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Nonprotein nitrogen compounds in harp seal (*Phoca groenlandica*) meat

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The composition of nonprotein nitrogen (NPN) compounds in manually deboned or mechanically separated seal meat (MSSM) unwashed, or washed, was studied. The NPN contents in manually or mechanically separated meat were 172 and 342 mg %, respectively. The enhanced NPN content in MSSM may influence the storage stability of the product and may contribute to flavour changes during thermal processing of meat. The NPN fraction in manually separated seal meat consisted of nitrogen from carnosine and anserine (79·4 mg %), free amino acids (30 mg %), nucleic acids (23·8 mg %), amines (1·36 mg %) and nucleotides (17·7 mg %). The contents of free amino acids in manually separated meat and unwashed MSSM were 0·21 and 0·35%, respectively. About 55% of the free amino acids consisted of taurine, alanine, glutamine, glutamic acid, leucine and lysine. Fresh seal meat contained 1·48 mg % of trimethylamine *N*-oxide, which after 8 months of storage at −20°C was almost completely exhausted, forming 0·73 mg % trimethylamine and 0·42 mg % of dimethylamine. Additionally, the presence of 0·30–0·57 mg % of spermidine and 2·98–3·41 mg % of spermine was determined. The nucleic acids content in MSSM varied from 291 to 336 mg %. Removal of both of the amines and nucleic acids enhances the quality of the products by reducing the chance of *N*-nitrosamine formation in the cured products and formation of crystals and stones in the human urinary tract, respectively.

INTRODUCTION

Seal meat is a rich source of nutritionally valuable proteins with a well-balanced essential amino acid composition (Shahidi *et al.*, 1990). However, full utilization of the meat is limited due to its dark colour and development of intense flavour during storage. High contents of hemoproteins, which act as prooxidants (Synowiecki & Shahidi, 1991), and presence of nonprotein nitrogen (NPN) compounds might be responsible. Among the NPN compounds, amines and peptides contribute to the flavour and taste of food (Maga, 1978). They are also precursors for aroma and colour compounds which are formed during thermal processing or enzymic reactions during storage of food. Additionally, the presence of secondary and tertiary amines in meat may lead to the formation of the hazardous carcinogenic *N*-nitrosamines during curing (Gray & Randall, 1979; Sen *et al.*, 1979). In marine species, the breakdown of trimethylamine *N*-oxide by endogenous enzymes forms dimethylamine and formaldehyde. It has been suggested that formaldehyde causes cross-linking of muscle proteins, rendering them insoluble and causing the flesh to toughen (Sikorski *et al.*, 1976).

Presence of excessive amounts of nucleic acids could potentially be harmful due to their influence on the formation of urate crystals in tissues as well as stone deposition in the urinary system. Although mechanical separation of seal meat from carcass gives a better product yield (Shahidi *et al.*, 1990), inclusion of bone marrow may lead to a high content of nucleic acids in the resultant meat. Therefore, the amounts of nucleic acids in MSSM and surimi-like products prepared from MSSM should be determined.

Sensory properties of meat products also depend upon adenosine triphosphate (ATP) conversion in the post-mortem state to different nucleotides, which can influence the flavour of meats. For example, hypoxanthine is known as a flavour enhancer in muscle foods (Hultin, 1985). This compound has a bitter flavour and may be responsible for off-flavour development in stored fish. The disappearance of inosine monophosphate (IMP) has been correlated with the loss of fresh fish flavour in some species (Fraser *et al.*, 1967; Jones, 1969).

The purpose of this study was to evaluate the amount of nonprotein nitrogen compounds in seal meat. These investigations are required for determining

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the importance and possible interactions of nonprotein nitrogen compounds in seal-based products.

MATERIALS AND METHODS

Harp seal (*Phoca groenlandica*) from 1 to 4 years of age, hunted in the coastal areas of Newfoundland during the month of April, were bled, skinned, blubber fat removed and eviscerated. A known amount of muscle was cut from each carcass and nucleotides were extracted with ice-cold perchloric acid as given below. The remaining carcasses, weighing up to 30 kg without head and flippers, were placed inside plastic bags and stored in containers with 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 and was trimmed of most of its subcutaneous fat. Mechanical separation of meat from the carcasses of 15 seals was carried out using a Poss deboner (Model PDE 500, Poss Limited, Toronto, Ontario, Canada). Small portions of mechanically separated seal meat (MSSM) were vacuum-packed in polyethylene pouches and kept frozen at −20°C or −60°C for up to 8 months before use.

The MSSM was washed one to three times with water (pH = 5·9–6·0) using a water-to-meat ratio of 3:1 (v/w). Other samples were washed with water and then with 0·5% NaCl or 0·5% $NaHCO_3$ solution at a solvent-to-meat ratio of 3 : 1 (v/w). Washings were carried out at 2°C for 10 min with manual stirring. The washed meat was then filtered through two layers of cheesecloth with 1-mm size holes.

Analyses

For determination of trimethylamine *N*-oxide (TMAO), trimethylamine (TMA), dimethylamine (DMA) and formaldehyde, 20 g of meat was homogenized with a 10% trichloroacetic acid (TCA) solution (1 : 2 (w/v)) using a Polytron homogenizer (Brinkmann Instruments, Mississauga, Ontario, Canada) and centrifuged for 20 min at 3000 × *g*. The extraction procedure was repeated and combined supernatants were diluted to 100 ml. Nonprotein nitrogen in the extract was assayed according to the AOAC (1990) methods of analysis.

The content of DMA in the samples was measured colorimetrically using a Beckman DU-8 spectrophotometer (Beckman, Palo Alto, CA, USA) at λ = 435 nm as a copper dimethyldithiocarbamate salt extracted from the TCA solution by benzene (Dyer & Mounsey, 1945). A calibration curve was prepared using DMA (Sigma Chemical Co., St Louis, Missouri, USA) in concentrations ranging from 0 to 3·2 μg/ml of solution.

Concentration of TMA in seal meat samples was determined using the picric acid procedure of Dyer (1945). The effect of DMA on the colour development was eliminated using a 25% KOH instead of a 50% K_2CO_3 solution (Tozawa *et al.*, 1970). The absorbance of TMA–picric acid in toluene was then recorded. The concentration of TMA was determined using a standard curve prepared for TMA . HCl (Sigma Chemical Co.) solutions in concentrations ranging from 0 to 50 μg/ml.

The amount of TMAO was determined as TMA produced from reduction of 1·5 ml of TCA extract with 0·5 ml 1% $TiCl_3$ solution in 4% TCA at 80°C (1·5 min). The amount of TMAO was calculated as the difference between the content of TMA before and after the reduction of the samples (Babbitt *et al.*, 1972). The content of formaldehyde in TCA extracts of seal meat was determined colorimetrically with acetylacetone (Sigma Chemical Co.) at λ = 415 nm, according to Nash (1953). The concentration of formaldehyde was assayed using the standard curve for formaldehyde solutions in concentrations ranging from 0 to 6·0 μg/ml.

For determination of free amino acids, imidazole dipeptides (carnosine and anserine), ethanolamine, polyamines (histamine, cadaverine, agmatine, putrescine, spermine and spermidine) and nucleotides, a 10 g seal-meat sample was homogenized in ice-cold 6% perchloric acid (1 : 2 (w/v)) using a Polytron homogenizer (Yamanaka, 1989). After 30-min incubation in ice, samples were centrifuged at 3000 × *g* for 10 min at 5°C. The procedure was repeated and supernatants were combined. The extract used for nucleotide analyses was kept at −60°C until use.

For determination of free amino acids and polyamines, the pH of the extract was adjusted to 7·0 using a 33% KOH solution. The precipitated potassium perchlorate was removed by centrifugation at 3000 × *g* for 10 min. The supernatant, acidified with 10 N HCl to pH 2·2, was diluted with 0·3 N lithium citrate buffer, pH = 2·2 (2 : 1 (v/v)). Free amino acids and peptides were analysed using a Beckman 121 MB amino acid analyser using Benson D-X 8.25 resin and a single column, according to the three-buffer lithium method as described in Beckman 121 MB-TB-0.17 application notes.

Polyamines were determined by introducing about 250 μl of the sample containing 3,3'-iminobispropylamine as internal standard, to a Beckman 121 MB amino acid analyser which was equipped with a linear recorder and a colorimeter with a 12-mm cuvette and a 60-mm column. Post-column ninhydrin reaction absorbance was monitored at λ = 440 and 570 nm (Hall *et al.*, 1978).

Nucleotides were determined using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of two Model LC-6A pumps with a mixing chamber, a Model SPD-6AV UV-vis spectrophotometric detector, a Model SIL-6B auto-injector, a Model SCL-6B system controller, and a Model CR501 Chromatopac (data processor). The column used was a 10 μm particle size LC-18T reversed-phase analytical column (4·5 mm × 24 cm) from Supelco (Oakville, Ontario, Canada) and the guard column (4·5 mm × 5 cm) was coupled with the analytical column. Nucleotide standards were obtained from Sigma Chemical Co. Before HPLC analysis, the extract was thawed at 0–4°C and the pH was adjusted to 6·5 by diluting it to 1/10 with 0·1 M K_2HPO_4

in order to avoid crystallization of perchloric acid during HPLC analyses. The pH-adjusted sample was then filtered through a 0·45 μm nylon filter (Cameo II; MSI, Westboro, USA) into the HPLC sampling vial. Nucleotides were determined by reversed-phase HPLC. A 20 μl sample of a standard or filtered solution was injected into the column using the auto-injector. The detector was calibrated daily by injecting a known mixture of the reference compounds. A modified Stocchi's method was used (Stocchi *et al.*, 1987). The chromatographic conditions were: 0·01–3·8 min at 100% of buffer B, 7·8 min at up to 20% buffer A, 15 min at up to 40% of buffer A, 19·5 min at up to 100% of buffer A, and holding until 27 min. The gradient was then immediately returned to 100% buffer B and held until stop for a 32-min time lapse. The flow rate was 1 ml/min and detection was carried out at 254 nm.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were extracted as described by Schmidt and Thannhauser (1945) and as modified by Munro and Fleck (1966). DNA in the extract was determined by the Ceriotti (1952) indole procedure. RNA was determined spectrophotometrically at 260 nm. Protein interference at this wavelength was eliminated by applying a correction factor of 0·001 absorbance unit per 1 μg/ml protein concentration in the RNA extracts. The Folin-phenol procedure of Lowry *et al.* (1951) was used to measure the protein concentrations. Calf-liver RNA and calf-thymus DNA (Sigma Chemical Co.) were subjected to the same treatment as the tissue extracts and were used as standards in this study.

Statistical analysis

Analysis of variance and Tukey's studentized range tests (Snedecor & Cochran, 1980) were used to determine differences in mean values based on data from 3–8 replications of each measurement. Significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Nitrogen from nonprotein compounds (NPN) amounted to 1·72 mg/g of manually separated seal meat and 3·41 mg/g in the case of mechanically separated seal meat (MSSM). Inclusion of bone marrow in MSSM might be responsible for this observation. However, the first, second and third washings with water or saline solution decreased the NPN content in MSSM by about 70·2, 83·2, 94·0 and 88·9%, respectively (Table 1). The NPN content in seal meat was 0·74% of total nitrogen, much less than in teleosts (9–18%) and elasmobranchs (33–38%). Low levels of NPN in seal meat are important because nonprotein nitrogen compounds may influence the development of undesirable changes in colour, flavour and taste of meat products. Among the nonprotein nitrogen compounds, seal meat contained nucleic acid, nucleotides, free amino acids, peptides, trimethylamine oxide and its derivatives as well as other amines. The distribution of nitrogen compounds in the samples is given in Table 1.

Except for imidazole dipeptides, free amino acids were the major fraction of NPN compounds in seal meat. Free amino acids contribute to flavour and taste of foods. Total content of free amino acids in manually separated seal meat and MSSM was 0·21 and 0·35%, respectively. However, the contribution of nitrogen from free amino acids to the total NPN in MSSM (15·2%) was lower than that in manually separated meat (17·4%). Existing differences between the free amino acid profiles of samples and their varying contributions to the contents of nitrogen from amino acids may be responsible for this observation. About 55% of free amino acids consisted of taurine, alanine, glutamine, glutamic acid, leucine and lysine. Free amino acid fractions of MSSM also contained 32·8% of sweet- and 22·1% of bitter-taste compounds (Table 2). Seal meat contained 0·09% histidine in the free amino acid fraction, which is similar to that in light-coloured flesh of fish (0·01–0·05%). This corresponds to 5·01% histidine in seal meat proteins (Synowiecki *et al.*, 1992). However, histidine is the predominant free amino acid in dark-flesh fish such as tuna and mackerel (Suyama & Yoshizawa, 1973; Konosu *et al.*, 1974). The low histidine content has nutritional significance, since bacterial decay of flesh can produce a large amount of histamine from histidine which may cause histamine poisoning. Similar to other nonprotein nitrogen compounds, free amino acids were effectively removed by aqueous or

Table 1. Nitrogen content (mg N/100 g sample) of nonprotein nitrogen (NPN) compounds of unwashed and washed seal meat[a]

Seal meat	Total NPN	Imidazole dipeptides	Free amino acids	Nucleic acids	Amines[b]	NH_3	Nucleotides[c]
Manually separated	172 ± 2·31	79·4	30·0	23·8	1·36	2·33	17·7
Unwashed MSSM[d]	342 ± 5·20	94·9	51·9	40·7	1·74	5·10	—
MSSM washed							
1 × H_2O	102 ± 0·50	28·0	18·5	34·2	1·01	1·74	—
2 × H_2O	57·5 ± 0·01	11·6	8·53	—	0·78	0·81	—
3 × H_2O	20·5 ± 0·12	2·87	3·76	—	0·75	0·39	—
1 × H_2O then 0·5% NaCl solution	38·0 ± 0·40	9·59	6·93	19·9	0·83	0·71	—

[a] Results are mean values of 3–4 replicates.
[b] Calculated from the contents of ethanolamine, trimethylamine, dimethylamine, spermidine and spermine.
[c] Calculated from the contents of adenosine tri-, di-, and mono-phosphate, inosine monophosphate, inosine, hypoxanthine and xanthine.
[d] MSSM = mechanically separated seal meat.

Table 2. Free amino acid content (mg/100 g sample) in unwashed and washed seal meat[a]

Amino acid	Unwashed MSSM	MSSM washed:			MSSM washed 1 × H_2O then:	
		1 × H_2O	2 × H_2O	3 × H_2O	0·5% NaCl	0·5% $NaHCO_3$
Alanine	41·4 ± 0·34	12·5 ± 0·39	4·63 ± 0·07	1·35 ± 0·02	4·08 ± 0·04	3·77 ± 0·01
β-Alanine	2·07 ± 0·01	0·21 ± 0·00	—	—	—	—
α-Aminoadipic acid	1·32 ± 0·01	0·10 ± 0·00		—	—	—
α-Aminobutyric acid	0·29 ± 0·01		—	—	—	—
γ-Aminobutyric acid	0·23 ± 0·00	0·08 ± 0·01	—	—	—	—
Arginine	17·2 ± 0·31	7·41 ± 6·13	4·08 ± 0·29	2·08 ± 0·04	3·39 ± 0·02	3·38 ± 0·08
Aspartic acid	9·07 ± 1·23	4·63 ± 0·12	2·17 ± 0·11	1·15 ± 0·02	1·66 ± 0·07	1·71 ± 0·01
Asparagine	3·12 ± 0·00	0·96 ± 0·06	0·42 ± 0·00	0·07 ± 0·00	0·27 ± 0·01	0·17 ± 0·00
Citrulline	1·61 ± 0·03	0·52 ± 0·03	0·08 ± 0·01		0·07 ± 0·01	0·04 ± 0·00
Cystathionine	0·43 ± 0·00	0·12 ± 0·01	0·02 ± 0·00		—	0·01 ± 0·00
Cystine	1·05 ± 0·14	0·54 ± 0·08	0·48 ± 0·01	0·45 ± 0·01	0·41 ± 0·01	0·36 ± 0·01
Glutamic acid	20·3 ± 0·64	9·98 ± 0·16	3·79 ± 0·01	1·14 ± 0·01	3·37 ± 0·02	3·32 ± 0·00
Glutamine	32·7 ± 0·00	8·19 ± 0·10	3·36 ± 0·11	0·99 ± 0·01	3·05 ± 0·01	2·86 ± 0·19
Glycine	14·0 ± 0·21	5·10 ± 0·08	2·19 ± 0·05	1·04 ± 0·07	1·88 ± 0·03	1·69 ± 0·01
Histidine	9·05 ± 0·31	4·13 ± 0·10	2·05 ± 0·11	1·24 ± 0·02	1·40 ± 0·03	1·39 ± 0·03
Hydroxyproline	2·98 ± 0·12	0·77 ± 0·08	0·15 ± 0·00		—	—
Isoleucine	8·93 ± 0·18	3·25 ± 0·10	1·39 ± 0·07	0·97 ± 0·01	1·20 ± 0·00	1·09 ± 0·01
Leucine	20·8 ± 0·35	7·55 ± 0·25	3·86 ± 0·12	1·85 ± 0·09	2·90 ± 0·00	2·51 ± 0·04
Lysine	18·8 ± 0·45	8·87 ± 0·10	4·38 ± 0·19	1·89 ± 0·06	3·27 ± 0·01	3·58 ± 0·13
Methionine	9·16 ± 0·15	3·79 ± 0·17	1·96 ± 0·08	0·78 ± 0·01	1·75 ± 0·04	1·57 ± 0·02
1-Methylhistidine	0·19 ± 0·00	0·15 ± 0·00	0·08 ± 0·00	0·08 ± 0·00	0·14 ± 0·00	0·08 ± 0·00
3-Methylhistidine	0·17 ± 0·00		—	—	—	—
Ornithine	2·15 ± 0·13	0·60 ± 0·03	0·33 ± 0·03	0·21 ± 0·02	0·26 ± 0·02	0·25 ± 0·01
Phenylalanine	10·2 ± 0·19	3·83 ± 0·03	1·89 ± 0·10	0·85 ± 0·03	1·48 ± 0·01	1·29 ± 0·01
Proline	9·60 ± 0·20	3·98 ± 0·25	1·47 ± 0·03	0·40 ± 0·00	1·06 ± 0·06	1·06 ± 0·05
Serine	17·1 ± 0·32	6·60 ± 0·24	3·17 ± 0·08	1·61 ± 0·04	2·48 ± 0·03	2·37 ± 0·01
Taurine	59·3 ± 3·43	16·22 ± 0·46	7·64 ± 0·35	3·68 ± 0·05	7·05 ± 0·04	5·79 ± 0·07
Threonine	12·9 ± 0·23	4·73 ± 0·13	2·19 ± 0·12	0·94 ± 0·02	1·60 ± 0·01	1·57 ± 0·03
Tryptophan	1·75 ± 0·05	0·78 ± 0·00	0·49 ± 0·00	0·17 ± 0·01	0·42 ± 0·01	0·30 ± 0·00
Tyrosine	9·86 ± 0·17	3·86 ± 0·14	1·96 ± 0·08	0·85 ± 0·01	1·59 ± 0·00	1·46 ± 0·01
Valine	15·4 ± 0·20	5·57 ± 0·37	2·79 ± 0·11	1·26 ± 0·12	1·86 ± 0·02	1·85 ± 0·08

[a] Results are mean values of 3 replicates ± standard deviation.

saline washing of seal meat (Table 2). Two washings of MSSM with water, or with water followed by 0·5% NaCl or $NaHCO_3$ solution, resulted in a decrease in the total free amino acid contents by 83·9, 86·8 and 87·7% of their initial amounts, respectively.

Manually separated seal meat contained 0·12% of carnosine and 0·03% of anserine as compared with corresponding amounts of 0·31% and 0·07% in MSSM. These peptides contain β-alanine bound to histidine or 1- (or 3-) methylhistidine. Carnosine is predominant in beef muscles (0·15–0·25%) while anserine is predominant in chicken meat (0·05–0·25%) (Belitz & Grosch, 1987). The physiological role of carnosine and anserine is not clear; however, they may be involved in revitalization of exhausted muscle or may serve as part of the buffer system in muscle.

The amount of trimethylamine *N*-oxide (TMAO) in fresh manually separated seal meat was 1·48 mg % (Table 3), considerably less than that in fresh fish which usually contains 100–1080 mg % of TMAO (Sikorski *et al.* 1990). After one month of storage at −20°C, levels of TMAO decreased to 0·38 mg % and were almost completely exhausted after 8 months (0·01 mg %). Similar to that in fish, enzymic degradation of TMAO in seal meat results in the production of trimethylamine (TMA) and dimethylamine (DMA). Thus, the concentrations of TMA and DMA in manually separated seal meat after 8 months of storage were 0·73 and 0·42 mg %, respectively. The amount of DMA in seal meat was similar to that in cooked ham, frankfurters and milk, which contain 0·22, 0·09 and 0·32 mg % of DMA, respectively (Singer & Lijinsky, 1976). The level of DMA in meats and its possible role as a precursor of *N*-nitrosamines in the curing process is an important factor. However, cured seal meats with up to 200 ppm of sodium nitrite and 550 ppm of sodium ascorbate did not produce any *N*-nitrosamines in model systems (Shahidi *et al.*, 1992). The formaldehyde produced concurrently with DMA can cause cross-linking of proteins, similar to that in cod (Synowiecki & Sikorski, 1983) and squid (Synowiecki & Sikorski, 1988). As a consequence, lysine availability and protein digestibility may be decreased. However, the importance of these effects is minor as low concentrations of formaldehyde, not exceeding 0·25 mg %, are present in seal meat. The TMA produced for TMAO can contribute to the fishy aroma of seal meat.

The distribution of ethanolamine and polyamines in unwashed and washed seal meat is given in Table 4. Although ethanolamine may only influence the sensory properties of seal meat, secondary amines which could be nitrosated include agmatine (Kawabata *et al.*, 1978),

Table 3. Trimethylamine oxide and its degradation products in seal meat (mg %)[a]

Seal meat	Trimethylamine oxide	Trimethylamine	Dimethylamine	Formaldehyde
Manually separated meat stored:				
5 days	$1{\cdot}48 \pm 0{\cdot}05^b$	$0{\cdot}04 \pm 0{\cdot}01^b$	ND	ND
1 month	$0{\cdot}38 \pm 0{\cdot}03^c$	$0{\cdot}61 \pm 0{\cdot}02^c$	$0{\cdot}19 \pm 0{\cdot}05^b$	$0{\cdot}13 \pm 0{\cdot}02^b$
8 months	$0{\cdot}01 \pm 0{\cdot}00^d$	$0{\cdot}73 \pm 0{\cdot}02^d$	$0{\cdot}42 \pm 0{\cdot}03^c$	$0{\cdot}24 \pm 0{\cdot}01^c$
MSSM unwashed stored for 3 months:	—	$0{\cdot}95 \pm 0{\cdot}08^e$	$0{\cdot}45 \pm 0{\cdot}02^c$	$0{\cdot}25 \pm 0{\cdot}02^c$
Washed:				
$1 \times H_2O$	—	$0{\cdot}29 \pm 0{\cdot}01^f$	$0{\cdot}19 \pm 0{\cdot}03^b$	$0{\cdot}14 \pm 0{\cdot}01^b$
$2 \times H_2O$	—	$0{\cdot}16 \pm 0{\cdot}04^g$	$0{\cdot}22 \pm 0{\cdot}06^b$	$0{\cdot}12 \pm 0{\cdot}00^{b,d}$
$3 \times H_2O$	—	$0{\cdot}21 \pm 0{\cdot}02^h$	$0{\cdot}19 \pm 0{\cdot}08^b$	$0{\cdot}10 \pm 0{\cdot}01^e$
$1 \times H_2O$ then 0·5% NaCl	—	$0{\cdot}11 \pm 0{\cdot}01^i$	$0{\cdot}18 \pm 0{\cdot}03^b$	$0{\cdot}08 \pm 0{\cdot}01^e$
$1 \times H_2O$ then 0·5% $NaHCO_3$	—	$0{\cdot}09 \pm 0{\cdot}00^j$	$0{\cdot}13 \pm 0{\cdot}01^d$	—

[a] Results are mean values of 4 replicates ± standard deviation.
[b–g] Values in each column with the same superscript are not significantly ($P > 0{\cdot}05$) different from one another.
ND—not detected.

a compound which was not detected in seal meat, as well as polyamines spermidine and spermine (Smith, 1980). The amounts of spermidine and spermine in seal meat were 0·30 and 2·98 mg % respectively. These values are similar to those in fresh pork which contained 0·3–0·8 mg % of spermidine and 3·3–6·9 mg % of spermine (Nakamura *et al.*, 1979). All amines were effectively extracted during washing of MSSM. One, two or three washings of MSSM with water, or first with water and then with 0·5% NaCl solution, removed 42·0, 55·2, 56·9 and 52·3% of the amine nitrogen contents, respectively (Table 1). The most effective washing agent for the removal of these polyamines was 0·5% sodium bicarbonate solution (Table 3 and 4).

Another minor nitrogen-containing constituent of seal meat is nucleic acid. In man, the purine portion of nucleic acid is degraded to uric acid, which is poorly extracted by the urinary system. This can result in the formation of urate crystals in tissues and joints as well as stone deposition in the urinary tract. The total content of nucleic acids in manually separated seal meat was 185–198 mg %, similar to those in bovine muscles (Trenkle *et al.*, 1978; Guenther *et al.*, 1979; Arasu *et al.*, 1981). The total nucleic acid content of MSSM varied from 291 to 336 mg % (Synowiecki & Shahidi, 1992) and was lower than that in mechanically separated beef (833 mg %) (Arasu *et al.*, 1981). This is perhaps due to the fact that a smaller amount of bone marrow was present in MSSM than that present in mechanically separated beef. The Protein Advisory Group (1970) has suggested that presence of 2 g nucleic acids per day in the normal diet is the upper safe limit for adults. This amount of nucleic acids is contained in about 600 g of MSSM. However, one washing of MSSM with water (pH=5·9) decreased the amount of nucleic acids in seal meat by about 16%, from 3·36 to 2·83 mg/g of sample. More effective for the removal of nucleic acids was the washing of MSSM with 0·06% and then 0·3% NaCl solutions. After these washings, the total nucleic acid content of MSSM decreased by 44%, from 2·91 to 1·63 mg/g of sample.

Nucleotides in seal meat are produced from adenosine triphosphate (ATP), which begins to degrade immediately after the death of the animal, thus forming, in order, adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (INO), hypoxanthine (HX), xanthine (X) and finally uric acid (U). The total nitrogen contribution from these compounds in manually separated seal meat, 8 h after the death of the animal, was 16·6 mg %, which remained essentially unchanged during further storage (Table 5). The amount of nucleotides formed in seal

Table 4. Ethanolamine and polyamine contents (mg/100 g sample) in unwashed and washed seal meat[a]

Species	Ethanolamine	Spermidine	Spermine
Manually separated meat	$0{\cdot}61 \pm 0{\cdot}01^b$	$0{\cdot}30 \pm 0{\cdot}04^b$	$2{\cdot}98 \pm 0{\cdot}14^b$
MSSM from flippers	$1{\cdot}16 \pm 0{\cdot}01^c$	$0{\cdot}52 \pm 0{\cdot}07^c$	$3{\cdot}24 \pm 0{\cdot}16^b$
MSSM from carcasses	$1{\cdot}17 \pm 0{\cdot}05^c$	$0{\cdot}57 \pm 0{\cdot}02^d$	$3{\cdot}41 \pm 0{\cdot}23^b$
MSSM from carcasses washed:			
$1 \times H_2O$	$0{\cdot}36 \pm 0{\cdot}03^d$	$0{\cdot}28 \pm 0{\cdot}01^e$	$2{\cdot}55 \pm 0{\cdot}05^c$
$2 \times H_2O$	$0{\cdot}18 \pm 0{\cdot}01^e$	$0{\cdot}23 \pm 0{\cdot}02^f$	$2{\cdot}06 \pm 0{\cdot}19^d$
$3 \times H_2O$	$0{\cdot}06 \pm 0{\cdot}00^f$	$0{\cdot}22 \pm 0{\cdot}01^f$	$2{\cdot}02 \pm 0{\cdot}13^d$
$1 \times H_2O$, then 0·5% NaCl	$0{\cdot}18 \pm 0{\cdot}00^e$	$0{\cdot}21 \pm 0{\cdot}07^f$	$2{\cdot}32 \pm 0{\cdot}03^e$
$1 \times H_2O$, then 0·5% $NaHCO_3$	$0{\cdot}14 \pm 0{\cdot}01^g$	$0{\cdot}18 \pm 0{\cdot}02^f$	$1{\cdot}69 \pm 0{\cdot}07^d$

[a] Results are mean values of 4 replicates ± standard deviation.
[b–g] Values in each column with the same superscript are not significantly ($P > 0{\cdot}05$) different from one another.
Cadaverine, putrescine and agmatine were not detected (detection limit of 0·01 mg %).

Table 5. The nitrogen content (mg/100 g sample) in the nucleotides of seal meat[a]

Nucleotide[b]	Storage time (days)			
	0·25	2·0	4·0	12·0
ATP	5·49 ± 1·20	ND	ND	ND
ADP	6·37 ± 0·95[c]	0·47 ± 0·11[d]	0·41 ± 0·12[d]	0·38 ± 0·09[d]
AMP	3·20 ± 0·72[c]	1·26 ± 0·22[d]	0·83 ± 0·15[e]	0·36 ± 0·11[f]
IMP	0·65 ± 0·15[c]	13·76 ± 0·54[d]	12·28 ± 0·73[d]	7·56 ± 0·54[e]
INO	0·44 ± 0·21[c]	1·67 ± 0·25[d]	2·45 ± 0·19[e]	4·40 ± 0·32[f]
HX	0·24 ± 0·10[c]	0·48 ± 0·08[d]	0·79 ± 0·05[e]	3·35 ± 0·86[f]
X	0·17 ± 0·09[c]	0·30 ± 0·10[c]	0·53 ± 0·19[c]	1·80 ± 0·47[d]
Total	16·56	17·94	17·29	17·85

[a] Results are mean values of 4 determinations ± standard deviation.
[b] ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; INO, inosine; HX, hypoxanthine; X, xanthine.
[c–f] Values in each row with the same superscript are not significantly ($P > 0·05$) different from one another.
ND = not detected.

meat seems to be smaller than that in muscles of other mammals. According to Hultin (1985), at the moment of slaughter, the average ATP-nitrogen content in muscles from different animals ranges from 41·3 to 68·8 mg %. Nucleotides are important flavour precursors and their profile may be used as an indicator of freshness quality of muscle foods (Shahidi *et al.*, 1992).

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