# Color Characteristics and Absence of N-Nitrosamines in Nitrite-Cured Seal Meat

Fereidoon Shahidi,<sup>\*,†</sup> Jozef Synowiecki,<sup>†,‡</sup> and Nrisinha Sen<sup>§</sup>

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9, and Health Protection Branch, Sir Frederick G. Banting Research Centre, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0K2

The effect of curing agents, nitrite and ascorbate, on the content of hemoproteins, their nitroso derivatives, color development, and possibility of N-nitrosamine formation in mechanically separated seal meat (MSSM) and seal surimi was investigated. Treatment of MSSM and washed MSSM with up to 200 ppm of sodium nitrite in the presence of 550 ppm of sodium ascorbate resulted in pigment conversions of 68.50 and 66.17%, respectively. The Hunter a (redness) values of cured MSSM as such, MSSM after one aqueous washing, or MSSM washed first with water and then with 0.5% NaCl were well correlated with the content of nitrosylheme in the meat (correlation coefficients: 0.952, 0.896, and 0.899, respectively). Although seal meat contains low amounts of trimethylamine, dimethylamine, spermidine, and spermine (0.73, 0.42, 0.30, and 2.98 mg %, respectively), no volatile N-nitrosamines were detected in the samples treated with sodium nitrite and sodium ascorbate at concentrations recommended by U.S. Department of Agriculture regulations.

# INTRODUCTION

Meat from harp seal (*Phoca groenlandica*) is available in approximately 2.0-2.5 million kilograms annually in Canada. The meat has a high content of protein and is well-balanced in its essential amino acid composition (Shahidi et al., 1990). However, very little is known about the quality, technological properties, and utilization potential of seal meat. Except for flippers, which are used for preparation of local foods, seal carcasses are generally reduced to low-grade silage.

Limited consumption of seal meat may be due to its dark color, which is related to the high myoglobin and hemoglobin contents of seal muscle tissues. Previous investigations (Synowiecki and Shahidi, 1991) have shown that the color of seal meat can be improved by partial extraction of hemoproteins with aqueous or saline solutions. The total content of hemoproteins present in seal meat was reduced from 5.94 to 3.00, 2.07, and 1.93% after the first, second, and third aqueous washings, respectively. Similarly, a reduction in the content of lipids in the washed mechanically separated seal meat (MSSM) was noticed (Synowiecki and Shahidi, 1991).

The color intensity of seal meat depends on its content of hemoproteins and oxidative changes that may occur in the muscles. The characteristic pink color of cured pork, beef, and poultry is generally associated with the high quality of such processed meat products. The color of cured meat is developed after addition of nitrite to raw meat and subsequent heat processing (Eakes and Blumer, 1975). In addition, nitrite may also contribute to the cured meat flavor and acts as a strong antioxidant (Sato and Hegarty, 1971) and possesses antimicrobial properties (Collins-Thompson et al., 1974; Holley, 1981). Thus, nitrite curing may lend itself as a processing option in preparing shelf-stable seal products. Concerns over the use of nitrite due to its possible reactions with amines and amino acids present in meat has been the subject of many investigations (Gray and Randall, 1979; Sen et al., 1973). N-Nitrosodimethylamine and N-nitrosopyrrolidine, examples of such reaction products, have been detected in some meat products, under certain heat-processing conditions (Gray, 1976; Sen et al., 1972), and have been shown to be carcinogenic and possibly mutagenic and teratogenic in experimental animals (Magee and Barnes, 1967; Preussmann and Stewart, 1984). Thus, it is essential to study the possibility of N-nitrosamine formation in cured seal meat products.

The objectives of this study were to examine the effects of nitrite curing on the color of seal meat, as such, and seal surimi under different processing conditions. Furthermore, the content of amines in seal meat and the possibility of N-nitrosamine formation as a result of nitrite curing were determined to investigate the potential use of nitrite curing of seal meat in the production of different varieties of seal-based products.

#### MATERIALS AND METHODS

Materials. Beater (3 weeks-1 year of age) and bedlamer (1-4 years of ages) harp seals (*P. groenlandica*), hunted in the coastal regions of Newfoundland/Labrador during May-July, were bled and skinned; blubber fat was removed, and the carcasses were eviscerated. Whole seal carcasses weighing 5-30 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 were trimmed of most of their subcutaneous fat. Mechanical separation of meat from carcasses of 15 seals was carried out using a Poss deboner (Model PDE500, Poss Limited, Toronto, ON). Small portions of mechanically separated seal meat (MSSM) were vacuum packed in polyethylene pouches and kept frozen at -20 °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<sub>3</sub> 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 diameter holes.

Samples of unwashed or washed MSSM were mixed with distilled water in amounts necessary to achieve a final water

<sup>&</sup>lt;sup>†</sup> Memorial University.

<sup>&</sup>lt;sup>‡</sup> On leave of absence from Department of Food Preservation and Technical Microbiology, Technical University of Gdansk, Gdansk, Poland.

<sup>&</sup>lt;sup>§</sup> Health and Welfare Canada.

Table I. Percentage of Total Hemoproteins from Unwashed and Washed MSSM Converted to a Nitroso Derivative in Curing with Different Levels of Nitrite and Sodium Ascorbate<sup>a</sup>

		NaNO <sub>2</sub> , ppm					
sodium ascor	bate, ppm	100	200	500	1000	2000	
0	a	$25.40 \pm 2.38^{at}$	31.27 ± 1.27 <sup>ex</sup>	$51.39 \pm 0.58^{ay}$	$64.27 \pm 0.76^{aw}$	$67.41 \pm 1.10^{az}$	
	b	$22.34 \pm 0.82^{at}$	$29.32 \pm 1.23^{nx}$	$37.92 \pm 1.09^{by}$	$45.46 \pm 0.68^{bw}$	$55.30 \pm 0.50^{bz}$	
	c	$24.81 \pm 0.91^{\text{st}}$	$37.50 \pm 1.52^{bx}$	$45.93 \pm 0.61^{cy}$	$51.73 \pm 1.23^{cw}$	$60.67 \pm 0.61^{cz}$	
100	а	$33.16 \pm 0.73^{bt}$	$49.45 \pm 1.18^{cx}$	$65.19 \pm 1.83^{dy}$	$79.99 \pm 0.82^{dw}$	$81.97 \pm 0.45^{dz}$	
	Ъ	$32.26 \pm 1.64^{bt}$	$44.20 \pm 1.23^{dx}$	$53.34 \pm 1.09^{ay}$	$61.30 \pm 1.36^{\text{ew}}$	$64.42 \pm 1.64^{ez}$	
	c	$29.80 \pm 1.22^{bt}$	$43.42 \pm 1.23^{dx}$	$49.62 \pm 0.61^{ey}$	$55.80 \pm 1.20^{\text{fw}}$	$63.68 \pm 1.49^{ez}$	
275	a	$39.67 \pm 2.48^{dt}$	$63.50 \pm 1.75^{ex}$	$75.71 \pm 1.01^{\text{fy}}$	80.33 ± 1.13 <sup>gw</sup>	$82.62 \pm 1.64^{dw}$	
	b	$38.62 \pm 0.68^{dt}$	$56.06 \pm 0.68^{fx}$	$64.07 \pm 2.01^{dy}$	$68.12 \pm 0.13^{hw}$	$72.29 \pm 0.51^{fz}$	
	c	$37.42 \pm 1.37^{dt}$	$52.69 \pm 0.88^{gx}$	$60.38 \pm 1.49^{dy}$	$65.08 \pm 1.48^{aw}$	$69.50 \pm 0.28^{gz}$	
550	а	$40.60 \pm 0.61^{\text{et}}$	$68.50 \pm 1.53^{hx}$	$80.50 \pm 0.94^{gy}$	$81.64 \pm 0.35^{gy}$	$84.21 \pm 1.65^{dw}$	
	b	$52.08 \pm 0.41^{ft}$	$66.17 \pm 0.13^{ix}$	$77.06 \pm 0.13^{hy}$	$79.14 \pm 0.26^{gw}$	$79.31 \pm 0.33^{hw}$	
	c	$46.22 \pm 1.80^{\text{gt}}$	$64.19 \pm 1.46^{ix}$	$69.75 \pm 0.73^{iy}$	$75.81 \pm 1.37^{iw}$	$77.00 \pm 0.90^{cw}$	
1100	а	$42.03 \pm 1.09^{ht}$	$74.16 \pm 1.28^{kx}$	$83.23 \pm 1.19^{iy}$	$84.36 \pm 1.37^{jy}$	$85.46 \pm 0.45^{dy}$	
	b	$53.01 \pm 0.24^{it}$	$74.12 \pm 0.27^{kx}$	$82.09 \pm 0.54^{jy}$	$83.99 \pm 0.41^{jw}$	$84.21 \pm 0.21^{dw}$	
	с	$50.89 \pm 0.51^{\text{jt}}$	$67.47 \pm 0.60^{ix}$	$75.05 \pm 1.83^{ky}$	$77.57 \pm 0.73^{kw}$	$79.79 \pm 0.28^{hz}$	

<sup>a</sup> Results are mean values of four replicates  $\pm$  standard deviation. a, Unwashed MSSM, total hemoproteins content 50.73 mg/g of meat; b, MSSM washed  $1 \times H_2O$ , total hemoproteins, 24.44 mg/g; c, MSSM washed  $1 \times H_2O$  then  $1 \times 0.5\%$  NaCl, total hemoproteins content 10.92 mg/g. Values with the same superscript are not significantly (P > 0.05) different from one another.

content in the meat of about 80%. Sodium ascorbate (0-1100 ppm) and sodium nitrite (0-2000 ppm) were added directly to meat samples. The mixtures were then thoroughly homogenized and heat processed at  $85 \oplus 2$  °C in a thermostated water bath for 45 min to reach an internal temperature of  $75 \pm 2$  °C with occasional stirring with a glass rod. After cooling to room temperature, cooked meat samples were homogenized in a Waring blender (Model 33BL73, Dynamics Corp., New Hartford) for 30 s, vacuum packed in polyethylene pouches, and stored at -60 °C for up to 2 days before analysis.

The tristimulus Hunter color parameters L (lightness, 100 = white; 0 = black), a (red, +; green, -), and b (yellow, +; blue, -) of top surfaces of cured meat samples were measured using a XL-20 colorimeter (Gardner Laboratory, Inc., Bethesda, MD). Standard plate No. XL-20-167C with Hunter L value of 92.0, a value of -1.1, and b value of 0.7 was used as a reference. The Hunter L, a, b color values were measured at eight different locations of the meat surface.

Analyses. The conversion of hemoproteins to their nitrosylheme derivatives was determined by triplicate extractions of the cured seal meat samples [meat to solvent ratio of 1:10 (w/v)] with a solution containing 80% acetone and 4% HCl for determination of total pigments or with acetone-water (4:1) for determination of nitrosylheme derivatives (Hornsey, 1956). Results were calculated using the equation

conversion yield (%) = 
$$\frac{A_{540\text{nm}} \times 290}{A_{640\text{nm}} \times 680} \times 100\%$$

where  $A_{540nm}$  and  $A_{640nm}$  are the absorbances of hemoproteins extracted by 80% aqueous acetone, without or with HCl, respectively.

For determination of trimethylamine N-oxide (TMAO), trimethylamine (TMA), and dimethylamine (DMA), 20 g of meat sample was homogenized with a 10% trichloroacetic acid (TCA) solution (1:2 w/v) using a Polytron homogenizer (Brinkmann Instruments, Rexdale, ON) and centrifuged for 15 min at 3000g. The extraction procedure was repeated, and combined supernatants were diluted to 100 mL.

The content of DMA in the samples were measured colorimetrically using a Beckman DU-8 spectrophotometer at  $\lambda = 435$ nm as a copper dimethyldithiocarbamate salt extracted from the TCA solution by benzene (Dyer and Mounsey, 1945). A calibration curve was prepared using DMA (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 0 to 3.2  $\mu$ g/mL of solution.

The concentration of TMA in seal meat samples was determined using the picric acid procedure of Dyer (1945). The effect of DMA on the color development was eliminated using a 25%KOH instead of a 50% K<sub>2</sub>CO<sub>3</sub> solution (Tozawa et al., 1970). The absorbance of TMA-picric acid in toluene was measured using a Beckman DU-8 spectrophotometer. The concentration of TMA was determined using the standard curve prepared for TMA-HCl (Sigma) solutions in concentrations ranging from 0 to 50  $\mu$ g/mL.

The amount of TMAO in each sample was determined as TMA produced after reduction of 1.5 mL of TCA extract with 0.5 mL of 1% TiCl<sub>3</sub> 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).

For determination of histamine, cadaverine, agmatine, putrescine, spermine, and spermidine, 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 a 30-min incubation in ice, samples were centrifuged at 3000g for 10 min at 5 °C. The procedure was repeated and supernatants were combined. The pH of the supernatant was adjusted to 7.0 using 33% KOH, and precipitated potassium perchlorate was removed by centrifugation at 3000g 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). About 250  $\mu$ L of the sample containing 3,3'-iminobis(propylamine), as internal standard, was introduced to a Beckman 121MB amino acid analyzer which was equipped with a linear recorder and a colorimeter with a 12-mm cuvette and a 6-mm column. Postcolumn ninhydrin reaction absorbance was monitored at  $\lambda = 440$  and 570 nm (Hall et al., 1978).

Volatile N-Nitrosamine Analysis. A 20-25-g sample aliquot was analyzed by a low-temperature vacuum distillation method, as described previously (Sen et al., 1985). A suspension of the sample, together with 100 ng of N-nitrosodi-n-propylamine (NDPA) as internal standard and 10 g of  $Ba(OH)_2$  in 200 mL of water, was distilled under vacuum at 45-50 °C in an all-glass flask evaporator. The aqueous distillate was made alkaline and was extracted with dichloromethane. The dichloromethane extract was concentrated and analyzed by gas-liquid chromatography-thermal energy analyzer (GLC-TEA) as described elsewhere (Sen et al., 1985). This procedure allowed for detection as well as quantitation of N-nitrosodimethylamine (NDMA), Nnitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP), Nnitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), and N-nitrosothiazolidine (NThZ). For samples that foamed excessively during vacuum distillation, the barium hydroxide solution was replaced with 190 mL of 1% sulfamic acid and 10 mL of 1  $N H_2 SO_4$ . The identify of NDMA in the sample containing the highest concentration was confirmed by GLC-high-resolution mass spectrometry (Sen et al., 1985).

Statistical Analysis. Analysis of variance and Tukey's Studentized range tests (Snedecor and Cochran, 1980) were used to determine differences in mean values based on data from three

Table II. Hunter a Values of Unwashed and Washed MSSM Cured with Different Concentrations of Nitrite and Sodium Ascorbate<sup>4</sup>

			NaNO <sub>2</sub> , ppm				
sodium ascor	bate, ppm	0	100	200	500	1000	2000
0	a	$7.47 \pm 0.15^{ss}$	$12.37 \pm 0.21^{at}$	$14.72 \pm 0.38^{ax}$	$17.66 \pm 0.14^{ay}$	$18.78 \pm 0.15^{aw}$	$18.34 \pm 0.12^{az}$
	b	7.26 ± 0.13™	$12.26 \pm 0.38^{at}$	$14.00 \pm 0.35^{ax}$	$16.02 \pm 0.30^{by}$	$16.96 \pm 0.12^{bw}$	$17.33 \pm 0.31^{bw}$
	c	$7.56 \pm 0.05^{ss}$	$9.95 \pm 0.20^{bt}$	$9.93 \pm 0.15^{bt}$	$10.83 \pm 0.13^{cx}$	$12.25 \pm 0.17^{ey}$	$13.53 \pm 0.16^{cw}$
100	a	$7.26 \pm 0.16^{as}$	$12.67 \pm 0.22^{\text{at}}$	$15.63 \pm 0.19^{cx}$	$17.80 \pm 0.12^{ay}$	$19.66 \pm 0.19^{dz}$	$18.76 \pm 0.24^{dw}$
	b	$7.16 \pm 0.15^{as}$	$12.51 \pm 0.19^{at}$	$15.07 \pm 0.39^{ex}$	$17.27 \pm 0.31^{ay}$	$17.96 \pm 0.38^{zy}$	$17.57 \pm 0.32^{by}$
	с	$7.13 \pm 0.09^{as}$	$11.16 \pm 0.28^{ct}$	$12.07 \pm 0.20^{dx}$	$13.63 \pm 0.32^{dy}$	$14.86 \pm 0.25^{\text{fw}}$	$15.52 \pm 0.14^{dz}$
275	a	$7.42 \pm 0.10^{as}$	$12.53 \pm 0.16^{at}$	$16.35 \pm 0.22^{ex}$	$18.77 \pm 0.15^{ey}$	$19.91 \pm 0.13^{dw}$	$19.93 \pm 0.22^{ew}$
	b	$6.61 \pm 0.08^{bs}$	$13.12 \pm 0.18^{bt}$	$16.01 \pm 0.20^{ex}$	$17.52 \pm 0.24^{ay}$	$18.12 \pm 0.32^{ew}$	$17.75 \pm 0.18^{by}$
	c	$7.25 \pm 0.16^{as}$	$12.57 \pm 0.34^{\text{at}}$	$13.10 \pm 0.33^{ft}$	$13.98 \pm 0.41^{dx}$	$15.17 \pm 0.32^{fy}$	$15.75 \pm 0.24^{dy}$
550	a	$6.95 \pm 0.12^{as}$	$15.10 \pm 0.20^{et}$	$17.61 \pm 0.25^{gx}$	$19.88 \pm 0.10^{\rm fy}$	$20.60 \pm 0.31^{hw}$	$20.92 \pm 0.24^{fw}$
	b	$6.52 \pm 0.10^{bs}$	$14.98 \pm 0.10^{ft}$	$17.60 \pm 0.05^{gx}$	$18.01 \pm 0.15^{gy}$	$18.61 \pm 0.15^{aw}$	$18.38 \pm 0.25^{ay}$
	с	$6.36 \pm 0.20^{\rm bs}$	$14.46 \pm 0.29^{\text{ft}}$	$15.23 \pm 0.35^{cx}$	$15.68 \pm 0.65^{hx}$	$16.16 \pm 0.13^{jx}$	$16.15 \pm 0.32^{dx}$
1100	a	$7.01 \pm 0.16^{as}$	$15.31 \pm 0.07^{\text{et}}$	$18.90 \pm 0.29^{hx}$	$19.82 \pm 0.34^{fx}$	$20.13 \pm 0.14^{ix}$	$20.43 \pm 0.12^{gy}$
	b	$6.36 \pm 0.05^{bs}$	$15.20 \pm 0.20^{et}$	$17.85 \pm 0.10^{ix}$	$17.92 \pm 0.05^{ax}$	$18.75 \pm 0.12^{ay}$	$18.47 \pm 0.17^{ay}$
	c	$6.01 \pm 0.19^{bs}$	$14.70 \pm 0.39^{ft}$	$15.22 \pm 0.58^{ct}$	$15.31 \pm 0.47^{ht}$	$16.13 \pm 0.10^{jx}$	$16.08 \pm 0.33^{dx}$

<sup>a</sup> Results are mean values of eight determinations  $\pm$  standard deviation. a, Unwashed MSSM, total hemoproteins content 50.73 mg/g of meat; b, MSSM washed  $1 \times H_2O$ , total hemoproteins, 24.44 mg/g; c, MSSM washed  $1 \times H_2O$  then  $1 \times 0.5\%$  NaCl, total hemoproteins content 10.92 mg/g. Values with the same superscript are not significantly (P > 0.05) different from one another.

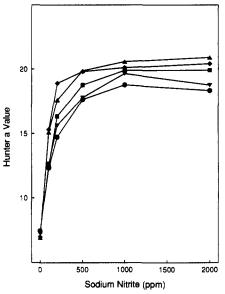
to eight replications of each measurement. Significance was determined at P < 0.05.

### **RESULTS AND DISCUSSION**

The effect of concentration of nitrite and ascorbate on the conversion of hemoproteins in comminuted seal meat to their nitrosylheme derivative was examined. The conversion yields depended on the concentration of both sodium nitrite and sodium ascorbate used in the curing process. In unwashed MSSM, the conversion ranged from 25.4% for samples cured with 100 ppm of sodium nitrite in the absence of sodium ascorbate to 85.46% for samples cured with 2000 ppm of sodium nitrite in the presence of 1100 ppm of sodium ascorbate (Table I).

The pigments' conversion was higher in cured unwashed MSSM than in washed samples. However, these results were not always significantly different from one another. After addition of up to 200 ppm of sodium nitrite in the presence of 550 ppm of sodium ascorbate, the amounts of hemoproteins converted to their nitroso form in unwashed MSSM (5.07% total pigment content) and MSSM after one aqueous washing (2.44% total pigment content) were 68.50 and 66.17%, respectively (Table I). The observed difference in the conversion of hemoproteins to their derivatives in the two samples were minor and was perhaps due to the existing differences in their hemoprotein concentration and possibly was affected by the ratio of myoglobin to hemoglobin in unwashed and washed meats. It should be noted that USDA (1978) regulations indicate that finished cured products of chopped and pickled pork should not contain more than 200 ppm of sodium nitrite.

The Hunter a (redness) values of cooked cured samples of unwashed MSSM and MSSM prewashed with water or water and then 0.5% NaCl solution were well correlated with the nitrosoheme content in the meat (correlation coefficients: 0.952, 0.896, and 0.899, respectively) (Table II). The Hunter L values of samples treated only with sodium ascorbate (0–1100 ppm) were not significantly different from one another, and as expected, these samples had a brown color after heat processing. Addition of sodium nitrite produced, upon heating, a red color which was visually too intense (beetlike color) in the case of unwashed meat, as judged by the authors. However, the color of the cured seal surimi obtained after aqueous and NaCl washings was visually similar to that of cured beef.



**Figure 1.** Hunter a (redness) values of unwashed MSSM after curing with different levels sodium nitrite and sodium ascorbate at 0 ( $\bullet$ ), 100 ( $\triangledown$ ), 275 ( $\blacksquare$ ), 550 ( $\blacklozenge$ ), and 1100 ppm ( $\blacktriangle$ ).

The Hunter L, a, b values of seal surimi cured with 200ppm of sodium nitrite in the presence of 550 ppm of sodium ascorbate were 29.6  $\pm$  0.3, 15.2  $\pm$  0.2, and 7.1  $\pm$  0.1, respectively. Corresponding values for cured pork and beef were  $58.2 \pm 0.4$ ,  $13.3 \pm 0.3$ , and  $8.6 \pm 0.2$  and  $4.6 \pm$ 0.3, 16.7  $\pm$  0.3, and 9.1  $\pm$  0.2, respectively (Shahidi and Pegg, 1990). The redness intensity of cured seal meat depended also on the concentration of sodium ascorbate in samples which were cured with the same concentration of nitrite. The intensities increased when higher amounts of ascorbate were used (Figure 1). Significantly different (P < 0.05) Hunter a values were observed when samples were cured in the absence or in the presence of 1100 ppm of sodium ascorbate (Table II). However, in cured samples containing 550 ppm of sodium ascorbate, the main color changes were achieved when 200 ppm of sodium nitrite was used.

The distribution of secondary and tertiary amines in unwashed and washed seal meat is given in Table III. Analysis of these compounds is important in view of their possible role in the formation of *N*-nitrosamines during

Table III. Major Secondary and Tertiary Amines and Polyamines in MSSM and As Affected by Washing (mg %)\*

species	trimethylamine	dimethylamine	spermidine	spermine
manually separated meat	$0.73 \pm 0.02^{a}$	$0.42 \pm 0.03^{\circ}$	$0.30 \pm 0.04^{a}$	$2.98 \pm 0.14^{\text{e}}$
MSSM from flippers			$0.52 \pm 0.01^{b}$	$3.24 \pm 0.16^{a}$
MSSM from carcasses	$0.95 \pm 0.08^{b}$	$0.45 \pm 0.02^{\circ}$	$0.57 \pm 0.02^{\circ}$	$3.41 \pm 0.23^{a}$
MSSM from carcasses				
washed $1 \times H_2O$	$0.29 \pm 0.01^{\circ}$	$0.19 \pm 0.03^{b}$	$0.28 \pm 0.01^{d}$	$2.55 \pm 0.05^{b}$
washed $2 \times H_2O$	$0.16 \pm 0.04^{d}$	$0.22 \pm 0.06^{b}$	$0.23 \pm 0.02^{\circ}$	$2.06 \pm 0.19^{\circ}$
washed $3 \times H_2O$	$0.21 \pm 0.02^{e}$	$0.19 \pm 0.08^{b}$	$0.22 \pm 0.01^{\circ}$	$2.02 \pm 0.13^{\circ}$
$1 \times H_2O$ , then 0.5% NaCl	$0.11 \pm 0.01^{\rm f}$	$0.18 \pm 0.03^{b}$	$0.21 \pm 0.07^{\circ}$	$2.32 \pm 0.03^{d}$
$1 \times H_2O$ , then 0.5% NaHCO <sub>3</sub>	$0.09 \pm 0.00^{g}$	$0.13 \pm 0.01^{\circ}$	$0.18 \pm 0.02^{\circ}$	$1.69 \pm 0.07^{\circ}$

<sup>a</sup> Results are mean values of four replicates  $\pm$  standard deviation. Values in each column with the same superscript are not significantly (P > 0.05) different from one another. Codaverine, putrescine, and agmatine were not detected (detection limit of 0.01 mg %).

curing. N-Nitrosodimethylamine is known to be a potent carcinogen (Smith, 1980). Its precursor, dimethylamine (DMA), is formed together with formaldehyde in the muscles of sea animals as a result of the activity of endogenous enzymes on trimethylamine N-oxide (TMAO). Seal meat frozen for 1 month at -20 °C contained 0.38  $\pm$  0.03 mg % of TMAO. After 8 months of storage, the concentration of TMAO was almost completely exhausted (0.01 mg %). In seal, TMAO probably serves as a part of their buffer system and as osmoregulator, as documented for other marine species.

The amount of dimethylamine in seal meat (Table III) was similar to its content in cooked ham, frankfurters, and milk, which contain 0.22, 0.09, and 0.32 mg % of DMA, respectively (Singer and Lijinsky, 1976). As compared with silver hake (Miller et al., 1972), cod (Singer and Lijinsky, 1976), and salmon (Gruger, 1972), seal meat had about 95.2, 176.2, and 1.6 times their content of DMA. Washings with water, 0.5% NaCl, or 0.5% bicarbonate solution decreased the DMA content by about 55, 57, and 69% of its initial amount (Table III). More recent work by Pensabene and Fiddler (1988) and Pensabene et al. (1991) reported that, if present, the nitrosamine contents of all-meat control and Alaska pollock surimi-meat frank-furters were similar.

Other secondary amines that could possibly be nitrostated include agmatine (Kawabata et al., 1978), a compound not detected in seal meat, and the polyamines spermidine and spermine (Smith, 1980). The amounts of spermidine and spermine in seal meat were 0.30 and 2.98 mg %, respectively, and these values are similar to those found in pork. Fresh pork contains 0.3–0.8 mg % of spermidine and 3.3–6.9 mg % of spermine (Nakamura et al., 1979). The spermidine and spermine content in seal meat was decreased during aqueous or saline washings (Table III); however, only volatile N-nitrosamine was qualitified in this work. Seal meat also contained TMA as a product of bacterial decomposition of TMAO. The amount of this compound in meat stored at -20 °C for 3 months ranged from 0.73 to 0.95 mg % (Table III).

Volatile N-nitrosamines were not detected in seal meat samples treated with 100 or 200 ppm of sodium nitrite (Table IV). The addition of 550 ppm of sodium ascorbate and the low levels of DMA originally present in MSSM were responsible for this observation. Only in unwashed MSSM stored for 8 months at -20 °C before treatment with 500 ppm of sodium nitrite was 4.2 µg of N-nitrosodimethylamine/kg of meat detected. However, even this amount was lower than the volatile N-nitrosamine contents of fried bacon and was comparable to those in other meat products (Sen et al., 1979, 1980). Thus, nitrite curing of seal meat with up to 200 ppm of sodium nitrite and 550 ppm of sodium ascorbate does not pose any health hazard connected with N-nitrosamine formation. The Hunter color parameters of seal surimi cured under these con-

#### Table IV. N-Nitrosodimethylamine (NDMA) Content (μg/kg) of Unwashed and Washed MSSM Cured with Different Amounts of Sodium Nitrite and 550 ppm of Sodium Ascorbate<sup>4</sup>

	NaNO <sub>2</sub> , ppm		
MSSM	100	200	500
stored 3 months, unwashed	ND	ND	ND
after washing: 1 × H <sub>2</sub> O	ND	ND	<0.2
2 × H <sub>2</sub> O	ND	ND	ND
3 × H <sub>2</sub> O	ND	ND	ND
$1 \times H_2O$ , then 0.5% NaCl	ND	ND	ND
$1 \times H_2O$ , then 0.5% NaHCO <sub>3</sub>	trace	ND	ND
stored 8 months, unwashed	ND	ND	4.2
after washing: $1 \times H_2O$	ND	ND	ND

<sup>a</sup> ND, not detected (detection limit, 0.1  $\mu$ g/kg). NDMA in the sample containing 4.2  $\mu$ g/kg was confirmed by GLC-MS.

ditions were similar to those of cured beef. Further improvement in the color of cured seal meat products may be achieved by combination of seal meat with pork, beef, or fish surimi in the preparation of hybrid products.

## ACKNOWLEDGMENT

We greatly appreciate financial assistance from the Newfoundland Department of Fisheries through a NIFDA grant. We thank P. A. Baddoo and D. Weber of the Food Research Division, Canadian Health Protection Branch, Ottawa, for N-nitrosamine and GLC-MS analyses, respectively.

## LITERATURE CITED

- Babbitt, J. K.; Crawford, D. L.; Law, D. K. Decomposition of trimethylamine oxide and changes in protein extractability during frozen storage of minced and intact Hake (Merluccius productus) muscle. J. Agric. Food Chem. 1972, 20, 1052-1054.
- Collins-Thompson, D. L.; Chang, P. C.; Davidson, C. M.; Larmond, E.; Pivnik, H. The effect of nitrite and storage temperature on the organoleptic quality and toxicogenesis by Clostridium botulinum in vacuum packed bacon. J. Food Sci. 1974, 39, 607-610.
- Dyer, W. J. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Board Can. 1945, 6, 351-358.
- Dyer, W. J.; Mounsey, Y. A. Amines in fish muscle. II. Development of trimethylamine and other amines. J. Fish. Res. Board Can. 1945, 6, 359-367.
- Eakes, B. D.; Blumer, T. N. Effect of nitrate and nitrite on color and flavor of country cured hams. J. Food Sci. 1975, 39, 607– 611.
- Gray, J. I. N-nitrosamines and their precursors in bacon: A Review. J. Milk Food Technol. 1976, 39, 686-689.
- Gray, J. I.; Randall, C. J. The nitrite/N-nitrosamine problem in meats. An update. J. Food Prot. 1979, 42, 168-179.
- Gruger, E. H. Chromatographic analyses of volatile amines in marine fish. J. Agric. Food Chem. 1972, 20, 781-785.
- Hall, D. E.; Symonds, G. W.; Symonds, D. L.; Brosnan, M. E. Analysis of spermidine and spermine in rat liver. Effect of

hypophysectomy on polyamine concentrations. Can. J. Biochem. 1978, 56, 291-295.

- Holley, R. A. Review of the potential hazard from botulism in cured meats. Can. Inst. Food Sci. Technol. J. 1981, 14, 183– 187.
- Hornsey, H. C. The color of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 1956, 7, 534-540.
- Kawabata, T.; Ohshima, H.; Ino, M. Occurrence of methylguanidine and agmatine, nitrosatable guanidine compounds in food. J. Agric. Food Chem. 1978, 26, 334–338.
- Magee, P. N.; Barnes, J. M. Carcinogenic nitroso compounds. Adv. Cancer Res. 1967, 10, 163-180.
- Miller, A.; Scanlan, R. A.; Lee, J. S.; Libbey, L. M. Quantitative and selective gas chromatographic analysis of dimethyl- and trimethylamine in fish. J. Agric. Food Chem. 1972, 20, 709– 711.
- Nakamura, M.; Wada, Y.; Sawaya, H.; Kawabata, T. Polyamine content in fresh and processed pork. J. Food Sci. 1979, 44, 515-517, 523.
- Pensabene, J. W.; Fiddler, W. Determination of volatile N-nitrosamines in frankfurters containing minced fish and surimi. J. Assoc. Off. Anal. Chem. 1988, 71, 839–843.
- Pensabene, J. W.; Fiddler, W.; Gates, R. A.; Hale, M.; Jahncke, M.; Gooch, J. A research note. N-nitrosothiozolidine and its 4-carboxylic acid in frankfurters containing Alaska pollock. J. Food Sci. 1991, 56, 1108–1110.
- Preussmann, R.; Stewart, B. W. N-Nitroso Carcinogen. In Chemical Carcinogen; Searle, C. E., Ed.; ACS Symposium Series 182; American Chemical Society: Washington, DC, 1984; pp 643-828.
- Sato, K.; Hegarty, G. R. Warmed-over flavor in cooked meats. J. Food Sci. 1971, 36, 1098-1100.
- Sen, N. P.; Seaman, S. W.; Miles, W. F. Volatile nitrosamines in various cured meat products: effects of cooking and recent trends. J. Agric. Food Chem. 1979, 27, 1354-1357.
- Sen, N. P.; Seaman, S.; McPherson, M. Further studies on the occurrence of volatile and non-volatile nitrosamines in foods. In N-nitroso-Compounds Analysis, Formation and Occur-

*rence*; Walker, E. A., Borzsonyi, M., Eds.; Publication 31; International Agency for Research on Cancer Science: Lyon, France, 1980; p 457.

- Sen, N. P.; Tessier, L.; Seaman, S. W.; Baddoo, P. A. Volatile and nonvolatile nitrosamines in fish and the effect of deliberate nitrosation under simulated gastric conditions. J. Agric. Food Chem. 1985, 33, 264-268.
- Shahidi, F.; Pegg, R. Color characteristics of cooked cured-meat pigment and its application to meat. *Food Chem.* **1990**, *38*, 61-68.
- Shahidi, F.; Synowiecki, J.; Naczk, M. Seal meat—A potential source of muscle food: Chemical composition, essential amino acids and color characteristics. *Can. Inst. Food Sci. Technol.* J. 1990, 23, 137–139.
- Singer, G. M.; Lijinsky, W. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. J. Agric. Food Chem. 1976, 24, 550-553.
- Smith, T. A. Amines in food. Food Chem. 1980, 81, 169-200.
- Snedecor, G. W.; Cochran, W. G. Statistical Methods, 7th ed.;
- The Iowa State University Press: Ames, IA, 1980. Synowiecki, J.; Shahidi, F. Lipid and pigment extraction from mechanically separated seal meat. J. Food Sci. 1991, 56, 1295– 1297.
- Tozawa, H.; Enokibara, K.; Amano, K. Effect of dimethylamine on the value of trimethylamine determined by Dyer's method. *Bull. Jpn. Soc. Sci. Fish.* **1970**, *36*, 606–603.
- USDA. Nitrates, nitrites and ascorbates (or isoascorbates) in bacon. U.S. Department of Agriculture; *Fed. Regist.* **1978**, *43*, 20992.
- Yamanaka, H. Changes in polyamines and amino acids in scallop adductor muscle during storage. J. Food Sci. 1989, 54, 1133– 1135.

Received for review December 10, 1991. Accepted May 11, 1992.

**Registry No.** TMA, 75-50-3; DMA, 124-40-3; NaNO<sub>2</sub>, 7632-00-0; sodium ascorbate, 134-03-2; spermidine, 124-20-9; spermine, 71-44-3.