INFLUENCE OF TEMPERATURE, TIME, AND SOLVENT ON COLLAGEN AND SALT-EXTRACTABLE MEAT PROTEINS

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Story in Brief

This study was conducted to gain some understanding of the functional attributes of fibrous hide collagen as a potential ingredient in processed meats. Fibrous collagen was used to replace either 0, 10, 20, 30 or 100% of minced muscle. The meat portion (meat and/or collagen) was treated with either 3% NaCl or 3% NaCl + 0.44% sodium tripolyphosphate and the resulting slurry was heated at either 50, 60 or 70C for either 0, 15, 30, 45, 60, 75 or 90 min and nitrogen solubility was determined.

Protein denaturation, as monitored by changes in nitrogen solubility, was manifested as a reduction in solubility for the 0% collagen (100% muscle) substitution level or an increase in solubility for the 100% collagen level. The degree of these responses was dependent on the solvent used as demonstrated by greater release of soluble nitrogen for 3% NaCl + 0.44% STPP than 3% NaCl at the 100% collagen substitution level and 50C.

(Key words: Collagen, Functional attributes, Meat proteins).

Introduction

The basic functional property of proteins is their solubility and in the case of muscle food the response to subsequent heat treatment. These two essential factors will determine the efficacy of using a novel protein, hide collagen, as an additive or extender in processed muscle foods.

Loss of nitrogen solubility is one of the most readily measurable properties of proteins and has been used widely as a criterion for denaturation. Monitoring changes in solubility for various fractions of muscle as a consequence of heat denaturation; to determine whether the functional performance of combinations of meat and extender proteins is greater in combination than the sum of their individual performance; were some of the earlier studies.

It was the purpose of this experiment to study changes in nitrogen solubility as a indication of protein response to various hydrothermal conditions and to gain more knowledge concerning the functional properties of hide collagen as a potential ingredient in processed meat items.

Materials and Methods

Materials

The Eastern Regional Research Center at Philadelphia, PA, provided

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comminuted native wet collagen, frozen in No. 10 cans and classified as product No. 1, based on pH, particle size, protein denaturation, and viscosity.

Methods

All collagen was removed from frozen storage (-13C) and thawed at 40C.It was strained through a Bunchner funnel to separate the fibrous and liquid portions of the material. A ratio of 2.18:1 (solids:liquid) was obtained. This ratio was determined so a constant proportion of solids to liquid could be maintained if more collagen were needed for conducting further experiments. Following filtration, the liquid and solids were recombined and thoroughly mixed. This standardized material was packaged in 250 g portions into 454 g freezer containers for -13C storage.

A beef inside round from a choice grade carcass was removed from frozen storage and thawed at 4C, manually freed of separable fat and superficial connective tissue, ground once through a 1.25 cm plate and ground twice through a 4.8 mm plate. Fifty gram portions of the minced muscle were packaged in plastic bags for storage at -13C. The individually packaged frozen meat and collagen were thawed (4C) as necessary for experimentation. Food-grade NaCl, granular food-grade sodium tripolyphosphate (STPP), and distilled water were used to formulate the extracting solutions.

Three percent NaCl and a combination of 3% NaCl and 0.44% STPP were used as the extracting solutions. Meat replaced with 0, 10, 20, 30 or 100% levels of collagen served as the meat portion. Fifteen and nine-tenths grams of the meat block were weighed into a 400 ml blender cup and blended with 159.1 gms of extractant at 6400 rpm for one minute using a Sorvall Omni-mixer. The resulting slurry was blended for an additional min following a three min rest period. This preparation of 175 gms of slurry was repeated four times to obtain sufficient sample for the heat treatment. The four blends were combined and the pH of the composite blend was adjusted to 6.00 with either 1N NaOH or 1N HCl.

Table 1. Effect of Solvent and Collagen Substitution on Meat Slurry pH Prior to Ajustment to pH 6.00a,b

Collagen Level (%)	3% NaCl (pH)	3% NaCl + 0.44% STPP (pH)
0	5.46 (0.15)	6.57 (0.07)
10	5.45 (0.06)	6.62 (0.15)
20	5.52 (0.13)	6.81 (0.11)
30	5.67 (0.08)	6.87 (0.13)
100	7.38 (0.17)	8.38 (0.22)

aValues in parentheses represent the standard deviation.

bMean of 8 observations.

Fifty grams of the resulting slurry were weighed into each of 12 test tubes for heat treatment in an oscillating water bath at either 50, 60 or 70C depending on the treatment conditions. Two tubes were removed at 15 min intervals until 90 min had elapsed. Upon removal from the water bath, the contents of each tube were immediately filtered through Watman No. 4 filter paper and the filtrate was placed in an ice bath.

The original slurry, in excess of that needed for heat treatment, was used for determination of the nitrogen content of the time-temperature control (0 min. heating time). The meat slurry was weighed into a 50 ml teflon tube and placed in an RC2-B refrigerated centrifuge for centrifugation at 10,000 rpm for 10 min. The resulting slurry supernatant was filtered through eight layers of cheese cloth and the filtrate was used in the nitrogen analyses.

Kjeldahl nitrogen was obtained using the Tecator 1013 digestion unit and the Kjeltec 1030 autoanalyzer and pH was measured. In addition to the 6 fifteen min intervals, nitrogen analysis was conducted in duplicate on the unheated filtrate obtained from the previously described centrifugation step.

Experimental Design and Data Analysis

The experiment was conducted using a Randomized Complete Block Design with a split-plot arrangement of treatments. In each block, 5 collagen levels, 3 temperature levels, and 2 phosphate levels denote the main-unit treatment factors for a total of thirty possible treatments (5 X 3 X 2) and within each treatment, seven time intervals represent the subunit treatment factors. Each treatment was replicated three times for a total of three blocks.

The data were anlyzed using Statistical Analysis System (SAS). F-tests from the analysis of variance was performed to determine if significant differences occurred between levels of each treatment and the significance of any two-way interactions among whole unit treatment factors, and between whole unit treatment factors and subunit treatment factors. The presence of quadratic trends in the data associated with time of heating was verified using the general linear models procedure of SAS.

Results and Discussion

Effect of Temperature and Time

The premise on which this experiment was based is that heat denaturation results in changes in solubility depending on the severity of the heat treatment. The quantity of soluble nitrogen was significantly reduced, when the meat slurry was exposed to heat, as a result of the heat induced denaturation and subsequent coagulation of meat proteins (Table 2). The major reduction in solubility occurred during the initial 15 min of heating for 50, 60 and 70C. From 30 to 90 min of heat treatment, the amount of soluble nitrogen maintained a relatively constant value for each of the three temperatures studied. However, a step-wise loss in solubility was observed as temperature increased for 50 to 70C at all the time intervals from 30 min onwards. This step-wise reduction indicated that sufficient energy became available to overcome the resistance to heat denaturation of more thermally stable proteins soluble in either of the solvents studied. Since myosin is the most abundant and one of the more heat sensitive

Table 2. Effect of temperature, time, and solvent on soluble nitrogen (%) for 0, 10, 20, 30 and 100% meat replacement with collagen[1,2]

Time (min)	50C		60C		70C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
			0% Meat Replacement			
0	0.128^{a}	0.160^{a}	0.124^{a}	0.099^{a}	0.111^{a}	0.156^{a}
15	0.094^{b}	0.115^{b}	0.080^{b}	0.092^{a}	0.058^{b}	0.069^{b}
30	0.094^{b,c}	0.098^{c}	0.068^{c}	0.079^{b}	0.055^{b}	0.061^{b,c}
45	0.092^{b,c}	0.094^{c,d}	0.065^{c}	0.075^{b}	0.054^{b}	0.060^{b,c}
60	0.088^{bc}	0.093^{c,d}	0.064^{c}	0.075^{b}	0.052^{b}	0.058^{c}
75	0.085^{c}	0.088^{d}	0.062^{c}	0.070^{b}	0.050^{b}	0.059^{c}
90	0.089^{b,c}	0.088^{d}	0.062^{c}	0.074^{b}	0.052^{b}	0.057^{c}
			10% Meat Replacement			
0	0.103^{a}	0.133^{a}	0.116^{a}	0.143^{a}	0.112^{a}	0.141^{a}
15	0.089^{b}	0.098^{b}	0.074^{b}	0.083^{b}	0.058^{b}	0.058^{b}
30	0.087^{b}	0.093^{b}	0.074^{b}	0.083^{b}	0.058^{b}	0.058^{b}
45	0.085^{b}	0.093^{b}	0.064^{c}	0.067^{c}	0.056^{b}	0.054^{b}
60	0.083^{b}	0.089^{b}	0.064^{c}	0.069^{c}	0.056^{b}	0.045^{b}
75	0.083^{b}	0.091^{b}	0.064^{c}	0.069^{c}	0.056^{b}	0.056^{b}
90	0.081^{b}	0.090^{b}	0.064^{c}	0.068^{c}	0.057^{b}	0.058^{b}
			20% Meat Replacement			
0	0.092^{a}	0.124^{a}	0.086^{a}	0.111^{a}	0.104^{a}	0.095^{a}
15	0.084^{a,b}	0.089^{b}	0.068^{b}	0.073^{b}	0.057^{b}	0.056^{b}
30	0.082^{a,b}	0.087^{b}	0.064^{c}	0.066^{b,c}	0.058^{b}	0.057^{b}
45	0.083^{a,b}	0.085^{b}	0.058^{b,c}	0.061^{c}	0.057^{b}	0.057^{b}
60	0.081^{b}	0.082^{b}	0.059^{b,c}	0.062^{c}	0.060^{b}	0.059^{b}
75	0.083^{a,b}	0.084^{b}	0.060^{b,c}	0.062^{c}	0.059^{b}	0.060^{b}
90	0.081^{b}	0.083^{b}	0.060^{b,c}	0.061^{c}	0.057^{b}	0.056^{b}
			30% Meat Replacement			
0	0.084^{a}	0.109^{a}	0.078^{a}	0.087^{a}	0.062^{a}	0.091^{a}
15	0.076^{b}	0.083^{b}	0.064^{b}	0.067^{b}	0.051^{b}	0.051^{b}
30	0.071^{b}	0.077^{b}	0.059^{b,c}	0.062^{b,c}	0.052^{b}	0.050^{b}
45	0.075^{b}	0.077^{b}	0.057^{b,c}	0.059^{b,c}	0.053^{a,b}	0.052^{b}
60	0.074^{b}	0.075^{b}	0.056^{b,c}	0.056^{c}	0.054^{a,b}	0.052^{b}
90	0.075^{b}	0.077^{b}	0.053^{c}	0.056^{c}	0.056^{a,b}	0.051^{b}
			100% Meat Replacement			
0	0.006^{e}	0.001^{e}	0.017^{d}	0.004^{e}	0.000^{e}	0.002^{c}
15	0.005^{e}	0.022^{d}	0.034^{c}	0.024^{d}	0.044^{d}	0.047^{b}
30	0.016^{d}	0.028^{c}	0.050^{b}	0.033^{c}	0.051^{c,d}	0.054^{a,b}
45	0.022^{c,d}	0.036^{b,c}	0.058^{a}	0.027^{c,d}	0.055^{c}	0.052^{a,b}
60	0.029^{b,c}	0.036^{b,c}	0.062^{a}	0.046^{b}	0.058^{b,c}	0.054^{a,b}
75	0.035^{a,b}	0.040^{a,b}	0.060^{a}	0.046^{b}	0.065^{a,b}	0.059^{a}
90	0.041^{a}	0.046^{a}	0.059^{a}	0.056^{a}	0.071^{a}	0.060^{a}

1 Means in columns not followed by the same letter are significantly different ($p < 0.05$).
2 Each value is a mean of six observations with predicted values

proteins, the majority of the initial reduction in solubility at the 15 min heating period for 50 and 60C could be accounted for by the denaturation of myosin with further reduction at 70C representing the denaturation of more thermally stable components of the salt-extractable fraction.

During the initial 15 to 30 min heating periods (Table 2), the loss of solubility is a time dependent response. The denaturation of protein constituents coagulable at 60C would be expected to be time-dependent at temperatures equal to or greater than 60C. The dependence on time was observed during the initial 15 to 30 minutes, any further changes in solubility would be temperature dependent as evidenced by decreased soluble nitrogen as temperature increased at these prolonged heating periods. Therefore, temperature dependency occurs as a result of increasing stability associated with the more thermally resistant proteins in solution.

Effect of Collagen Replacement

The heat treatment control (0 min time interval) illustrates that replacing portions of the minced muscle with hide collagen reduced the quantity of nitrogen soluble in either 3% NaCl (Table 2) or 3% NaCl + 0.44% STPP. Quantitative determinations of salt-soluble protein in various meats used in the processing industry revealed that approximately 7% of muscle is salt-extractable.

There are two opposing reactions affecting the quantity of soluble nitrogen as a result of heat denaturation; precipitation of heat coagulable muscle proteins and thermal solubilization of collagen. At 50C the loss in solubility attributed to the heat denaturation of salt-extractable muscle proteins influenced to a greater extent the quantity of nitrogen in the filtrate whereas at 60C with 3% NaCl as the extractant and at 70C for both solvent conditions (Table 2) and disparity associated with collagen substitution levels was not as distinct after 30 min of heating. This pattern for these three hydrothermal conditions may be due to more complete coagulation of the salt-extractable muscle proteins and an opposing increase in solubility associated with thermal hydrolysis of collagen and consequently reducing the disparity associated with greater dilution of muscle proteins by collagen.

Table 2 illustrates that at all hydrothermal conditions studied for 100% collagen substitution, the amount of nitrogen in the filtrate increased with increasing temperature and time at a given temperature. In the presence of 3% NaCl at 50C there was no significant increase in solubility until 30 min, whereas at 70C under the same solvent conditions there was a marked increase in solubility attributable to the thermal hydrolysis of collagen after heating for 15 min.

The initial decrease in solubility at 50C may be due to a release of newly synthesized tropocollagen molecules that have not undergone extensive crosslinking. The shrinkage temperature of collagen (Ts = 39C) is marked by a sudden release of soluble collagen as a consequence of the disruption of the secondary structure. The primary bond responsible for stabilizing the collagen triple helix is extensive hydrogen bonding associated with structural water and the hydroxyl group of hydroxyproline. At 70C, sufficient heat may be present to break the continuity of the interchain waterbridges, the hydroxyproline related stability, and the aldol type crosslinkages associated with hydroxylysine.

The initially sharp increase in soluble nitrogen at 70C and 15 min of heating time for 100% collagen (Table 2) changed to a more gradual increase for the remaining time increments. This initial increase may represent more extensive rupture of collagen crosslinks of mature collagen in addition to the soluble material released at 50C. At the remaining heating time any increase in collagen solubility may be due strictly to the denaturation of more mature collagen fibrils. The same response for 100% collagen with 3% NaCl as the solvent was observed at 60C. There was a gradual increase in the percent soluble nitrogen from 0-45 min, after which the soluble nitrogen leveled off at approximately 0.060% nitrogen that was comparable to the values for 70C under the same solvent conditions.

Effect of Solvent

STPP increased the amount of protein solubilized in the presence of 3% NaCl as evidenced by more soluble nitrogen in the filtrate. This effect was evident for all heating conditions for 0% collagen substitution (100% meat). A comparison of the unheated sample (0 min time interval) and the 15 min time interval reveals that the difference associated with the use of STPP was greater before heat treatment than following 15 min of heating at 50C (Table 2). This observation suggested that the protein components, (i.e. myosin) most susceptible to heat coagulation,were affected more by the action of STPP. This may be due to the larger portion of myosin available for the action of STPP as compared to other proteins rather than a preferential association of STPP and myosin.

The improved solubility attributable to the action of STPP was not observed for 10, 20 and 30% collagen substitution levels at 70C for heating periods longer than 15 min (Table 2). Therefore, it is understandable why replacing portions of muscle tissue with a rather insoluble protein such as hide collagen reduces tha total amount of protein extractable with either solvent. This dilution effect is consistent with other research workers in that they reported a decrease in the quantity of sarcoplasmic and myofibrillar proteins as collagen replacement of meat increased. The equality of the values for the two solvent conditions at 70C and heating times longer than 15 min may be due to the opposite manner in which collagen and salt-extractable proteins manifest protein denaturation and solubilization in 3% NaCl and 3% NaCl + 0.44% STPP. At 70C those factors contributing to a decrease in soluble nitrogen (i.e. muscle protein dilution by collagen and heat coagulation of muscle proteins) are no longer predominant and subsequently thermal hydrolysis of collagen dominates and consequently increases the quantity of nitrogen in the filtrate. Therefore, thermal hydrolysis reduces the disparity between the two solvent conditions.

The solubility increase associated with the use of STPP at 0, 10, 20 and 30% collagen substitution levels is a result of the effect of increased pH and ionic strength on the muscle tissue component of the meat block. Table 1 illustrates that pH, prior to adjustment to 6.00, was significantly greater in the presence of STPP and it is also correct to assume that ionic concentration was greater when STPP was combined with NaCl than when NaCl was used alone. Also, the initially small particle size, the mechanical action of the omni-mixer and the high ratio of extractant to the meat block would facilitate the diffusion and action of the solvent in regard to the solubilization of the meat proteins.

In regard to solvent effects on the fibrous collagen used in this experiment, Table 2 reveals that at 5OC, 3% NaCl and 0.44% STPP produced an increase in soluble nitrogen for the 100% collagen above that found using 3% NaCl alone as the extractant. The collagen solubilized at 5OC may represent newly synthesized tropocollagen since this substituent has not undergone significant crosslinking and is stabilized by weak hydrogen bonds. Therefore, the addition of STPP and subsequent pH and ionic strength effects may destabilize the hydrogen bonds to the point that allows more complete release of the soluble component after heating at 5OC for 15 min. The increased pH from 7.38 to 8.38 produced by STPP (Table 1) would change the net negative charge on the protein and thus affect the solubility of tropocollagen by increasing the repulsive forces on the protein and consequently improve hydration.

At 60 and 7OC, solubility increased less with time in the presence of STPP. Other researchers using native hide collagen reported that at 6% NaCl, hydration was reduced at pH values greater than 5.00. This was attributed to a shielding of water by either Na or Cl ions at these extreme pH's. The reduction in hydration may explain the difference in solubility associated with pH changes. However, the temperature effect was preeminent since at both solvent conditions more collagen is solubilized at 7OC followed by 6OC and 5OC respectively. A consideration of what portion of the thermally solubilized collagen was due to newly synthesized collagen and what portion was due to mature collagen would aid in understanding the mechanism of action on fibrous hide collagen.

Conclusion

The degree of response was dependent upon the solvent, as demonstrated by a greater release of soluble nitrogen, for 3% NaCl + 0.44% STPP, at the 0, 10, 20, and 30% collagen substitution levels; where as, 3% NaCl elicited the greatest release of soluble nitrogen for 100% collagen at 5OC.