THE USE OF BOVINE HIDE COLLAGEN

IN COARSE BOLOGNA

By

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CHAPTER I

INTRODUCTION

A need to develop all third world nations has led to the exporting of labor intensive industries, among them leather. This action has caused the hide market to experience unstable and exorbitant prices for the "raw" hides needed to manufacture leather goods. This problem has encouraged research in the use of hide for products other than leather. The USDA's Eastern Regional Research Center in Pennsylvania, developed a food grade collagen from the flesh split of young bovine hide. All hides are alkaline and acid treated before being processed into five separate collagen products which vary in particle size, shape, and density. The water content of the collagen also varies according to the process used. It is anticipated that new markets may open when this food grade product is commercially introduced, and may help to alleviate the unstable hide market and meet the need of an alternative food-grade protein for developing nations.

At the present, research is being conducted concerned with the use of bovine hide collagen in a wide variety of products, some of which include bread, muffins, cookies, meatloaf, and hamburger. Since the diet of the developing nations is primarily grain based, bovine hide collagen may have a use in the protein fortification of grain based products; however, it is an incomplete protein therefore its use may

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be limited. In the United States fibrous collagen's greatest potential lies in the meat industry, specifically in the area of processed meats. It can be added to processed meats as an extender and the product can be labeled as an all beef product. Collagen occurs in conventional processed meats since it is found in varying amounts in muscle depending upon type of muscle, age, sex, and species. It is the predominant protein of connective tissues. The use of bovine hide collagen in processed meats is a new concept. Collagen might be expected to have little if any benefit in sausage emulsions due to its ability to gelatinize after exposure to heat at approximately 80°C. However, some reports suggest that collagen exhibits the same water binding and fat binding characteristics as myosin and actin, the primary proteins that stabilize emulsions in conventional processed meats.

The objective of this study was to determine the effect of bovine hide collagen on coarse bologna. The fat, moisture, freewater, crude protein, shrinkage, emulsion stability, amount of soluble collagen and color of the processed product were studied. These areas were chosen since they are deemed most important to the sausage industry.

CHAPTER II

REVIEW OF LITERATURE

Collagen Review

Collagen is a protein found in biological systems where it is utilized as a structural material for a wide variety of cells and membranes. The basic molecular unit of collagen is composed of a triple helix containing three similar, but not identical, polypeptide chains (Wang and Vieth, 1973; Lehninger, 1975). The collagen fiber is about 2800°A long, with a diameter of about 14°A, and a molecular weight of about 300,000 (Wang and Vieth, 1973). The molecule is rod-like in shape and the three polypeptide chains termed alpha-chains, are wound around each other to form a three-strand fiber and each alpha-chain has about 1050 amino acid residues (Miller, 1976).

Collagen contains nineteen different alpha-amino acids, thirty-three percent of which are glycine. The glycine residues are located at every third position along the polypeptide chain (Gustavson, 1956; Wang and Vieth, 1973; Lehninger, 1975). Approximately twenty-five percent of the amino acid residues are proline and hydroxyproline (Gustavson, 1955; Lehninger, 1975; Stryer, 1975). Of this combination hydroxyproline compromises about thirteen percent of the total amino acid content (Lehninger, 1975; Schut, 1976; Piez, 1976). Collagen also contains a very rare amino acid hydroxylysine. Unlike hydroxyproline,

the hydroxylsine content of collagen is highly variable from tissue to tissue depending on the type of collagen involved (Miller et al., 1967; Butler, 1968; Barnes et al., 1974). There are four major types of collagen (type I, II, III, and IV) depending on the tissue involved, and there are subdivisions within each type (Piez, 1976). The hydroxylysine content will vary from 0.5 percent to 4.5 percent depending on the type of collagen involved (Bornstein and Wolfie, 1979). The hydroxylysine and hydroxproline residues stablize the collagen molecule by the formation of covalent linkages. Two kinds of cross-links are formed in the collagen fiber; intramolecular and intermolecular. The intramolecular cross-links in collagen are derived from lysine and hydroxylysine sidechains (Stryer, 1975). The hydroxyproline stabilizes through the formation of interchain hydrogen crosslinks with keto-imide groups (Gustavson, 1955). The hydroxylysine residues in collagen serve as sites of attachments of galactosyl and galatosylglucose moieties (Bornstein and Wolfie, 1979).

Most of the amino acids in collagen are considered nonessential. Collagen is deficient in cysteine and tryptophan. It has been determined to have a PER (Protein Efficiency Ratio) of <0 and contains seventeen grams of essential amino acids per one hundred grams of amino acid residue (Happich et al., 1975). The NPU (Net Protein Utilization) of beef with varying amounts of collagen in the cuts was investigated using rats as study animals (Bender and Zia, 1976). Beef shanks that contained twenty-four percent collagen had an NPU value of sixty-nine compared to loin eye that contained two and one half percent collagen, which had an NPU of eighty-two. Other investigations indicated that the

content of the individual amino acids as well as the total available essential amino acids in meat decrease with increasing amounts of connective tissue (Dvorak and Vognorova, 1969). A meat cut or processed meat product containing more than 28.5% collagen has an undesirable PER (Lee et al., 1978).

Collagen is characterized by undergoing a sharply defined thermal shrinkage at a specified temperature, which is characteristic of the species at a given age. Prolonged heat treatment above the thermal shrinkage temperature converts collagen to soluble gelatin (Kramlick et al., 1973). It has been reported that at a temperature of 70°C or higher, the endomysial sheath remained intact, but swelling and crimping of the endomysial sheath were evident. Degradation of collagen fibers in the perimysium was initiated at 70°C and intense disintegration was observed at 80°C (Gross, 1964; Field et al., 1970a; Kamlich et al., 1973; Cheng and Parrish, 1976; Schut, 1976). The amount of heat extractable collagen in meat has an effect on the tenderness of the cut. The location of the collagen and the age of the animal influences the amount of soluble collagen. When the percent of soluble collagen in the epimysium was compared to percent of heat extractable collagen in intramuscular connective tissue, much larger amounts of soluble collagen were found in the epimysium. During cooking, less collagen is solubilized in meat from older animals than in meat from younger animals due to an increase in cross-linkages in the older tissues (Goll et al., 1964; Hill, 1966; Field et al., 1970b; Williams and Harrison, 1978). There are three fractions of collagen; (1) the fraction soluble in dilute salt solutions, (2) the fraction soluble in citrate buffer, and (3) the insoluble collagenous fraction. In the earlier

stages of collagen fibril formation the soluble forms of collagen can be extracted using the aforementioned procedure. However, as the tissue develops even these reagents become ineffective and an insoluble form of collagen is formed due to extensive cross-linkages (Bowes et al., 1955; Bowes et al., 1957; Reed et al., 1963; Bowes and Raistrck, 1968).

Manufacture of Bovine Hide Collagen

The bovine skin is divided into three distinct layers: (1) the epidermis, a thin outer layer of epithelial tissue; (2) the true leather-forming derma, a much thicker layer of connective tissue; and (3) the subcutaneous tissue known as the "flesh" which attaches the skin to the main body (Gustavson, 1956). A process was developed by the USDA's Eastern Regional Research Center in which five different comminuted products from (18-24 months) bovine hide trimmings and flesh splits are produced. This process requires the limed hides to be acidified to the isoelectric point of collagen. The acidified hides are then comminuted in successive stages, utilizing a strip cutter, a rotary knife cutter, and an Urschel Comitrol high speed cutter grinder. A disc mill is utilized to shear hide particles at different stages of comminution.

Early work in the process demonstrated that limed hides could be successfully ground through an 0.023 centimeter head of the Comitrol with little change in the percent denaturation in product number one (Komanowsky et al., 1974). However, the manufacture of the other four products using limed hides involves a greater danger of denaturation unless excessive amounts of water are added to reduce friction. For this reason an acidification step is involved so less water is required.

The comminuted collagen products include very little denaturation and are chemically and microbiologically acceptable for use in human foods and animal feeds. The collagen has an adjusted pH of 5.3 and ranges in moisture content from seventy-eight to eighty-seven percent (Turkot et al., 1978).

Product number one that was utilized in this study has an average moisture content of seventy-eight percent and has an estimated factory selling price of ninety-two to thirty cents per pound depending on the cost of the lime splits and the steps involved (Turkot et al., 1978). The hide collagen is presently being used in sausage casing (Elias et al., 1970). Slurries with high solids content might be used in foods as meat binders and/or extenders, vegetable protein texturizers, or components of high protein synthetic meat (Elias et al., 1970).

Theory of Sausage Emulsions

Until recently sausage processing was considered more of an art than a science. However, with the growth of the processed meat industry a need to understand the principles of sausage emulsions became apparent. Schut (1976) described a sausage emulsion as a, "two-phase system, consisting of a dispersion of a solid in a liquid in which the solid is not miscible with the liquid." The liquid phase is made up of salts and proteins in an aqueous solution, and it also serves as a medium in which the insoluble proteins and connective tissue are dispersed. The fat molecules make up the solid phase.

Hansen (1960) reported that salt extractable myofibrillar proteins, myosin and actomyosin appear to concentrate at the fat globule surface

and form a stabilized membrane. Swift et al. (1961) observed that the salt extractable proteins were more effective than those extracted by water. The participation of the water soluble proteins was dependent on the addition of NaCl, and required the addition of an unacceptable level of NaCl before they were functional in the emulsion. Swift and Sulzbacher (1963) reported that pH and NaCl content are extremely critical factors affecting the capacity of meat to stabilize emulsions. It is the poor retention of fat that produces problems in comminuted meat products. Hegarty et al. (1963) reported similar results to Hansen's (1960) in that myosin and actomysin produced emulsions with superior stability. It was also observed that the amount of protein utilized in the formation of an interface appeared to be related to the stability of an emulsion. It is the shape of the myosin molecule which increases the viscosity of the emulsion and spreads a protein film over more fat surface area (Carpenter and Saffle, 1965). The myosin molecule consists of a heavy meromyosin "head" and a light meromyosin "tail." The "head" is hydrophilic and the "tail" is hydrophobic. Due to this property the "tail" of the meromyosin molecule is oriented toward the fat particle while the "head" is oriented toward the liquid phase of the emulsion (Schut, 1976).

There has been work in the area of comparing animal proteins with plant proteins to determine their ability to stabilize an emulsion. Crenwelge et al. (1974) noted that hemoglobin when compared with soy, cottonseed, and non-fat dry milk proteins had the highest viscosity and greater emulsifying ability at a lower concentration of proteins to oil. While the ability of a protein to stabilize an emulsion by encapsulating fat particles is of great importance, the interaction of

the protein with water is also of interest since it will directly
effect the proteins emulsifying ability. Chou and Morr (1979)
reported that water interacting with a protein molecule exhibits
different properties from those of "free" water. Protein functional
properties such as swelling, solubility, gelation, and water holding
capacity are directly related to the manner in which the protein
interacts with water. The area of functional properties of proteins
in sausage emulsions is still relatively new and progress in this area
is still to be made.

Functional Properties of Collagen

The use of collagen in sausage has been discouraged in the past
due to its ability to gelatinize after being heated above 70°C
(Schut, 1976). This gelatinization of the collagen was thought to
have caused emulsion instability and breakdowns. Boedtker and Doty
(1956) reported that the viscosity of collagen drops to about four
percent of its original value upon thermal denaturation. Saffle et
al. (1964) using pork skins in sausage emulsions observed that the
"peeling score" was lowest for the emulsion containing the pork skins
with an initial smokehouse temperature of 54°C. However, he also
indicated that the second-best panel mean peeling score was for the
emulsion containing pork skin but processed at 81°C. He hypothesized
that at the higher initial cooking temperature the time needed to form
gelatin wasn't sufficient. Maucer and Baker (1966) found that the
collagen content of poultry meat was a reliable estimator of emulsifying
capacity when dealing with meat and skin mixtures. Collagen was detri-
mental to the process of making poultry meat emulsions due to the

inability of collagen to dissolve and form stabilizing membranes needed for emulsion formation. Connective tissue proteins (collagen) utilize water-binding mechanisms similar to those used by myofibrillar proteins (Wiley et al., 1979). He further reported that gel-pocket formation in frankfurter-type sausages was minimal when the product was processed in the temperature range of 65-80°C. Satterlee et al. (1973) used beef or pork skin hydrolyzates to replace non-fat dried milk in a sausage formulation and reported that they produce a sausage with a slightly greater water- and fat-holding ability. Even though the emulsion capacity of the various skin hydrolyzates is slightly lower than the capacity of NFDM (on a per 100 mg protein basis), the greater protein content of the hydrolyzates gave the sausage emulsion improved stability during cooking, when compared to an emulsion containing NFDM (Satterlee et al., 1973). The skin from bovine is reported to be eighty-seven percent protein of which approximately seven percent is noncollagenous protein the major protein fraction of which resembles serum albumin (Cooper et al., 1967).

It was observed by Cerny et al. (1970) that reported changes in the viscosity of collagen were age related. Collagen from young animals have less cross linkages and showed a greater overall change in viscosity when exposed to heat but had a greater viscosity value than collagen from older animals. It was thus hypothesized that it was the amount of soluble collagen that determines its functional properties in a sausage emulsion and not the total amount of collagen. Peptide bonds of collagen which have received alkaline pretreatment and have thus been exposed to swelling forces should be capable of functioning

as centers of hydration (Gustavson, 1956). While this is accepted, it is the percentage of soluble collagen that is observed to be highly related to the binding characteristics of meat. The more soluble the collagen the greater the binding characteristics when compared to the increase in insoluble collagen (Carpenter et al., 1979; Wiley et al., 1979).

CHAPTER III

MATERIALS AND METHODS

Bovine Hide Collagen

Bovine hide collagen from the USDA's Eastern Regional Research Center in Philadelphia, Pennsylvania, was utilized in the study. Product number one, as described by Turkot et al. (1978), having an average moisture content of 78.2% was chosen. Komanowsky et al. (1974) found this product to have the greatest density of the five prepared collagen products. The density of this product closely corresponds with the density of lean to be replaced. The collagen was manufactured in July, 1978, and delivered to the Meat Science Laboratory, sealed in number ten size cans in a frozen state, in August, 1979. The cans were stored at -20°C. The collagen was then removed as needed and thawed by placing it in a 3.3°C room for 48 hours. Thawed collagen was then placed in a Buchner funnel in an 8°C room for 30 minutes to allow the excess moisture to drain.

Experimental Ground Beef

Ground beef used in these experiments was from a Hereford cow of approximately canner/cutter grade. The animal was delivered to the university abattoir 24 hours prior to slaughter, and was slaughtered in the conventional manner. After a 48 hour shrinkage period the

carcass was boned in the conventional manner. The "cold" boned meat was then ground through a 1.27 centimeter plate using a Biro grinder. The ground meat was mixed in a 45.4 kilogram Reliance sausage mixer for two minutes. Five samples were obtained from the meat in the mixer using a core 2.54 centimeter in diameter sampler to determine the fat, moisture, protein, and ash content of the beef. The sampling procedure was repeated four times until a final yield of 168 kilograms of product was obtained. The product was then divided into thirty-seven 4.54 kilogram units, double wrapped in freezer paper, and a number was assigned to each unit (1 through 37). The wrapped units were stored at -20°C until needed. Sample units were randomly removed (by selection of a number 1-37) from the freezer two at a time, and thawed for 48 hours in a 3.3°C room before preparing experimental sausages.

Sausage Preparation

Grinding was done at 21°C while all other work was accomplished in a 8°C room. Twenty-four hours prior to preparation two Reliance sausage mixers were cooled in the 8°C room. Two randomly drawn (based on the flip of a coin) ground beef units were used in the preparation of sausage containing 0% and 7.5% collagen or 15% and 22.5% collagen (collagen substituted for lean meat at 0%, 10%, 20% and 30% levels). The units were then weighed using an Electronic Model MKII digital balance to the nearest hundredth of a pound. The amount of ground beef corresponding to the desired percentage of collagen was removed and the appropriate amounts of wet collagen and fat tissue were added (see Table I). The collagen, fat and lean tissue were mixed by hand using a plastic spatula, in separate stainless steel pans.

The pans were removed from the 8°C room one at a time and the collagen-ground beef mixture was ground twice through a 0.32 centimeter plate utilizing a Biro grinder. The grinder was cleaned between each batch. The ground mixture was then returned to the 8°C room and placed into one of the cooled Reliance mixers. Spices and water were added (see Table II) and seasoned emulsion mixed for five minutes. The resulting emulsion was then stored for 22 hours at 3.3°C, in covered stainless steel pans to allow the spices and salt to distribute throughout the mixture.

TABLE I

FORMULATION FOR COLLAGEN-GROUND BEEF MIX

| Collagen | 25/75 Fat-Lean Ratio Before Addition of Water-In Grams* | | |
	Collagen Added	Ground Beef	Fat Tissue Added
0	0.	4471.90	68.10
7.5	340.50	4063.30	136.20
15.0	681.00	3586.60	272.40
22.5	1021.50	3132.60	385.90

*1026.04 grams of 5°C water were added to each batch.

TABLE II

INGREDIENTS*

Salt	136.0 grams
White Pepper	16.8 grams
Coriander	5.6 grams
Allspice	2.8 grams
Sage	2.8 grams
Garlic Powder	.7 grams
Onion Powder	1.4 grams
Sodium Nitrite	.45 grams
Erythorbate	2.50 grams
Water	1026.04 grams

*Added to 4.54 kilograms of ground beef.

Stuffing and Cooking Procedure

The sausage mixtures were stuffed into number 2.5 Union Carbide fibrous casings using a 11.35 kilogram Vogt hand stuffer. The stuffed uncooked sausages were then marked with water soluble red and blue ink (see Figure 1) to facilitate the removal of samples for color and texture studies after thermal processing and identify the sausages. The front (the end first stuffed) and back end of the sausages were also marked to insure the sausages were placed in the oven in the same manner. Care was taken to number the sausages in the order they were stuffed (one through four). The sausages were then randomly placed (see Figure 2) by drawing numbers, in a Blodget convection oven. The internal temperature of each sausage was recorded using a Honeywell
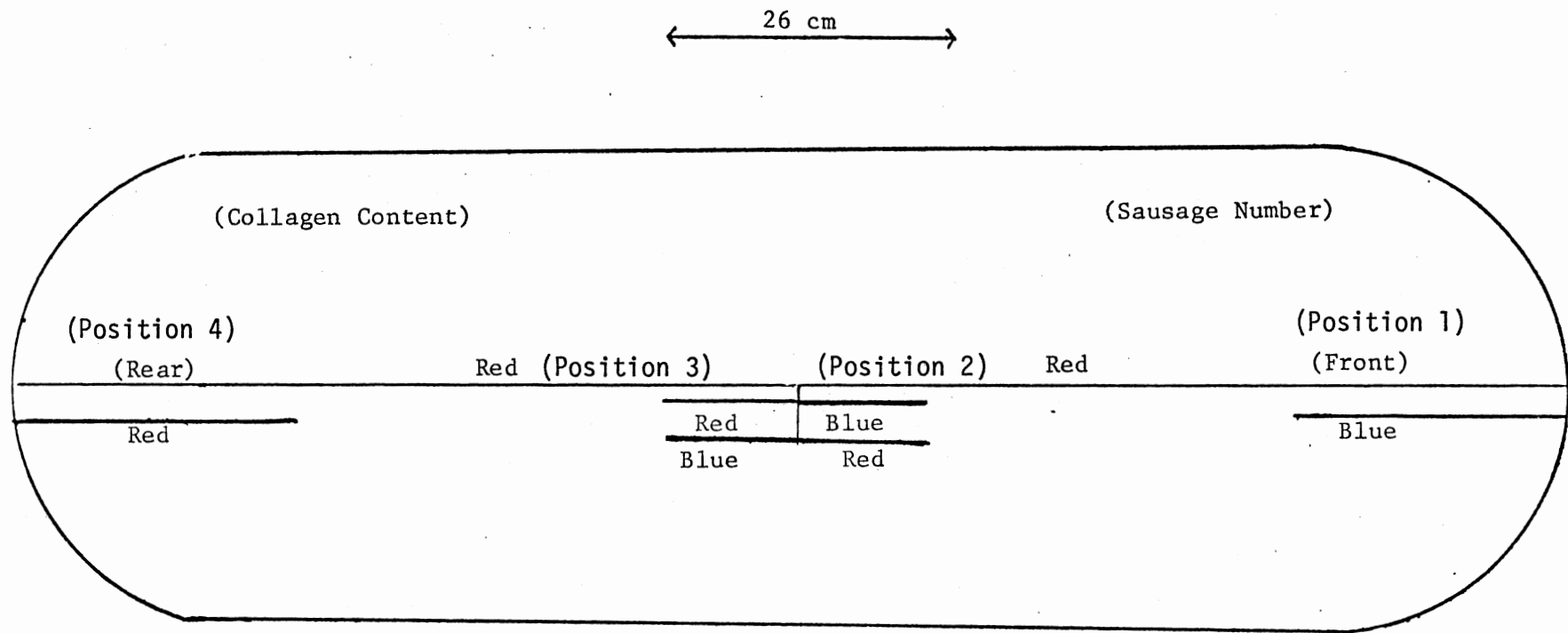
Figure 1. Sausauge Marking Scheme Used for Oven Placement and Sample Identification

17
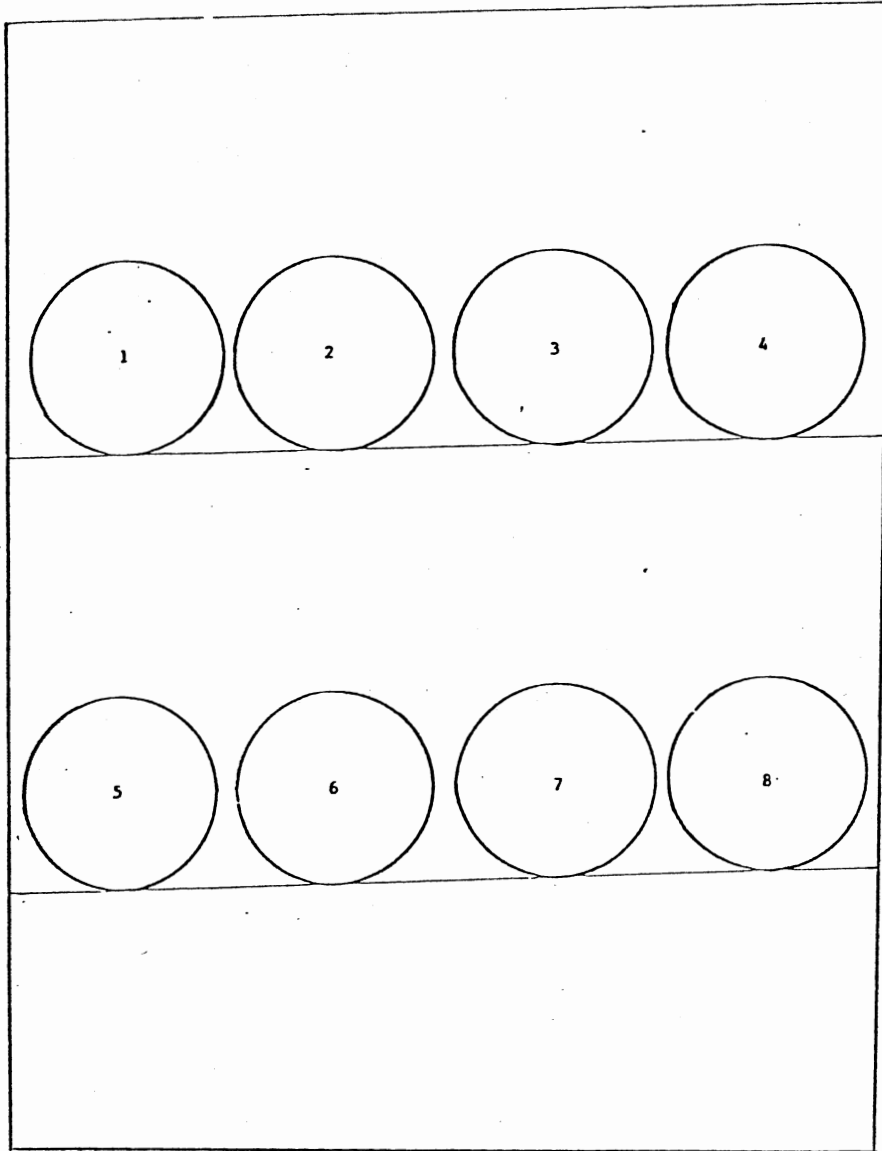
Figure 2. Oven Placement Used for Random Sausage
Placement

Recorder with thermocouples. A four hour cooking schedule was followed (see Table III) until an internal temperature of 68°C was reached. All sausages were then removed and placed in a 4.4°C water bath for one hour. The bath was 30.54 centimeters deep, 61.08 centimeters wide and 61.08 centimeters long. After cooling, they were dried with a towel and placed in a 3.3°C cooler for 16 hours.

TABLE III

COOKING SCHEDULE

Temperature (°C)	Time (Minutes)
54.4	90
65.5	60
71.1	60
76.6	30

Sampling Scheme and Preparation

The raw samples were all randomly removed using a transparent grid square marked with 30.25 centimeter numbered divisions (see Figure 3) placed over a pan containing the mixture. Samples of cooked sausage for the color and texture measurements were removed after the volume test using a Sanitary Meat Slicer in a manner illustrated in Figure 4. The remaining sausage was diced into approximately .65 square centimeter

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	...				
				...	48

Figure 3. Transparent Grid Square Used to
Remove Raw and Cooked Blended
Samples
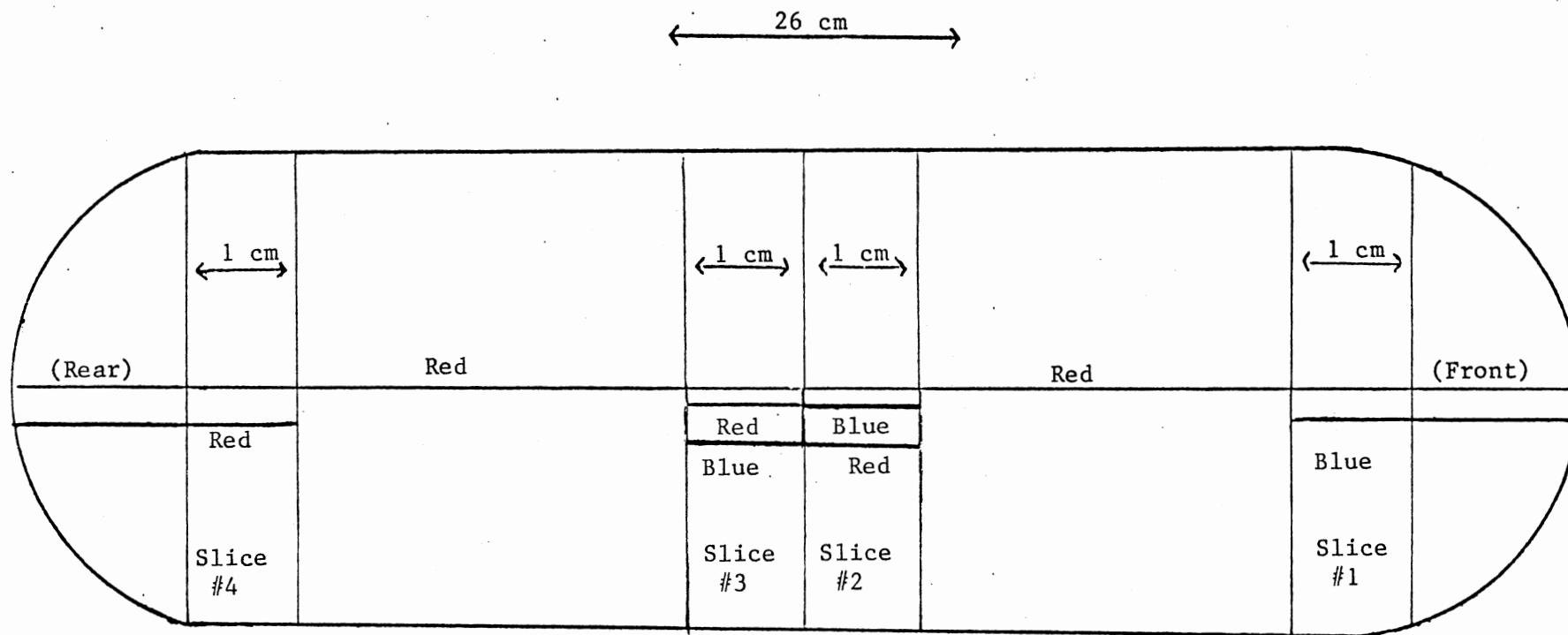
Figure 4. Sample Removal Scheme for Color and Texture, Sample Removal After Cooking

sections, placed in a Waring Blender and blended for 30 seconds. The sides of the blender were scraped and the mixture was reblended for 30 seconds. The scraping step was repeated a second time for a total blending period of 90 seconds. The blended cooked sausage was then placed in a plastic pan and a conventional home mixer was used to mix the blended sausage for one minute. A grid square, smaller than the previously mentioned grid, was placed over the pan and a 9 square centimeter sample was randomly chosen from each sausage. The remaining blended sausage from the four sticks in each collagen level was placed in a stainless steel pan and mixed for two minutes with a conventional home mixer, and random samples were chosen. The raw samples were prepared according to methods for meat products (Horwitz, 1970). After an adequate sample was removed for analysis the remaining sample was sealed in Whirl-Pak bags and stored at -20°C.

Shrinkage

Shrinkage was determined by taking pre- and post-cooked weights of each sausage using a Mettler PL1200 Balance. This was done to determine the percent cooking loss for each treatment level. The formula for calculating percent cooking loss was:

$$\frac{A-B}{A} \times 100 = \text{Percent Cooking Loss (Shrinkage)}$$

A = Raw Sausage Weight

B = Cooked Sausage Weight

Volume Change

Change in the volume of the sausage was noted in the preliminary

study of collagen in sausage, on visual inspection. A 10.2 centimeter diameter cylinder was constructed to determine the volume loss by water displacement as by Archimedes (287BC-212BC). The cylinder was constructed of plastic pipe, sealed at one end,which measured 50.8 centimeters in length and had an opening 37.5 centimeters up from the bottom to allow the displaced water to drain (see Figure 5). The cylinder was filled with 5°C water until it flowed out the drain opening. After waiting until the water stopped flowing, a sausage was placed in the cylinder and the water was collected in a plastic bottle, sealed and weighed on an Electronic Scale Model MKII. Pre- and post-cooked weights were taken on the water displaced for each sausage, to compare the difference in percent volume loss. The formula for calculating the percent volume loss was:

$$\frac{A-B}{A} \; X \; 100 = \text{Percent Volume Loss}$$

A = Raw Sausage Volume

B = Cooked Sausage Volume

Moisture

The percent moisture for both the raw and the cooked samples was determined by the oven drying method of meat (Horwitz, 1970).

Fat Analysis

Chemical fat analysis was determined by the method of Horwitz (1970) as modified by Falk (1974), the modification being the use of non-absorbent cotton.
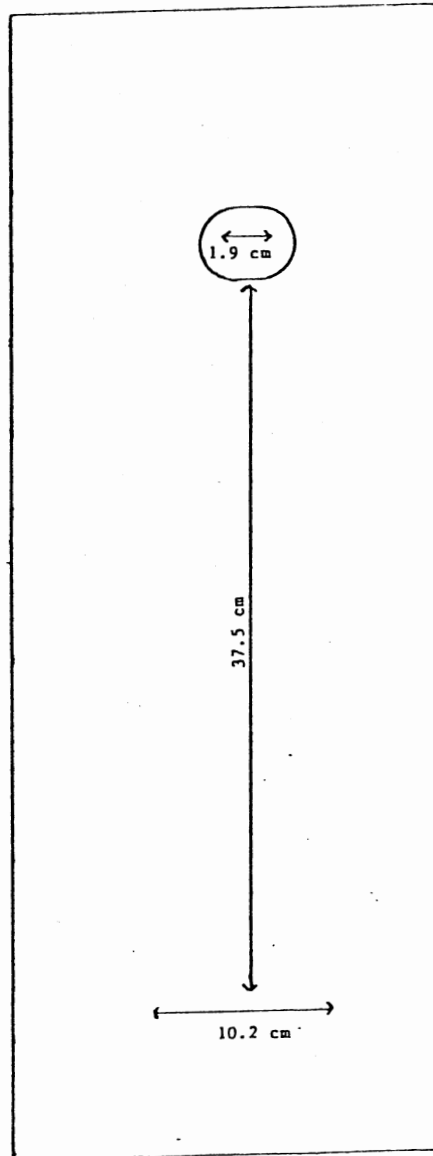
Figure 5. Water Displacement
Tank Used to
Determine Volume
Change

Pressed Fluids

The amount of free water or "juice" retaining capacity of the cooked sausage chilled 16 hours was measured by the filter-paper moisture-absorption technique of Grau and Hamm (1953) as modified by Urbin et al. (1962) and was expressed as the ratio of total area to meat-film area (Sayre et al., 1963).

Color Difference

Difference in the color of the cooked bologna among the four collagen levels, within the levels and within each sausage was examined using a Hunter Lab Tristimulus Colorimeter. The method used to evaluate the color using L, a, and b values was described by Hunter (1976) for bologna products.

Crude Protein

The amount of crude protein was determined by the Kjeldahl method as modified by Schefer (1969).

Emulsion Stability

Stability of the raw product was determined using the Georgia test for emulsion stability from Saffle et al. (1967).

Texture Studies

A table model, Instron Universal Testing Instrument Model 1122, was utilized. The chart speed was set at 200 mm per minute and the crosshead speed at 50 mm per minute. A L.E.E. Kramer Shear Cell was

used. Four samples from each sausage were tested for texture after being analyzed for color. The samples were prepared by placing them in a 3.3°C room for 24 hours. Four and eight hundreths centimeters in diameter core samples one centimeter thick were removed and the resistance force met by the cell was expressed as kilograms of peak force as illustrated in Figure 6. The peak force represents force exerted to shear the 4 1/8 centimeter in diameter core.

Collagen Analysis

The amount of soluble collagen, insoluble collagen and the total collagen were analyzed. Five cans of the product number one collagen were randomly chosen. The collagen was thawed in a 3.3°C room for 48 hours, and drained for 30 minutes in a Buchner funnel. Collagen from each can was placed in separate stainless steel pans and mixed and random samples were drawn. The samples were mixed in an Omni-Mixer for 30 seconds. Samples were then stored at -20°C until needed. Hydroxyproline determinations were made by heating four grams of the mixture in one-fourth strength Ringer's solution for 77 minutes in a 70°C water bath according to the procedures of Hill (1966). Values for hydroxyproline were determined for the soluble and insoluble fractions employing the procedures outlined by Hill (1966). Values for total collagen (mg/gm), soluble collagen, and insoluble collagen were obtained by multiplying the hydroxyproline content of the supernatant plus residue, supernatant alone, and residual along fractions, respectively, by 7.25 (Goll et al., 1964).

The fat, moisture, and ash content were determined by the methods of Horwitz (1970). The crude protein was determined by the Kjeldahl
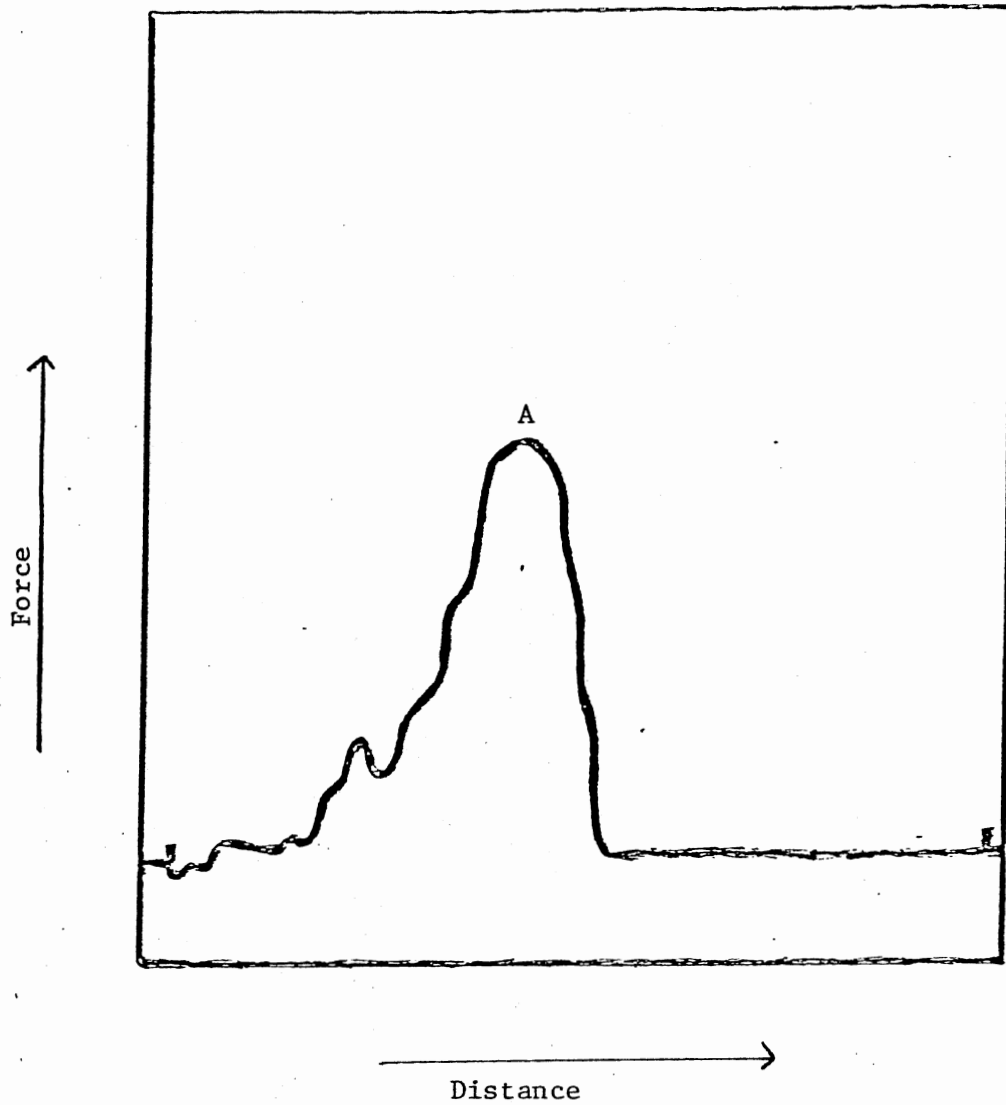
Figure 6. An Instron Reading Depicting the Peak Force, A, Required to Shear a 4 1/8 Centimeter Core

method of Schefer (1969). The percent protein was calculated by multiplying the percent nitrogen by 5.55 which is the conversion factor used for gelatin (Pomeranz and Meloan, 1978).

Statistical Analyses

In this study all the 0% and 7.5% collagen levels were cooked together and were identified as A-low, and all the 15% and 22.5% collagen levels were cooked together and were identified as A-high. The collagen levels within cookings were referred to by the factor b; i.e. B-low is 0% or 15% and B-high is 7.5% or 22.5%. Since the 0% and 7.5% levels were always cooked together and the 15% and 22.5% levels were always cooked together, a split plot design was used. This split plot design involved AB interaction. AB interaction determines if the B-low to B-high means at A-high and the B-low to B-high means at A-low values run in parallel lines when plotted on a graph. This split plot design was used for shrinkage, volume change, fat percentage, moisture percentage, and free water percentage. In each of these parameters, there were eight cookings. Each cooking consisted of eight sausages from either A-low (0% and 7.5%) or A-high (15% and 22.5%), four sausages from each level in A-low or A-high. Within each of these levels there were four observations (B-low and B-high) and the AB interaction. The split plot design was used for the color and texture studies. The A, B, and AB interaction as described above was used; however, it also involved four observations from each sausage, from each level within each cooking. The position of each observation within each sausage was important. Therefore, the interaction of A with position,

and interaction of B with position was determined. Crude protein evaluation involved the split plot design A-low (0% and 7.5%), A-high (15% and 22.5%), B-low (0% and 15%), B-high (7.5% and 22.5%), and AB interaction. There were eight cookings. Each cooking consisted of eight sausages, four from each level in A-low or A-high. There was one observation from each level within each cooking.

Emulsion stability was analyzed according to a one-way classification design, classification being based on the four collagen levels (0%, 7.5%, 15%, and 22.5%). The samples were randomly drawn before the product was cooked by using a grid square. The randomly drawn samples from each level were then analyzed and consisted of one observation from each level of collagen in the eight cookings.

Analysis of variance for the product number 1 collagen consisted of a one-way classification design; this one-way classification was based on arbitrarily assigning each can a number (1 through 5). There were nine observations from each can, and they were analyzed for fat, crude protein, ash, moisture, soluble collagen, insoluble collagen, and total collagen. This analysis was done to determine if variability was being introduced into the product from a parameter within the collagen.

The split plot design and one-way classification are described by Steel and Torrie (1960). Calculations for analysis of variance for color and textural studies were performed using the Statistical Analysis System (Barr et al., 1976).

CHAPTER IV

RESULTS AND DISCUSSION

Shrinkage Evaluation

Results of the percent shrinkage of the cooked product, as deter-
mined by weight difference, indicate there is no significant (P=.85)
difference between the two cookings (A-low and A-high). The A-low
cooking (0% and 7.5%) had a mean value of 7.38% shrink while A-high
(15% and 22.5%) had a mean value of 7.49% shrink. The percent shrink
within the B levels (B-low concerns 0% or 15% and B-high concerns
7.5% or 22.5%) was insignificant (P=.59). In the B-low range (within
the low levels 0%-15%) the 0% collagen level had a mean value of
7.19% and the 15% level had a mean value of 7.51%. B-high (within the
high levels 7.5%-22.5%) values were 7.58% shrinkage for the 7.5% level
and 7.47% for the 22.5% level of added collagen. The AB interaction of
the shrinkage means was found to be insignificant (P=.20) (see Appendix
A, Table IV). The addition of collagen to the sausage appears to cause
no undesirable shrinkage as previously reported by Saffle et al. (1964).

Volume Loss Evaluation

Volume loss between cookings (A-low and A-high) was found to be
insignificant (P=.40). The mean value for A-low (0% and 7.5%) was
10.10% volume loss, and A-high (15% and 22.5%) had a mean value of

9.28% volume loss. The loss within the B levels (B-low, 0% or 15% and
B-high 7.5% or 22.5%) were insignificant (P=.36). Mean values for the
four levels were 9.37% for the 0% level, 9.07% for the 15% level
(B-low), 10.84% for the 7.5% level, and 9.50% for the 22.5% level
(B-high). AB interaction was also found to be insignificant (P=.60) for
volume loss (see Appendix A, Table V).

Fat Evaluation

Fat percentage of the cooked product as determined by the modified
ether extract procedure (Falk, 1974) indicates there was no significant
difference (P=.29) for percent fat between the cookings. The A-low
cooking had a mean fat percentage of 20.52% and the A-high cooking had
a mean value of 19.51% fat. The difference between the B levels
(B-low, 0% or 15% and B-high, 7.5% or 22.5%) was insignificant (P=.97)
for percent fat and the AB interaction was also insignificant (P=.52)
(see Appendix A, Table VI).

Free Water Evaluation

Free water evaluation, as determined by the pressed fluids method,
indicates there was no significant difference between the cookings
(P=.72). A-low (0% and 7.5%) had a mean value of 54.70% and A-high
(15% and 22.5%) had a mean value of 55.45% for free water. There was
no significant difference (P=.43) within the B levels (B-low, 0% or
15% and B-high, 7.5% or 22.5%). The 0% level had a mean value of
54.62%, 15% level a mean value of 54.64% (B-low), 7.5% level a mean
value of 54.78%, and 22.5% level a mean value of 54.26% (B-high) for
free water. AB interaction was found to be insignificant (P=.74) for

the levels tested (see Appendix A, Table VII).

Crude Protein Evaluation

The crude protein of the cooked product was found to be insignificant (P=.20) between the cookings. A-low had a mean value of 13.79% and A-high a mean value of 13.94%. The difference within cookings was found to be insignificant (P=.73) within the levels tested. The B-low levels had mean values of 13.92% (0%) and 13.84% (15%), and the B-high levels had mean values of 13.67% (7.5%) and 14.04% (22.5%) for crude protein. The AB interaction was found to be insignificant (P=.26) for the levels tested (see Appendix A, Table VIII).

Moisture Evaluation

Percent moisture between the cookings was found to be insignificant (P=.53). A-low (0% and 7.5%) had a mean value of 62.14% and A-high (15% and 22.5%) had a mean value of 62.79%. The difference between levels was also insignificant (P=.63). The B-low levels had mean values of 62.23% (0%) and 62.36% (15%) while the B-high levels had mean values of 62.05% (7.5%) and 63.22% (22.5%) for percent moisture. AB interaction was found to be insignificant (P=.73) for the levels tested (see Appendix A, Table X).

Emulsion Stability

The stability of the emulsions, as determined by the Georgia Test, were found to be insignificant in the levels tested. This would support the findings of the shrink percentage which is also an indication of the emulsion stability. The OSL was 0.57 in the levels tested.

The 0% level had a mean value of 5.63, 7.5% level a mean value of 6.63, 15% level a mean value of 6.75, and the 22.5% level an emulsion stability value of 7.15 (see Appendix A, Table IX).

The use of collagen in the cooked bologna appears to be feasible. Parameters tested in this study were found to be insignificant for the quality contributing characteristics. The emulsion breakdown and excess shrinkage that have been reported in the literature did not occur; however, since the sausages were coarse emulsion products the fat and water binding ability of the collagen wasn't as important as in a fine emulsion product (see Appendix B, Table XVI).

Color Evaluation

Using the methods of Hunter (1976) the L value for color which measures lightness-darkness of the product was insignificant (P>.08) for the comparison of A levels. A-low (0% and 7.5%) had a mean value of 41.66, and A-high (15% and 22.5%) had a mean value of 42.59 for L color values. The difference within levels was significant (P<.005), B-low (0% or 15%) had mean L values of 41.34 (0%) and 42.66 (15%). The B-high levels (7.5% or 22.5%) had mean values of 41.98 (7.5%) and 42.52 (22.5%) for L color values (Figure 7). The AB interaction was very significant (P<.0001) (Figure 8) and the position of the sample within the sausage was also significant (P>.0001). The mean values for lightness-darkness were 41.69 (Position 1), 42.35 (Position 2), 42.38 (Position 3), and 42.07 (Position 4). Since the L value measures lightness-darkness, it can be expected that as lean is removed and replaced with collagen the product will lighten. The significance of the sample position in the sausage was expected since two samples were
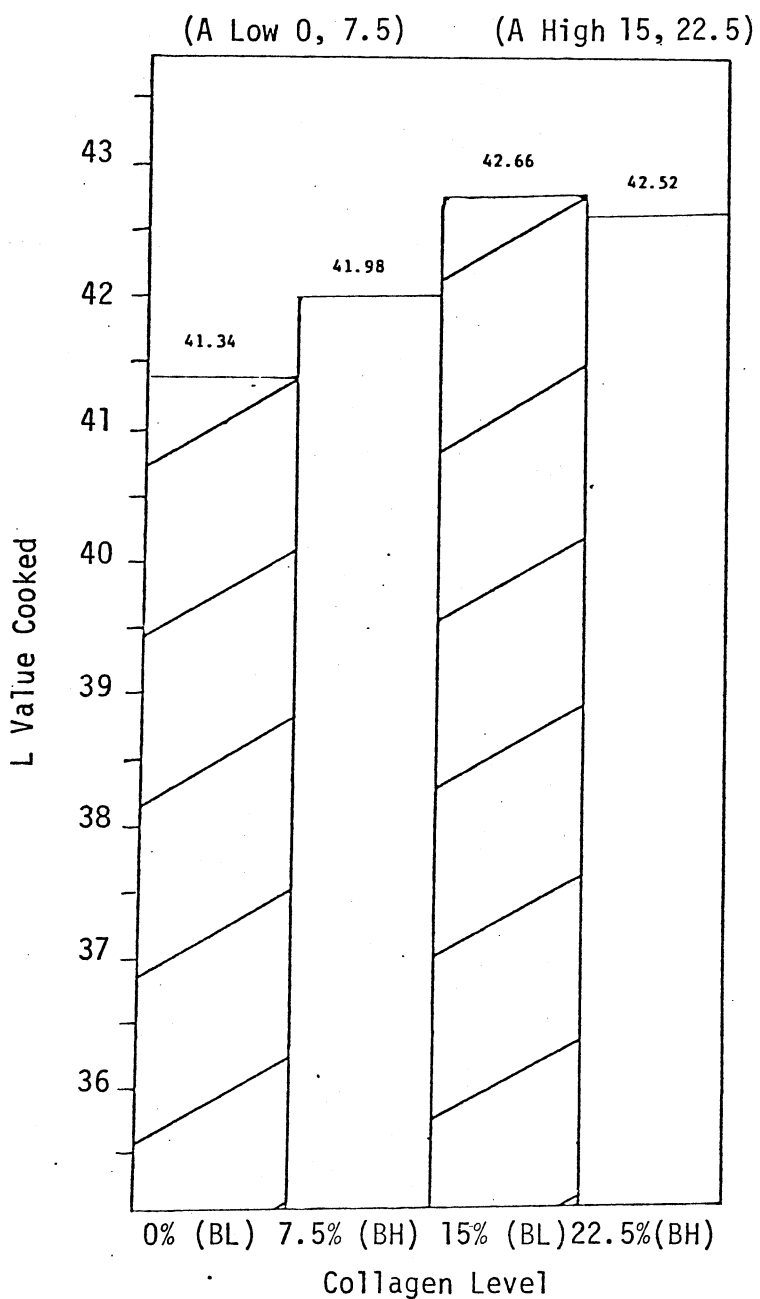
Figure 7. Effect of Collagen Levels on
Lightness-Darkness Values of
Collagen-Bologna
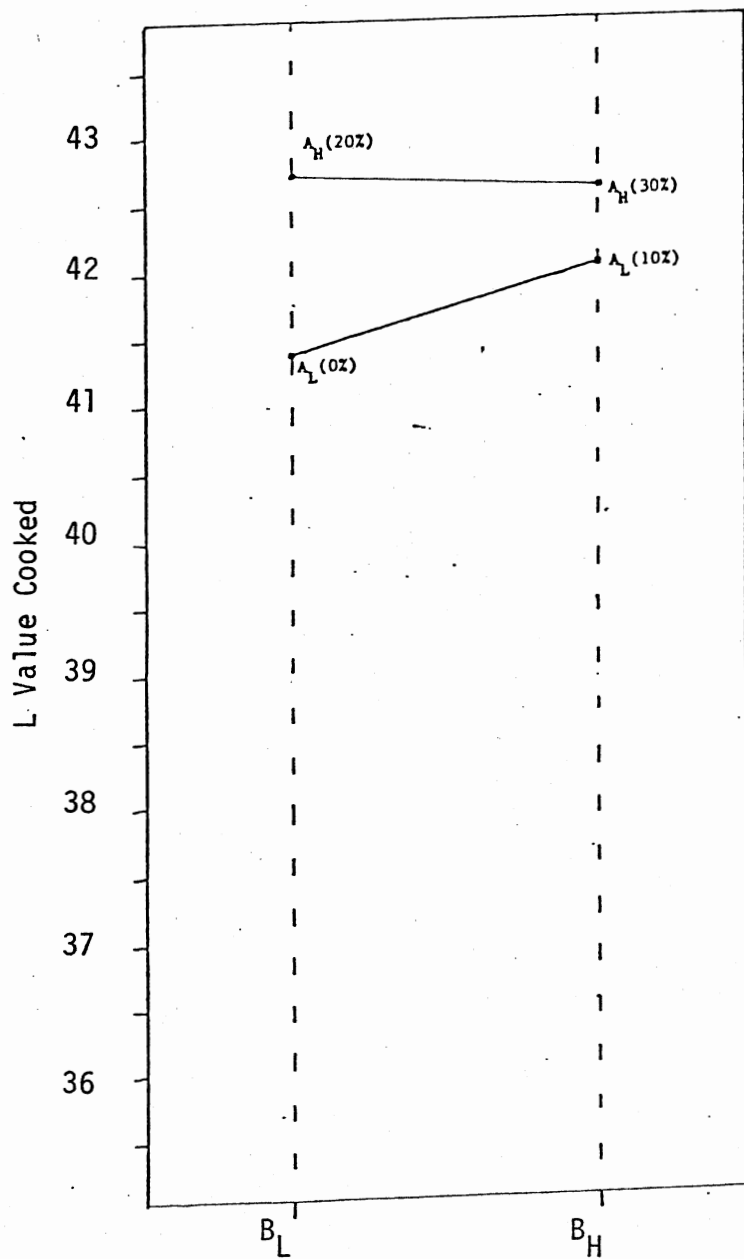
Figure 8. AB Interaction of Lightness-Darkness
Values in Collagen-Bologna

located on each end of the sausage, thus exposed to heat penetration and drying longer than the two middle samples (Figure 9).

The a value which measures redness-greenness was significant (P<.0005) between the cookings. A-low (0% and 7.5%) had a mean value of 10.60 and A-high had a mean value of 9.64 (Figure 10). The difference within levels was significant (P<.0004). The mean values for the B-low levels were 10.67 (0%), 9.86 (15%), and B-high levels had mean values of 10.52 (7.5%), and 9.43% (22.5%) for a color values (Figure 11). There was no significant AB interaction (P>.05) for the levels tested; however, the position of the slice in the sample was significant (P<.0001) as might be expected due to heat penetration and exposure differences (Figure 12). The a value for the low level cooking (A-low) is higher than the a value for the high level cooking (A-high) as could be expected since as more collagen is added the less the value for red. Samples located in the center of the sausages had lower mean a values (10.06 and 10.00) than samples located on either end of the sausages (10.28 and 10.14). It would seem that the longer the heat exposure and penetration the greater the a value as related to sample position.

The b value , a measure of yellowness-blueness was found to be significant (P<.0001) between the two cookings. The A-low mean (0% and 7.5%) was 7.66, and the A-high mean (15% and 22.5%) was 7.45 (Figure 13). The difference between the B levels was insignificant (P>.18) and had mean values of 7.64 (0%), 7.67 (7.5%), 7.50 (15%),and 7.40 (22.5%). AB interaction was significant (P<.05) for the levels tested (Figure 14). There was a significant (P<.0001) difference in the position of the sample as might be expected due to heat exposure
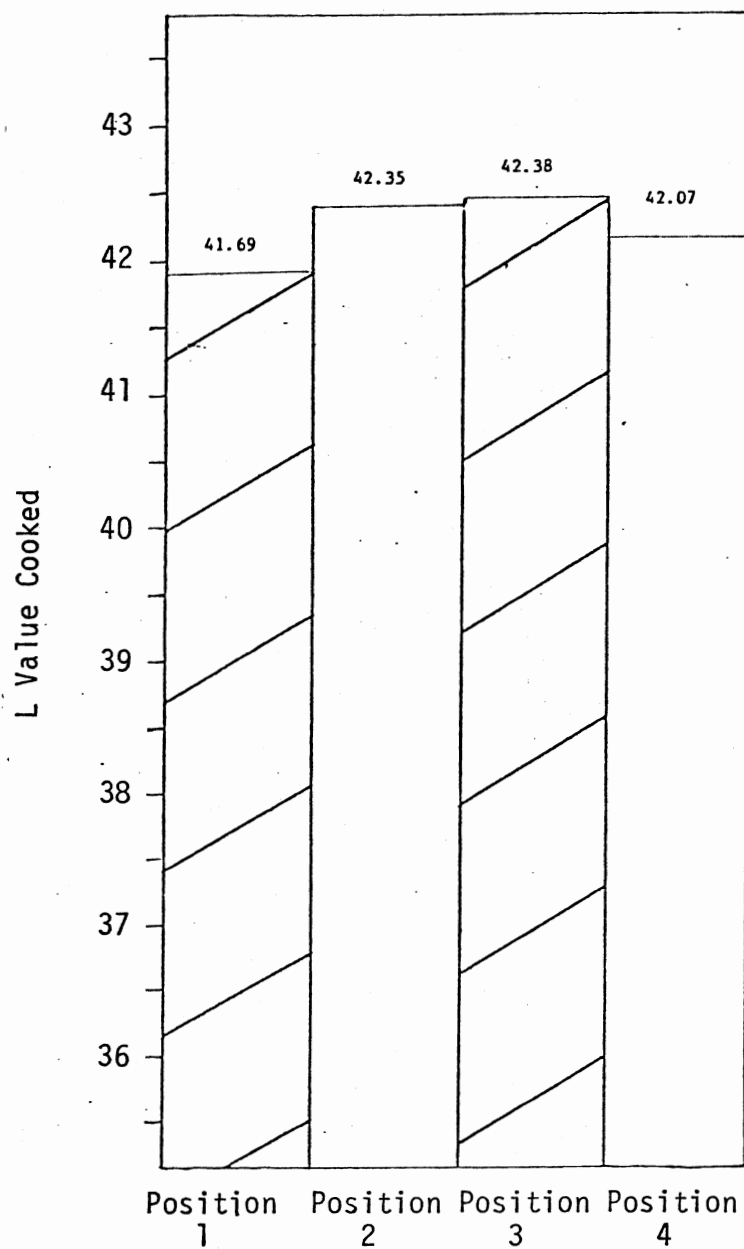
Figure 9.   Effect of Sample Position Within
Sausage on Lightness-Darkness
Values
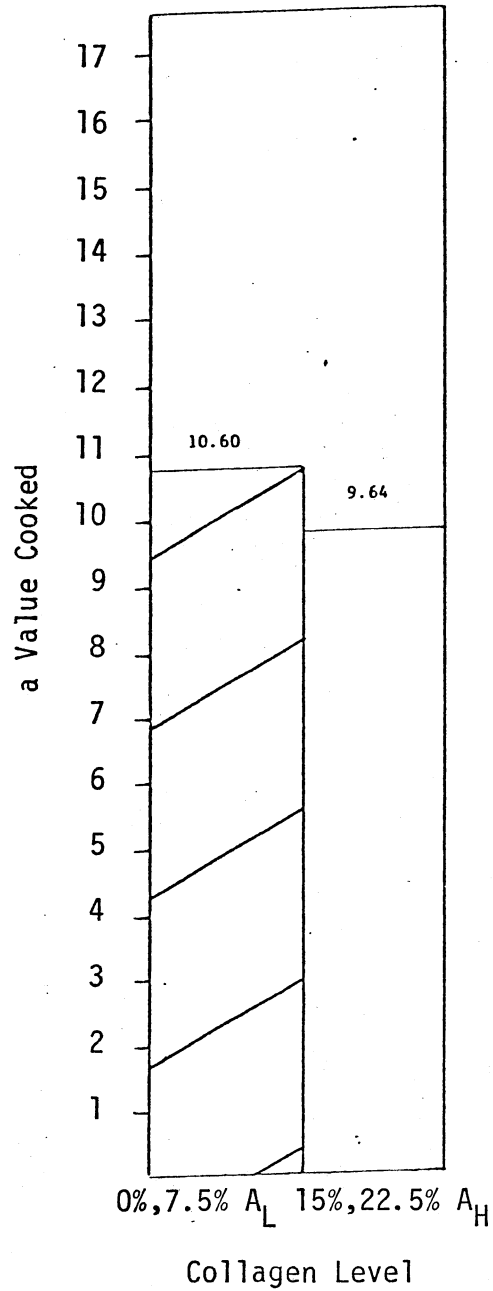
Figure 10. Effect of Collagen Level
            on Redness-Greenness
            Color Values in Collagen
            Bologna
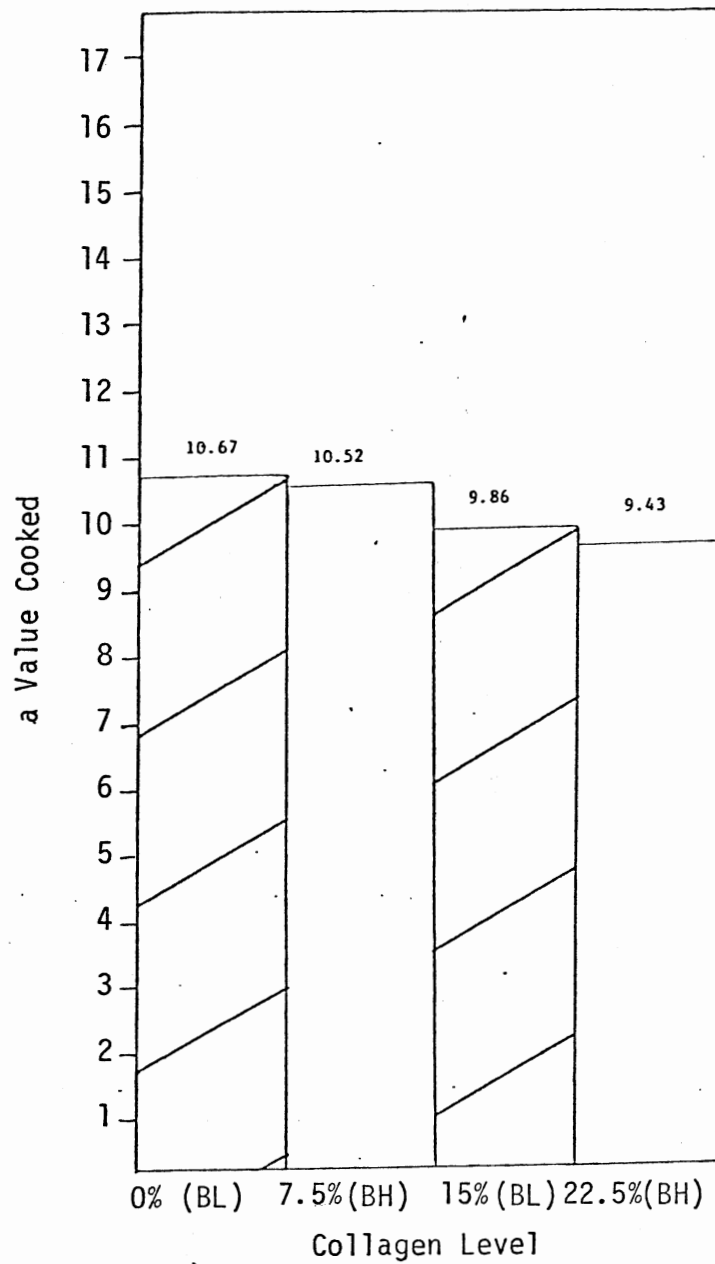
Figure 11.   Effect of Collagen Level on Redness-
              Greenness Values in Collagen
              Bologna
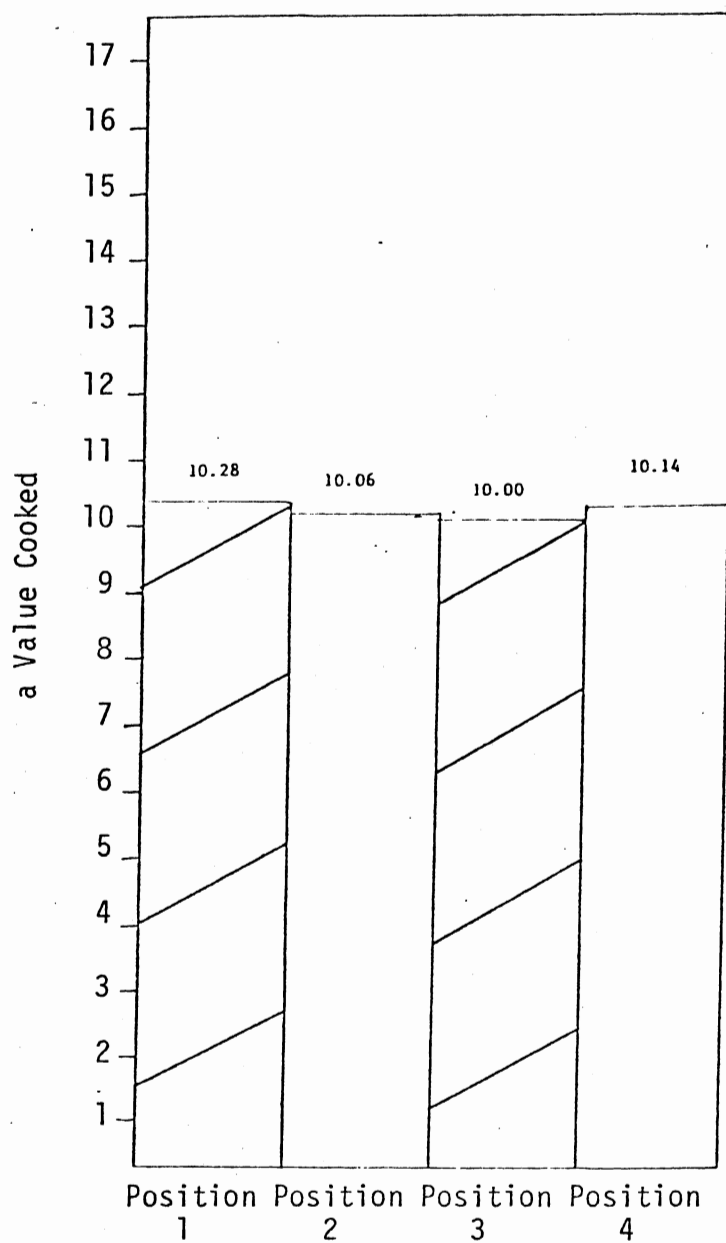
Figure 12. Effect of Sample Position on
Redness-Greenness Color
Values in Collagen Bologna
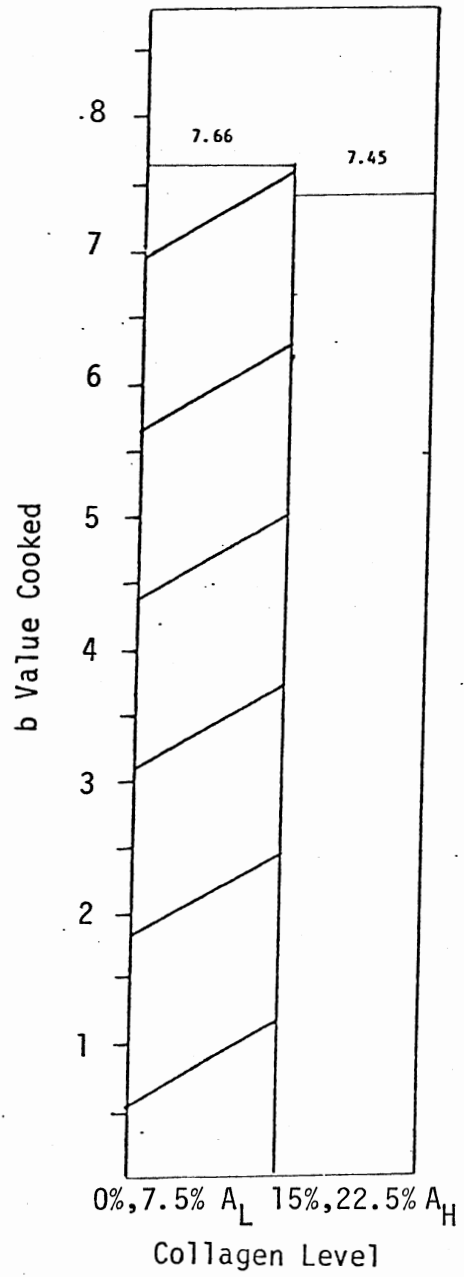
Figure 13. Effect of Collagen Level on
Yellowness-Blueness Values
Collagen Bologna
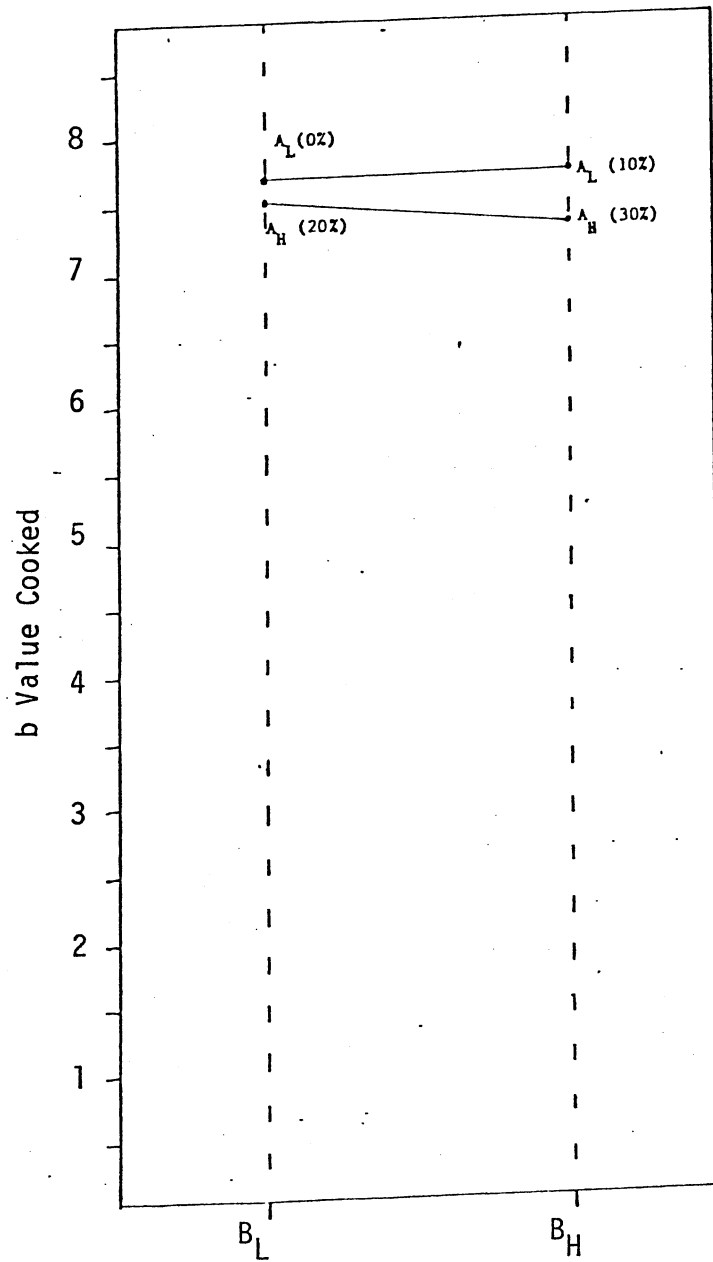
Figure 14.   AB Interaction of Yellowness-
Blueness Values in Collagen
Bologna

and penetration (Figure 15). The difference in the b value in A is probably due to the increased addition of collagen which resulted in a decreased b value mean for A-high. As before the position of the sample was important, but in this case the sample positioned in the rear of the convection oven had the greatest mean b value. This may suggest that there was a temperature gradiant in the oven that affected the b color value in such a way that it increased significantly over the other three sample positions in each sausage. AB interaction in this study is difficult to explain, but is linked to the chemical changes brought about by the exposure of the collagen levels to heat (see Appendix A, Tables XI, XII, XIII).

Textural Studies

Results of the Instron studies indicated a significant difference (P<.0001) within the B levels (B-low 0% or 15% and B-high 7.5% or 22.5%) of the peak force needed to shear the 4 1/8 centimeters in diameter core sample. B-low had mean values of 22.69 (0%), 21.40 (15%), and B-high had mean values of 19.38 (7.5%) and 20.19 (22.5%) kilograms of peak force, the higher the kilograms of peak shear the firmer the texture (Figure 16). There was also a significant (P<.05) AB interaction (Figure 17) and as in the case of color values a very significant (P<.0001) difference in position (Figure 18). The difference in kilograms of peak force for shear within the B levels is not unexpected since with the increase in collagen, the more gelatinization upon thermal exposure. This gelatinization would result in decreasing shear force values for each successive increase in collagen level. However, in this study there was a marked decrease in the peak
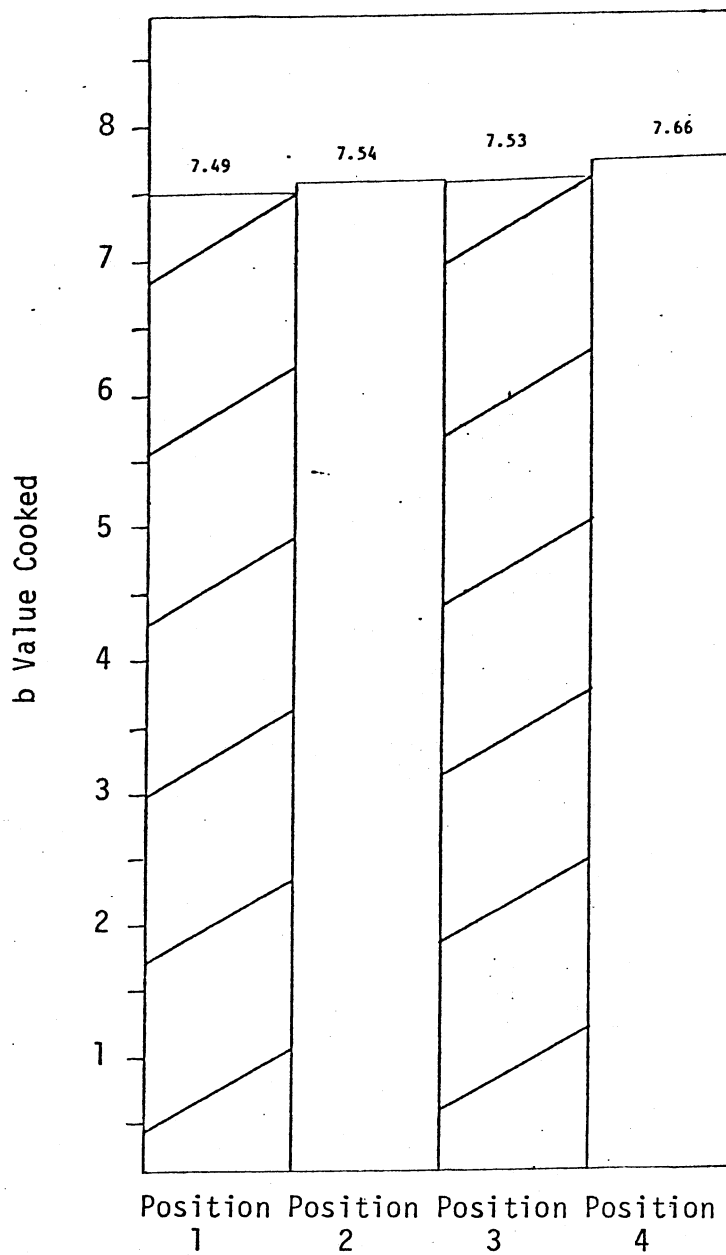
Figure 15. Effect of Sample Position on
Yellowness-Blueness Color
Values in Collagen Bologna
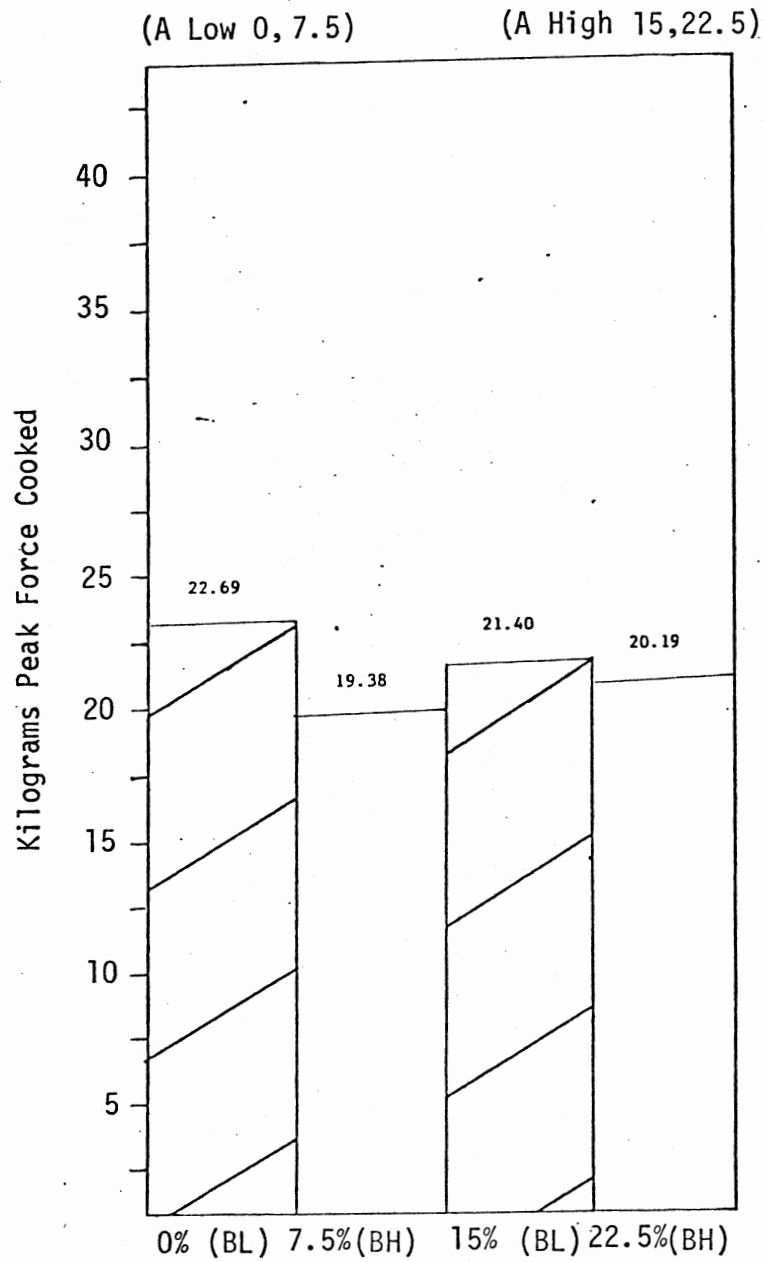
Figure 16.   Effect of Collagen Level on
Peak Force in Collagen-
Bologna
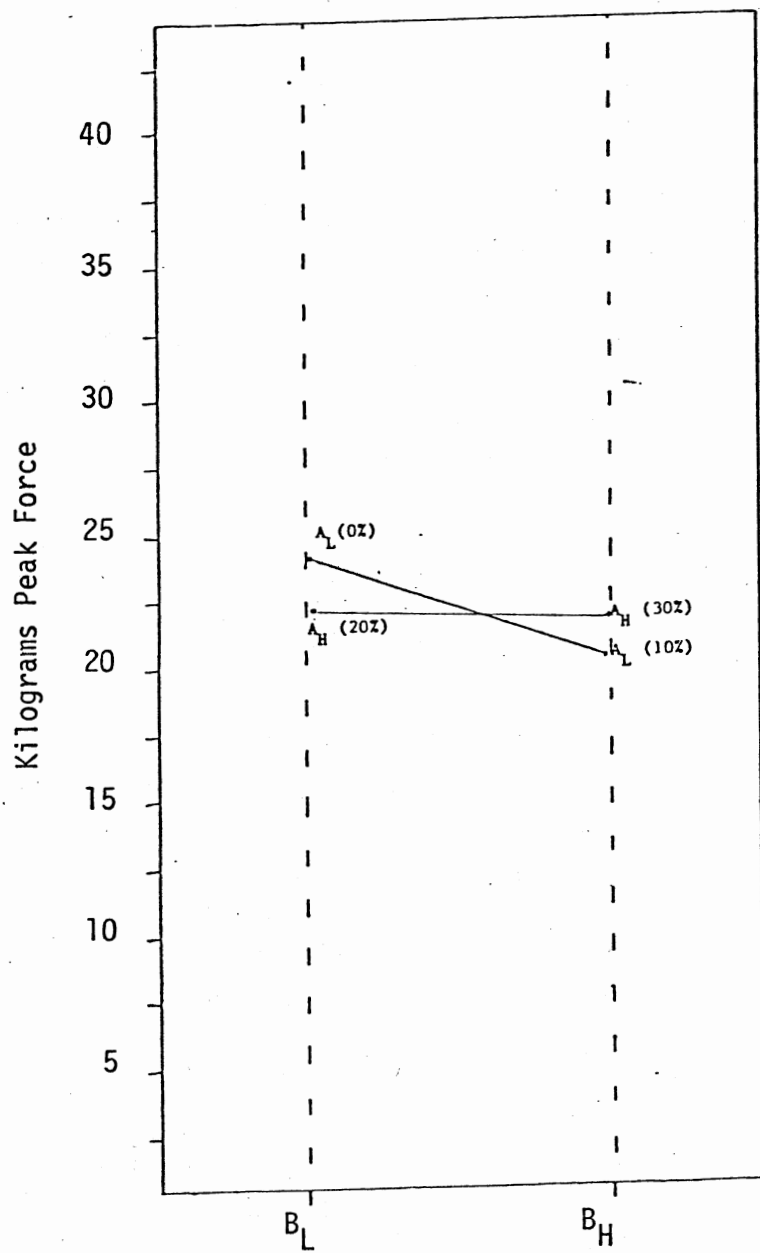
Figure 17. AB Interaction of Peak Force in
Collagen-Bologna

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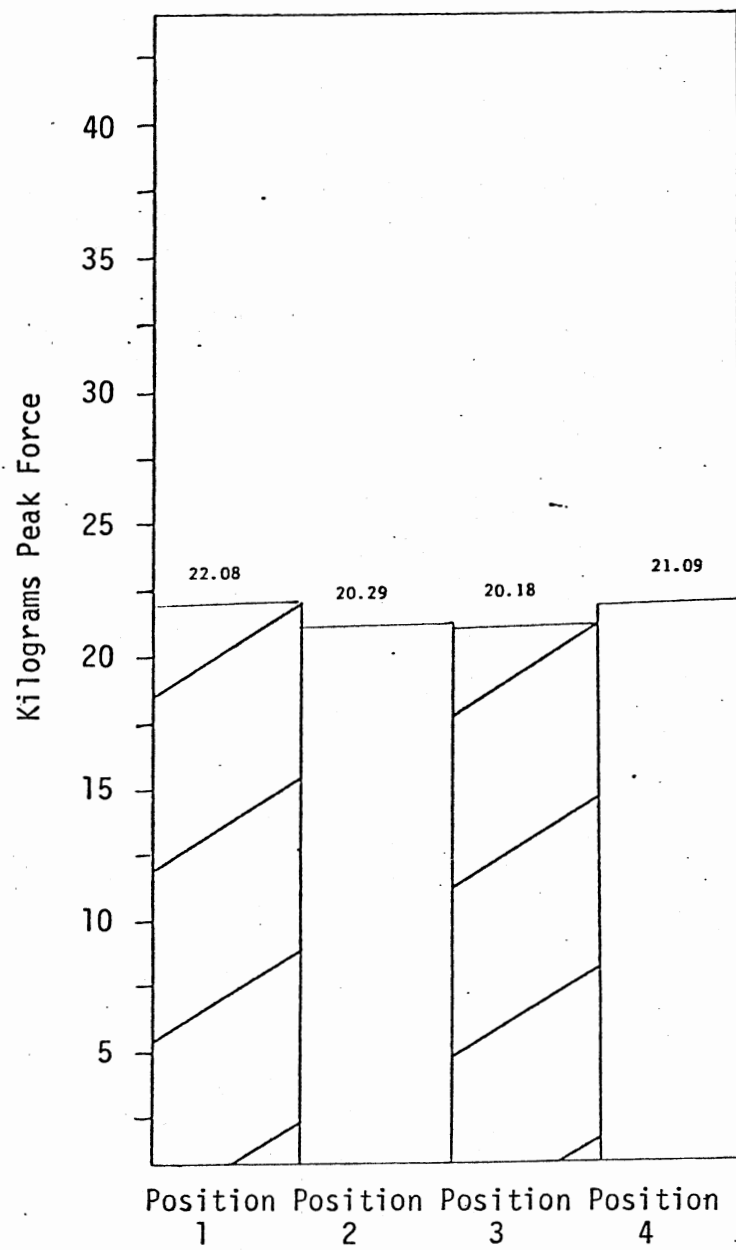
Figure 18.   Effect of Sample Position With-
in Sausage on Peak Force for
Shear

shear values at the 7.5% level (19.38%) compared to 22.69 (0%), 21.40 (15%), and 20.19 (22.5%) followed by an increase at the 15% level and a decrease at the 22.5% level. The AB interaction may be linked to these conditions or a chemical change brought about at a specific collagen level. Position of sample here would indicate that the number 1 position was exposed to a higher heat than position 4, however, since position 1 was the end of the sausage that was first stuffed it may have greater density. The center samples having less thermal penetration time have lower shear values.

The addition of collagen appears to have no detrimental effect on the quality contributing attributes; however, collagen effects the color in what appears to be a negative way with increasing levels. As the color values approach a zero value they indicate a grey color. In this study for color values, with the exception of the L color values, the addition of collagen resulted in decreases in the a and b color values approaching a value of zero indicating a grey color value (Appendix A, Table XIV).

Collagen Evaluation

Evaluation of the collagen was done for the purpose of determining if, by adding the collagen, variability was being introduced into the product from a parameter within batches of collagen.

Crude protein percentage of the bovine collagen was not significant ($P>.10$) and the crude protein had a mean value of 21.09%. Fat percentage in the bovine collagen was determined to be non-significant ($P>.10$), and the fat was determined to have a mean value of .26% fat. Ash for the collagen had a mean value of .17% and was insignificant

(P>.10). Moisture for the bovine collagen used in the experiment was significant (P<.01). The mean value of the moisture was 76.60% the standard deviation was 2.38% (see Appendix A, Table XV).

Soluble collagen content of the bovine collagen had a mean value of 22.38 mg per gram and was insignificant (P>.10) in the product tested. Since the amount of soluble collagen is reported to be important in the functional properties of collagen by Wiley et al. (1979) the amount of soluble collagen in this particular study does not appear to be a source of variation.

Insoluble collagen in the product had a mean value of 159.17 mg per gram, and was insignificant in the product tested (P>.10). The total amount of collagen in the product was determined to have a mean of 183.58 mg per gram and was insignificant (P>.10) (see Appendix B, Table XVII for mean values of collagen).

The amount of moisture that was found to be significant in the collagen may be one of the sources of variation that is responsible for the weak OSL values in the collagen-bologna. This variation of moisture in the collagen may have resulted from the fact that the collagen had been frozen for over a year before being used. Moisture variability may be present only in the batch tested due to a problem in processing that was present only during the manufacture of that particular batch. Handling of the product prior to testing may have also added to the variability of the moisture or a combination of two or more of the above mentioned circumstances.

CHAPTER V

CONCLUSIONS AND SUMMARY

Four levels of bovine-hide collagen were added to ground beef and four collagen-bologna products were produced. The functional properties of the collagen-bologna at 0%, 7.5%, 15% and 22.5% collagen levels were studied. Shrinkage, volume change, emulsion stability, fat, free water, moisture, and crude protein were all determined to be insignificant.

Due to the design, weak OSL values resulted; however, it was expected that there would be no significant difference in the above parameter. In a coarse-bologna product, gel pockets that would form from the gelatinized collagen would have little effect on emulsion stability since the gel would have difficulty migrating to the outer area of the sausage due to the coarseness of the mixture. Collagen could readily form as gel pockets located throughout the product. In a fine emulsion sausage, the level of collagen will be much more critical. A fine emulsion sausage requires more fat and water binding ability due to its texture than does a coarse emulsion.

The addition of collagen to coarse beef bologna resulted in a change in the color of the product as expected. The greater the collagen content the greater the L value which is a measure of lightness-darkness. As the collagen level increases, so does the lightness of

the product. The value which measures redness-greenness decreased with increased levels of collagen. The b value, which is a measure of yellowness-blueness, was higher in the products with 0 and 7.5% collagen than in the 15% and 22.5% levels when compared to the standard.

Textural studies revealed that with increasing amounts of collagen lower peak force values are observed with the exception of the 7.5% level which had the lowest peak force value of the four levels tested.

The position of the sample within the sausage was very significant ($P<.0001$) in all color and textural studies. The end samples exposed to thermal penetration longer than the samples in the center were significantly different. AB interaction encountered in this study was unexpected and is difficult to explain. This interaction may result from differences between the cookings of the sausages, differences in collagen levels, or chemical change brought about at a specific collagen level that is not present in the other three levels.

In conclusion, while values for color differences were significantly different, the actual change may not be detected with the unaided eye. Shear force values while significantly different also may not be detected in sensory evaluation. It would be of interest, therefore, to conduct sensory panel studies in these areas.

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APPENDIXES

APPENDIX A

ANALYSES OF VARIANCE

TABLE IV

ANALYSIS OF VARIATION FOR PERCENT SHRINK

Source	DF	Sum of Squares	Mean Squares	F	PR>F
Total	47	31.51			
Among Cookings	5	13.09			
A	1	0.14	0.14	0.04	0.85
Error (a)	4	12.95	3.24		
Within Cookings	42	18.42			
B	1	0.35	0.35	1.46	0.58
AxB	1	0.56	0.56	2.34	0.20
Error (b)	4	0.96	0.24		
Sample Error	36	16.55			

TABLE V

ANALYSIS OF VARIATION FOR PERCENT VOLUME LOSS

Source	DF	Sum of Squares	Mean Squares	F	PR>F
Total	63	389.49			
Among Cookings	7	85.70			
A	1	10.28	10.28	0.81	0.40
Error (a)	6	75.42	12.57		
Within Cookings	56	303.79			
B	1	14.56	14.56	0.99	0.36
AxB	1	4.40	4.40	0.30	0.60
Error (b)	6	88.56	14.76		
Sample Error	48	196.27			

TABLE VI

ANALYSIS OF VARIATION FOR FAT PERCENTAGE-COOKED

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	63	214.01			
Among Cookings	7	123.81			
A	1	21.97	21.97	1.29	0.30
Error (a)	6	102.02	17.00		
Within Cookings	56	90.02			
B	1	0.29	0.29	0.03	0.97
AxB	1	3.02	3.02	0.28	0.52
Error (b)	6	63.52	10.58		
Sample Error	48	23.19			

TABLE VII

ANALYSIS OF VARIATION FOR FREE WATER PERCENT-COOKED

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	63	1776.03			
Among Cookings	7	402.19			
A	1	9.36	9.36	0.14	0.72
Error (a)	6	392.83	65.47		
Within Cookings	56	1373.84			
B	1	12.73	12.73	0.17	0.43
AxB	1	8.84	8.84	0.12	0.74
Error (b)	6	442.04	73.67		
Sample Error	48	910.23			

TABLE VIII

ANALYSIS OF VARIATION FOR CRUDE PROTEIN PERCENTAGE-COOKED

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	15	1.20			
Among Cookings	7	0.66			
A	1	0.15	0.15	1.87	0.20
Error (a)	6	0.51	0.08		
Within Cookings	8	0.54			
B	1	0.01	0.01	0.14	0.72
AxB	1	0.11	0.11	1.57	0.26
Error (b)	6	0.42	0.07		

TABLE IX

ANALYSIS OF VARIATION FOR EMULSION STABILITY

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	15	33.80			
Among Treatments	3	5.05	1.68	0.70	0.40
Experimental Error (W/n Trts)	12	28.75	2.39		

TABLE X

ANALYSIS OF VARIATION FOR PERCENT MOISTURE-COOKED

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	63	180.49			
Among Cookings	7	57.85			
A	1	3.97	3.97	0.44	0.53
Error (a)	6	53.88	8.98		
Within Cookings	56	122.64			
B	1	3.92	3.92	0.26	0.62
AxB	1	2.02	2.02	0.13	0.73
Error (b)	6	90.16	15.03		
Sample Error	48	26.54			

TABLE XI

ANALYSIS OF VARIATION FOR L COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	255	212.86			
Among Cookings	7	131.12			
A	1	55.37	55.37	4.39	.08
Error (a)	6	75.75	12.62		
Within Cookings	248	81.73			
Among Sausage	56	38.74			
B	1	4.07	4.07	8.82*	<.005
AxB	1	9.73	9.73	21.08*	<.0001
Error (b)	54	24.93	0.46		
Within Sausage	192	42.99			
Position	3	19.66	6.55	52.59*	<.0001
AxPosition	3	0.10	0.03	0.28	
BxPosition	3	0.42	0.14	1.123	
Error (c)	183	22.81	0.12		

*Significant P<.05).

TABLE XII

ANALYSIS OF VARIATION FOR A COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	255	106.15			
Among Cookings	7	65.36			
A	1	58.03	58.03	47.47*	<.0005
Error (a)	6	7.33	1.22		
Within Cookings	248	40.78			
Among Sausage	56	27.36			
B	1	5.52	5.52	14.41*	<.0004
AxB	1	1.15	1.15	3.00	
Error (b)	54	20.69	0.38		
Within Sausage	192	13.42			
Position	3	2.87	0.96	17.56*	<.0001
AxPosition	3	0.43	0.14	2.61	
BxPosition	3	0.15	0.05	0.92	
Error (c)	183	9.97			

*Significant P<.05.

TABLE XIII

ANALYSIS OF VARIATION FOR b COLOR VALUE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	255	11.89			
Among Cookings	7	4.62			
A	1	2.74	2.740	8.76*	<.0001
Error (a)	6	1.88	0.310		
Within Cookings	248	7.27			
Among Sausage	56	3.48			
B	1	0.09	0.090	1.84	.18
AxB	1	0.25	0.250	4.78*	<.05
Error (b)	54	3.14	0.050		
Within Sausage	192	3.79			
Position	3	1.10	.366	24.21*	<.0001
AxPosition	3	0.02	.008	0.52	
BxPosition	3	0.01	0.004	0.25	
Error (c)	183	2.66	0.014		

*Significant P<.05.

TABLE XIV

ANALYSIS OF VARIATION FOR KILOGRAMS PEAK FORCE

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	255	2274.63			
Among Cookings	7	545.18			
A	1	3.72	3.72	0.04	0.85
Error (a)	6	541.46	90.24		
Within Cookings	248	1729.45			
Among Sausage	56	1052.41			
B	1	327.61	327.61	27.03*	<.0001
AxB	1	70.25	70.25	5.79*	<.025
Error (b)	54	654.56	12.12		
Within Sausage	192	677.04			
Position	3	148.08	49.36	18.65*	<.0001
AxPosition	3	24.69	8.22	3.11	
BxPosition	3	19.87	6.62	2.50	
Error (c)	183	484.41	2.65		

*Significant P<.05).

TABLE XV

ANALYSIS OF VARIATION FOR PERCENT MOISTURE-COLLAGEN

Source	DF	Sum of Squares	Mean Square	F	PR>F
Total	44	107.94			
Among Cans	4	32.77	8.19	4.36*	<.01
Within Cans	40	75.17	1.88		

*Significant P<.05.

APPENDIX B

MEANS

TABLE XVI

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

Collagen Level[a]	Shrinkage (%)	Volume Loss (%)	Fat (%)	Free Water (%)	Crude Protein (%)	Moisture (%)	Emulsion Stability Value
0%	7.19	9.37	20.24	54.62	13.92	62.23	5.63
7.5%	7.58	10.84	20.81	54.78	13.67	62.05	6.63
15.0%	7.51	9.07	19.81	54.64	13.84	62.36	6.75
22.5%	7.47	9.50	19.21	56.26	14.04	63.22	7.15

[a]Collagen level based on percentage of lean meat replaced with collagen. Before the addition of water collagen levels were 0, 10, 20, and 30%.

TABLE XVII

MEAN VALUES OF COLLAGEN PARAMETERS IN COLLAGEN PRODUCT #1

Collagen Can	Fat (%)	Moisture (%)	Ash (%)	Crude Protein (%)	Soluble Collagen (mg/g)	InSoluble Collagen (mg/g)	Total Collagen (mg/g)
1	0.23	74.01	0.17	21.98	22.51	161.08	184.09
2	0.28	78.81	0.16	20.41	23.18	157.10	184.77
3	0.26	75.48	0.18	19.89	21.10	159.14	182.75
4	0.25	78.97	0.19	22.07	22.14	158.91	184.00
5	0.27	74.50	0.15	21.08	23.01	159.61	182.31

VITA

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