THE USE OF BOVINE HIDE COLLAGEN IN FINE EMULSION BOLOGNA

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CHAPTER I

INTRODUCTION

Collagen is the most abundant protein in many animal tissues. One-third of all the protein in the animal body or six percent of the total body weight is collagen. The highest concentration of the protein collagen is found in the hide. Currently, the slaughter of cattle and calves in the United States exceeds 40 million head per year. Each animal yields a hide that amounts to over seven percent of its live body weight. This would annually equate to about 1.5 million tons of hide. The outer or "grain" split of the hide is used to make smooth leathers. The inner or "flesh" split is used to make suede leathers and sausage casings.

In the past, the market for cattlehide "flesh" splits has experienced a large degree of price instability. Periods of low demand have resulted in the underutilization of large quantities of the "flesh" splits. It was during one such period (1960s), that scientists working at the United States Department of Agriculture's Eastern Regional Research Center, Waymoor, Pennsylvania, became interested in the feasibility of making food-grade collagen from "flesh" splits. They theorized that alternative uses would help stabilize the demand and price paid for splits.

Since that time, five different hide collagen products have been developed. These products vary with respect to average solids content and particle size. They hoped this variety of products would facilitate a wide range of food applications. Presently, the five products are being studied as food extenders, binders, moisturizers and texturizers. Scientists at Oklahoma State University have incorporated collagen into hamburger, meatloaf and bakery products.

The use of collagen as a food is still the subject of extensive study. While the hide collagen is "microbiologically acceptable" (Komanowsky, Sinnamon, Elias, Heiland and Aceto, 1974, p. 410), it is still an incomplete protein source. Further, functional properties such as thermal shrinkage and gelatinization make additive levels critical.

The purpose of this study was to determine the effect of bovine hide collagen on the lipid, crude protein, moisture, cookloss, emulsion stability, texture, pressed fluid and color of fine emulsion bologna. Criteria for study were selected on the basis of economic value to the sausage industry.

CHAPTER II

REVIEW OF LITERATURE

Collagen

Collagen is the most abundant protein in the mammalian body. It occurs in nearly all organs, but is most prevalent in bone, cartilage, tendons, teeth and hide. Insolubility and high tensile strength are responsible for its role as the primary structural component in mature and developing tissues (Stryer, 1975).

Tropocollagen, with a molecular weight of 285,000 daltons, is the basic structural unit. Each molecule of tropocollagen is 2800 angstroms long and 15 angstroms wide (Gross, 1961). The rodlike molecule is composed of a three chain coiled helix. The three chains are all in alpha helix conformation. Two chains are always identical. The third is identical or very similar. The two identical alpha helices are called alpha one; the other, if different, is termed alpha two (Piez, 1976; Stryer, 1975). Tropocollagen molecules are arranged in quarter-stagger array to form collagen fibrils. Molecular overlaps of 700 angstroms account for the high tensile strength of the fibrils and their striated appearance (Gross, 1961; Lehninger, 1975).

The amino acid composition of collagen is very different from that of other proteins. One-third of the amino acid residues are glycine. In the sequencing of the collagen molecule, glycine residues occur in nearly every third position of the tropocollagen molecule chains (Gustavson, 1956; Lehninger, 1975; Stryer, 1975). This regular spacing is extremely important to the molecular structure, as glycine is the only amino acid small enough to accommodate the interior positions of the triple helix (Stryer, 1975). Collagen also contains 11 percent alanine, 12 percent proline, 13 percent hydroxyproline and varying amounts of hydroxylysine. The latter two amino acids are characteristic to and occur almost exclusively in collagen (Gustavson, 1956). The amount of hydroxylysine depends on the type of collagen (I, II, II or IV) and tissue from which it originates (Barnes, Constable, Morton and Royce, 1974). The remaining amino acid residues are present in much smaller amounts and include: histadine, phenylalanine, isoleucine, tyrosine, leucine, arginine, lysine, methionine, valine, threonine, serine, glutamic acid and aspartic acid (Asghar and Henrickson, 1980).

The tropocollagen helix is highly ordered in the direction of the long axis of the rod. The triple stranded helix of the rod is the consequence of many noncovalent reinforcing bonds, each of which is relatively weak. This stability is related to the content of proline and hydroxyproline (imino acids). The higher the imino acid content, the more

stable the helix (Stryer, 1975). Collagen fibers are also strengthened by intramolecular and intermolecular covalent cross-linkages. These cross-links are derived from the lysine side chains (Lehninger, 1975; Stryer, 1975).

The two most important factors of collagen in meat products are the characteristic thermal shrinkage and subsequent conversion to gelatin (Kramlich, Pearson and Tauber, 1973). Rates of shrink and gelatinization (solubilization) are dependent on specie, age, sex, rate of growth and tissue location (Gross, 1961). All collagen is composed of three functional fractions: 1) dilute salt soluble, 2) citrate buffer soluble and 3) insoluble. As animal age increases, fraction 3 increases with the concomitant decrease of fractions 1 and 2 (Bowes, Elliott and Moss, 1957; Reed, Stainsby and Ward, 1963). Molecular cross-linking is responsible for this age related decrease in solubility (Goll, 1964; Hill, 1966). Collagen from young animals will therefore undergo more change upon heating (Cerney, Groug and James, 1970).

The value of collagen as a human food or as a nonruminant animal feed is limited because of its poor amino acid balance; collagen is low in methionine and contains no tryptophan (Whitmore, Jones, Windus and Naghski, 1970). Accordingly, the protein efficiency ratio (PER) is negative (Happich, Whitmore, Feairheller, Taylor, Swift, Nagbski, Booth and Alsmeyer, 1975; Vincent, 1969). Meat

cuts high in collagen containing connective tissue have shown less desirable nutritional profiles than those low in collagen (Dvorak and Vognarova, 1969; Bender and Zia, 1976). Arginine is the only essential amino acid which is not limiting in collagen (Ramo Rao, Norton and Johnson, 1964). It has been calculated, however, that meat products with up to 28.5% collagen should not be considered as having a low PER value (Lee, Elliott, Rickansrud and Hagberg, 1978).

Hot Boning and Electrical Stimulation

Excision of skeletal muscle before chilling and the onset of rigor (hot boning) produces meat with significantly more emulsifying capacity than conventional processing (Acton and Saffle, 1969; Van Eerd, 1972). Prerigor meat has also been shown to have superior protein binding when used in restructured products (Pepper and Schmidt, 1975; Solomon and Schmidt, 1980). Other studies have indicated that hot boning increased the total meat yield (Schmidt and Keman, 1974), decreased the cost of refrigeration (Kastner, Henrickson and Morrison, 1973) and decreased bacterial counts (Acton and Saffle, 1969). In the form of ground beef, prerigor meat has shown decreased cookloss and increased juiciness over conventional processing (Jacobs and Sebranek, 1980). Presently, the consensus of scientific opinion is that these effects are

caused by higher muscle pH level resulting in the extraction of a larger fraction of soluble protein (Goll, Henderson and Kline, 1965; Acton and Saffle, 1969; Johnson and Henrickson, 1970; Van Eerd, 1972).

The effects of carcass electrical stimulation (ES) on tenderness and quality have been well established and are summarized by Cross (1979). Examinations of muscle microstructure revealed that ES resulted in swelling of cellular components and myofibrillar disruption, possibly resulting in protein release (Savell, Dutson, Smith and Carpenter, 1978; Will, Ownby and Henrickson, 1980). This is supported by the finding of Whiting, Strange, Miller, Benedict, Mozersky and Swift (1980) that hot boned electrically stimulated lamb had emulsion stability ratings superior to conventionally processed lamb, but that other functional properties were unchanged. The combined effects of ES and hot boning have also been shown to "prolong the shelf life of ground beef" (Raccach and Henrickson, 1978, p. 957).

Manufacture of Bovine Hide Collagen

Scientists at the United States Department of Agriculture's Eastern Regional Research Center (ERRC) in Philadelphia, Pennsylvania became interested in collagen, the major protein in cattle hides, when limed "flesh" splits became underutilized in the 1960s (Turkot, Komanoswky and Sinnamon, 1978). A "flesh" split is that portion of the

bovine hide which lies below the leather-forming derma. This connective tissue layer (corium) functions as a skin-to-body attachment (Gustavson, 1956). It was realized that the collagen derived from these splits by comminution, had many potential food applications. One use had been explored in depth; that of sausage casing manufacture. Therefore, research was directed toward developing a process to produce food grade comminuted collagen products from "flesh" splits that had a minimum amount of protein denaturation (Turkot et al., 1978). Since that time, five forms of hide collagen have been produced. These products are made from the limed "flesh" splits of bovine animals 18 to 24 months of age (Komanowsky et al., 1974). The average solids content varies from 13.3% (product five) to 21.8% (product one) (Turkot et al., 1978). For the work discussed here, product one was used.

Limed splits are prepared for conversion to food grade collagen by size reduction. First, the splits are cut into strips. Then, the strips are carried by conveyor to a rotary knife for further shredding. Following shredding, the pieces of "flesh" split are adjusted to the isoelectric point of collagen (pH 5.3) by the addition of a solution of 0.3% propionic and 0.1% benzoic acid. The pieces were then drained, fresh water added and a final partical size of 0.023 cm was achieved using an Urschel Comitrol. This series of particle reductions yielded product number one. Other products are produced by using different particle

reduction methods and rates of water addition. Product one produced in this manner is a relatively undenatured product selling for 30 to 92 cents per pound (depending on the price of hides) (Turkot et al., 1978).

Sausage Emulsion Theory

There have been many reviews of sausage emulsion technology (Saffle, 1968; Gordon 1969a and 1969b; Sulzbacher, 1973; Webb, 1974; Schut, 1978). Many of these reviews point out that until the 1960 study by Hansen, the structure of sausage emulsions was poorly understood. The broad definition of an emulsion is "the dispersion of one liquid in a second immiscible liquid" (Webb, 1974, p. 1). A stable emulsion consisting of only two components could not exist. Some type of emulsifying agent must also be present (Webb, 1974). Meat emulsions are dispersions of solid fats (discontinuous phase) in water (continuous phase) with proteins as emulsifying agents. These agents function to reduce the interfacial tension between the two immiscible phases (Saffle, 1968). In a typical meat emulsion, the fat droplets are primarily encapsulated by salt soluble (myofibrillar) proteins (Hansen, 1960). The salt soluble proteins are more effective emulsion stabilizers than water soluble (sarcoplasmic) proteins (Swift, Lockett and Fryar, 1961; Hegarty, Bratzler and Pearson, 1963). Of the myofibrillar proteins, myosin is the best

agent because it is an amphipathic molecule. It has one hydrophillic (the "head") and one hydrophobic (the "tail") end. These molecules orient themselves around each fat droplet (tails in) and reduce surface tension between the two phases (Schut, 1976). Upon cooking, myosin molecules overlap into a three-dimensional network of fibers (Siegel and Schmidt, 1979), functioning to hold the fat droplet in place.

High levels of collagen-containing meats in sausage have been associated with unstable emulsions, poor peelability, gel-pocket formation and wrinkling of the outer skin upon cooking (Saffle, Christian and Moore, 1964). Consequently, a "rule of thumb" used by many sausage makers has been to keep high collagen meats at fractions less than 15% of the total formulation (Carpenter, Wiley, Reagan and Campion, 1979). In 1972 Hamm reported that collagen protein utilized water binding mechanisms similar to those of myofibrillar proteins. This would directly involve collagen with the emulsion stabilization by myosin.

CHAPTER III

MATERIALS AND METHODS

Lean Tissue

The source of lean tissue for this experiment was a 417 kilogram Angus bullock, 270 days of age. Dressed right and left sides weighed 129 and 130 kilograms, respectively. The carcass was split 18 minutes after exsanguination. The left and right sides were electrically stimulated 29 and 31 minutes after exsanguination, respectively. Stimulation included an input of 300 volts direct current with a 0.5 millisecond square wave pulse duration at 300 cycles per second for a one minute period.

Both sides of the carcass were then boned and separated into lean and fatty tissue. The carcass provided 156 kilograms of lean skeletal muscle and 31 kilograms of fatty tissue.

The lean tissue was ground through a 1.27 centimeter plate and then divided into three plastic tubs containing approximately 52 kilograms each. The lean tissue from each tub was mixed for two minutes in a chilled 45.4 kilogram Reliance sausage mixer. After the lean tissue in each tub had been mixed once, one-half of the material from tub number one was mixed with one-half of the material

from tub number two using the same mixing schedule. The remainder of the meat from tub number two was combined with one-half of the mixture from tub number three and mixing was repeated. Finally, the remaining meat from tubs one and three was combined and mixed in the same manner. This entire procedure was repeated twice.

The lean from all three tubs was divided into 37 portions each containing 3.6 kilograms. A sample weighing approximately 75 grams was removed from each portion. Each 3.6 kilogram quantity was then double wrapped in heavy duty Reynolds aluminum foil and frozen in a blast freezer at -20°C. Total time from exsanguination to freezer was 3 hours and 53 minutes.

Fatty Tissue

The fatty tissue from the same carcass was ground through a 1.27 centimeter plate and then placed directly into a chilled 45.4 kilogram Reliance sausage mixer. All fatty tissue was mixed for two minutes then divided into 12 portions of approximately 2.6 kilograms. A sample weighing approximately 75 grams was removed from each portion for proximate analysis. The 12 portions of fatty tissue were each double wrapped in heavy duty Reynolds aluminum foil and frozen in an air blast freezer at -20°C. Total time from exsanguination to freezer was 4 hours and 10 minutes for the fatty tissue.

Collagen

Bovine hide collagen product number one, as described by Turkot et al. (1978) was used in this experiment. The number one product was selected because its composition most closely resembled the lean meat to be replaced (Schalk, 1980). The collagen product used was manufactured in July of 1978 and delivered from the United States Department of Agriculture's Eastern Regional Research Center, Philadelphia, Pennsylvania, in August of the same year.

The hide collagen was delivered frozen in number 10 cans and stored at -20°C. Prior to use in bologna and for proximate analysis, the material was thawed at 3.3°C for 48 hours then allowed to drain 30 minutes in a Buchner funnel to remove excess moisture.

Sampling

A 75 gram sample was removed from each portion of lean and fatty tissue, sealed in an individual Whirl-Pak plastic bag and stored at 1°C until analyzed, usually within 16 hours. Each sample was removed from its bag, mixed for a total of 60 seconds, using a Sorvall type OM Omnimixer, and returned to its bag. Following omnimixing, two samples were removed from each bag for each of the following analyses: protein, lipid, moisture and ash content.

Formulation

The proximate analyses of fat, lean and hide collagen are each shown in Table I. The hide collagen analysis was taken from the work by Schalk (1980). Based on the collagen analyses, four different, 9.08 kilogram, sausage recipes were formulated using hide collagen to replace fractions of the nonlipid constituents. The lipid:nonlipid ratio was held at 25:75, thus making the nonlipid collagen levels 0.0, 4.7, 8.9 and 12.8 percent prior to the addition of water. Water was added to bring the moisture up to a level of four times the percent protein plus ten percent. The lean tissue, fat, collagen and water content for each of the four products is shown in Table II.

TABLE I

PROXIMATE ANALYSIS OF LEAN, FATTY AND COLLAGEN TISSUES

Constituent	Percent in Lean Tissue	Percent in Fatty Tissue	Percent in Collagen
Crude Protein	19.80	1.70	21.09
Moisture	71.71	20.57	76.60
Lipid	7.06	77.24	0.26
Ash	0.93	0.20	0.17
Carbohydrate*	0.50	0.29	1.88

*By difference

TABLE II

BOLOGNA FORMULATIONS

Nonlipid Replacement Level (Percent)	Lean Tissue (kg)	Fatty Tissue (kg)	Collagen (kg)	Water (kg)
0.0	6.760	2.320	0.000	1.094
4.7	6.397	2.361	0.318	1.085
8.9	6.084	2.388	0.608	1.080
12.8	5.766	2.447	0.867	1.067

Sodium chloride, sodium nitrite, sodium erythorbate and spices (Table III) were added to all four formulations in the same amounts.

Bologna Manufacture

Bologna was manufactured on Mondays and Tuesdays of each week for six consecutive weeks. The four treatments were randomly assigned to cooking dates subject to two restrictions: 1) that products of a given collagen replacement level were not manufactured on consecutive dates and 2) that products of a given level were made on three occasions. The cooking dates for the corresponding products appear in Table IV.

Prior to product manufacture, two 3.6 kilogram portions of the lean tissue and one 2.6 kilogram portion of the fatty tissue were allowed to thaw 24 hours at 1°C.

TABLE III

BOLOGNA INGREDIENTS*

Item	Grams
Sodium Chloride	272.0
White Pepper	33.6
Coriander	11.2
Allspice	5.6
Sage	5.6
Garlic Powder	1.4
Onion Powder	2.8
Sodium Nitrite	0.9
Sodium Erythorbate	5.9

*Added to each 9.08 kilogram formulation.

TABLE IV

MANUFACTURING SCHEDULE FOR BOLOGNA PRODUCTS

Week	Day	Non Lipid Replacement Level
1	Monday	0.0
1	Tuesday	12.5
2	Monday	4.7
2	Tuesday	0.0
3	Monday	12.8
3	Tuesday	4.7
4	Monday	8.9
4	Tuesday	4.7
5	Monday	8.9
5	Tuesday	12.8
6	Monday	8.9
6	Tuesday	0.0

The two portions of lean were combined, hand mixed and four 10 gram samples were removed at random for pH determinations. The bowl of a Kramer-Grebe Schneidmicher-45 silent cutter was iced one hour, then wiped dry.

All ingredients except the fatty tissue were weighed and added to the dried silent cutter bowl prior to the initial chopping. Water was added to the meat as flaked ice. The non fatty tissue ingredients were chopped at high speed until the mixture reached 8°C. The silent cutter was then stopped, and the fatty tissue added. Chopping was resumed at high speed until the emulsion temperature reached 13°C.

The fine emulsion was then loaded into a number 12-1/2 Vogt Ideal hand stuffer and stuffed into eight, 2.5 x 16 inch Union Carbide Clear fibrous casings. Before each sausage was stuffed, a sample of approximately 100 grams was removed from the stuffing horn for later analyses.

Sausages were randomly assigned dated tags numbered 1 through 8. Tags were tied to the end and the weight determined. Sausages numbered through 4 were cooked first. Sausages marked 5 through 8 were stored at 1°C and then cooked immediately after sausages 1 through 4 were removed from the oven using the same numerical oven placement.

Cooking Procedure

Sausages were assigned positions in the oven as shown

in Figure 1. Tagged ends were positioned nearest to the door. The thermocouples of a Honeywell model Electronik 15 recorder were inserted into the tagged ends of each sausage. All cooking was done in a Blodgett model CTB-1 convection oven, preheated to 54°C. Cooking for both groups of four sausages followed the schedule in Table V. After cooking, the bologna sticks were immersed in an ice water bath for 10 minutes. Following removal from the ice water, each bologna was towel dried, wrapped in Loxol freezer paper and stored for 48 hours at 1°C.

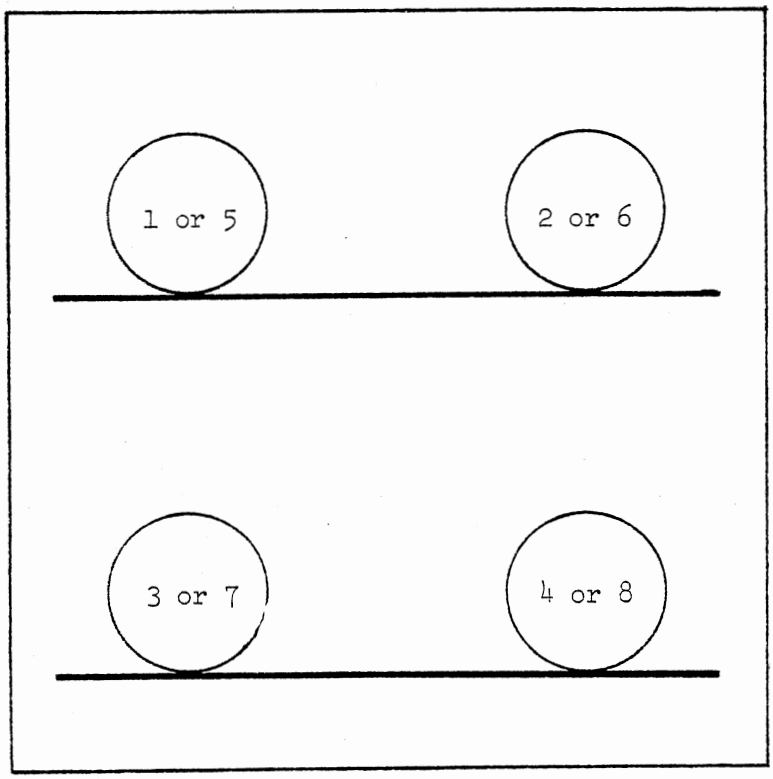

Figure 1. Oven Placement for Sausage Cooking

TABLE V

BOLOGNA COOKING SCHEDULE

Oven Temperature	Time
54°C	1 hour 20 minutes
66°C	1 hour 00 minutes
77°C	Until the average internal temperature of all four sausages was 67°C

Raw Emulsion Sample Preparation

The eight raw emulsion samples of approximately 100 grams were placed in a single 12 ounce labled Whirl-Pak bag immediately following sausage stuffing. The bag was kneaded well to insure adequate mixing and stored at 1°C prior to the removal of 24 subsamples for emulsion stability tests and four subsamples for pH evaluation (usually within 20 minutes). After sampling for pH and emulsion stability, the bag was stored at -20°C prior to removal of 30 subsamples for lipid and moisture analyses and four subsamples for protein analyses.

Cooked Sausage Sample Preparation

After 48 hours storage at 1°C, the cooked sausages were unwrapped, weighed, peeled and sliced into one centimeter thick slices using a Sanitary model S-4 meat slicer.

A single slice was removed from the center of each quadrant of each sausage for color evaluation and shear testing. The slices were labeled A, B, C and D with the A slice being that which was removed from the quadrant nearest the oven door during cooking, as illustrated in Figure 2. The A, B, C and D slices were wrapped in Loxol freezer paper, labeled and stored one hour at 1°C prior to color and shear testing to allow temperature equilibration. The remaining slices from each sausage were then ground in a Sunbeam Le Chef food processor equipped with a shredding head. The ground cooked sausage was hand mixed, placed in individual plastic bags and stored at 1°C prior to removal of four subsamples for pressed fluid tests. Four 1.25 gram samples of the ground bologna were also removed from each bag at this time to make four composite pH tests. The bagged emulsions were frozen at -20°C until lipid, moisture and protein determinations were completed.

Emulsion Stability

The emulsion stability of the raw sausage was determined by the method of Saffle, Christian, Carpenter and Zirkel (1967).

Extractable Lipid

All analyses of extractable lipid were determined by the method of Horwitz (1970), as modified by Falk (1974). The modification was the substitution of non absorbent cotton for sand.

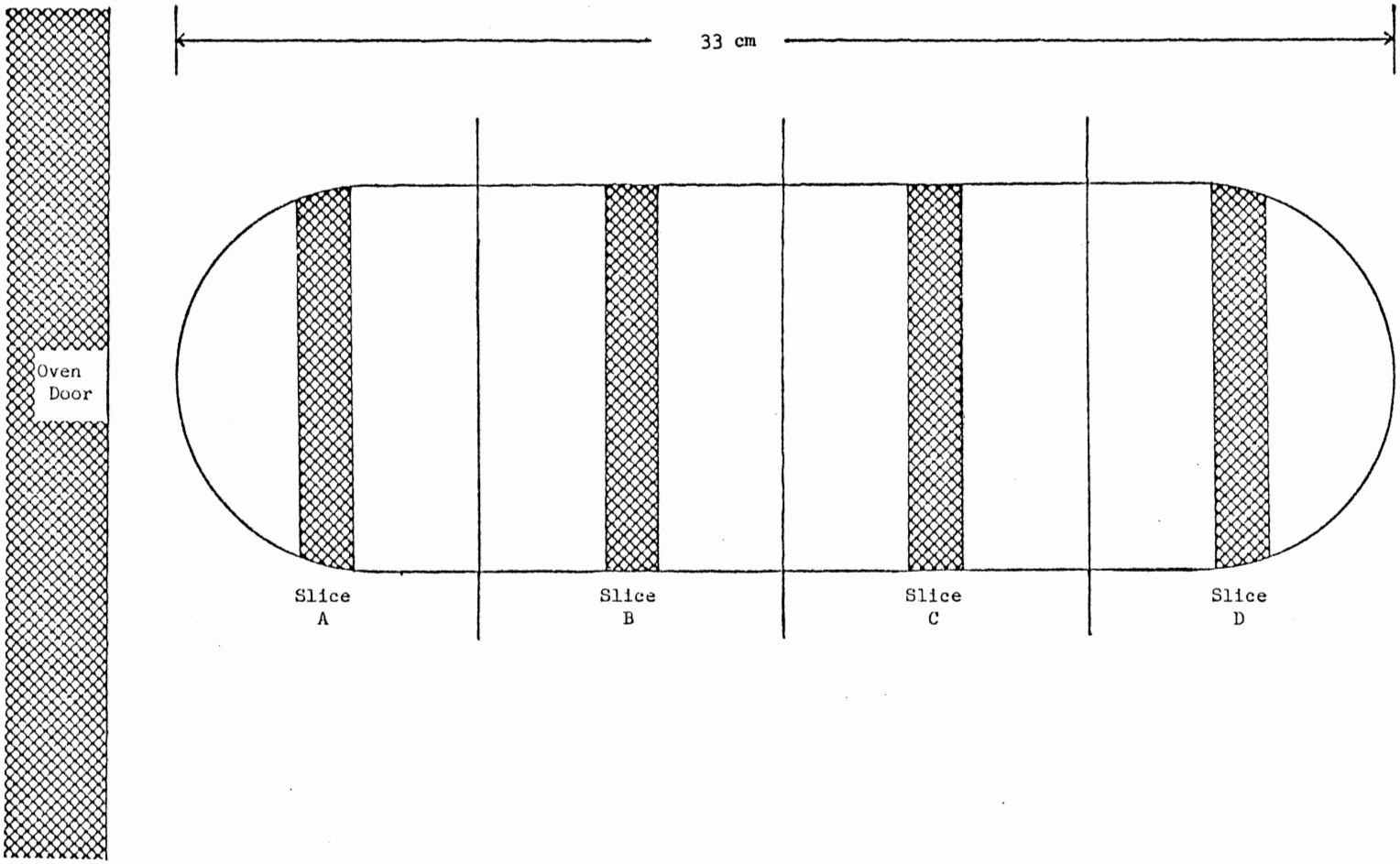

Figure 2. Position of Slices Used for Color and Shear Evaluations

Ash

The muffle furnace method for determining ash content of meat was used (Horwitz, 1970).

Crude Protein

All analyses for crude protein except those performed on the lean and fatty tissues were determined by the macro Kjeldahl titration method as modified by Schefer (1969). Crude protein analyses of the lean and fatty tissues were determined by the micro Kjeldahl Nesslerization method of Bock and Benedict as outlined by Hawk (1954).

pH

pH was determined by the method of Sebranek (1978), using a Corning model 130 pH meter.

Moisture

The method of Horwitz (1970) for determining the moisture content of meat by oven drying was used.

Cooking Loss

Cooking loss was determined by the method of Schalk (1980), using a Mettler PL 1200 electronic balance.

Color

The four quadrant center slices removed from each sausage were evaluated for color with a Hunter Lab

colorimeter model D25M/L-9 calibrated to a white porcelain and a red-brown ceramic standard as described by Hunter (1976). A three millimeter thick glass plate (provided by Hunter Labs) was positioned over each slice to prevent pillowing. Results were recorded as L, a, b values.

Shear Value

Peak shear force for the four quadrant center slices was determined by the method of Schalk (1980), with two modifications: The chart speed was set at 20 millimeters per minute and the samples were prepared by placing them in a 1°C room for two hours. Results were recorded as kilograms of peak shear force.

Pressed Fluids

The water holding capacity of the cooked sausage was determined 51 hours after sausage manufacture by the method of Grau and Hamm (1953) as modified by Urbin, Darrel and Wilson (1962). Results were expressed according to the method of Sayre, Briskey and Hoekstra (1963).

Statistical Analysis

A completely randomized design was used for the analysis of the data generated by the proximate analysis of lean and fatty tissues, the classification being lots. Two observations were made of the sample removed from each lot for lipid, moisture, crude protein and ash content.

A completely randomized design was used for all analyses of the raw emulsion, the pH of the lean and the pH of the cooked emulsion. This was based on the four levels of collagen replacement.

The statistical analyses of the cooked sausage tests were done using a split-plot design. Main-plot treatments for cooking loss, crude protein, lipid, moisture and pressed fluid content were the four replacement levels. Main plot treatments were in a completely randomized design. Subplot treatments were cooking order (first or second cooking) and position in the oven (1, 2, 3 or 4).

The data generated by the tests on the four quadrant center slices from each sausage (color and peak shear force) were analyzed using a split split-plot design. Main-plot treatments were the collagen replacement levels and again they were in a completely randomized design. Subplot treatments were cooking order and oven position. Sub subplot treatments were the four slice positions within each sausage (A, B, C or D).

The completely randomized and split-plot designs are described by Snedecor and Cochran (1967). Calculations were performed using the Statistical Analysis System of Barr and Goodnight (1972).

CHAPTER IV

RESULTS AND DISCUSSION

Lean and Fatty Tissues

Chemical analyses were performed on each subsample taken from the 37 portions of lean and 12 portions of fatty tissue to determine the effectiveness of mixing and to facilitate bologna formulation. The analyzed data revealed no significant differences ($P>0.41$) in the lipid, crude protein, moisture or ash content of the fatty tissue. The 37 portions of lean tissue also showed no significant differences ($P>0.36$) between portions when evaluated in the same manner. All bologna formulations were therefore based on the mean analytical values for the lean and fatty tissues and on the composition of the bovine hide collagen as reported by Schalk (1980).

Two lean tissue portions were used to manufacture each batch of sausage. They were (hand) mixed prior to being added to the formulation. Four ten gram samples were then randomly removed from the combined portions and evaluated for pH in the manner previously described. No significant difference ($P=0.38$) was found in the mean hydrogen ion concentration of the lean tissue portions used. The mean hydrogen ion concentration resulted in pH values

of 5.65, 5.58, 5.52 and 5.50 for the 0.0, 4.7, 8.9 and 12.8% collagen replacement levels, respectively. This was felt to be important since many of the functional properties of the lean tissue (e.g., water binding) are pH dependent (Van Eerd, 1972). By establishing the raw material lots to be similar, any difference in the functional properties between collagen replacement levels could then be attributed to influences other than pH of the lean or the composition of the lean or fatty tissue.

Raw Emulsions

The bologna containing the four collagen replacement levels (0.0, 4.7, 8.9 and 12.8%) were formulated to have identical percents of lipid, crude protein and moisture. Chemical analyses were performed on each raw sausage emulsion to verify the formulation accuracy. When analyzed on the basis of collagen replacement level, a mean lipid content of 20.14, 19.73, 19.87 and 19.60 percent was determined for the 0.0, 4.7, 8.9 and 12.8 percent collagen replacement products, respectively. Differences were determined to be nonsignificant ($P=0.56$). An evaluation of the raw emulsion for moisture content provided similar results. The 0.0, 4.7, 8.9 and 12.8 percent collagen replacement products had mean moisture content of 62.17, 62.29, 62.30 and 62.30, respectively. Differences were again deemed nonsignificant ($P=0.79$). The crude protein content of the raw emulsions was also evaluated. On the

basis of collagen level, differences in protein content were not found to be significant (P=0.42). The 0.0, 4.2, 8.9 and 12.8 percent collagen replacement levels had respective crude protein contents of 14.07, 14.26, 14.18 and 14.16 percent. Lipid and moisture analyses were based on 30 observations from each batch of raw emulsion. Crude protein analysis was based on four observations of each batch of raw emulsion. This established that the formulation was uniform.

Emulsion stability testing revealed significant differences (P<0.0001) between the four levels of collagen replacement based on 24 observations of each batch of raw emulsion. The zero percent replacement level, with a mean stability value of 0.77 (read as percent fat released), was significantly more stable than the other three levels. The 12.8 percent collagen replacement level, with a mean stability value of 2.86, was significantly less stable than the other three levels. The 4.7 and 8.9 percent collagen bologna, with mean stability values of 1.84 and 1.57, respectively, were not significantly different from each other. This agreed with the finding of Saffle et al. (1964) that collagen tends to destabilize meat emulsions.

The reason that the 12.8 percent replacement product had a less stable, but an acceptable stability rating is probably due to the small fraction of soluble collagen in the collagen used (Schalk, 1980). It is the soluble fraction of the collagen, not the absolute amount, that

adversely affects emulsion stability (Wiley, Reagan, Carpenter and Campion, 1979). Saffle et al. (1967) reported that stability values up to 4.00 were acceptable.

Four 10 gram samples of each batch of raw emulsion were evaluated for pH. No significant differences (P=0.80) were found in the mean hydrogen ion concentrations of the four replacement levels. The mean hydrogen ion concentration provided pH values of 5.78, 5.79, 5.78 and 5.79 for the 0.0, 4.7, 8.9 and 12.8% collagen replacement bologna, respectively.

Cooked Sausage

After cooking and cooling, bologna sticks were weighed, peeled and sliced. Selected slices were removed for color and shear testing. The remainder was ground to facilitate composition analysis. Before composition analysis was begun, four composite samples of 10 grams each were evaluated for pH. No significant differences (P=0.88) were found in the mean hydrogen ion concentrations of the four replacement levels. The mean hydrogen ion concentrations resulted in pH values of 6.07, 6.09, 6.04 and 6.06 for the 0.0, 4.7, 8.9 and 12.8 percent collagen replacement bologna, respectively.

Determinations of the crude protein content of cooked bologna showed no significant differences between the collagen replacement levels (P=0.12). The 0.0, 4.7, 8.9 and 12.8 percent replacement levels each had a mean protein

content of 14.77, 14.96, 14.76 and 14.71 percent, respectively. This was based on two observations of each of the 24 sausages manufactured at each of the four replacement levels. Differences due to cooking order (P=0.27) and oven position (P=0.51) were also deemed nonsignificant. Interactions of the effects of collagen replacement with cooking order (P=0.12) and collagen replacement with oven position (P=0.13) also caused no significant differences in protein content. There was, however, a significant difference due to the interaction of the effect of cooking order with that of oven position (P=0.03). This interaction is illustrated in Figure 3. As can be seen, changing from first to second cooking order resulted in a decrease in the percent protein for positions one and two. The same change resulted in an increase in protein content for positions three and four. This trend should be accompanied by an opposite trend in the effects of the interaction of cooking order and oven position on moisture or lipid content. In fact, no such trend existed. It was reasoned, therefore, that this significance resulted from a low number of observations and some random sampling error. A similar occurrence is believed to have been the cause of the significant three-way interaction of collagen level, cooking order and oven position (P=0.03).

The moisture content of the cooked sausages was not significantly affected by collagen replacement (P=0.82). The mean moisture content of the 0.0, 4.7, 8.9 and 12.8

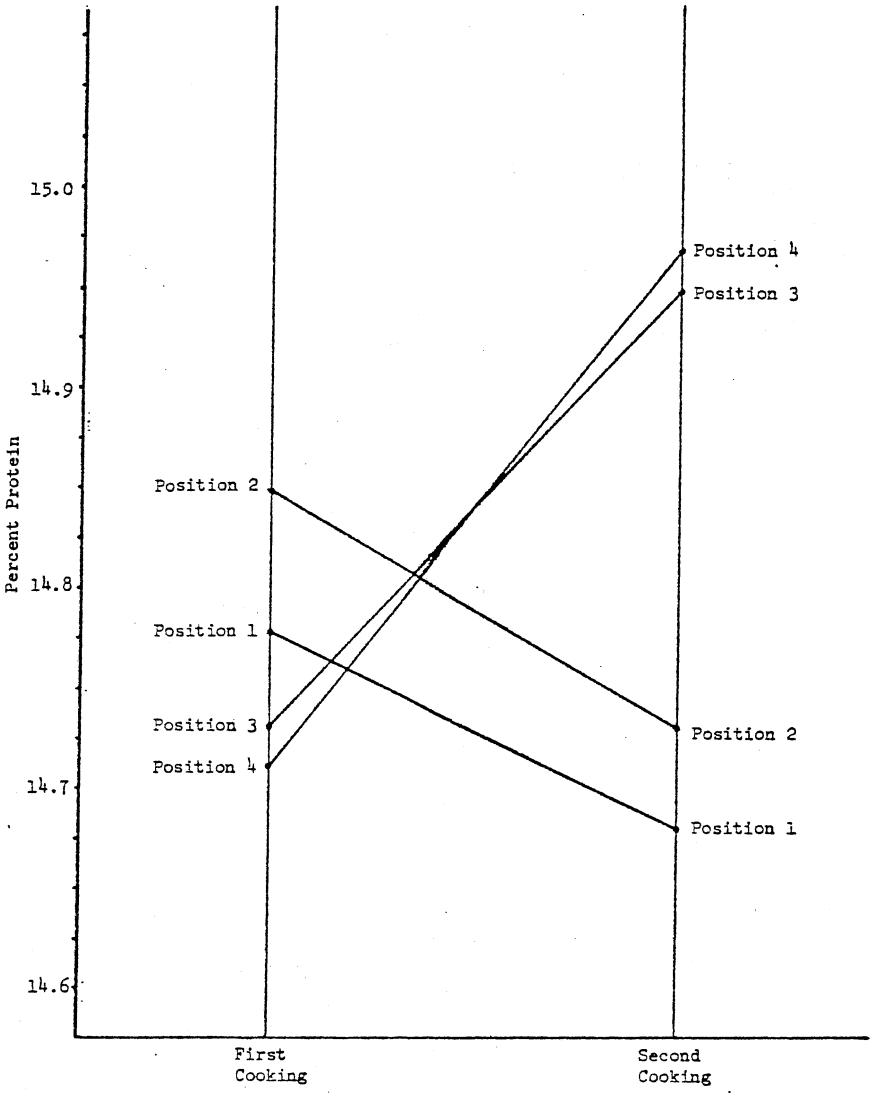

Figure 3. Effect of Cooking Order--Oven Position Interaction on Protein Content of Cooked Bologna

collagen replacement products was 60.18, 60.28, 60.12 and 60.21 percent, respectively. This was based on four observations of each of the 24 sausages manufactured at each replacement level. Differences in the moisture content due to the cooking order (P=0.28) and oven position (P=0.76) were not significant. Two-way interactions of the treatments of cooking order with oven position (P=0.60), collagen level with cooking order (P=0.07) and collagen level with oven position (P=0.26) produced nonsignificant differences. The three-way interaction of collagen level, cooking order and oven position also did not produce significant differences (P=0.53) in moisture content.

Evaluation of the lipid content of the cooked sausages showed results similar to those for moisture. The mean lipid content, based on collagen replacement was 20.75, 20.65, 20.59 and 20.66 percent for the 0.0, 4.7, 8.9 and 12.8 percent levels, respectively. This followed the same sampling scheme as that used for moisture content. Collagen replacement (P=0.85), cooking order (P=0.97) or oven position (P=0.09) did not cause significant differences in the percent lipid of the cooked sausages. Lipid content was also unaffected by the interaction of the treatments of cooking order with oven position (P=0.25), collagen level with cooking order (P=0.39) or collagen level with oven position (P=0.38). No significant differences in lipid content existed due to the three-way interaction of the treatments of collagen replacement, cooking order and oven position (P=0.90).

The percent weight loss of sausages upon cooking was not found to be significantly affected by collagen replacement ($P=0.16$), cooking order ($P=0.31$) or oven position ($P=0.09$). This was based on the observation of the weight loss of each of the 24 sausages manufactured at each of the four collagen levels. The respective mean cooking loss for the 0.0, 4.7, 8.9 and 12.8 percent replacement levels was 5.78, 6.04. 6.12 and 6.00 percent. There were also no significant effects due to any two-way interactions ($P>0.10$) of these three treatments. There was, however, a significant difference ($P=0.02$) due to the three-way interaction of collagen replacement, cooking order and oven position.

Collagen replacement of lean tissue had no significant effect ($P=0.08$) on the pressed fluid content (a measure of water holding capacity) of the bologna. This was based on four observations per sausage. The mean pressed fluid content was 47.68, 46.18, 43.03 and 44.40 percent for the 0.0, 4.7, 8.9 and 12.8 percent levels, respectively. The cooking order also exhibited no significant effect ($P=0.68$) on pressed fluid content. The treatment of oven position showed a significant effect ($P=0.02$) on the pressed fluid content of the sausages. Sausages in positions 1, 2, 3 and 4 had mean pressed fluid contents of 45.92, 45.44, 44.18 and 45.74 percent, respectively. A similar, but nonsignificant trend in the cookloss due to oven position seems to support this. As previously stated, the pressed fluid content is a measure of water holding capacity.

Also, water holding capacity is an indirect measure of protein denaturation (Grau and Hamm, 1953). It was therefore reasoned that the sausages in position three (the deviant in both instances) were exposed to a more gentle heat increase, resulting in less protein denaturation and more water retention.

The interaction of the effects of collagen replacement and cooking order also produced significant differences ($P=0.003$) in pressed fluid content. It was reasoned that the high waterbinding capacity of collagen became important to sausages that underwent holding periods prior to cooking. No other two-way or three-way interactions of the treatments of collagen replacement, cooking order or oven position had a significant effect ($P>0.30$) on the pressed fluid content of sausages.

Slice Evaluations

The four slices removed from each of the 24 cooked sausages at each level were used for textural and color evaluations. Slices were first evaluated for color. Color was scored by the L, a, b system as prescribed by Hunter (1976). Values for the lightness-darkness (L), redness-greenness (a) and yellowness-blueness (b) were analyzed separately. This analysis was based on collagen replacement level, cooking order, oven position and slice position within the bologna stick.

There were no significant differences in the L values of color scores due to collagen replacement (P=0.13) or oven position (P=0.74). The respective L values for 0.0, 4.7, 8.9 and 12.8 percent collagen replacement products were 44.80, 44.82, 45.81 and 44.67, respectively. There were, however, significant differences in the lightness-darkness values for the slices due to the separate effects of cooking order (P=0.0001) and slice position (P=0.0001). It is believed that the differences due to cooking order are related to the significant effects found in the interactions of cooking order with other treatments involving cooked sausage evaluations. It is possible that sausages held in cold storage prior to the second cooking underwent changes (e.g., protein denaturation) due to presence of salt (Johnson and Henrickson, 1970). The significant change (P=0.002) in lightness due to the interaction of collagen level and cooking order seems to support this. The effect of slice position on lightness-darkness is believed to be caused by the rate of heat penetration into the bologna sticks. As can be seen in Figure 2, heat penetration would not affect all four slices evenly. The interaction of slice position and cooking order also produced significant differences (P=0.03) in L values. The reason for this is believed to be a combination of the aforementioned factors. No other interactions of the four treatments resulted in significant effects (P>0.25).

The redness-greenness (a) values were not significantly ($P>0.51$) affected by collagen replacement, cooking order or oven position. The respective a values for the 0.0, 4.7, 8.9 and 12.8 percent replacement levels were 10.70, 10.72, 10.44 and 10.19. There was a significant difference in a values due to slice position within each sausage. Those slices furthest from the oven door (slices C and D) were redder, with mean a values of 10.38 and 10.43. This is probably due to higher air turbulence resulting in increased heat penetration at the back of the oven. This disagrees somewhat with the more plausible finding by Schalk (1980) that the end slices were redder. There was also a significant ($P=0.0049$) difference in the redness-greenness values resulting from the three-way interaction of collagen level, cooking order and oven position. The cause of this is unknown. All other interactions of the treatments of collagen replacement, cooking order, oven position and slice position produced nonsignificant difference ($P>0.09$) in mean a values.

The b values (yellowness-blueness) for color were significantly affected ($P=0.02$) by slice position within the bologna sticks. Slice A, with a mean b value of 8.71, is more yellow than the other three slices. This tends to agree with the findings for a values that color effects show differences between the A and B versus the C and D slices, rather than the A and D (end slices) versus the B and C (center slices). The treatments of collagen

replacement (P=0.24), cooking order (P=0.13) and oven position (P=0.56) had nonsignificant effects on b values, as did the interactions of the same treatments (P>0.22). The 0.0, 4.7, 8.9 and 12.8 percent levels had mean b values of 8.49, 8.55, 8.61 and 8.74, respectively.

The texture of the cooked sausage was evaluated by shearing cores of selected slices and recording the kilograms of peak force required. A significant difference in peak shear force resulted from the treatments of collagen replacement (P=0.04), cooking order (P=0.0001), oven position (P=0.006), slice position (P=0.0001) and the interaction of collagen replacement and cooking treatments (P=0.0001). The mean peak force required to shear area was 16.16, 16.91, 20.93 and 22.30 kilograms for the 0.0, 4.7, 8.9 and 12.8 percent colalge replacement products, respectively. As can be seem from the data, higher levels of collagen increase the kilograms of peak force required to shear slices. This would seem to be inconsistent with the reported effects of collagen on sausage, but in fine emulsion bologna the opposite may be true. It was reasoned that since the collagen added to the bologna in this study was of a fibrous nature and had a low soluble fraction (Schalk, 1980), it could easily increase the force required to shear slices. The collagen is incorporated into the aqueous phase of the emulsion, thereby being included in the web of myofibrillar proteins. Cooking would fix the insoluble collagen in the three dimensional

overlap of myosin molecules. The high tensile strength would help strengthen the cooked product. This effect would not be expected in a coarse meat product (e.g., meatloaf) where the aqueous phase was not continuous and little of the myosin had been extracted.

Mean peak force required to shear was lower for slices from second-cooking sausages. The reason for this is believed to be the protein denaturation cited for the change in lightness-darkness values. As stated, there was a significant effect due to the slice position within each sausage. Positions A, B, C and D had respective mean peak shear values of 19.84, 18.92, 18.80 and 18.74 kilograms. This is the same A to D trend that occurred in the color evaluations. It was reasoned that the heat effect believed to be responsible for the color differences also affected the texture of the sausages.

Peak shear force was also affected by oven position. Bologna slices from the top two positions (one and two) had mean shear values of 19.33 and 19.32 kilograms. Slices from positions three and four had mean shear values of 18.81 and 18.85 kilograms. It is unknown why this occurred. Trends in other tests do not tend to support these findings.

There was a significant effect on texture due to the interaction of collagen replacement and cooking order ($P=0.0001$). This is believed to be related to the significant effects in cooked sausage tests that also involve

interactions with the treatment of cooking order. No other interactions of the treatments of collagen replacement, cooking order, oven position or slice position had a significant effect on the peak shear force ($P=0.11$).

It is important to note that none of the aforementioned changes in the bologna were obvious upon casual inspection. Color changes due to slice position were not detectable with the naked eye and collagen-caused texture changes were unnoticed in day to day tasting of the bologna slices.

CHAPTER V

SUMMARY AND CONCLUSIONS

The nonlipid fraction of an all beef fine emulsion bologna was replaced with bovine hide collagen at 0.0, 4.7, 8.9 and 12.8 percent levels. Significant differences in the texture and emulsion stability resulted from collagen replacement. Differences in lipid, moisture, protein, cookloss, pressed fluid and color due to collagen replacement were deemed nonsignificant.

Emulsion stability remained in acceptable ranges, even at the highest replacement level. An increase in peak force required to shear slices had the beneficial effect of increasing the "bite" of the bologna product. The minimal change in the functional properties is attributed to the low fraction of soluble collagen in the hide product and the relatively low oven temperatures employed.

There were significant effects due to the cooking order, oven position, slice position and interactions of these treatments throughout this study. Most of these involved cooking order in some way. It was suggested that the raw sausages may have undergone protein denaturation during storage. Color and texture studies were also highly affected by slice position, but in a manner

inconsistent with previous reports. Several interactions did not show trends that could be explained relative to other findings.

In conclusion, the use of bovine hide collagen in fine emulsion bologna would seem to be acceptable at levels up to and including 12.8 percent of the nonlipid fraction. Hide collagen did not affect the composition and the textural change is an improvement. Decreases in emulsion stability, while significant, are not critical and should present few problems to the sausage manufacturer. It is suggested that additional studies be undertaken to determine the maximum collagen replacement level that would result in a stable emulsion. Further, sensory panel studies should be initiated to determine if the significant effects on the color and texture of the product are humanly detectable.

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APPENDIXES

APPENDIX A

ANALYSES OF VARIATION

TABLE VI

ANALYSIS OF VARIATION FOR PERCENT ASH--LEAN TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	73	0.003058			
Lots	36	0.001567	0.000044	1.08	0.4125
Error	37	0.001491	0.000040		

TABLE VII

ANALYSIS OF VARIATION FOR PERCENT LIPID--LEAN TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	73	6.72804			
Lots	36	2.20600	0.061278	0.50	0.9718
Error	37	4.52204	0.122217		

TABLE VIII

ANALYSIS OF VARIATION FOR PERCENT MOISTURE--LEAN TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	73	95.1641			
Lots	36	34.7061	0.964060	0.59	0.9493
Error	37	60.4580	1.633999		

TABLE IX

ANALYSIS OF VARIATION FOR PERCENT PROTEIN--LEAN TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	73	190360.2			
Lots	36	79056.0	2196.00	0.73	0.8760
Error	37	111304.2	3008.22		

TABLE X

ANALYSIS OF VARIATION FOR PERCENT ASH--FATTY TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	23	0.005595			
Lots	11	0.001218	0.000111	0.30	0.9698
Error	12	0.00437	0.000364		

TABLE XI

ANALYSIS OF VARIATION FOR PERCENT LIPID--FATTY TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	23	0.973328			
Lots	11	0.400928	0.036448	0.76	0.6675
Error	12	0.572400	0.047700		

TABLE XII

ANALYSIS OF VARIATION FOR PERCENT MOISTURE--FATTY TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	23	0.039613			
Lots	11	0.021421	0.001947	1.28	0.3600
Error	12	0.018192	0.001516		

TABLE XIII

ANALYSIS OF VARIATION FOR PERCENT PROTEIN--FATTY TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	23	0.018333			
Lots	11	0.007133	0.000649	0.69	0.7289
Error	12	0.011200	0.000933		

TABLE XIV

ANALYSIS OF VARIATION FOR HYDROGEN ION CONCENTRATION--LEAN TISSUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	3.726×10^{-10}			
Collagen	3	1.127×10^{-10}	3.756×10^{-11}	1.16	0.3846
Error	8	2.599×10^{-10}	3.249×10^{-11}		

TABLE XV

ANALYSIS OF VARIATION FOR PERCENT LIPID--RAW EMULSION

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	65.6380			
Collagen	3	14.2862	4.7621	0.74	0.5564
Error	8	51.3518	6.4190		

TABLE XVI

ANALYSIS OF VARIATION FOR PERCENT MOISTURE--RAW EMULSION

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	8.4578			
Collagen	3	0.9762	0.3254	0.35	0.7919
Error	8	7.4816	0.9352		

TABLE XVII

ANALYSIS OF VARIATION FOR HYDROGEN ION CONCENTRATION--RAW EMULSION

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	1.292×10^{-10}			
Collagen	3	1.445×10^{-11}	4.815×10^{-12}	0.33	0.8009
Error	8	1.148×10^{-10}	1.435×10^{-11}		

TABLE XVIII

ANALYSIS OF VARIATION FOR PERCENT PROTEIN--RAW

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	0.7851			
Collagen	3	0.2238	0.0746	1.06	0.4169
Error	8	0.5613	0.0702		

TABLE XIX

ANALYSIS OF VARIATION FOR EMULSION STABILITY

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	172.884			
Collagen	3	161.128	53.709	36.55	0.0001
Error	8	11.756	1.470		

TABLE XX

ANALYSIS OF VARIATION FOR HYDROGEN ION CONCENTRATION--COOKED BOLOGNA

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	11	3.570×10^{-11}			
Collagen	3	2.746×10^{-12}	9.153×10^{-13}	0.22	0.8781
Error	8	3.296×10^{-11}	4.120×10^{-12}		

TABLE XXI

ANALYSIS OF VARIATION FOR PERCENT PROTEIN--COOKED BOLOGNA

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	191	29.90225			
Among Main Units	11				
C	3	1.79661	0.59887	2.65	0.1202
Error (a)	8	1.80941	0.22617		
Within Main Units	180				
O	1	0.20410	0.20410	1.24	0.2704
P	3	0.38786	0.12928	0.78	0.5114
OP	3	1.58412	0.52804	3.19	0.0297
CO	3	0.99436	0.33145	2.01	0.1222
CP	9	2.43719	0.27079	1.64	0.1263
COP	9	3.44403	0.38267	2.32	0.0269
Error (b)	56	9.25205	0.16522		
Residual	96	7.99250	0.08326		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven.

TABLE XXII

ANALYSIS OF VARIATION FOR PERCENT MOISTURE--COOKED BOLOGNA

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	176.19125			
Among Main Units	11				
C	3	1.26737	0.42246	0.31	0.8170
Error (a)	8	10.81164	1.35146		
Within Main Units	372				
O	1	0.73028	0.73028	1.19	0.2801
P	3	0.71548	0.23849	0.39	0.765
OP	3	1.17588	0.39196	0.64	0.5977
CO	3	4.62831	1.54277	2.51	0.0669
CP	9	7.10429	0.78936	1.28	0.2657
COP	9	5.42896	0.60322	0.98	0.5337
Error (b)	56	34.43346	0.61488		
Residual	288	109.89558	0.381582		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven.

TABLE XXIII

ANALYSIS OF VARIATION FOR PERCENT LIPID--COOKED BOLOGNA

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	168.07019			
Among Main Units	11				
C	3	1.33081	0.44360	0.26	0.8527
Error (a)	8	13.65284	1.70660		
Within Main Units	372				
O	1	0.00093	0.00093	0.002	0.9661
P	3	3.71171	1.23723	2.27	0.0892
OP	3	2.29437	0.76479	1.40	0.2506
CO	3	1.68914	0.56304	1.03	0.3862
CP	9	5.38169	0.59796	1.10	0.3801
COP	9	2.22461	0.24718	0.45	0.8993
Error (b)	56	30.53330	0.54523		
Residual	288	107.25079	0.37239		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven.

TABLE XXIV

ANALYSIS OF VARIATION FOR COOKING LOSS

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	95	20.09438			
Among Main Units	11				
C	3	1.55889	0.51963	2.23	0.1615
Error (a)	8	1.86157	0.23269		
Within Main Units	84				
O	1	0.16170	0.16170	1.05	0.3108
P	3	1.03672	0.34558	2.24	0.0920
OP	3	1.00429	0.33476	2.17	0.1001
CO	3	0.33297	0.11099	0.72	0.5470
CP	9	1.99323	0.22147	1.44	0.1941
COP	9	3.51910	0.39101	2.54	0.0160
Error (b)	56	8.62590	0.15403		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven.

TABLE XXV

ANALYSIS OF VARIATION FOR PRESSED FLUID

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	7751.63435			
Among Main Units	11				
C	3	1188.49612	396.16537	3.32	0.0773
Error (a)	8	953.94479	119.24310		
Within Main Units	372				
O	1	3.06378	3.06378	0.18	0.6803
P	3	178.24758	59.41586	3.40	0.0235
OP	3	46.73570	15.57857	0.89	0.5463
CO	3	277.33591	92.44530	5.29	0.0031
CP	9	118.86857	13.20762	0.76	0.6583
COP	9	190.22753	21.13639	1.21	0.3075
Error (b)	56	978.94187	17.48110		
Residual	288	3815.77250	13.24921		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven.

TABLE XXVI

ANALYSIS OF VARIATION FOR
L COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	273.602966			
Among Main Units	11	172.114522	15.646775		
C	3	83.458884	27.819628	2.51	0.1323
Error (a)	8	88.655638	11.081955		
Within Main Units	84	41.635044	0.495655		
O	1	10.036267	10.036267	28.82	0.0001
P	3	0.446578	0.148859	0.43	0.7377
OP	3	0.226458	0.075486	0.22	0.8846
CO	3	6.245819	2.081940	5.98	0.0016
CP	9	1.130478	0.125609	0.36	0.9486
COP	9	4.050965	0.450107	1.29	0.2610
Error (b)	56	19.498479	0.348187		
Within Subunits	288	59.853400	0.207824		
S	3	19.880693	6.626898	48.02	0.0001
CS	9	1.434922	0.159436	1.16	0.3239
OS	3	1.276781	0.425594	3.08	0.0274
PS	9	1.578920	0.175435	1.27	0.2522
OPS	9	0.488285	0.054254	0.39	0.9377
Error (c)	255	35.193799	0.1380149		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven; S=Slice Position.

TABLE XXVII

ANALYSIS OF VARIATION FOR
a COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	196.486866			
Among Main Units	11	65.887922	5.989811		
C	3	17.646514	5.882171	0.98	0.5476
Error (a)	8	48.241408	6.030176		
Within Main Units	84	34.005295	0.404825		
O	1	0.168338	0.168338	0.49	0.5059
P	3	0.369978	0.123326	0.36	0.7860
OP	3	1.726042	0.575347	1.67	0.1821
CO	3	1.371215	0.457072	1.33	0.2735
CP	9	1.593382	0.177042	0.51	0.8582
COP	9	9.509748	1.056639	3.07	0.0049
Error (b)	56	19.266592	0.344046		
Within Subunits	288	96.593649	0.335395		
S	3	5.539080	1.846360	5.82	0.0011
CS	9	4.593997	0.510444	1.61	0.1126
OS	3	2.058977	0.686326	2.16	0.0915
PS	9	2.357399	0.261933	0.82	0.5944
OPS	9	1.104135	0.122682	0.39	0.9408
Error (c)	255	80.940061	0.317412		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven; S=Slice Position.

TABLE XXVIII

ANALYSIS OF VARIATION FOR
b COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	74.464237			
Among Main Units	11	7.943421	0.722129		
C	3	3.109161	1.036387	1.72	0.2404
Error (a)	8	4.834260	0.604282		
Within Main Units	84	17.948341	0.213671		
O	1	0.519940	0.519940	2.30	0.1309
P	3	0.470744	0.156915	0.70	0.5622
OP	3	0.417784	0.139261	0.62	0.6108
CO	3	0.408851	0.136284	0.60	0.6193
CP	9	0.784794	0.087199	0.39	0.9367
COP	9	2.703188	0.300354	1.33	0.2420
Error (b)	56	12.643040	0.225768		
Within Subunits	288	48.572474	0.168654		
S	3	1.627763	0.542588	3.28	0.0213
CS	9	1.173892	0.130432	0.79	0.6296
OS	3	0.612544	0.204182	1.23	0.2977
PS	9	1.996742	0.221860	1.34	0.2162
OPS	9	0.916977	0.101886	0.62	0.7851
Error (c)	255	42.244556	0.165665		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven; S=Slice Position.

TABLE XXIX

ANALYSIS OF VARIATION FOR PEAK SHEAR FORCE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	383	4916.10372	377.59378		
Among Main Units	11	4153.53154	377.59378		
C	3	2596.99341	865.66447	4.45	0.0405
Error (a)	8	1556.53813	194.56727		
Within Main Units	84	413.33468	4.92065		
O	1	157.72190	157.72190	95.31	0.0001
P	3	23.24341	7.74780	4.68	0.0057
OP	3	1.77529	0.59176	0.36	0.7866
CO	3	112.37695	37.45898	22.64	0.0001
CP	9	14.05065	1.56118	0.94	0.5036
COP	9	11.49294	1.27699	0.77	0.6440
Error (b)	56	92.67354	1.65488		
Within Subunits	288	349.23750	1.21263		
S	3	76.54216	25.51405	27.28	0.0001
CS	9	13.63357	1.51484	1.62	0.1093
OS	3	0.34820	0.11607	0.12	0.9450
PS	9	12.74690	1.41632	1.51	0.1425
OPS	9	7.48336	0.83148	0.89	0.5361
Error (c)	255	238.48331	0.93523		

Note: C=Collagen Replacement Levels; O=Cooking Order; P=Position in Oven; S=Slice Position.

APPENDIX B

MEANS

TABLE XXX

MEAN VALUES OF COOKED COLLAGEN-BOLOGNA PARAMETERS AS INFLUENCED BY COLLAGEN LEVELS

Collagen Level[1]	Cookloss[3] (%)	Lipid[4] (%)	Moisture[4] (%)	Crude Protein[5] (%)	pH[2,6] (Cooked)	Pressed Fluid[4] (%)	Peak Shear[4] (kg)
0	5.78	20.75	60.18	14.77	6.08	47.67	16.16
5	6.04	20.65	60.28	14.96	6.08	46.18	16.91
10	6.12	20.59	60.12	14.76	6.04	43.03	20.93
15	6.00	20.66	60.21	14.71	6.08	44.40	22.30

[1]Nonlipid Replacement Level.

[2]Based on the mean hydrogen ion concentration.

[3]Based on 24 observations at each replacement level.

[4]Based on 96 observations at each replacement level.

[5]Based on 48 observations at each replacement level.

[6]Based on 12 observations at each replacement level.

TABLE XXXI

MEAN VALUES OF RAW COLLAGEN-BOLOGNA PARAMETERS AS INFLUENCED BY COLLAGEN LEVELS

Collagen Level	Lipid[2] (%)	Moisture[2] (%)	Protein[3] (%)	Emulsion Stability[4]	pH[3] (raw)
0.0	20.14	62.17	14.07	0.77	5.78
4.7	19.73	62.29	14.26	1.84	5.79
8.9	19.87	62.30	14.18	1.57	5.78
12.8	19.60	62.30	14.16	2.86	5.79

[1]Nonlipid Replacement Level

[2]Based on 90 observations at each replacement level.

[3]Based on 12 observations at each replacement level.

[4]Based on 72 observations at each replacement level.

TABLE XXXII

MEAN VALUES FOR COOKED COLLAGEN-BOLOGNA COLOR TESTS

Collagen Level[1]	L Color Value[2]	a Color Value[2]	b Color Value[2]
0.0	44.80	10.70	8.49
4.7	44.82	10.72	8.55
8.9	45.81	10.44	8.61
12.8	44.67	10.19	8.74

[1]Nonlipid Replacement Level.

[2]Based on 96 observations at each replacement level.

VITA

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