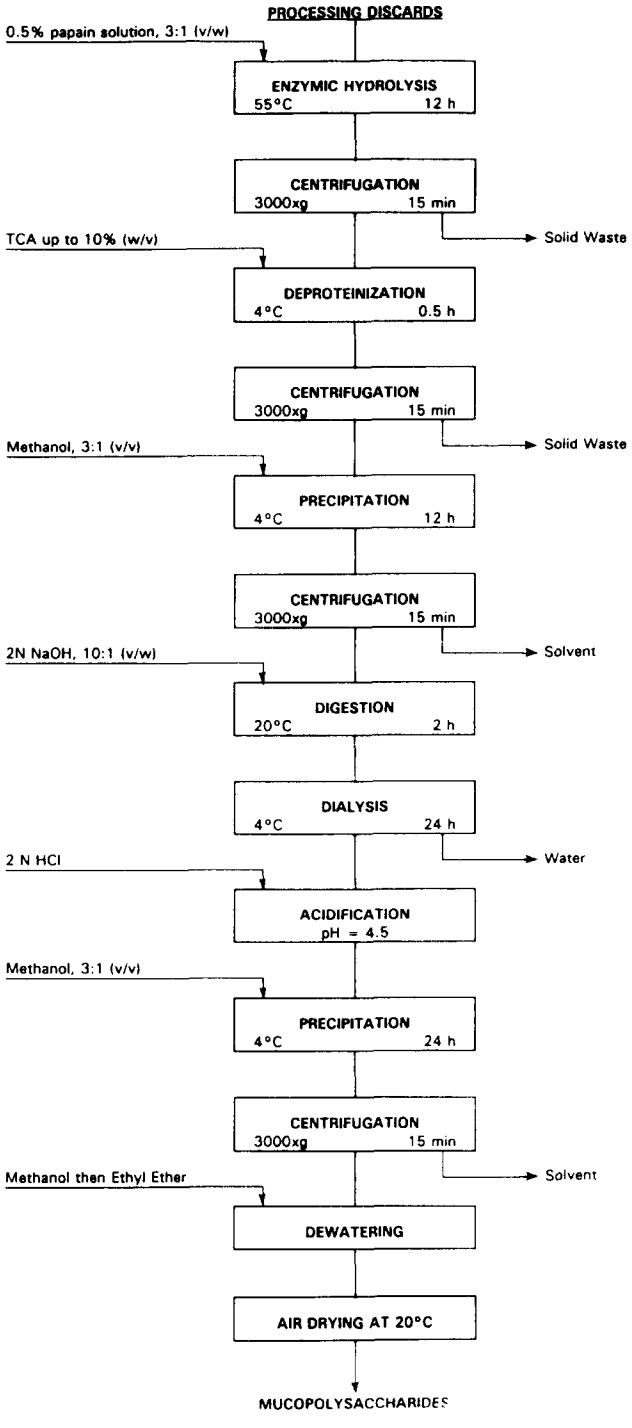


**Fig. 1.** Flowsheet for the isolation of mucopolysaccharides from animal tissues.

Dynamics Corp., New Hartford). MPS from seal and bovine samples were isolated according to the flowsheet given in Fig 1. Dialysis was carried out using a Spectrapor membrane tubing No. 2 with a molecular weight cut-off of 12000-14000 D (Spectrum Medical Industries, Inc., Los Angeles, CA, USA).

## Analyses

The content of crude protein (P) in the tissues was calculated from their total nitrogen (N) content (AOAC, 1990) using the equation:

$$P = (N - N_{hexosamines}) \times 5{\cdot}56$$

Ash content in the samples was determined according to the AOAC (1990) method of analysis. Total lipids were extracted by a chloroform–methanol–water mixture as described by Bligh and Dyer (1959). The content of residual peptides in crude MPS was assayed according to the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as a standard.

The individual amino acids in samples were determined after their digestion in 6M HCl at 110°C as described by Blackburn (1978). The HCl was then removed under vacuum and dried samples were reconstituted using a lithium citrate buffer at pH 2·2. The amino acid composition of the hydrolysate was determined using a Beckman 121 MB amino acid analyser (Beckman Instruments Inc., Palo Alto, CA., USA) Cysteine and methionine were determined by performic acid oxidation prior to their digestion in 6M HCl and were measured as cysteic acid and methionine sulphone, respectively (Blackburn, 1978). Analysis of tryptophan was carried out by hydrolysis of the sample under vacuum in 3M mercaptoethane sulphonic acid at 110°C, as described by Penke *et al.* (1974).

Glucosamine and galactosamine contents were determined after hydrolysis of the samples with 4M HCl at 110°C for 8 h. The HCl was then removed in vacuo over sodium hydroxide and the dried samples were reconstituted using a pH 2·2 citrate buffer. Analysis was performed on a modified Beckman Model 121 amino acid analyser equipped with 7 cm × 0·6 cm Beckman W2 resin bed column at 65°C. The elution buffer was borate/citrate, pH 7·24. The detection agent was ninhydrin.

The total content of MPS which is the sum of hyaluronic acid and chondroitin sulphates was calculated according to the equation:

$$MPS\% = \text{Glucosamine} \times f_1 + \text{galactosamine} \times f_2$$

The contents of glucosamine and galactosamine are determined experimentally and $f_1$ is the percentage of molecular weight of glucosamine residue in molecular weight of disaccharide composed of *N*-acetylglucosamine and glucuronic acid (hyaluronic acid is a polymer of this disaccharide) and $f_2$ is the percentage of molecular weight of subphonated *N*-acetylgalactosamine residue in molecular weight of disaccharides composed of glucuronic acid and sulphonated *N*-acetylgalactosamine (monomer of chondroitin sulphates).

## Statistical analysis

Analysis of variance and Tukey's studentized range test (Snedecor & Cochran, 1980) were used to determine differences in mean values based on the data presented in the tables. Significance was determined at 95% probability.

**Table 1. Proximate composition of mucopolysaccharide (MPS)-containing tissues, % on a dry weight basis[a]**

| Source | Protein | MPS | Lipids | Ash |
|---|---|---|---|---|
| Seal bone residues | 57·3 ± 0·97 | 0·79 ± 0·14 | 10·7 ± 0·47 | 31·8 ± 3·82 |
| Seal hide | 75·1 ± 3·04 | 0·37 ± 0·10 | 20·1 ± 5·09 | 1·44 ± 0·25 |
| Bovine hide | 86·3 ± 0·52 | 0·52 ± 0·11 | 12·9 ± 0·09 | 1·22 ± 0·07 |
| Connective tissue from bovine diaphragm | 77·6 ± 0·82 | 0·91 ± 0·12 | 17·5 ± 1·94 | 0·93 ± 0·14 |
| Bovine trachea cartilage[b] | 54·7 ± 1·86 | 10·1 ± 0·53 | 24·0 ± 1·62 | 8·53 ± 0·11 |

[a] Results are mean values of three to five determinations ± standard deviation.
[b] Corresponding result for MPS of seal trachea cartilage was 10·9%.

**Table 2. Isolation yield of mucopolysaccharides (MPS)[a]**

| Source | Content of MPS in wet material A | Amounts of Crude Isolate | Content of MPS in preparation B | Yield[b](%) |
|---|---|---|---|---|
| Seal bone residues | 0·16 ± 0·04 | 0·18 ± 0·03 | 0·11 ± 0·02 | 68·7 |
| Bovine skin | 0·20 ± 0·05 | 0·22 ± 0·02 | 0·13 ± 0·04 | 65·0 |
| Connective tissue from bovine diaphragm | 0·35 ± 0·06 | 0·34 ± 0·03 | 0·20 ± 0·03 | 57·1 |
| Bovine cartilage[c] | 3·57 ± 0·11 | 3·67 ± 0·24 | 2·61 ± 0·15 | 72·9 |

[a] Results are mean values of data from three separate preparations ± standard deviation.
[b] Yield = $(B/A) \times 100\%$ where $A$ is calculated from the amount of glucosamine in the sample and $B$ is the amount of isolated MPS.
[c] Corresponding results for seal cartilage from one animal were 3·72, 3·89, 2·68, and 72·0%, respectively.

## RESULTS AND DISCUSSION

The connective tissues of diaphragms, cartilage and bone joints contain MPS in the form of mucoprotein complex. These complexes are composed of proteins and different MPS molecules such as hyaluronic acid, chondroitin sulphates A and C and keratan or dermatan sulphates. Structure of these complexes and types of protein-MPS interactions have been reviewed by Bettelheim-Jevons (1958) and Lindahl and Roden (1972). The best source of chondroitin sulphates was bovine trachea cartilage which contained 10·12% MPS, on a dry weight basis (Table 1). However, the content of MPS in this tissue was reported to be approximately 2·5% higher than that in this study (Ebinder & Schubert, 1950). Existing differences in the age group of animals studied may be responsible for this variation. The amount of MPS in seal bone residues was 0·79%, on a dry weight basis. This is similar to that in bovine diaphragm and somewhat greater than that in seal and bovine hides.

Isolation of MPS involved basic operations such as their release by enzymic digestion of proteins, precipitation of enzyme with trichloroacetic acid (TCA), separation of components dissolved in TCA and products of protein hydrolysis by dialysis, and precipitation of MPS in methanol (Fig. 1). The yield of isolation of MPS depended on their initial amount in the raw material (Table 2); the best yield of 72·9% was obtained for bovine trachea cartilage. However, the recovery of MPS from other sources such as seal bone residues, bovine hide and connective tissues from bovine diaphragm were 68·7, 65·0, and 57·1%, respectively (Table 2). Preliminary investigations (Synowiecki, 1988) have shown that the best method for the release of MPS from tissues is enzymic hydrolysis at 55°C using a 0·5% papain solution activated with addition of 0·1% cysteine.

The enzyme and macromolecular peptides produced during protein hydrolysis were removed by precipitation with a TCA solution. This method was more effective than deproteinization with chloroform and amyl alcohol mixture used by Meyer and Chaffe (1941). The total nitrogen content of crude MPS deproteinized by a TCA solution was 4·53 ± 0·08% which is lower than that of the sample deproteinized with a mixture of

**Table 3. Influence of NaOH digestion on chemical composition of mucopolysaccharides (MPS)[a]**

| Component | % of Dry MPS | |
|---|---|---|
| | Undigested | Digested[b] |
| Hexosamines | 25·3 ± 0·22[a] | 28·6 ± 0·31[b] |
| Protein | 24·6 ± 0·11[a] | 13·1 ± 0·25[b] |
| Total nitrogen (N) | 6·62 ± 0·01[a] | 4·51 ± 0·12[b] |
| Ash | 1·34 ± 0·23[a] | 16·4 ± 0·11[b] |
| $\frac{\text{Hexosamines nitrogen}}{\text{Total nitrogen}} \times 100\%$ | 18·9 | 31·2 |

[a] Results are mean values of five determinations ± standard deviation. Values in each row with different following letter are significantly ($P < 0.05$) different from one another.
[b] Digested for 2 h in 2 M NaOH.

**Table 4. Mass balance of mucopolysaccharides (MPS) isolated from bovine cartilage[a]**

| Component | Weight of the component (g) | Content of MPS | | % of initial amount of MPS in the material |
|---|---|---|---|---|
| | | % | g | |
| Starting material | 50·0 ±0·10 | 3·57 ± 0·11 | 1·78 ± 0·08 | 100 |
| Solution of hydrolysate after papain digestion | 180 ± 2·01 | 0·91 ± 0·17 | 1·64 ± 0·22 | 92·13 |
| Precipitate after TCA treatment | 0·09 ± 0·02 | 11·9 ± 0·08 | 0·01 ± 0·00 | 0·56 |
| Mucoproteins before digestion with 2M NaOH | 2·52 ± 0·10 | 64·0 ± 0·19 | 1·62 ± 0·15 | 91·01 |
| Final product | 1·83 ± 0·12 | 70·3 ± 0·22 | 1·30 ± 0·17 | 73·03 |

[a] Results are mean values of three different processes ± standard deviation.

chloroform and amyl alcohol due to a better deproteinization efficiency with the TCA solution. The enzyme was incapable of removing some of the peptides which were directly attached to the MPS molecules (Lindahl & Roden, 1972). The amount of these peptides was decreased during a 2 h digestion with 2M NaOH solution at 20°C and subsequent dialysis. Effectiveness of this method was confirmed by enhanced percentage of hexosamine nitrogen in total nitrogen content of crude MPS, from 18·9% in preparations obtained without NaOH treatment to 31·2% after digestion (Table 3). The NaOH treatment produced sodium salts of MPS and therefore increased the ash content of products from 1·34 to 16·43% (Table 3). Mass balance of the process given in Table 4 shows that the greatest loss of MPS, about 18% of their initial amount, was observed during NaOH digestion, dialysis and precipitation of the final product. The contents of MPS in crude preparations isolated from seal bone residues, seal and bovine hides, connective tissue of bovine diaphragm and bovine trachea cartilage were 61·6, 54·6, 58·2, 53·4 and 73·3%, respectively. The corresponding total hexosamines content of these samples ranged from 20·8, to 28·6% (Table 5).

The hexosamines fraction of mucopolysaccharides of bovine trachea cartilage and seal processing discards contained 5·05 and 5·55% glucosamine (from hyaluronic acid) and 95·0 and 94·5% galactosamine (from chondroitin sulphates), respectively. Preparations isolated from various seal and bovine tissues contained some residual peptides which were strongly connected to MPS molecules and were not removed under mild conditions of enzymic hydrolysis and basic digestion. Their contents in the preparations depended on the energy of complexation (Lindahl & Roden, 1972) and ranged from 13·1% in case of MPS from bovine trachea cartilage to 29·3% for preparations obtained from bovine diaphragm (Table 5). The main components of peptides connected with MPS were arginine, aspartic acid, glutamic acid, glycine, lysine and proline (Table 6). Their amounts in peptide fraction of MPS of seal processing discards and bovine trachea cartilage were 66·9 and 72·1% of the total amino acid contents, respectively.

In conclusion, seal processing discards and bovine tissues may be used as good sources of MPS which

**Table 5. Chemical composition of harp seal and bovine mucopolysaccharides on a dry weight basis (%)[a]**

| Source | Hexosamines | Proteins | Ash |
|---|---|---|---|
| Seal bone residues | 24·2 ± 0·36 | 15·7 ± 0·34 | 17·5 ± 0·31 |
| Seal skin | 21·3 ± 0·43 | 19·9 ± 0·14 | 18·0 ± 0·41 |
| Bovine skin | 22·7 ± 0·81 | 21·4 ± 0·60 | 18·4 ± 0·22 |
| Connective tissue from bovine diaphragm | 20·8 ± 0·12 | 29·3 ± 0·75 | 16·7 ± 0·25 |
| Bovine trachea cartilage | 28·6 ± 0·31 | 13·1 ± 0·25 | 16·4 ± 0·11 |

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

**Table 6. Amino acid composition (%) of residual proteins of mucopolysaccharides of harp seal and bovine[a]**

| Amino acid | Seal bone residues | Seal hide | Bovine cartilage |
|---|---|---|---|
| Alanine | 4·71 ± 0·01 | 4·95 ± 0·08 | 4·45 ± 0·02 |
| Arginine | 12·8 ± 0·37 | 12·5 ± 0·17 | 14·6 ± 0·03 |
| Aspartic acid | 11·3 ± 0·43 | 12·0 ± 0·07 | 11·5 ± 0·18 |
| Cysteine | 1·69 ± 0·02 | 1·62 ± 0·03 | 1·52 ± 0·03 |
| Glutamic acid | 10·5 ± 0·06 | 9·91 ± 0·01 | 10·4 ± 0·06 |
| Glycine | 13·5 ± 0·03 | 14·6 ± 0·29 | 15·4 ± 0·10 |
| Histidine | 5·56 ± 0·11 | 6·23 ± 0·13 | 4·06 ± 0·02 |
| Hydroxyproline | 0·62 ± 0·01 | 0·80 ± 0·00 | 1·59 ± 0·02 |
| Isoleucine | 1·32 ± 0·29 | 1·99 ± 0·09 | 1·46 ± 0·03 |
| Leucine | 2·13 ± 0·11 | 2·15 ± 0·08 | 1·78 ± 0·03 |
| Lysine | 11·4 ± 0·09 | 9·47 ± 0·03 | 10·1 ± 0·04 |
| Methionine | 0·75 ± 0·01 | 1·01 ± 0·05 | 0·32 ± 0·01 |
| Phenylalanine | 1·98 ± 0·02 | 2·45 ± 0·01 | 1·71 ± 0·01 |
| Proline | 7·45 ± 0·33 | 6·42 ± 0·21 | 10·0 ± 0·02 |
| Serine | 5·06 ± 0·27 | 4·31 ± 0·00 | 4·89 ± 0·03 |
| Threonine | 2·63 ± 0·03 | 2·12 ± 0·03 | 2·03 ± 0·01 |
| Tryptophan | 0·18 ± 0·01 | 0·21 ± 0·00 | 0·19 ± 0·01 |
| Tyrosine | 1·61 ± 0·09 | 1·98 ± 0·04 | 1·34 ± 0·02 |
| Valine | 3·26 ± 0·05 | 4·17 ± 0·07 | 2·09 ± 0·02 |

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

could be recovered with a yield of up to 68·7%. The best source of MPS was bovine and seal trachea cartilage. The purity of MPS obtained in this work may potentially allow their use in cosmetic applications.

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## REFERENCES

AOAC (1990). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC., USA.

Bettelheim-Jevons, F. R. (1958). Protein–carbohydrate complexes. In *Advances in Protein Chemistry* (Vol. 13) eds C. B. Anfinsen, M. L. Anson & J. T. Edsall. Academic Press, New York, USA.

Blackburn, S. (1978). Sample preparation and hydrolytic methods. In *Amino Acid Determination Methods and Techniques.* Marcell Dekker Inc., New York, USA, pp. 7–37.

Bligh, E. G. & Dyer, H. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–17.

Ebinder, J. & Schubert, M. (1950). Separation of chondroitin sulfate from cartilage. *J. Biol. Chem.*, **185**, 725–30

Lindahl, U. & Roden, L. (1972). Carbonhydrate-peptide linkages in proteoglycans of animal, plant and bacterial origin. In *Glycoproteins,* ed. A. Gottschalk. Elsevier, New York, USA.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. (1951). Protein measurement with the Folin–phenol reagent. *J. Biol. Chem.* **193**, 265–303.

Meyer, K. & Chaffe, E. (1941). The mucopolysaccharides of skin. *J. Biol. Chem.*, **138**, 491–9.

Nieduszczycki, J. A. (1985). Connective tissue polysaccharides. In: *Polysaccharides Topics in Structure and Morphology*. Ed. E. D. T., Atkins, VCH Publishers, Weinheim, Germany.

Penke, B., Ferenczi, R. & Kovacs, K. (1974). A new acid hydrolysis method for determining tryptophan in peptides and proteins. *Anal. Biochem.*, **60**, 45–50.

Shahidi, F., Synowiecki, J. & Naczk, M. (1990). Seal meat. A potential source of muscle food: Chemical composition, essential amino acids and colour characteristics. *Can. I. Food Sci. Technol. J.*, **23**, 137–9.

Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods.* (7th edn). The Iowa State University Press, Ames, IA. USA.

Synowiecki, J. (1988). Isolation of mucopolysaccharides from connective tissue and cartilage. *Przem. Spoz.*, **6**, 182-5.