Enzymatic Modification of the Structure and Functional Properties of Mechanically Deboned Fowl Proteins

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Partial proteolysis of mechanically deboned fowl (MDF) myofibrillar proteins with an acid protease, Milezyme AFP 2000, improved protein solubility, emulsifying capacity, and gelation compared to myofibrillar protein controls. The extent of proteolysis needed for optimum improvement varied with the functional property examined. Partial proteolysis of MDF improved functionality as determined in an emulsified meat test system where half of the MDF used was enzyme treated. Modified MDF samples, with and without 3% added salt, had greater cooked yields, less cook-out fat, and similar texture to controls containing 3% salt. Protein fragments of 144 000, 96 000, 78 000, and 36–40 000 daltons were produced when MDF myofibrillar proteins were hydrolyzed. The source of the protein fragments was identified. The protein fragments differed in solubility from the parent protein molecules.

INTRODUCTION

Recent interest in the use of mechanically deboned poultry meat in processed meat products such as frankfurters and bologna has opened up a new and potentially large market for spent fowl. Mechanical deboning enables efficient use of whole spent fowl carcasses, which cannot be economically deboned by hand. The high fat and collagen content of mechanically deboned fowl (MDF) (Froning, 1976; Randall, 1977) reduces the quality of products containing this tissue compared with products containing meat from younger chickens and turkeys, limiting its use by the processed meat industry. MDF quality is adversely affected by protein denaturation which occurs during deboning (McMahon and Dawson, 1976) and long-term frozen storage (Khan et al., 1963; Matsumoto, 1979). One means of improving the quality of products containing MDF is to increase the functionality of the fowl meat proteins.

Enzymatic modification has been used extensively to improve the functional properties of proteins and to tailor the functionality of certain proteins to meet specific needs (Richardson, 1977). Partial hydrolysis with proteases is the most common method to enzymatically modify proteins (Brekke and Eisele, 1981). Proteolysis was effective in improving protein functionality of beef (DuBois et al., 1972) and fish (Spinelli et al., 1972) skeletal muscle proteins and of beef heart proteins (Smith and Brekke, 1984).

The most important protein functional properties in processed meat products are solubility, emulsifying capacity, gelation, and water binding (Kinsella, 1982). Proteolysis alters these properties by changing the molecular size, conformation, solubility, and strength of the inter- and intramolecular bonds of the protein molecules (Kinsella, 1976; Ryan, 1977).

Salt (NaCl) is generally added to processed meat products at a concentration of 2.25–2.75% of the formulation (Olson and Terrell, 1981). Salt contributes flavor, influences shelf life, and affects the functional attributes of the myofibrillar proteins in processed products (Olson, 1982). The salt-soluble myofibrillar proteins must be solubilized in order to be functional.

Some evidence indicates that a reduction in sodium consumption may lessen hypertension in individuals genetically predisposed to the disease (IFT, 1980), and therefore, many meat processors are trying to reduce the sodium content in their processed products (Olson, 1982). Enzymatic modification can increase protein solubility and has potential as a partial substitute for salt in processed meat products (Brekke and Eisele, 1981). Smith and Brekke (1984) showed that low-salt enzyme-modified 30% beef heart/70% beef skeletal frankfurters had significantly greater smokehouse and consumer cooked yields than 30% beef heart/70% beef skeletal control frankfurters produced with 2.3% salt.

The objectives of this research were to determine the effect of partial proteolysis on MDF protein functionality in three model system functional tests by using MDF myofibrils and actomyosin and in an emulsified meat test. Associated changes in protein structure were investigated by using hydrolysates of MDF actomyosin and myofibrils and purified hen myosin fractions.

MATERIALS AND METHODS

Substrate. Blocks of frozen mechanically deboned fowl (MDF) were purchased from Tony Downs Foods Co. (St. James, MN). The MDF was cut into 1-kg blocks, wrapped in polyethylene, and stored at −30 °C no longer than 6 months. Pork back fat was obtained from the Washington State University Meat Science Laboratory (Pullman, WA), ground through a 6-mm plate, and stored at −30 °C. MDF and fat were thawed 24 h at 4 °C before use in experiments. Meat ingredients were analyzed for fat, moisture, and protein following AOAC (1980) procedures.

Preparation of MDF Myofibrillar Proteins. MDF myofibrils were isolated as described by Eisele and Brekke (1981) for beef heart myofibrils. MDF actomyosin was prepared as described by Briskey and Fukazawa (1971). Actin was purified (Pardee and Spudich, 1982) from muscle obtained after exsanguination of a spent hen. The final myofibril, actomosin, or actin pellet was resuspended and centrifuged in 0.1 M NaCl, 0.05 M K phosphate buffer, pH 3.0, and then resuspended in the buffer to obtain a final protein concentration of 35–45 mg of protein/mL of buffer.

Myosin Extraction. A spent laying hen or pullet was sacrificed, the breast and leg muscles were quickly removed and chilled in ice for 30 min. Myosin was extracted as described by Wagner and Yount (1975). Myosin from MDF was extracted following the same procedure.

Purified myosin was diluted with glycerol to 50% and stored at −20 °C. Myosin was prepared for use by adding 10 volumes of cold water and removed by centrifuging at 12 000g at 0 °C for 15 min. The myosin pellet was then suspended in the desired buffer. The concentration of myosin in the supernatant was determined by using an extinction coefficient of $E_{280}^{1\%} = 6.66$ cm^{-1}.

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Myosin ATPase Activity. The Ca^{2+} ATPase activity was assayed as described by Wells et al. (1979). The K^+–EDTA ATPase activity was assayed similarly except the assay solution was composed of 5 mM ATP, 5 mM EDTA, 0.6 M KCl, and 100 mM Tris-HCl, pH 7.5, instead of 7.6 mM ATP, 15 mM $CaCl_2$, 150 mM KCl, and 180 mM Tris-HCl, pH 7.4.

Model System Functional Tests. *Hydrolysis Conditions.* Acid protease, Milezyme AFP 2000 (Miles Laboratories, Elkhart, IN), standardized to contain 2000 spectrophotometric acid protease units/g, was used to hydrolyze the MDF proteins. AFP 2000, from *Aspergillus niger* var., randomly hydrolyzes most plant and animal proteins. According to the manufacturer, optimum pH for activity is 3.0, and inactivation occurs at pH 7.0 or greater. The optimum temperature range is 50–60 °C at pH 3.0. In addition to protease activity, AFP 2000 contains amylase, cellulase, hemicellulase, and pectinase activities.

Hydrolysates were prepared with 3.5–4.5% (w/v) suspensions of myofibrils or actomyosin in 0.1 M NaCl, 0.05 M phosphate buffer, pH 3.2. Hydrolysis was initiated by adding AFP 2000 at an enzyme–protein ratio of 1:100 (w/w). Hydrolysis was performed at 20 °C with slow stirring. Hydrolysates were removed as desired intervals between 0 and 100 min. The enzyme reaction was terminated by increasing the pH to 7.0 with 3 N KOH. The modified myofibril suspensions were stored no longer than 3 days at 0 °C before use.

Protein Solubility. Solubility of the proteins in the suspension was determined either in the reaction buffer (containing 0.1 M NaCl) or by adding NaCl to bring the concentration to 0.6 M and readjusting the pH to 7.0. Ten milliliters of the protein suspension was centrifuged at 10000*g* for 15 min. Protein in the supernatant was estimated by using biuret reagent (Gornall et al., 1949). Percentage solubility was determined by dividing the protein content of the supernatant by the total protein content and multiplying by 100.

Protein solubility of myofibrils in 0.1 M NaCl as affected by pH was also determined after various times of hydrolysis (enzyme treated at pH 3.2, inhibited at pH 7.0). The pH was reduced from 7.0 to 3.0, in 0.5 unit increments, with 2 M HCl. An appropriate amount of NaCl was added to adjust ionic strength for all samples to that of the pH 3.0 sample after HCl addition (μ = 0.3). Aliquots were removed at each pH increment and protein solubility was determined.

Emulsifying Capacity. Emulsifying capacity of the partially proteolytically degraded myofibrils and actomyosin was determined by using a procedure similar to that of Webb et al. (1970), as modified by Eisele and Brekke (1981). Hydrolysates were tested at a concentration of 1.0 mg/mL in 0.1 M NaCl, 0.05 M K phosphate, pH 7.0. An ohmmeter (Simpson 260, Simpson Electrical Co., Chicago, IL) was used to monitor an increase in electrical resistance which occurred upon emulsion collapse. Results were expressed as milliliters of oil emulsified per milligram of protein.

Preparation of Myofibril Gels. Gels were prepared by adjusting suspensions of the modified and control myofibrils to 4.0% (w/v) protein, 0.6 M KCl, pH 6.0. An aliquot (250 mL) of each myofibril suspension was transferred to a 400-mL beaker, heated on a hot plate to 40 °C while being stirred, and then placed in a 70 °C water bath for 45 min. The gels were held overnight at 4 °C. The apparent gel viscosity was determined at 20 °C with a Brookfield Viscometer (Model RVT, Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a helipath stand and T-bar spindles.

Preparation of Actomysoin Gels. Actomyosin gels were prepared in 5 × 100 mm test tubes with 5 mL of a 4.0% (w/v) actomysin suspension which had been adjusted to 0.6 M KCl, pH 6.0. The actomysin suspensions were heated in a 70 °C water bath for 45 min and held overnight at 4 °C. The resultant actomyosin gels were equilibrated to 20 °C before being evaluated.

The semiqualitative method of Pour-El and Swenson (1976) was used to describe the characteristics of the actomyosin gels. The strength of the gel was indicated by a number from zero to five depending on the gel characteristics observed upon shaking the test tube. Syneresis, which occurred during and subsequent to gel formation, was monitored by measuring the volume of free liquid which could be removed from the test tube with a Pasteur pipet. Gel strength was also monitored at 20 °C with an inversion test. The gels were inverted, and the time for the gels to fall from the bottom of the test tube was determined.

Scanning Electron Microscopy. Myofibril gels were prepared for scanning electron microscopy as described by Yasui et al. (1979). The gels were observed with a Perkin-Elmer ETEC autoscan electron microscope with an accelerating voltage of 20 kV.

Emulsified Meat Test. MDF meat equilibrated to 20 °C was placed in a Kitchen Aid Stand Mixer (Model KS-A, Hobart Corp., Troy, OH) and the pH reduced to 3.2 with 3 N HCl while mixing with the paddle attachment at the lowest speed setting for 2 min. Hydrolysis was initiated by adding AFP 2000, at an enzyme–protein ratio of 1:100(w/w). The meat was mixed for 1 min and thereafter for 30 s at 10-min intervals. Aliquots of MDF were removed at the desired time intervals, and the pH was adjusted to 7.0 with 3 N KOH to inactivate the enzyme. The modified MDF meat was stored at 4 °C until used the next day.

Solubility changes were assayed by mixing 2 g of modified MDF meat with 8 mL of 0.1 M NaCl, 0.05 M K phosphate, pH 7.0, adjusting the pH and NaCl concentration to that of the buffer, and assaying as previously described for protein suspensions.

The meat component of the emulsified meat test system (Randall et al., 1976, as modified by Smith and Brekke, 1984), was composed of a 50:50 blend (based on the protein content of the MDF) of modified and control MDF. Pork back fat and water were added to the system to achieve the desired formulation of 10.5% protein, 33.0% fat, with or without 3.0% salt. The prepared emulsion (ca. 36–39 g) was packed into 29 × 103 mm preweighed polypropylene centrifuge tubes. The samples were heated for 30 min in a 75 °C water bath. After heating, the meat plug was removed and weighed. The cook-out liquid was transferred to a 10-mL graduated cylinder and fat, water, and total cook-out volume determined upon standing and phase separation.

A Fudoh Rheometer (Fudohkogyo Co., LTD, Tokyo, Japan) was used to evaluate the meat plugs for cohesiveness and firmness. A meat plug was cut cross-wise into 10-mm pieces. The central core was removed along the longitudinal axis with a cork borer to produce a core of meat 10 mm in length by 7 mm in diameter. The core was placed upright on its long axis on the sample shelf of the rheometer and compressed between two flat parallel surfaces under a crosshead speed of 6 cm/min to the maximum 19.6 N force allowed by the rheometer. Force was recorded against time with a chart recorder. The applied force at which the meat plug ruptured was defined as

cohesiveness and was indicated by a sudden decrease in the slope of the force–time curve. Firmness was calculated from the slope of the force–time curve before rupture (Voisey et al., 1975).

Identification of Proteolytic Fragments. *Preparation of Myosin Fragments.* Heavy meromyosin (HMM) was prepared as described by Margossian and Lowey (1982) with a tryptic digest of at-death hen myosin. Trypsin (Type III, 11 000 BAEE units/mg of protein) and soybean trypsin inhibitor (Type 1-S) were purchased from Sigma Chemical Co. The solubilized HMM was dialyzed 20 h against 0.1 M NaCl, 0.05 M K phosphate, pH 3.2, and used as a source of HMM for hydrolysis by AFP 2000. The concentration of HMM was estimated by using an extinction coefficient of $E_{280}^{1\%} = 6.47$ cm^{-1} (Weeds and Pope, 1977). The concentration of HMM in the supernatant was ca. 5–6 mg/mL.

Myosin rod was prepared from a papain digest of at-death hen myosin as described by Margossian and Lowey (1982). Papain (Type IV, 23 units/mg of protein) was purchased from Sigma Chemical Co. The purified myosin rod was suspended in 0.1 M NaCl, 0.05 K phosphate, pH 3.2, in preparation for hydrolysis by AFP 2000. The concentration of the rod in solution was estimated by using an extinction coefficient of $E_{280}^{1\%} = 3.5$ cm^{-1}.

Fluorescent Labeling of Myosin. Purified at-death hen myosin was specifically labeled at its SH_1 group by using N-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-IAEDANS) as described by Reisler (1982). The time course of fluorescent labeling was followed by monitoring the Ca^{2+} and K^+–EDTA myosin ATPase activities. Labeled myosin was hydrolyzed by AFP 2000 and protein fragments separated by SDS–polyacrylamide gel electrophoresis. Relative mobility of fluorescent protein fragments was calculated.

Electrophoresis of Hydrolysates. Hydrolysates for SDS–polyacrylamide gel electrophoresis were prepared as described by Porzio and Pearson (1979). Molecular weight standards were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoresis were performed with a LKB 2001 Vertical Electrophoresis Unit (Bromma, Sweden) and a constant voltage power supply (Model 38201, Gelman Instrument Co., Ann Arbor, MI) by using the Tris-glycine buffer system described by Laemmli (1979). A 10% acrylamide gel (0.25% bis(acrylamide)) or an 8–16% acrylamide (0.25% bis(acrylamide)) linear gradient (O'-Farrell, 1975) was used as the resolving gel.

Protein hydrolysates (100 μg) or molecular weight standards (45 μg) were loaded on the gel and the current set at 1.0 ma/channel. When the tracking dye had entered the gel, the current was increased to 2.0 ma/channel. Electrophoresis was stopped when the tracking dye reached the bottom of the gel (5–6 h). The gels were stained overnight in 0.2% Coomassie Brilliant Blue R-250 in 9/45/46 acetic acid–methanol–H_2O (v/v/v) and destained in 7.5/25/67.5 acetic acid–methanol–H_2O (v/v/v).

The molecular weight of the protein fragments separated on nongradient gels was estimated (±10%) as described by Weber and Osborn (1969). Molecular weights were confirmed on 8 and 12% acrylamide resolving gels. The molecular weights of the protein fragments on the gradient gels were estimated (±7%) as described by Poduslo and Rodbard (1980).

Replications. All data represent a mean value of at least duplicate analyses on each of three MDF protein or meat preparations.

RESULTS AND DISCUSSION

Inhibition of AFP 2000. When partial proteolysis to improve protein functionality is used, it is critical to inactivate the enzyme after the desired effect. Heat causes denaturation and insolubilization of the modified proteins, and chemical enzyme inhibitors may alter protein structure or be unsafe for human consumption. AFP 2000 was confirmed in this study to be inactivated by adjusting the pH to 7.0, which is slightly greater than the pH of MDF, pH 6.4. Thus, pH 7.0 was used in all experiments to stop proteolysis.

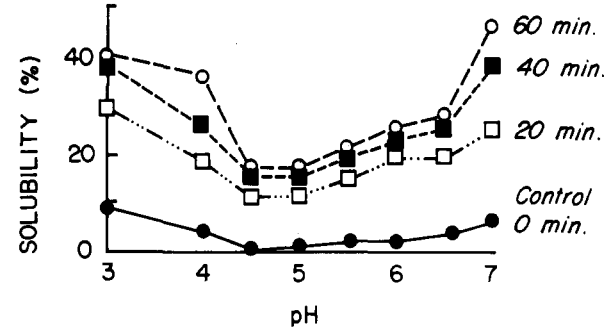

Figure 1. Solubility of a 3.5% (w/v) MDF myofibril suspension in 0.1 M NaCl, 0.05 M K phosphate, pH 7.0 as affected by pH and duration of enzyme treatment.

Protein Solubility. Myofibrillar proteins are insoluble at low ionic strength. There was no difference in solubility for either myofibrils or actomyosin suspended in 0.05 M K phosphate, pH 7.0 after extraction and those suspensions adjusted to 3.2, and readjusted to pH 7.0. Proteolysis for 1 h increased the solubility of MDF myofibrils to 46% and of actomyosin to 57% at pH 7.0 in 0.1 M NaCl. Myofibril solubility increased at a lower initial rate than actomyosin solubility. Susceptibile bonds in the myofibril were probably less available for proteolysis, due to intact sarcomere structure, than bonds in extracted actomyosin. Solubility of the modified myofibrils was greater than control myofibrils at pH 3.0–7.0 (Figure 1), suggesting improved functionality over this pH range.

Myofibrillar proteins are generally soluble in 0.6 M or greater NaCl (Forrest et al., 1975). MDF myofibrils were only 39% solubilized in 0.6 M NaCl at pH 7.0, indicating denaturation. The Ca^{2+} and K^+–ATPase activities of MDF myosin were 2.0% or less of the ATPase activities in myosin prepared from at-death hen muscle, further confirming that MDF proteins were denatured. Limited solubility and low ATPase activity may be caused by protein denaturation during mechanical deboning (Froning, 1976; McMahon and Dawson, 1976) and long-term frozen storage (Khan et al., 1963). Proteolysis increased myofibrillar protein solubility in 0.6 M NaCl up to a maximum of 84% after 40 min of enzyme treatment. Limited proteolysis may increase solubility by disrupting the aggregated, denatured structure of MDF proteins. Since protein solubility varied slightly with the time of enzyme treatment in each experimental replication, functionality tests were based on percentage solubility instead of proteolysis time.

Emulsifying Capacity. Emulsifying capacity (EC) for myofibril or actomyosin controls suspended directly in 0.05 M K phosphate, pH 7.0 after extraction did not differ from EC for controls adjusted to pH 3.2 and readjusted to pH 7.0. Proteolysis of MDF myofibrils or actomyosin by AFP 2000 increased EC compared to controls (Figure 2). Maximum EC of myofibrils occurred at 42% solubility, while the maximum EC of actomyosin occurred at 28% solubility. Actomyosin had a greater EC than the myofibrils. Proteolysis of the regulatory proteins in the myofibril preparation may have contributed to increased

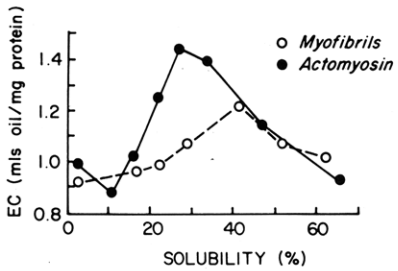

Figure 2. Effect of duration of enzyme treatment (solubility) on the emulsifying capacity of 1.0% (w/v) MDF protein suspensions in 0.1 M NaCl, 0.05 M K phosphate, pH 7.0.

Table I. Characteristics of 4.0% (w/v) Mechanically Deboned Fowl Actomyosin Gels Prepared in 0.6 M KCl, pH 6.0, 70 °C[a]

actomyosin solubility,[b,c] %	semiqualitative score[d]	expressed liquid,[e] %	inversion test, s to drop
4 ± 1 (control)	4	44	0
19 ± 2	4	0	60
23 ± 3	3	0	60
31 ● 3	2	10	30
45 ● 2	2	16	0
66 ● 4	1	24	0

[a] Values are means and standard deviations of three determinations. [b] Solubility measured in 0.1 M NaCl, pH 7.0 after enzyme treatment, but before the adjustment to 0.6 M KCl. [c] Solubility (%) = (protein in supernatant after centrifugation/total protein concentration) × 100. [d] Semiqualitative score: 5 = strong gel (withstands shaking); 4 = good gel (hard to break up); 3 = medium gel (clumps stick together); 2 = weak gel (clumpy); 1 = very weak gel (viscous); 0 = no gel (liquid). [e] Syneresis (%) = (volume of free liquid after gel formation/total volume in tube) × 100.

solubility, but contributed little to functionality. Increases in EC following proteolysis have also been reported for fish myofibrillar proteins (Spinelli et al., 1972), salt-extractable proteins of beef muscle (DuBois et al., 1972), and beef heart myofibrillar proteins (Smith and Brekke, 1984). DuBois et al. (1972) reported that EC increased or decreased from the controls, depending on the extent of proteolysis and type of protease.

The EC of meat proteins decreases to a minimum at the pI and increases as the pH is adjusted away from the pI (Randall, 1977). The pH solubility profile (Figure 1) indicates that proteolysis increased the EC of samples compared to controls in the pH region of low solubility, pH 4.5–5, due to the increases in solubility with proteolysis in this region.

Heat-Induced Gelation. There was no difference in gel strength in 0.6 M KCl between myofibril controls suspended directly in 0.05 M K phosphate, pH 7.0 and myofibril controls suspended in 0.05 M K phosphate, pH 3.2 and readjusted to 7.0. Proteolysis of myofibrils to 18% solubility increased gel apparent viscosity from 175 000 cps for the control myofibrils to 200 000 cps. A rapid decrease in apparent gel viscosity was observed with further proteolysis and solubility increase.

Actomyosin gels were evaluated with the semiqualitative evaluation technique (Table I). The control gel was given a score of four, to indicate a good gel which withstood shaking. Upon gel formation the control shrunk to form a very compact plug with 44% syneresis. Actomyosin modified to 19% solubility produced a gel as strong as the control, but there was no syneresis and the gel did not fall from the test tube on inversion, indicating improved gelation characteristics. The actomyosin gels became progressively weaker and less able to hold water with increasing proteolysis.

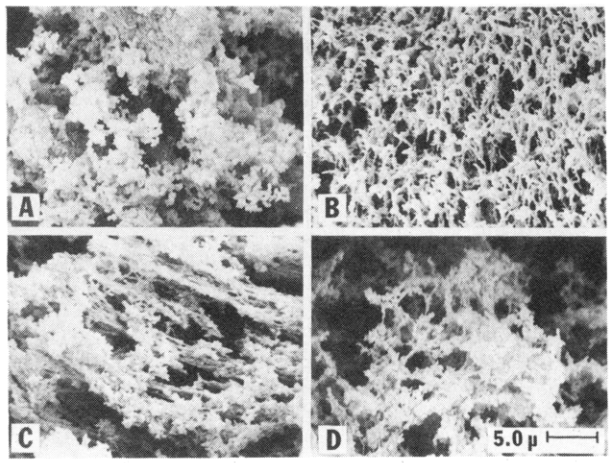

Figure 3. Scanning electron micrographs of heat-induced 4.0% (w/v) MDF myofibril gels modified by different times of enzyme treatment (A: control, 4% solubility; B; 18% solubility, 15 min; C: 31% solubility, 30 min; D: 46% solubility, 90 min).

Myofibril gels were examined by scanning electron microscopy to assess ultrastructural changes which resulted from proteolysis. The myofibril control (Figure 3 part A) formed a gel with a compact, globular microstructure. This microstructure explains the high gel strength and large quantity of syneresis observed (Johnson and Zabik, 1981). Myofibrils modified to 18% solubility produced a gel with a microstructure very different from the control (Figure 3 part B). This gel had a fibrous protein network, which explains the water holding ability and the high gel strength observed (Johnson and Zabik, 1981; Hermansson, 1982). As the extent of proteolysis increased, there was progressive breakdown in gel microstructure (Figure 3 parts C and D).

Emulsified Meat Test. In preliminary experiments, it was determined that cooked yield was maximized, and changes in cohesiveness or firmness minimized when meat plugs were produced with a 50:50 blend of modified and control MDF. Greater proportions of modified to control MDF in the test system caused decreases in cohesiveness and firmness without any significant improvement in cooked yield. The effect of proteolysis time on cooked yield of a 50:50 blend of modified and control MDF is shown in Table II. In meat plugs made without salt, the cooked yield increased from 76.5% for the control at 25% solubility to 94.6% yield for the meat plug containing MDF modified to 40% solubility. When 3.0% salt was used in the formulation, the cooked yield increased from 85.3% for the control at 34% solubility to 94% yield at 40 and 60 min of proteolysis. There was a decrease in the percentage fat in the cook-out liquid in both formulations, indicating increased fat binding ability. Firmness and cohesiveness of control meat plugs was significantly greater for those containing salt. Proteolysis reduced firmness of the salt-containing meat plugs to that of the plugs made without salt but increased the cohesiveness of plugs without salt to that of plugs containing salt. Partial proteolysis of MDF was beneficial to meat plug texture only when salt was not included.

Cooked yields for emulsions containing modified MDF without salt were significantly greater than yields for controls made with 3% salt and equal to yields for emulsions containing modified MDF with salt. Using this same meat test, Smith and Brekke (1984) reported improved cooked yields for meat plugs containing 30% proteolytically degraded beef heart/70% beef skeletal compared to 30% beef heart/70% beef skeletal controls with 0 and 3% salt. These findings indicate that partial

Table II. Mean Cooked Meat Yield, Cook-Out Composition and Texture Values of Meat Test Emulsions Prepared with a 50:50 Blend of Enzyme-Modified and Untreated Mechanically Deboned Fowl[a]

sample	solubility,[b] %	cooked yield,[c] %	cook-out composition[d] water, %	cook-out composition[d] fat, %	firmness, N/s	cohesiveness, N
			No Salt			
control	25 ± 2.5	76.5[a] ± 1.7	87 ± 2	13 ± 2	1.02[a] ± 0.01	4.8[a] ± 0.1
pH control	16 ± 1.2	81.9[b] ± 1.1	83 ± 3	17 ± 3	0.97[b] ± 0.02	3.8[b] ± 0.1
15 min	26 ± 1.4	82.8[b,c] ± 2.5	83 ± 2	17 ± 2	1.04[a] ± 0.02	5.7[c] ± 0.3
40 min	32 ± 1.5	91.3[d,e] ± 2.6	89 ± 4	11 ± 4	1.07[a] ± 0.02	5.8[c] ± 0.1
60 min	40 ± 0.5	94.6[d] ± 1.3	99 ± 1	1 ± 1	1.01[a] ± 0.01	5.8[c] ± 0.1
			3% Salt			
control	34 ± 1.3	85.3[c] ± 1.8	85 ± 2	15 ± 2	1.16[c] ± 0.02	5.9[c] ± 0.2
pH control	22 ± 2.1	82.0[c] ± 0.9	88 ± 3	12 ± 3	0.92[b] ± 0.04	4.4[a] ± 0.3
15 min	35 ± 1.2	87.8[c,e] ± 1.6	87 ± 3	13 ± 3	1.14[c] ± 0.03	6.0[c] ± 0.3
40 min	40 ± 0.3	94.1[d] ± 1.1	91 ± 5	9 ± 5	1.08[a] ± 0.03	5.6[c] ± 0.3
60 min	48 ± 1.1	94.3[d] ± 0.9	97 ± 2	3 ± 2	1.08[a] ± 0.03	5.8[c] ± 0.1

[a] Means in a column followed by the same letters did not differ significantly ($P < 0.05$). Values are means and standard deviations of six determinations. [b] Solubility (%) = (protein in supernatant after centrifugation/total protein concentration) × 100. [c] Cooked yield (%) = (weight of meat emulsion after cooking/weight of raw meat emulsion) × 100. [d] Proportion of fat and water in the cook-out liquid.

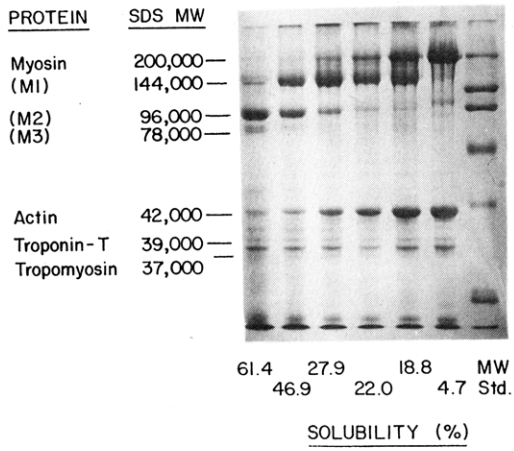

Figure 4. Progression of an AFP 2000 digestion of MDF myofibrils as resolved on 10% SDS–polyacrylamide gels (M1, M2, M3: myosin fragments, myosin:myosin heavy chain).

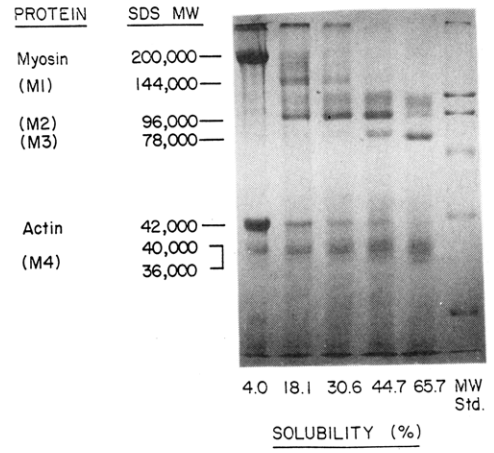

Figure 5. Progression of an AFP 2000 digestion of MDF actomyosin as resolved on 10% SDS–polyacrylamide gels (M1, M2, M3, M4: myosin fragments, myosin:myosin heavy chain).

proteolysis of meat proteins may have potential as a partial substitute for salt in the production of processed meat products.

Comparison of solubility values for control and pH control treatments in Table II indicates that addition of acid and base during proteolysis of MDF decreased meat protein solubility. The solubility decrease may be attributed to denaturation of the sarcoplasmic proteins which occurs easily under acidic conditions (Lawrie, 1979). Myofibrillar protein solubility was not affected by acid and base addition, as shown by the model system functional tests.

Identification of Proteolytic Fragments. The four major fragments which were produced when AFP 2000 hydrolyzed MDF myofibrils and actomyosin are designated M1, M2, M3, and M4 (Figures 4 and 5). Similar patterns occurred when AFP 2000 hydrolyzed at-death hen myosin, beef heart myofibrils, and rabbit skeletal myosin. Pepsin and AFP 2000 produced identical proteolytic fragmentation patterns upon hydrolysis of MDF myofibrils. The identical proteolysis of MDF myofibrils by the two acid proteases may indicate similar substrate specificity. Likewise, the myosin molecule may be folded into a conformation at low pH in which only specific domains are available for proteolysis. Protein fragments produced by neutral proteases (Cardinaud, 1979) differ from the protein fragments produced by AFP 2000 and pepsin.

M1 was identified as originating from heavy meromyosin (HMM) based on molecular weight, solubility, and fluorescent labeling studies. The heavy chain of HMM isolated from a trypic digest of at-death hen myosin and literature values for rabbit HMM (Balint et al., 1978; Margossian and Lowey, 1982) showed a molecular weight similar to M1 from MDF (Figure 6). M1 from MDF actomyosin was insoluble in 0.1 M NaCl (Figure 7), but M1 from hydrolyzed at-death hen actomyosin was soluble under the same conditions. Since native HMM is soluble in low-salt solutions (Margossian and Lowey, 1982), it is probable that

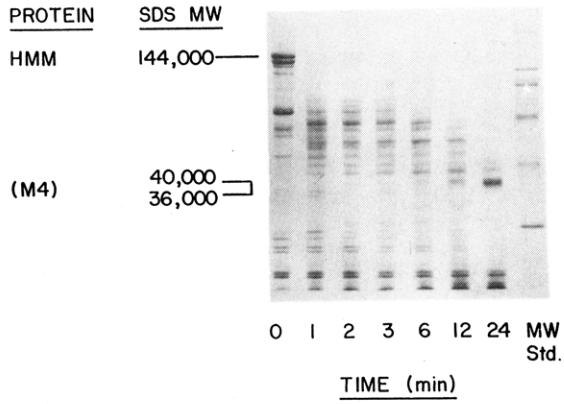

Figure 6. Time course of an AFP 2000 digestion of at-death hen heavy meromyosin as resolved on a 8–16% gradient SDS–polyacrylamide gel (HMM: heavy meromyosin; M4: myosin fragment).

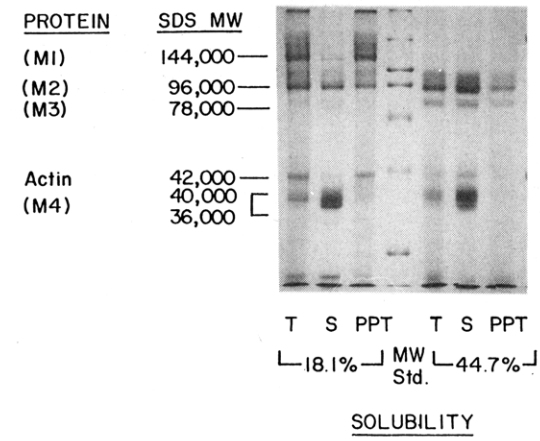

Figure 7. Solubility in 0.1 M NaCl, pH 7.0 of proteolytic fragments produced from an AFP 2000 digest of MDF actomyosin as resolved on 10% SDS–polyacrylamide gels (T, total hydrolyzate; S, soluble fraction; PPT, insoluble fraction).

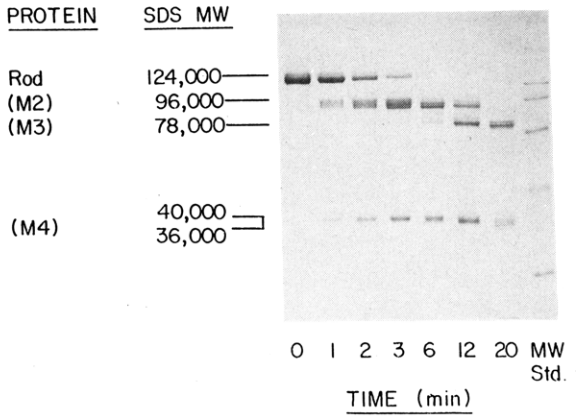

Figure 8. Time course of an AFP 2000 digestion of at-death hen myosin rod as resolved on a 10% SDS–polyacrylamide gel.

HMM from MDF was insoluble because of denaturation during mechanical deboning and frozen storage. The identity of M1 was also confirmed by labeling the SH_1 sulfhydryl group of myosin, purified from at death hen muscle, with a fluorescent reagent, *N*-[[(iodoacetyl)-amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-IAEDANS) (Reisler, 1982). SH_1 is located in the globular head region of the myosin heavy chain which is part of HMM. Myosin and M1 were the only protein bands which exhibited fluorescence.

Proteolytic fragments M2 and M3 originated from the rod portion of the myosin molecule. The source of M2 and M3 was determined by isolating the myosin rod from a papain digest of myosin and treating the purified rod with AFP 2000. The myosin rod was hydrolyzed into fragments of 96 000, 78 000, and 36 000 daltons (Figure 8). Purified HMM was hydrolyzed to many nonspecific fragments and did not produce major bands at 96 000 and 78 000 daltons (Figure 6) and was not a source of M2 and M3.

M4 protein fragments have molecular weights between 36 000 and 40 000 daltons. The M4 band, resulting from the hydrolysis of myofibrils, may be partially composed of troponin-T (Porzio and Pearson, 1977) with a molecular weight of 37 000. Otherwise, the proteolytic fragments at M4 are breakdown products of HMM (Figure 6) and the rod (Figure 8). M4 also contains an actin fragment based on the experiments in which purified hen actin was hydrolyzed by AFP 2000.

Several of the MDF proteolytic fragments produced by AFP 2000 were more soluble than actomyosin when assayed in 0.1 M K phosphate, 0.1 M NaCl, pH 7.0 (Figure 7). MDF actomyosin, myosin heavy chain, actin, and M1 are insoluble in the low-salt buffer. M2 solubility in 0.1 M NaCl increased with longer hydrolysis times. M3 is partially soluble, while proteins at M4 are soluble in low-salt buffer. Increases in solubility and decreases in the molecular weight of the major protein fragments compared to actomyosin may contribute to the increased protein functionality observed after proteolysis (Du Bois et al., 1972; Kinsella, 1976; Ochiai et al., 1982).

Relationships of Functional Properties and Proteolysis. Proteolytic patterns where maximum EC occurred were similar for actomyosin and myofibrils (Figures 4 and 5), although solubility of the preparations differed; myosin was completely broken down, M1 partially broken down, and the M2 protein had appeared. A positive correlation between solubility and EC has been reported (Ochiai et al., 1982). DuBois et al. (1972) and Ochiai et al. (1982) suggested that a certain molecular size or axial ratio may maximize EC. Kinsella (1976) and Nakai (1983) suggested that an increase in the rate or extent of protein unfolding at the fat/water interface may improve the EC. Therefore, an increase in protein solubility and decrease in molecular size, allowing for easier migration of protein to the fat/water interface, may be two reasons for the improved EC of modified MDF proteins. MDF myofibril protein fragments may unfold easier than the parent molecules due to fewer intramolecular forces, resulting in an improved EC.

Myofibril gel strength was maximum at 18% protein solubility in low-salt buffer and 68% protein solubility in 0.6 M NaCl. At this extent of proteolysis myosin is partially broken down to HMM (Figures 4 and 5). Cleaving HMM from myosin may cause a change in the aggregated, denatured structure of the myofibrillar proteins, allowing for dissociation and improved solubility in 0.6 M KCl during gel formation. It appears that a very short time of proteolysis of MDF dramatically affects protein solubility.

There was no relationship between the extent of proteolysis necessary to maximize EC in model system tests (Figure 3) and cooked yields in the emulsified meat system (Table II). Differences between the EC test and the meat model system in rate of shear, type of fat, temperature, protein concentration, and ingredients would have affected the results obtained (Acton et al., 1983). Also, differences in functionality between extracted meat proteins and intact meat tissue cannot be ignored.

Cooked yields in the meat system were maximum when proteolysis produced an increase in protein solubility of 15% in the no-salt meat plugs and 6–14% in the meat plugs containing 3.0% salt (Table II), and maximum gel strength occurred at 18% solubility (Table I). Thus, the extent of proteolysis necessary to maximize gel strength in functional tests is closely related to the extent of proteolysis necessary to maximize cooked yields in the emulsified meat system. Gelation model system tests may, therefore, be a better indicator than EC model system tests of meat protein functionality in an actual meat system.

The changes in myofibrillar protein structure due to proteolysis and their relationship to protein functionality are not clear. Increased functionality can be only partially explained by the increased solubility of the new fragments compared to the unhydrolyzed parent proteins. Additional experiments are in progress to examine the relationship between actomyosin functionality and structure. The results of this study indicate that partial proteolysis of MDF improves protein functionality in functional tests and in

an emulsified meat test, and that partial proteolysis of MDF may allow reduction in salt concentration in processed meat products.

LITERATURE CITED

Acton, J. C.; Ziegler, G. R.; Burge, D. L. *Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 99.
AOAC. "Official Methods of Analysis", 13th ed.; Association of Official Analytical Chemists: Washington, D.C., 1980.
Balint, M.; Wolf, I.; Tarcsafalvi, A.; Gergely, J.; Sieter, F. *Arch. Biochem. Biophys.* **1978**, *190*, 793.
Brekke, C. J.; Eisele, T. A. *Food Technol.* **1981**, *35*(5), 231.
Briskey, E. J.; Fukazawa, T. *Adv. Food Res.* **1971**, *19*, 279.
Cardinaud, R. *Biochimie* **1979**, *61*, 807.
DuBois, M. W.; Anglemier, A. F.; Montgomery, M. W.; Davidson, W. D. *J. Food Sci.* **1972**, *37*, 27.
Eisele, T. A.; Brekke, C. J. *J. Food Sci.* **1981**, *46*, 1095.
Forrest, J. C.; Aberle, E. D.; Hedrick, H. B.; Judge, M. D.; Merkel, R. A. "Principles of Meat Science"; W. H. Freeman and Co.: San Francisco, CA, 1975.
Froning, G. W. *Food Technol.* **1976**, *30*(9), 50.
Gornall, A. G.; Bardawill, C. J.; David, M. M. *Biol. Chem.* **1949**, *177*, 751.
Hermansson, A.-M. *J. Food Sci.* **1982**, *47*, 1965.
Institute of Food Technologists' Expert Panel on Food Safety and Nutrition and the Committee on Public Information. *Food Technol.* **1980**, *34* (1), 85.
Johnson, T. M.; Zabik, M. E. *Poultry Sci.* **1981**, *60*, 2071.
Khan, A. W.; Van den Berg, L.; Lentz, C. P. *J. Food Sci.* **1963**, *28*, 425.
Kinsella, J. E. *Crit. Rev. Food Sci. Nutr.* **1976**, *8*, 219.
Kinsella, J. E. In "Food Protein Deterioration, Mechanism and Functionality"; Cherry, J. P., Ed.; American Chemical Society: Washington D.C., 1982; *ACS Symp. Ser. 206*, p 301.
Laemmli, U. K. *Nature (London)* **1979**, *227*, 680.
Lawrie, R. A. "Meat Science"; Pergamon Press: New York, 1979.
Margossian, S. S.; Lowey, S. *Methods Enzymol.* **1982**, *85B*, 55.
Matsumoto, J. J. In "Proteins at Low Temperature"; Fennema O., Ed.; American Chemical Society: Washington, D.C., 1979; *Adv. Chem. Ser. 180*, p 205.
McMahon, E. F.; Dawson, L. E. *Poultry Sci.* **1976**, *55*, 573.
Nakai, S. *J. Agric. Food Chem.* **1983**, *31*, 767.
Ochiai, K.; Kamata, Y.; Shibaski, K. *Agric. Biol. Chem.* **1982**, *46*, 91.
O'Farrell, P. H. *J. Biol. Chem.* **1975**, *250*, 4007.
Olson, D. G. *Natl. Provis.* **1982**, *186* (April 17), 7.
Olson, D. G.; Terrell, R. N. In "Proceedings of the Meat Industry Research Conference"; American Meat Institute: Arlington, VA, 1981; p 59.
Pardee, J. D.; Spudich, J. A. *Methods Enzymol.* **1982**, *85B*, 164.
Poduslo, J. F.; Rodbard, D. *Anal. Biochem.* **1980**, *101*, 394.
Porzio, M. A.; Pearson, A. M. *Biochem. Biophys. Acta* **1977**, *490*, 27.
Porzio, M. A.; Pearson, A. M. *Meat Sci.* **1979**, *3*, 255.
Pour-El, A.; Swenson, T. S. *Cereal Chem.* **1976**, *53*, 438.
Randall, C. J. *Can. Inst. Food Sci. Technol. J.* **1977**, *10*, 147.
Randall, C. J.; Raymond, D. P.; Voisey, P. W. *Can. Inst. Food Sci. Technol. J.* **1976**, *9*, 216.
Reisler, E. *Methods Enzymol.* **1982**, *85B*, 84.
Richardson, T. In "Food Proteins. Improvement through Chemical and Enzymatic Modification"; Feeney, R. E., Whitaker, R. R., Eds.; American Chemical Society: Washington, D.C., 1977; *ACS Adv. Chem. Ser. 160*, p 185.
Ryan, D. S. In "Food Proteins. Improvement through Chemical and Enzymatic Modification"; Feeney, R. E., Whitaker, J. R., Eds.; American Chemical Society: Washington, D.C., 1977; *ACS Adv. Chem. Ser. 160*, p 67.
Smith, D. M.; Brekke, C. J. *J. Food Sci.* **1984**, *49*, 1525.
Spinelli, J.; Koury, B.; Miller, R. *J. Food Sci.* **1972**, *37*, 604.
Voisey, P. M.; Randall, C. J.; Larmond, E. *Can. Inst. Food Sci. Technol. J.* **1975**, *8*, 23.
Wagner, P. D.; Yount, R. G. *Biochemistry* **1975**, *14*, 1900.
Webb, N. B.; Ivey, F. J.; Craig, H. B.; Jones, V. A.; Monroe, R. J. *J. Food Sci.* **1970**, *35*, 501.
Weber, K.; Osborn, M. *J. Biol. Chem.* **1969**, *244*, 4406.
Weeds, A. G.; Pope, B. *J. Mol. Biol.* **1977**, *111*, 129.
Wells, J. A.; Werber, M. M.; Yount, R. G. *Biochemistry* **1979**, *18*, 4800.
Yasui, T.; Ishioroshi, M.; Nakano, H.; Samejima, K. *J. Food Sci.* **1979**, *44*, 1201.

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