Heat-Induced Changes in Sulfhydryl Groups of Harp Seal Muscle Proteins

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The effect of time–temperature variations during heat processing on changes in sulfhydryl groups and disulfide bonds in seal meat was investigated. The content of free SH groups in raw meat was about 63 μmol/g of protein, and after 40 min of heating at 99 °C, it decreased by about 50%. The total content of sulfhydryl groups after reduction of disulfide bonds was 84.7 μmol/g of protein in the raw sample and did not change significantly in the cooked meat. The correlation coefficients between the amount of disulfide bond formation in heated seal meat and their solubility in 0.035 M sodium dodecyl sulfate solution or the degree of thermal coagulation of the proteins were −0.982 and +0.897, respectively.

INTRODUCTION

Changes in proteins due to heating, mainly in solubility, water and fat binding capacities, thermal coagulation, and gel forming ability, have a significant effect on the properties of cooked muscle foods (Kinsella, 1976). Sulfhydryl (thiol) groups and disulfide bonds are important in maintaining structure and functional properties of native proteins. During thermal processing of meat, disulfide cross-linking of protein molecules due to oxidation of sulfhydryl groups may occur. This has been demonstrated by heat processing of fish (Opstvedt et al., 1984), meat (Hamm and Hofmann, 1965), and squid (Synowiecki and Sikorski, 1988). Changes in sulfhydryl and disulfide groups in muscle proteins and possible formation of H_2S are also important for both the taste and texture of canned meat products (Hamm and Hofmann, 1965).

It has been shown that denaturation of myosin and actomyosin occurs with formation of hydrogen, ionic, hydrophobic, and disulfide bonds (Jiang et al., 1988, 1989). These would in turn affect viscosity, emulsifying, and coagulation properties of protein preparations (Voutsinas et al., 1983). Furthermore, changes in rheological properties of eggs, meats, and kamaboko products due to heat processing are also brought about by hydrogen and disulfide bond formation (Buttkus, 1974). According to Hamm and Hofmann (1965), the increase in the toughness of meat during prolonged cooking may be due to the formation of intermolecular disulfide linkages between the peptide chains of the actomyosin.

The presence of disulfide cross-linking has been thought to hamper the attack of proteolytic enzymes which decrease protein digestibility (Friedman et al., 1982). The content of sulfhydryl groups in foods is also found to be important in preventing *N*-nitrosamine formation in the stomach. The reaction between nitrite and sulfhydryl groups at pH conditions mimicking the gastric juice is over 10 000 times faster than that of nitrite with amines and amino groups. Thus, the presence of large amounts of sulfhydryl groups in foods prevents the formation of *N*-nitrosamines in the stomach (Cantoni and Cattaneo, 1974). Interaction between disulfide/sulfhydryl groups and heme compounds helps to convert and keep the heme iron in its ferrous state (Ellman, 1959). This may have an important effect on changes of meat color, especially in the case of seal muscles which contain high levels (about 5.3%) of hemoproteins (Shahidi and Synowiecki, 1990).

The importance of disulfide bond formation on the nutritional quality of proteins and its technological properties is well demonstrated. Therefore, studies on changes of sulfhydryl groups and disulfide bonds of seal muscle proteins are part of the information needed for their quality evaluation. This paper presents data on the effect of processing of seal meats on their sulfhydryl groups and disulfide bonds and makes an attempt to correlate them with changes of the meat's protein solubility and thermal coagulation properties.

MATERIALS AND METHODS

Materials. Beater (3 weeks to 1 year) and bedlamer (1–4 years) harp seals (*Phoca groenlandica*) hunted in the coastal regions of Newfoundland/Labrador during May–July were bled and skinned, the blubber fat was removed, and the carcasses were eviscerated. Whole seal carcasses, 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. Seal meat was then separated either by hand or by mechanical means from the entire carcass. In manual separation, the deboned cuts of meat from three whole carcasses were ground twice using an Oster meat grinder (Braun AG, Model KGZ3, Frankfurt, Germany) through a 7-mm and then a 4-mm grind plate. 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 comminuted seal meats, separated by manual or mechanical means, were vacuum packed in polyethylene pouches and kept frozen at −20 °C for about 3 weeks until use to obtain samples similar to the meat available in the market. Seal meat is usually available in the frozen state, since the season when animals can be hunted is short.

The mechanically separated seal meat (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). Higher ratios of water to meat, 5:1 (v/w), were also attempted in the preliminary experiments; however, extensive loss of proteins occurred under these conditions. Smaller amounts of water were not sufficiently effective for the removal of pigments. Each washing was carried out at 5 °C for 10 min, while stirring manually. The washed meat was then filtered through two layers of cheesecloth with 1 mm diameter holes.

For determination of disulfide bond formation and changes in protein solubility of seal meat in sodium dodecyl sulfate, samples were heated in sealed polyethylene pouches in a controlled water

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Table I. Sulfhydryl Groups, Disulfide Bonds, and Cysteine Content (Micromoles per Gram of Protein) in Seal Muscle Proteins[a]

seal meat	sulfhydryl groups		disulfide bonds	cysteine
	initial sample	after reduction of disulfide bonds		
manually separated from female beater	62.93 ± 0.81[a]	83.49 ± 0.22[b]	9.97 ± 0.49[c]	83.36 ± 0.16
manually separated from male beater	62.03 ± 1.78[a]	82.00 ± 0.71[c]	9.68 ± 0.89[c]	
mechanically separated (MSSM)	63.80 ± 1.24[a]	84.68 ± 0.27[a]	10.12 ± 0.63[c]	78.81 ± 0.99
MSSM washed				
1 × H_2O	55.70 ± 0.73[b]	78.96 ± 0.35[d]	11.28 ± 0.35[bc]	75.44 ± 0.08
2 × H_2O	53.23 ± 0.59[bc]	78.85 ± 0.30[d]	12.42 ± 0.44[ab]	78.41 ± 0.16
3 × H_2O	52.48 ± 2.59[c]	81.92 ± 0.38[c]	14.27 ± 1.23[a]	81.71 ± 0.08

[a] Results are mean values of six determinations ± standard deviation. Values with the same superscript in each column are not significantly ($P > 0.05$) different from one another.

bath over 40 min at temperatures ranging from 40 to 99 °C or at 80 °C over a 60-min period.

Analyses. Moisture content was determined by oven drying at 105 °C to a constant weight (AOAC, 1990). Protein content was calculated on the basis of total nitrogen (N) determined according to the AOAC (1990) methods (i.e., N × 6.25).

For determination of protein solubility, 5 g of meat sample was homogenized in ice for 60 s using a Polytron homogenizer (Brinkmann Instruments, Rexdale, ON) with 100 mL of 5% NaCl solution in 0.003 M $NaHCO_3$, 0.035 M sodium dodecyl sulfate (SDS) from Sigma Chemical Co. (St. Louis, MO) in 0.003 M $NaHCO_3$, or 0.035 M SDS plus 0.15 M 2-mercaptoethanol (Sigma) in 0.003 M $NaHCO_3$ at pH 7.0. After 30 min of solubilization of meat sample with intermittent mixing and 10 min of centrifugation at 3000*g*, total proteins in the supernatant were determined according to the Kjeldahl method (AOAC, 1990). The degree of thermal coagulation as loss of solubility was assayed by heating of the MSSM extracts in a 5% NaCl solution in 0.003 M $NaHCO_3$ for 40 min at 40, 50, 60, 75, and 99 °C. The degree of thermal coagulation was expressed as $[(C_1 - C_2)/C_1] \times 100$, where C_1 and C_2 are the concentrations of proteins before and after the heat treatment, respectively.

Free sulfhydryl groups (SH) were determined in 0.10–0.13 g of meat sample dissolved in 8 mL of 0.75% ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and 0.035 M SDS solution in a Tris buffer, pH 8.2, according to the procedure given by Opstvedt et al. (1984). After the solution had stood for 2 h, 0.5 mL of a 0.016 M solution of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma) in methanol and 31.5 mL of methanol were added with mixing. The solution was allowed to stand at 20 °C for 15 min and centrifuged at 3000*g* for 15 min, and the absorbance was read at $\lambda = 412$ nm according to the method of Ellman (1959). The calibration curve for sulfhydryl groups was prepared using reduced glutathione (Sigma) in concentrations ranging from 0 to 0.1 mg/mL of sample. A linear correlation in the range of absorbance values from 0 to 1.5 was noticed. From this curve the molar extinction coefficient at 412 nm was calculated as 13 612; this value compared well with that of 13 600 reported by Ellman (1959).

Cysteine was determined by digestion of 10–12 mg of lyophilized meat sample in 6 N HCl at 110 °C for 24 h followed by performic acid oxidation (Blackburn, 1968). Cysteine was then separated from other amino acids and identified as cysteic acid using a Beckman 121MB amino acid analyzer.

Disulfide bonds in meat samples containing about 35 mg of proteins were determined. These samples were then reduced with 4 mL of 0.6 M $NaBH_4$ in 8 M urea (Opstvedt et al., 1984) or with 0.15 M 2-mercaptoethanol in 8 M urea. The disulfide bonds were calculated from the difference between the content of SH groups in the reduced samples and those in the original sample.

Statistical Analysis. Analysis of variance and Tukey's Studentized range test (Snedecor and Cochran, 1980) were used to determine differences in mean values based on the data collected from four to six replications of each measurement. Significance was determined at 95% probability.

RESULTS AND DISCUSSION

The sulfhydryl content of manually separated seal meat was about 63 μmol/g of protein (Table I), as compared with beef longissimus dorsi (Hamm and Hofmann, 1965), Pacific mackerel, Alaska pollock (Opstvedt et al., 1984), squid (Synowiecki and Sikorski, 1988), and mechanically separated chicken meat (Shahidi and Onadenalore, unpublished data) containing 88, 72, 70, 79, and 58 μmol of SH/g of protein, respectively. Mechanical separation did not influence the amount of free SH groups present in the samples (Table I).

The accuracy of sulfhydryl group determination may depend on the possibility of steric effects that might block the test reagent. According to Kalab (1970) and Sedlak and Lindsay (1968) the SH groups occur either in exposed and unhindered form, which can immediately react with the DTNB reagent, or in the masked form, which cannot be detected unless unmasked. To liberate these groups, protein molecules have to be denatured. The most common denaturants used for this purpose are SDS, guanidine hydrochloride, and urea. Sedlak and Lindsay (1968) tested the suitability of 0.035 M SDS in Tris buffer (pH 8.2) for unmasking of sulfhydryl groups. Their method was re-examined by Opstvedt et al. (1984). These authors found that in undenatured fish proteins only 90% of the total SH groups were reactive toward DTNB in Tris buffer without SDS. However, adding SDS to Tris buffer increased the measurable sulfhydryl groups in the samples by about 8–10%. Thus, SDS addition makes the SH groups available to the test reagent. The SH content in samples after reduction of disulfide bonds with $NaBH_4$ was in good agreement with cysteine content determined in seal meat (Table I). It also demonstrates that possible steric effects were almost eliminated during determination of SH groups. The sulfhydryl groups in seal meat are mainly cysteine residues of proteins. The SH content of low molecular weight compounds which did not precipitate in 0.6 M trichloroacetic acid was only 1.07 ± 0.11 μmol/g of protein.

Raw seal meat contained about 10 μmol of disulfide bonds/g of proteins, and after their reduction, the amount of free SH groups increased from 63.8 μmol/g in untreated samples to 84.7 μmol/g of protein (Table I). Aqueous washing resulted in a decrease in the content of free SH groups. However, the disulfide bonds in washed MSSM increased proportionally (Table I). This effect may be explained by consideration of the difference in the content of sulfhydryl groups and disulfide bonds between proteins removed during washings and the resultant insoluble meat residue. Table II shows that about 34.75% of proteins were removed after three washings of MSSM with water. These proteins are mostly sarcoplasmic, and their removal

Table II. Protein Extraction from MSSM during Washing with Water[a]

MSSM	moisture, %	protein, %	protein removed after washings, as % total amount
unwashed	70.84 ± 0.11	23.21 ± 0.13	
washed			
1 × H_2O	82.98 ± 0.16	13.90 ± 0.39	23.01 ± 1.14
2 × H_2O	83.70 ± 0.26	13.40 ± 0.36	29.50 ± 0.93
3 × H_2O	83.99 ± 0.10	13.24 ± 0.15	34.75 ± 1.33

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

Table III. Effect of Heat Processing (40 min) at Various Temperatures on Sulfhydryl Groups and Disulfide Bonds in Seal Meat Proteins[a]

temp, °C	sulfhydryl groups, μmol/g of protein	disulfide bonds, μmol/g of protein
20	63.80 ± 1.24[a]	10.12 ± 0.99[e]
40	57.51 ± 2.31[b]	13.17 ± 1.15[d]
50	54.09 ± 0.92[bc]	14.83 ± 0.44[cd]
60	49.66 ± 2.53[c]	16.98 ± 1.23[c]
75	44.05 ± 1.39[d]	19.70 ± 0.69[b]
99	32.34 ± 1.07[e]	25.38 ± 0.45[a]

[a] Results are mean values of five or six determinations ± standard deviation. Values with the same superscript in each column are not significantly ($P > 0.05$) different from one another. The total SH content in MSSM after reduction of disulfide bonds was 84.68 ± 0.27 μmol/g of protein.

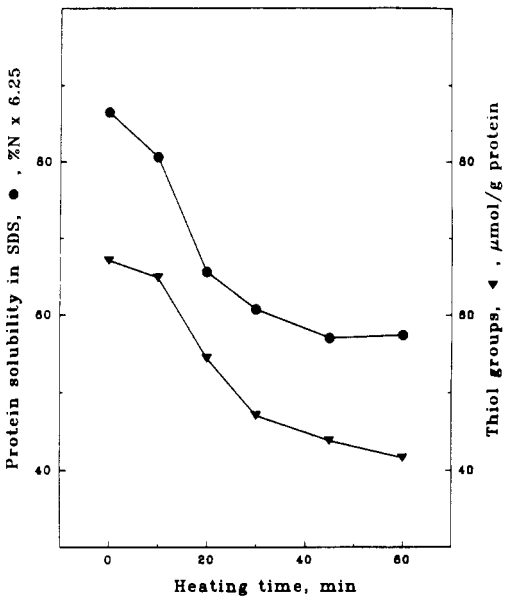

Figure 1. Effect of heat processing time of MSSM at 80 °C on protein solubility in 0.035 M SDS (●) and free sulfhydryl groups content (▲). Results are mean values of four replicates, and standard deviations did not exceed 3.0% of the recorded mean values.

concentrated the myofibrillar and connective tissue proteins in the washed meat.

Heating resulted in a decrease in the content of sulfhydryl groups of seal meat proteins and an increase in the content of disulfide bonds (Table III). Increasing the temperature from 20 to 99 °C increased the content of disulfide bonds by a factor of 2.5. However, after 40 min of heating at 80 °C, the content of free SH groups in seal meat was decreased by approximately 50% (Figure 1).

The extent of thermal denaturation of proteins during heating was also monitored as changes in their solubility in 5% NaCl solution. A large decrease in the solubility of seal meat proteins and an increase in their degree of

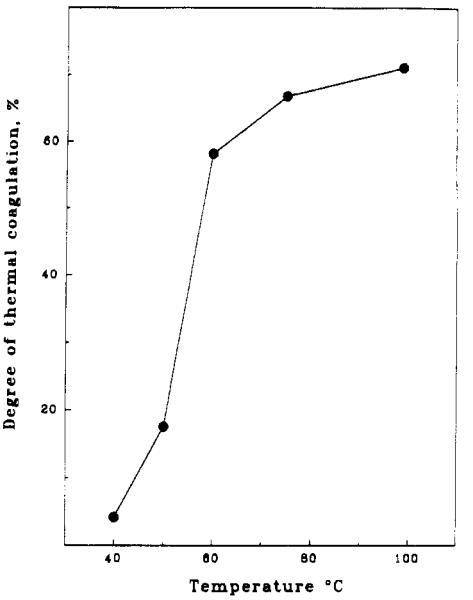

Figure 2. Degree of thermal coagulation of proteins extracted from MSSM by 5% NaCl in 0.003 M $NaHCO_3$ solution. Results are mean values of four replicates, and standard deviations did not exceed 3.7% of the recorded mean values.

thermal coagulation was observed in temperatures ranging from 50 to 75 °C (Figure 2). In the process of thermal denaturation and aggregation of protein molecules, different functional groups of proteins are involved (Madovi, 1980). However, the influence of disulfide bond formation was evidenced by comparison of protein solubility in SDS with or without the addition of 2-mercaptoethanol, which reduces the disulfide bonds. Solubilities of seal meat proteins, expressed as the percent of their amount in the meat (23.2%), after 20 min of heating at 80 °C in SDS without or with 2-mercaptoethanol were 65.67 ± 0.31% and 76.32 ± 0.20%, respectively. This difference in protein solubility is perhaps due to the formation of disulfide bonds.

The disulfide bonds formed during heating at different temperatures (X) correlated with the degree of thermal coagulation of seal proteins (Y). A correlation coefficient of $r = 0.897$ was observed for the regression equation $Y = 5.38X$. The content of sulfhydryl groups also depended on the duration of heating period (Figure 1). The reduction of SH groups (X) in seal meat heated at 80 °C for up to 60 min and the decrease in the solubility of proteins in 0.035 M SDS (Y) were correlated ($r = 0.982$) for the regression equation $Y = 7.70 + 1.13X$. The decrease in the SH groups was due to disulfide bond formation. No H_2S was produced in this process as no significant ($P > 0.05$) difference in the content of SH groups was found between raw (84.68 ± 0.27 μmol/g) and cooked (83.61 ± 0.97 μmol/g) samples after reduction of disulfide bonds. Excellent correlations between the content of sulfhydryl groups and solubility of proteins in SDS solution as well as the degree of their thermal coagulation were observed. These indicate that the formation of disulfide bonds due to heat processing may affect the rheological properties of seal meat.

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