FOOD GRADE COLLAGEN AS A LEAN OR FAT REPLACEMENT IN PORK SAUSAGE

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REPLACEMENT IN PORK SAUSAGE

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CHAPTER I

INTRODUCTION

Pork sausage is one of the more common meat items on the breakfast menu. Throughout the years, it has been one of the leading pork items to be served by the institutional food service. In fiscal year 1981 alone, 1,114,663,000 pounds were prepared and processed under federal inspection (United States Department of Agriculture (USDA), 1982). This represents a product with great potential for the incorporation of non-meat protein products such as collagen.

Food grade collagen has been used in a variety of products ranging from coarse and fine-emulsion bologna to an assortment of bakery products. However, the use of bovine collagen has not been approved as a food ingredient by the United States of Agriculture Meat Inspection Service.

Soy protein, meanwhile, has been incorporated into pork sausage at levels as high as 40% (Andres, 1976). These extended products have received favorable consumer acceptance due to the reduced cost and the perceived better nutritional quality attributed to the reduced fat content and less shrinkage during frying.

The purpose of this study was to determine the feasibility of substituting food grade collagen for varying portions of the total lean tissue or the fat tissue of pork sausage in order to form products comparable to or better than the more common pork sausage in terms of overall quality. The effect of storage on the total aerobic plate count, the color of the uncooked and cooked patties, the texture, the cook yield and the level of rancidity of the pork sausage were also studied.

CHAPTER II

REVIEW OF LITERATURE

Pork Sausage

Pork sausage is commercially prepared with unfrozen and/or frozen meat, or meat by-products and seasoned with condimental substances. It shall not be made with any amount of products which, in the aggregate, contains more than 50% trimmable fat; i.e. fat which can be removed by thorough practicable trimming and sorting. Water or ice may be used in an amount not to exceed 3% of the total ingredients used. Extenders or binders may be used to the extent of 3.5% of the finished sausage (de Holl, 1981).

Collagen

Collagen is the most abundant protein in mammals comprising about 25% of the total protein (Stryer, 1981). It functions primarily as the principal supporting element in a wide variety of connective tissues (Gay and Miller, 1978). It is first synthesized in specialized connective tissue cells and the molecules are then released into the intercellular spaces, where they self-assemble into fibrils. At first, these fibers are soluble in neutral salt solutions or in dilute acids. They gradually develop, however, into an insoluble state by the formation of covalent intermolecular bonds, which crosslink the collagen molecules within the fibrils. In this form, the mature collagen fibrils fulfill their physiological function as a skeletal and supporting substance (Kuhn, 1969).

Collagen is a high-molecular weight, insoluble, fibrous protein. It may range in various sausage meats, such as pork shoulders, from 8 to 11% of the total protein (Porteous, 1981). Collagen can serve as a food texturizing agent since it will absorb and bind a large quantity of water (Happich, 1975).

The Primary and Secondary Structures of Collagen

Collagen has an unusual amino acid composition and sequence. The proportion of glycine residues in all collagen molecules is nearly one-third of the total amino acids (Ramachandran and Ramakrishnan, 1976; Gay and Miller, 1978; Stryer, 1981) while proline and hydroxyproline make up approximately 25% (Gross, 1961; Ramachandran and Ramakrishnan, 1976; Gay and Miller, 1978). Amino acids with polar side chains such as arginine, lysine, aspartic acid, and glutamic acid account for 20% of the total amino acid residues while alanine, a non-polar amino acid, makes up 10% (Ramachandran and Ramakrishnan, 1976). Low amounts of methionine, isoleucine, tyrosine and histidine are present (Gay and Miller, 1978) while tryptophan is virtually absent

(Satterlee and Zachariah, 1973; Asghar and Henrickson, 1982). Hydroxylysine also is present in smaller amounts than hydroxyproline. Hydroxylysine also serves as the attachment site for the principal carbohydrate components namely, glucose and galactose (Bornstein and Traub, 1979).

One distinctive feature of the collagen molecule is that every fourth carbon position is occupied by glycine and is followed immediately by proline or hydroxyproline (Gross, 1961). The general amino acid sequence is (Gly-X-Y)n (Bornstein and Traub, 1979) where X and Y represent the positions occupied by amino acids such as proline and hydroxyproline. The sequence of glycine - proline - hydroxyproline recurs frequently (Stryer, 1981).

The Tertiary Structure of Collagen

The collagen molecule is shaped like a small rod, 2900-3000 Angstroms long, 14-15 Angstroms in diameter and with a molecular weight of 300,000 (Kuhn, 1969; Gay and Miller, 1978; Asghar and Henrickson, 1982). It consists of three polypeptide alpha chains, each having a molecular weight of 95,000 (Asghar and Henrickson, 1982) and about 1000 amino acid residues (Gross, 1961; Kuhn, 1969; Bornstein and Traub, 1979). Each alpha chain is coiled into a left-handed helix with about three amino acids per turn over a distance of 9 Angstroms (Gross, 1961; Gay and Miller, 1978; Asghar and Henrickson, 1982). The three alpha chains intertwine about a common central axis to form a right-handed triple helix with a repeat distance of 100 Angstroms (Gross, 1961; Kuhn, 1969; Gay and Miller, 1978; Asghar and Henrickson, 1982). The three spirals are so arranged that the glycine residues, which have no side chains, lie inside the triple helix, while the bulky rings of proline and hydroxyproline and the side chains of the heavy and polar amino acids are on the outside (Kuhn, 1969). The whole structure is held together by hydrogen bonds established between oxygen atoms, located where amino acids are joined by peptide linkages in one chain and the nitrogen atoms, located at peptide linkages in an adjacent chain (Gross, 1961; Kuhn, 1969). Each three-residue repeating element of a given chain participates in two peptide hydrogen bonds, one to each of the two neighboring chains (Josse and Harrinton, 1964). Aside from these hydrogen bonds, the proline and hydroxyproline residues prevent easy rotation of the regions in which they are located and thus impart rigidity and stability to the molecule (Gross, 1961).

Preparation of Food Grade Collagen

The food grade collagen must come from inspected slaughter and identity with acceptable carcasses must be established for all hides intended for food use (Whitmore et al., 1970). Hides are limed and then split into two layers: the outer "grain" and the inner "flesh" layer. These "flesh splits" are fed to strip cutters, and the resulting pieces go into a rotary cutter which reduces them to about 9.5 mm particle size. The collagen particles are transferred using a conveyor to a hide processor containing water, propionic acid, and benzoic acid (1000:3:1 by weight), tumbled there for 4 hr, and then drained on a screen conveyor. These processed pieces are fed to either a comitrol or disc mill by cavity pumps, and then to the microcut depending on the type of desired product. The temperature is reduced to 1.7 C before packing, and the products are stored at -18 C to keep them microbiologically safe.

Nutritional Aspects

Collagen is an incomplete protein since it is limited in some essential amino acids such as methionine, lysine, and threonine while it is practically devoid of tyrptophan (Asghar and Henrickson, 1982). These amino acids can be supplied by many other foods. Mixture of various foods with collagen could constitute a product of well-balanced protein and caloric value (Whitmore et al., 1970).

In rat-feeding experiments, researchers found collagen to be completely digestible with 86% of the caloric or energy value of casein. Its protein efficiency ratio (PER) compared to casein is low (Whitmore et al., 1970). Delimed, washed, fibrous, insoluble hide collagen when fed to rats also was found to be well digested (90%) and served as a source of energy. It is not toxic when fed at a high percentage of the diet for relatively long periods (Whitmore et al., 1975).

The degree of incorporation of bovine hide collagen depends greatly on the food system involved. When added to corn meal muffins at a 10% level, the organoleptic qualities were rated equal to the reference samples but the overall quality tended to decline (Ebro et al, 1980). Similarly, Maurer and Baker (1966) considered a high collagen content (>15%) to be a causative factor of gel pockets, wrinkling of the outer skin, poor peelability in poultry meat sausages. On the other hand, Schalk et al. (1980) used as much as 30% in coarse bologna and found no significant difference in the texture, emulsion stability, shrinkage and volume change of coarse-beef bologna compared to the control samples.

Potential Uses of Food Grade Collagen

Collagen has potential applications in food systems as a binder, filler, extender, moisturizer, texturizer and nutrient enhancer (Henrickson et al., 1980).

Satterlee and Zachariah (1973) used hydrolysates of beef or pork skin to replace non-fat dry milk (NFDM) in a sausage formulation. The sausages had a greater water- and fat-holding ability. The emulsion capacity of various skin hydrolysates was slightly lower than the capacity of NFDM (on a per 100 mg protein basis). The greater protein content of the hydrolysates also gave the sausage emulsion improved stability during cooking.

Food-grade collagen was used in various bakery products such as whole wheat muffins, sweet wheat loaf, corn meal muffins, plain cakes, carrot cake, oatmeal cookies and in plain and whole wheat spatzle (Ebro et al., 1979, 1980). There was little adverse effect of the five USDA types (Turkot et al., 1978) of collagen products based on the juiciness and chewability of beef loaves. Overall scores for texture and flavor were higher for loaves containing collagen. A 20% addition level gave more firmness to the beef loaf than 10 or 30% levels.

Air-dried collagen also was substituted into a plain muffin formulation (Ebro et al., 1980). The cellular structure was comparable with the reference samples but the aroma was not acceptable. Whole wheat muffins with 5% collagen had a grainy texture but were as good as the reference samples in terms of aroma. The same was true for sweet whole wheat loaf. Corn meal muffins with 10% collagen rated equal to the reference samples in the organoleptic characteristics at higher levels. White cake was not a suitable medium for collagen supplementation since the granular nature of air-dried collagen resists proper blending with plain cake.

The effect of food-grade collagen substitution on the functional properties of coarse beef bologna by replacing lean meat at 10, 20 and 30% levels was studied (Schalk, 1981). The functional characteristics, e.g., the raw emulsion stability, pH, cook yield, water activity density and expressible juice were not altered by the replacement of lean meat with hide collagen. Bologna with collagen was less tender, as shown by increased shear force values. Bologna with collagen possessed (P<.05) less red color than the control as the fat content decreased. Textural and color changes were not perceptible on visual examination of the product but were detected on intrumental analysis.

Collagen also was used in fine-emulsion bologna sausages (Gielissen, 1981). Collagen was used at 5, 10, 15% levels, replacing lean meat and keeping fat content constant at 25%. In each case, the emulsions were stable but at higher levels, this stability tended to decline slightly.

Chavez (1983) added food grade wet collagen to ground beef at 0, 10 and 20% levels as a lean meat replacement, and stored the mixed products at -15 C for up to two weeks to evaluate the effect of collagen level and storage period on the quality characteristics. Significant differences (P<0.05) in flavor, juiciness, texture and overall acceptability due to the collagen level were found by a 6-9 member semi-trained panel. Overall acceptability and flavor decreased as the level of collagen increased but beef patties with collagen were superior in texture and juiciness. However, no significant differences (P>0.05) were found for these attributes due to storage time. Collagen level and storage period did not significantly affect (P>0.05) the cooking loss of ground beef patties, which showed that collagen did bind moisture during cooking. Hunter 'L' values indicated that the addition of collagen caused a lighter colored patty. The color was influenced by both the collagen level and storage period. The product tended to become less cohesive upon collagen replacement, decreasing the texture, but there was an increase in cohesiveness due to storage time reflecting hardening of collagen and muscle fibers. The development of rancidity as measured by the Thiobarbituric Acid (TBA) test demonstrated that as the collagen levels increased, oxidation of the unsaturated lipids significantly decreased ($P<0.05$).

CHAPTER III

MATERIALS AND METHODS

Preparation of the Lean and Fat

Forty-five kg of raw pork shoulders and ten kg of pork backfat were purchased from Ralph's Packing Co. (Perkins, Oklahoma) for each of the four replications in this experiment. After manually separating the fat tissue from the pork shoulders, the fat trimmings, the lean trimmings, and the pork backfat were ground separately once through a 1.27 cm plate (Globe Slicing Machine Co., Inc., Stamford, Conn., Model 5028) into separate containers.

The resulting ground lean tissue and ground fat tissue were each mixed thoroughly and sampled at random locations in their containers for fat determination by the modified Babcock method for cream (AOAC, 1980). Three samples were obtained from each of the lean and fat tissues. The average fat content for each was computed. The lean and the fat tissues were packaged separately into 2.0 kg batches in freezer wrapping paper and frozen at -15 C until used.

Preparation of Food Grade Collagen

Eight cans of food grade collagen (Product No. 3) were used throughout this study (Turkot et al., 1978). This product was manufactured in July, 1978 by the United States Department of Agriculture's Eastern Regional Research Center (Philadelphia, PA 19118), of which 2.7 kg were sealed in number ten size cans and kept at -20 C until used. For each replication, two cans were thawed by placing them in a cooler (4 C) for 48 hours prior to use. The contents of the cans were filtered through a Buchner funnel in the cooler (4 C) for 12 hours. The weight of each portion was measured and the ratio of the liquid portion to the solid portion was calculated. A 6:5 ratio (w/w) of the liquid to the solid was obtained for each can and was used throughout this study. After filtration, the solid portions from the two cans were thoroughly mixed with the Hobart Model AS-200 paddle-type mixer for two minutes to obtain a homogeneous mixture. The liquid portions were likewise combined.

Preparation of the Pork Sausage

The nine different formulations prepared for this experiment are shown in Table I. Based on the measured fat percentage, the amount of ground lean tissue and ground fat tissue needed to produce four kg batches for each formulation were calculated using the Pearson's square method (Church and Pond, 1974). The total amount of ground lean tissue and ground fat tissue needed for each replication were thawed by placing them in a cooler at 4 C for 12 hours.

The calculated amounts of ground lean tissue, ground fat tissue, and bovine hide collagen for each formulation were mixed with the spices (Table II) using a Hobart Model AS-200 paddle-type mixer for two minutes. After mixing, the resulting sausage dough was ground once through a 0.635 cm grinder plate in order to provide a uniform distribution of the fat tissue, lean tissue, and collagen.

Table I

NINE FORMULATIONS OF PORK SAUSAGE

Code or Symbol	Tissue Replaced	Level %	Amount of Lean Tissue (g)(1)	Amount of Fat Tissue (g)(2)	Amount of Collagen (g)	
					Solid (3)	Liquid (4)
C00	None	0%	2776	1224	0	0
L05	Lean	5%	2576	1224	91	109
L10	Lean	10%	2376	1224	182	218
L15	Lean	15%	2176	1224	273	327
L20	Lean	20%	1976	1224	364	436
F05	Fat	5%	2776	1024	91	109
F10	Fat	10%	2776	824	182	218
F15	Fat	15%	2776	624	273	327
F20	Fat	20%	2776	424	364	436

(1) Based on the 10.0% average fat content of the four lots of lean tissue.
(2) Based on the 75.4% average fat content of the four lots of fat tissue.
(3) Solid obtained from filtration.
(4) Aqueous portion obtained from filtration.

Supralon casings (Union Carbide, Size 90 mm, Stuff Diameter 6.6 cm) were prepared by cutting 25 cm long strips. One of the two open ends of the casings was sealed by tying a knot about 2 cm from the end with a 25 cm long piece of string. The ground sausage dough was then stuffed into these casings using a Vogt mechanical stuffer to produce twelve 300 g chubs. The dough was compressed to remove the excess air pockets using a mechanical wringer after which the open end of the casing was fastened securely with string to form the whole chub. These chubs were stored in a FREAS 815 Low Temperature Incubator at 0 C. The incubator was used in order to maintain a constant temperature (0 C) throughout the study.

Table II

SPICE FORMULATION USED IN THE PREPARATION OF PORK SAUSAGE

Ingredient	Amount (g/4 kg)
Salt	61.6
Sage	2.2
Ground red pepper	4.4
Ground black pepper	8.8

The grinder, mixer, stuffer and wringer were all allowed to equilibrate in the cooler (4 C) for 12 hours prior to use. All the operations involved in the preparation of the product were conducted inside the cooler (4 C).

Chemical Analyses

During stuffing of the sausage dough, approximately one hundred gram samples were taken at random from each formulation and stored in Whirl-pak bags in order to minimize moisture loss due to evaporation. Moisture, fat and protein were determined on the day after manufacture according to the Official Methods for meat and meat products (A.O.A.C., 1980). Moisture content was determined as the weight loss from a 2-3 g sample after drying for 16-18 hours at 100-102 C. Extractable lipid was determined as the weight loss of the dried samples after 16 hours of extraction with petroleum ether in a Soxhlet apparatus. The amount of crude protein was determined by the Kjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator, Herndon, Virginia 22070). The percentage of protein was calculated as percentage of nitrogen times 6.25. Triplicate samples from each formulation were used to determine the amount of moisture, protein and fat.

The proximate analysis of the solid portion of the food grade collagen was also made following the Official Methods (A.O.A.C., 1980), for meat and meat products in order to determine the moisture, crude protein and crude fat (ether extractable) content. The percentage of protein for food grade collagen was calculated as percentage of nitrogen times 5.56 (Henrickson and Turgot, 1983).

The Analyses During Each Storage Period

The twelve chubs prepared for each formulation were randomly assigned to four storage periods. These storage periods were 0, 2, 4 and 6 weeks. At the end of each storage period, samples from each formulation were obtained for the total aerobic plate count, taste panel evaluation, color measurements of both the uncooked and cooked patties, texture measurements, cook yield, and the Thiobarbituric Acid Test (TBA). The schedule of the analyses is shown in Table III.

Microbiological Assay

The external surface of each chub was disinfected by rinsing with 70% ethanol solution before removing a 1 cm thick cross section from one portion near the midsection (Figure 1) labelled B. The two parts obtained by the removal of the cross section (Figure 1), labelled A and C were returned to the FREAS 815 Low Temperature Incubator (0 C) for latter use (Table III) in Thiobarbituric acid test and the cook yield test, respectively.

The patty was obtained by using a sterile spatula after which one-half of the patty was weighed into a sterile Waring blender jar. A volume of 0.1% peptone water (Difco

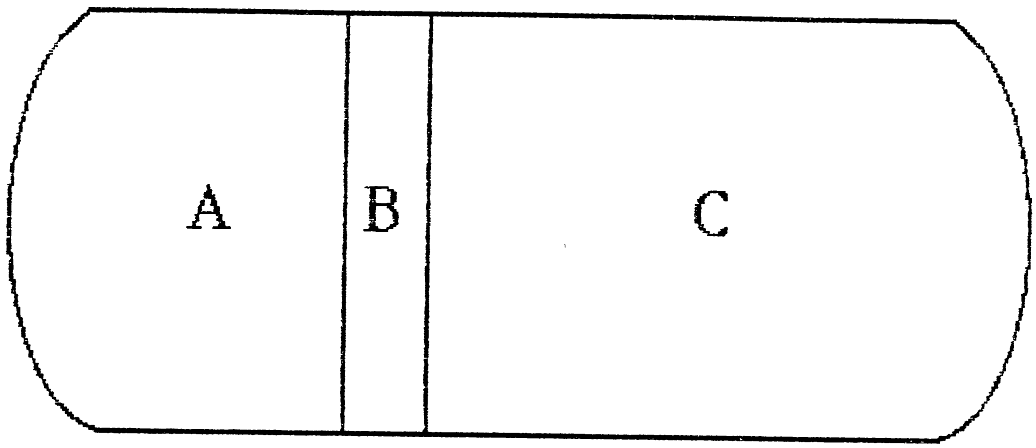

Figure 1. Schematic Diagram of a Sausage Chub Illustrating the Portions Used for the Thiobarbituric Acid Test (A), Total Aerobic Plate Count (B), and Cook Yield Determination (C)

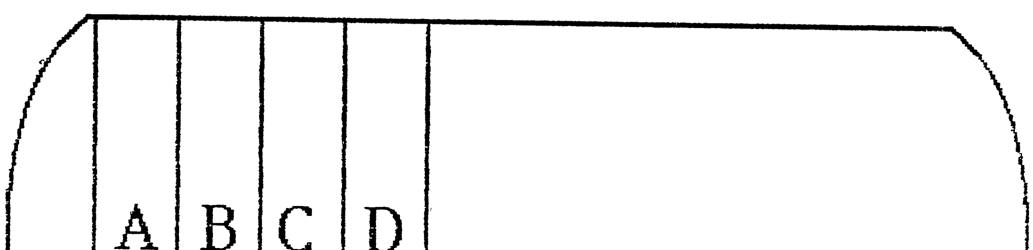

Figure 2. Schematic Diagram of a Sausage Chub Illustrating the Patties Used for Taste Panel Evaluation

Laboratories, Detroit, Michigan, USA) equal to nine times the weight of the sample was added to the sterile Waring blender jar and subsequently the mixture was blended at high speed for 30 seconds. Appropriate subsequent dilutions were prepared using 99 ml volumes of peptone water and plated with standard plate count agar (Difco Laboratories, Detroit, Michigan) using the pour plate method. These dishes were subsequently incubated at 32 C for 48 hours. The colonies were counted with a Darkfield Quebec Colony Counter (American Optical Company Instrument Division, Buffalo, New York) and the total plate count was reported as the logarithm (base 10) of the colony forming units/gram of sample.

Table III

WEEKLY SCHEDULE FOLLOWED FOR THE DIFFERENT ANALYSES OF PORK SAUSAGE

Activity (1)	Day
Pork Sausage Manufacture	Tuesday
Chemical Analyses (A.O.A.C.)	Wednesday
Total Aerobic Plate Count	Thursday
Taste Panel Evaluation	Friday
Color Determination of Uncooked and Cooked Patties	Saturday
Texture Measurement	Saturday
Cook Yield	Sunday
Thiobarbituric Acid Test (TBA)	Sunday

(1) The manufacture of the pork sausage and its chemical analyses are conducted during Week 0 only.

Cooking Method

One centimeter thick patties were cut from two chubs from each formulation and were used for sensory evaluation (Figure 2, patties A, B, C, and D) and texture measurements and color determination of cooked patties (Figure 3, patties D, E, and F). Patties of the same treatment were randomly assigned to one of five rows on the griddle (Figure 4). These patties were cooked on a preheated Toastmaster Deluxe Electric Griddle (Model 1208, Toastmaster, Inc., Boonville, Mo 65233 set at 162.8 C) and turned over every five minutes until each side had been cooked for a total of 10 minutes.

Sensory Evaluation

Panel members, Food Science graduate students and technicians from the Animal Science Department were instructed on the interpretation of the rating scale prior to actual testing. They were instructed to chew the sample and then spit out the residue. Panelists were provided with water for oral rinsing between samples and white bread for removing flavor carryover.

The four samples for the morning session and the five samples for the afternoon session were assigned at random using a table of random numbers. In each taste panel session, four patties from each formulation (Figure 2, patties A, B, C, and D) were cooked using the cooking method described above. Each patty was halved and kept on the

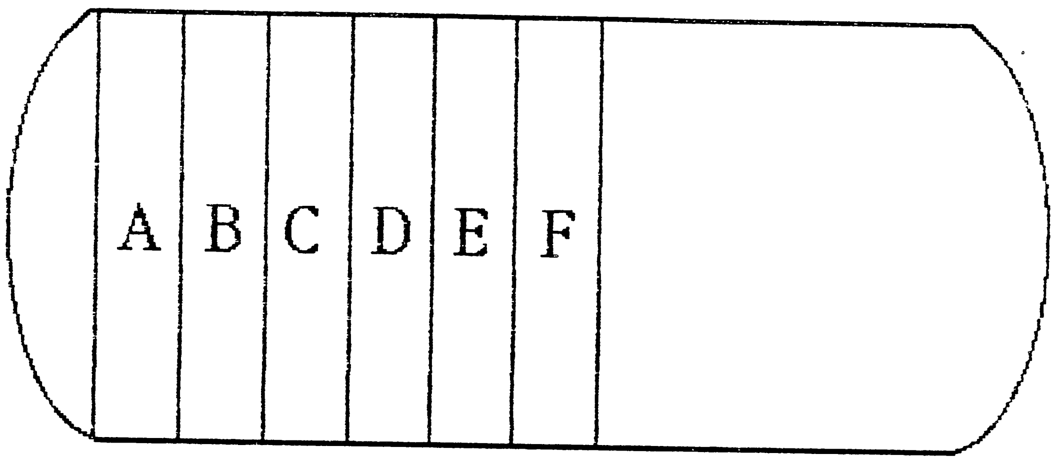

Figure 3. Schematic Diagram of a Sausage Chub Illustrating the Patties Used for Color Determination of Uncooked Patties (A,B, and C) and of Cooked Patties (D,E, and F)

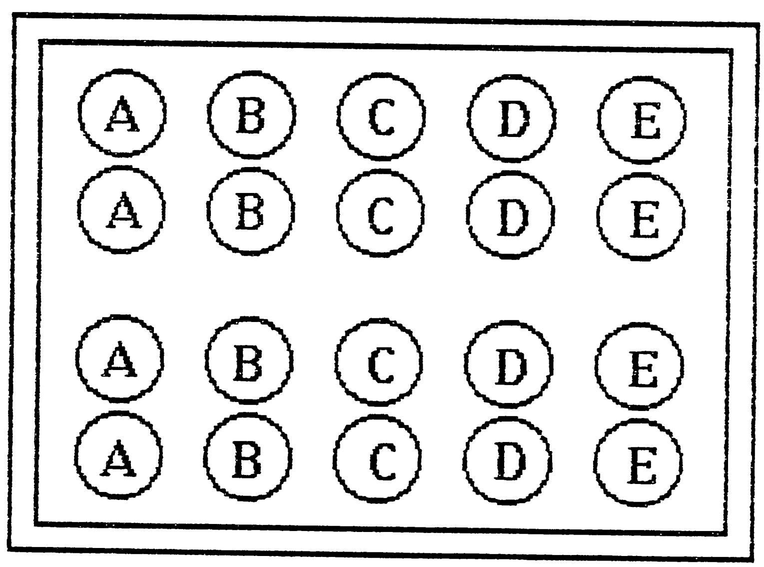

Figure 4. Schematic Diagram of a Griddle Illustrating Locations of Patties During Cooking. Circles with the same letters indicate that patties belong to the same treatment.

griddle set at 65.5 C for no more than 15 minutes to keep the patties at the serving temperature. The samples were then randomly served on paper saucers and evaluated on a descriptive scale (Figure 5) based on the degree of color, juiciness, texture, flavor and overall acceptability.

The descriptive scale used was a modification of a scorecard suggested by Stone et al. (1974). The modified descriptive scale is an interval scale with the following features: the lines are 14 cm long with anchor points after every 2 cm beginning at one end. The vertical lines marked by each panelist on each scale were converted to numerical values using a template. The left endpoint of the line was given a value of 0.00 with each succeeding anchor point to the right being assigned values in increments of 1.00. The right endpoint was given a value of 7.00.

Color Determination

Color measurements were determined for both the uncooked and cooked patties with a Hunterlab D25-9 Optical Sensor (Hunter Associates Laboratory, Inc., Fairfax, Virginia) using a white tile as the standard (No. C2-12544) to calibrate the instrument. Three uncooked patties (Figure 3, patties A, B, and C) from each formulation were allowed to stand for one hour at room temperature to allow the exposed surface to oxygenate. Four readings were then taken at randomly selected locations on each patty.

For the color measurements of the cooked patties, three

NAME____________________________DATE_____________CODE___________

Please taste the sausage sample and answer each question in sequence by placing a vertical line across the horizontal line at the point that best describes that property in the sample. Take sufficient sample and time to evaluate each characteristic.

After you have answered all the questions, return this sheet, and the sample, and wait for the next sample.

If you have any questions or need anything else, please ask the experimenter.

Thank you.

1. Color

Very Undesirable — Moderate — Very Desirable

2. Juiciness

Very Dry — Moderate — Very Juicy

3. Texture

Very Coarse — Moderate — Very Fine

4. Flavor

Extremely Off Flavor — Bland — Intense Pork Flavor

5. Overall Acceptability

Dislike Moderately — Neither — Like Moderately

Figure 5. Sensory Evaluation Score Sheet Used for Cooked Pork Sausage.

patties from each formulation (Figure 3, patties D, E, and F) were cooked using the procedure described under Cooking Method. After cooking, the same procedure used in the color determination of uncooked patties was followed. The data were reported in the 'L' (lightness - darkness), 'a' (redness - greenness), and 'b' (yellowness - blueness) values.

Texture Measurements

Texture was determined using a modification of the method of Kastner et al. (1973). Immediately after the color determination of three cooked patties from each formulation, each patty was cut in half and each half was weighed and individually placed in a Kramer shear cell. The shear force was measured by the Instron Universal Testing Instrument (Model 1122) with the crosshead speed, full scale load, and the chart speed set at 100 mm/min, 50 kg, and 100 mm/min, respectively. The results were reported in kg/g of sample.

Cook Yield

The cook yield of each formulation was determined using the method of Evans and Ranken (1972). A one centimeter slice was cut from the exposed end of the portion allotted for cook yield determination (Figure 1, patty C) after which the remaining portion was divided into quarters. Small increments of approximately five grams each were randomly obtained from each of the four quarters and added onto a preweighed 2.0 x 3.3 cm perforated plate (Figure 6, labelled B) in a preweighed 3.3 x 11.5 cm test tube (Figure 7, labelled A) until a total of approximately 40 grams have been placed into the test tube. No air pockets between the dough and the test tubes were allowed in order to maximize the heat transfer during cooking. After the 40 grams were packed into the tube, the upper surface was shaped into a dome (Figure 6, labelled F) to facilitate the easy flow of fat and moisture down the sides of the test tube during cooking. The condenser (Figure 6, labelled C) was attached and subsequently the test tubes were immersed in an 80 C water bath for 30 min. The level of the water in the water bath was not allowed to decrease below the level of the upper dome of the sample in the tubes.

After cooking, the condenser (Figure 7, labelled C) was detached and the perforated plate along with the cooked sausage (Figure 7, labelled B and E, respectively) was elevated out of the drippings (Figure 7, labelled F and G) until the bottom surface of the perforated plate was at least 1 cm above the upper surface of the drippings. The condenser was reattached and the fat layer (Figure 7, labelled F) was allowed to solidify at room temperature before placing the tubes in a cooler (4 C) for 24 hours. The test tube (Figure 7, labelled A) and the drippings (labelled F and G) were weighed after removing the condenser, the perforated plate, the cooked sausage sample,

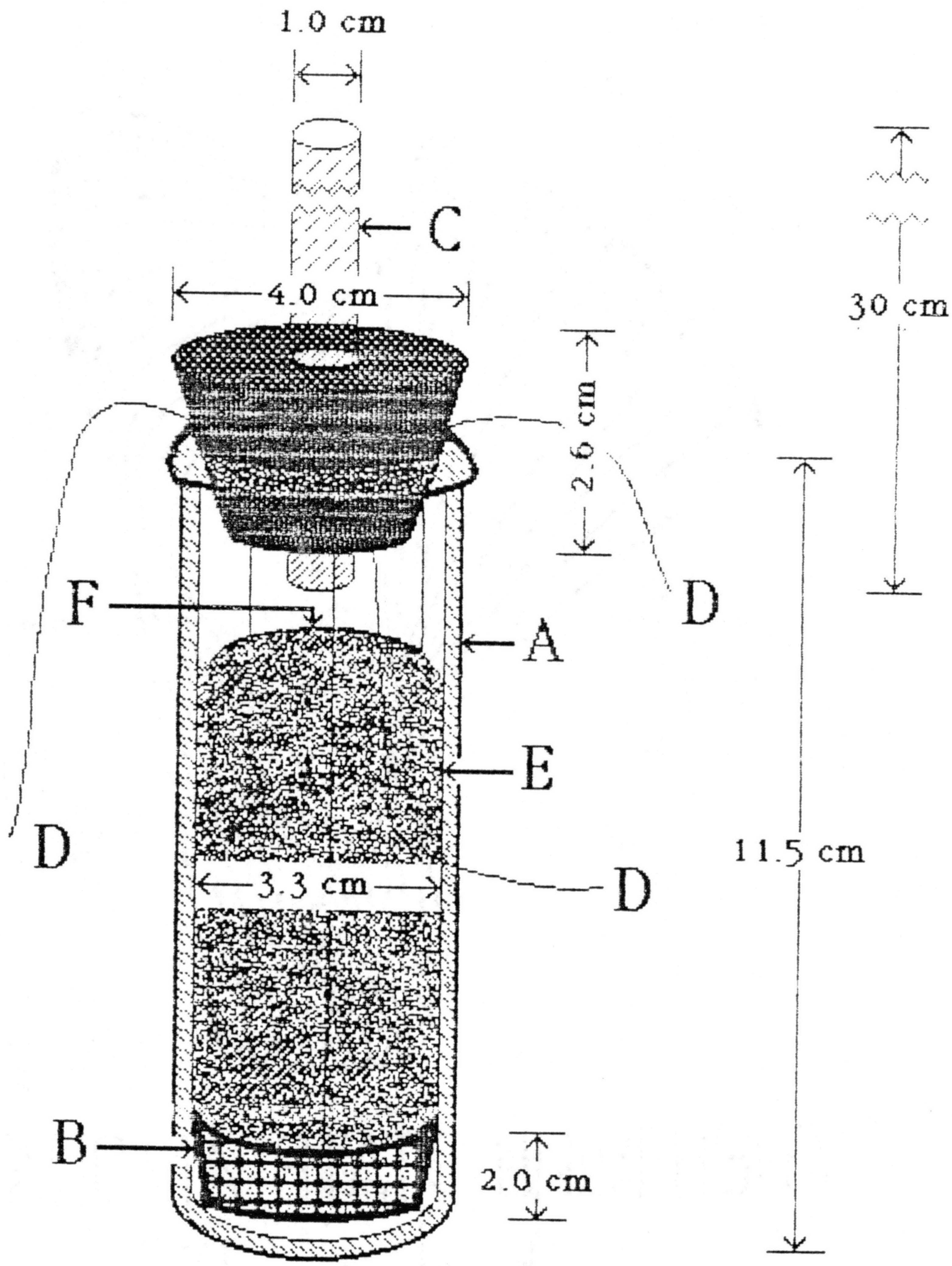

Figure 6. Illustration of the Cook Yield Apparatus (Before Cooking)

Note: A=Test Tube; B=Perforated Plate; C=Condenser; D=String; E=Uncooked Sausage Sample; F=Dome

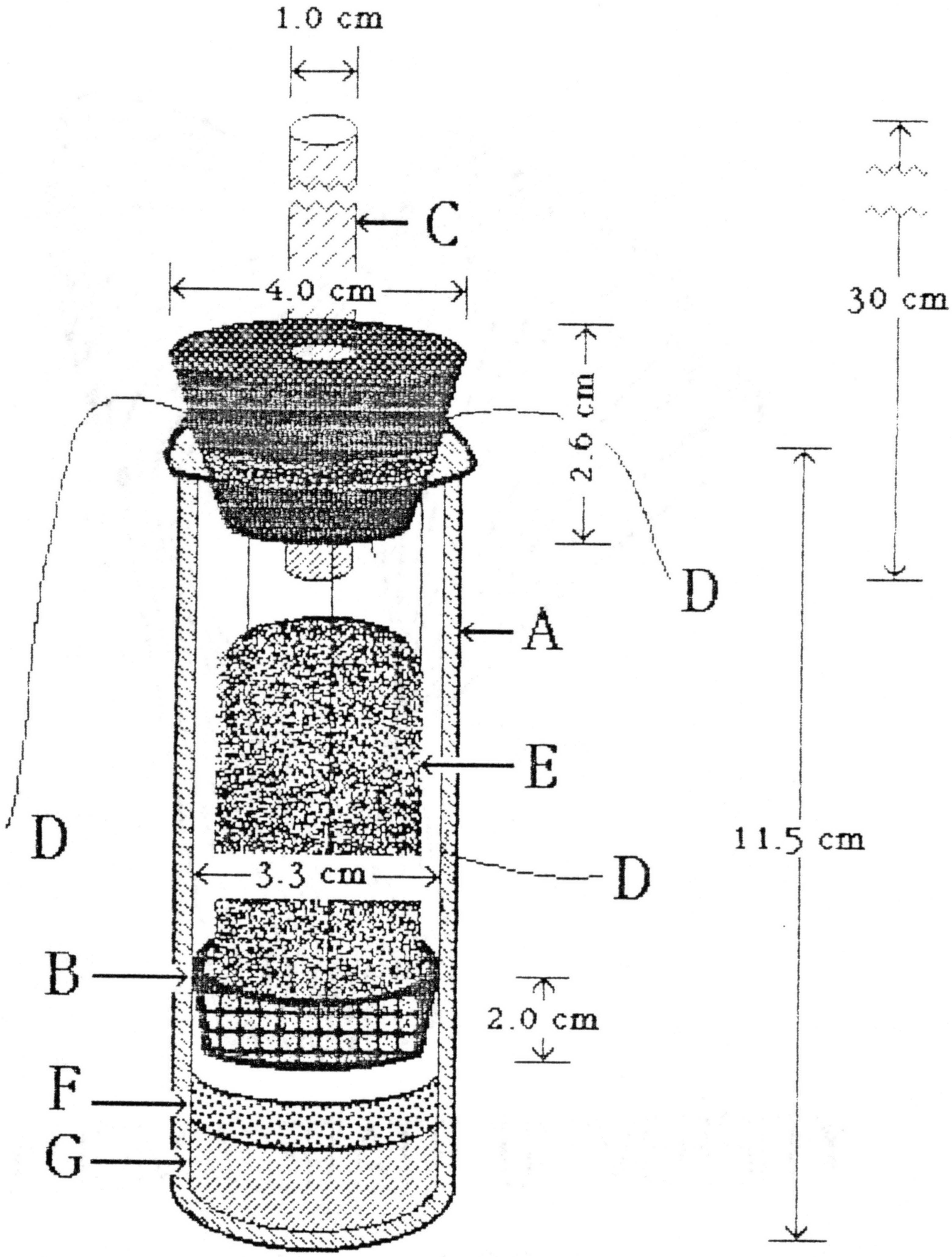

Figure 7. Illustration of the Cook Yield Apparatus (After Cooking)

Note: A=Test Tube; B=Perforated Plate; C=Condenser; D=String; E=Cooked Sausage Sample; F=Fat Layer; G=Liquid Layer and the string (Figure 7, labelled C, B, E, and D, respectively). The average percentage yield of the triplicate samples was calculated by the following equation:

$$\% \text{ cook yield} = \frac{100(\text{Cooked Sample Weight})}{(\text{Uncooked Sample Weight})} \quad \text{where}$$

Cooked Sample Weight = Uncooked Sample Wt. - Drip Wt.

Thiobarbituric Acid Test

Thiobarbituric acid values (TBA) were determined for the raw sausages by the distillation method described by Ockerman (1980). Reagents were freshly prepared prior to each TBA determination.

Ten grams from each formulation was homogenized in 50 ml of 50 C distilled water in an OMNI-MIXER (Model 17150, Sorvall, Inc., Newtown, Connecticut) for 2 min set at maximum speed. The homogenate was then transferred to a Kjeldahl flask to which 47.5 ml of 50 C distilled water and 2.5 ml of 3.99 N HCl were added. The mixture was digested using the Kjeldahl digester (setting at number 6) until about 50 ml of the distillate was collected. Five ml of the distillate was transferred to a test tube to which 5 ml of 0.02 M 2-thiobarbituric acid aqueous solution was added. The test tube was capped, heated in a boiling water bath for 35 minutes, and cooled in tap water at room temperature for 10 min. The absorbance was then measured at 530 nm using the Gilford Spectrophotometer 240. TEP (1, 1, 3, 3-tetraethoxypropane) standards were run along with each group of sausage samples for the standard curve.

A standard curve was prepared with each TBA determination by using a 0.001 M stock solution of tetraethoxypropane (malonaldehyde) to prepare solutions with concentrations of 2.0, 4.0, 6.0, 8.0 and 10.0 nanomoles/liter. Five ml. of these solutions were transferred to a test tube to which 5 ml of 0.02 M 2-thiobarbituric acid aqueous solution was added. The test tube was capped, heated in a boiling water bath for 35 minutes, and cooled in tap water at room temperature for 10 min. The absorbance was measured at 530 nm using the Gilford Spectrophotometer 240. The absorbance was plotted versus the concentration and the slope of the curve was calculated using regression analysis (Steel and Torrie, 1980). The slope was used in calculating the constant K (Ockerman, 1980). The TBA numbers for the sausage samples were calculated by multiplying the absorbance by the constant K and the value was reported in mg of malonaldehyde per 1,000 g of sample. All analyses were done in triplicate.

Statistical Analyses

The data from the analyses of fat, protein, and moisture of the nine treatments involving the two types of replacement (lean tissue and fat tissue) and the five levels of collagen (0, 5, 10, 15, and 20%) were analyzed in a completely randomized design (CRD). Three observations from each treatment were taken. The data from the analyses for fat, protein, and moisture of food grade collagen were also analyzed in a completely randomized design with three observations per batch of food grade collagen used.

A 4 x 5 factorial arrangement of treatments (FAT) in a randomized block design (RBD) was used for the analyses of the data for the total aerobic plate counts, TBA numbers, cook yield, color values of both the uncooked and cooked patties, and Instron shear force values. The four levels of storage were 0, 2, 4, and 6 weeks while the five collagen levels were 0(control), 5, 10, 15, and 20%. Three subsamples per cell (storage period x collagen) were obtained for the total aerobic plate counts, TBA numbers, and cook yield. Four subsamples per cell were obtained for the color determination of both the uncooked and cooked pattes while for the Instron shear force values, duplicate measurements were made. These analyses were performed on both the lean tissue and fat tissue replaced sausages.

A split plot model in a randomized block design was used for the analyses of the data for the sensory variables in the taste panel. Storage periods were the main plot treatment factors while collagen levels and panelists were the subunit treatment factors.

The Statistical Analysis System (Barr and Goodnight, 1972) and Steel and Torrie (1980) were used in calculating the analysis of variance for the completely randomized design, randomized block with factorial arrangement of treatments, and randomized block with split-model. Comparison of mean values of results from the taste panel was accomplished using the methods of Duncan (1955).

CHAPTER IV

RESULTS AND DISCUSSION

Chemical Analyses

The chemical analyses of nine different pork sausage samples are shown in Table IV. For the treatments with lean tissue replacement, the percentages of fat, crude protein, and moisture were similar. The percentage of fat decreased while the percentage of water increased for the treatments with fat tissue replacement. The decrease in the amount of fat in the sausages could be attributed to the fact that food grade collagen has a very minimal amount of fat (Table V). Only the percentage of protein remained relatively constant probably as a result of the replacement of the protein in the fat tissue with approximately the same amount of protein from the collagen.

The chemical analysis of the food grade collagen (Table V) revealed similar percentages of fat, moisture, and protein for all four lots. Statistical analysis of the data revealed no significant variation among batches ($P>0.05$)(Appendix Tables XXIII-XXV).

The factor 5.56 was used to convert the percent nitrogen to percent protein in the collagen. Although the

TABLE IV

CHEMICAL ANALYSIS OF PORK SAUSAGE AS INFLUENCED BY LEAN TISSUE AND FAT TISSUE REPLACEMENT AND BY COLLAGEN LEVELS (1)

Treatment	Fat %	Crude Protein %	Moisture %
C00	29.86(1.61)	13.68(0.81)	55.19(1.89)
L05	30.02(1.00)	13.34(0.63)	55.18(3.19)
L10	28.66(1.09)	12.94(1.00)	56.39(2.29)
L15	29.56(0.72)	12.49(1.26)	56.59(2.75)
L20	28.88(1.34)	12.74(1.50)	58.12(3.08)
F05	27.44(1.50)	13.87(1.08)	56.61(2.11)
F10	24.66(0.94)	13.58(1.46)	58.30(1.96)
F15	20.29(0.66)	14.60(1.97)	63.90(2.30)
F20	17.17(0.94)	14.42(0.79)	67.45(2.07)

(1) Means from 12 observations.
Values in parentheses indicate the standard deviation.

TABLE V

CHEMICAL ANALYSIS OF COLLAGEN (1)

Block	Fat %	Crude Protein %	Moisture %
1	0.40(0.15)	19.53(0.47)	79.09(0.44)
2	0.31(0.31)	18.00(1.04)	79.15(1.02)
3	0.33(0.19)	17.56(1.58)	78.27(0.55)
4	0.31(0.14)	19.97(1.42)	78.80(0.60)
Avg	0.34(0.18)	18.76(1.47)	78.82(0.69)

(1) Means from 12 observations.
Values in parenthesis indicate the standard deviations.

addition of collagen may affect the total protein in the pork sausages, the factor 6.25 was still used to calculate the percent protein. The amount of protein contributed by collagen on a weight basis is a small fraction of the total protein contributed by the other components of the sausage, i.e., the lean and fat tissues. Therefore, any calculation involving a different factor to account for the incorporation of the collagen would yield protein values that would be slightly but not significantly higher than the protein values of the pork sausages previously calculated.

Total Aerobic Plate Count

Increasing the collagen levels of sausage did not affect ($P>0.05$) the total microbial count in either the lean tissue-replaced or fat tissue-replaced sausages (Appendix Table XII). However, the total microbial counts were significantly affected ($P<0.05$) by the storage periods in both replacements (Appendix Table XIII). Figures 8 and 9 show similar microbial counts for the different replacement levels at Week 0. From Week 0 and Week 2, there was an increase of nearly 2 log cycles in the microbial counts with the fat tissue-replaced sausages yielding slightly higher counts compared to the lean tissue-replaced sausages probably due to the higher moisture content and lesser fat content of the fat tissue-replaced sausages. Between the second and fourth weeks, the increase in the microbial count was only about one-half of a log cycle for both types of

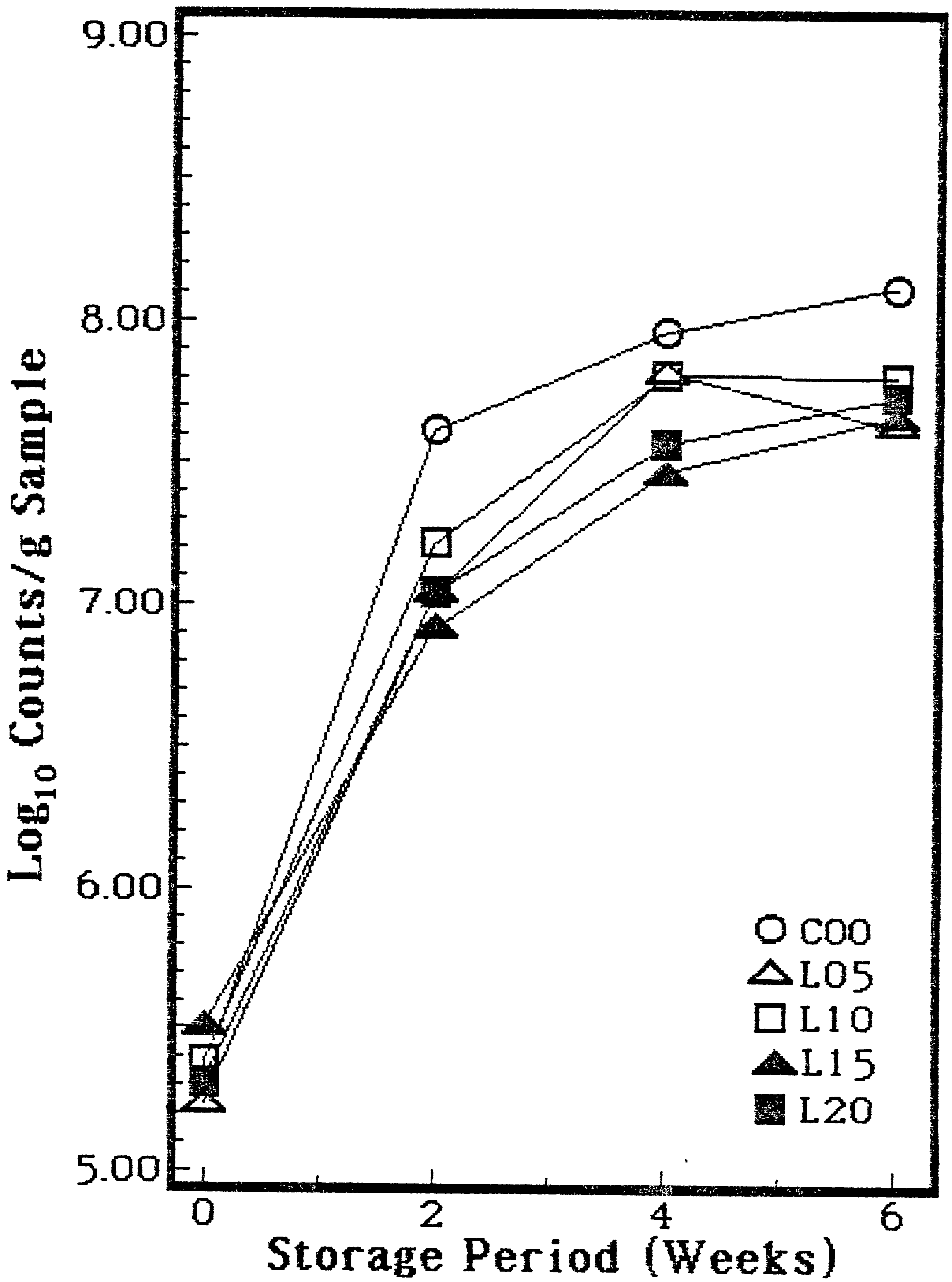

Figure 8. Effect of Storage Period on the Total Aerobic Plate Counts of Pork Sausages when Collagen Replaced Portions of the Lean Tissue

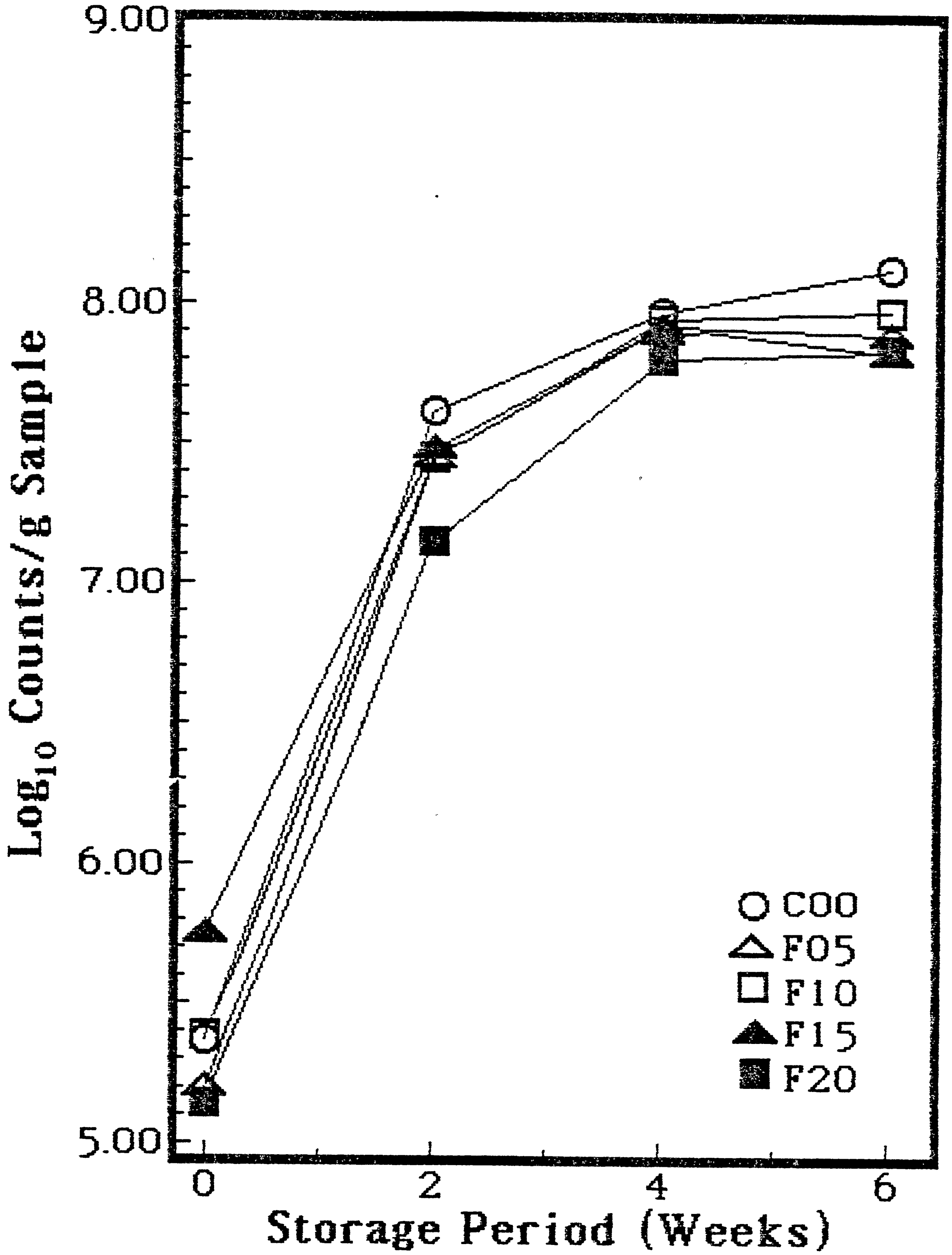

Figure 9. Effect of Storage Period on the Total Aerobic Plate Counts of Pork Sausages when Collagen Replaced Portions of the Fat Tissue

replacement. This would suggest that the microbial growth had already begun to taper off probably because the microorganisms have begun to approach the stationary phase of growth. Finally, the microbial count in both instances started to level off between the fourth and sixth week of storage suggesting that bacterial growth may have approached their maximum growth and thus have reached the stationary phase.

TBA Number

Increasing the levels of collagen significantly decreased ($P<0.05$) the TBA numbers of both the lean tissue and fat tissue replaced sausages (Appendix Table XII). Figures 10 and 11 show that the sausages involving both types of replacement at the 10, 15, and 20% levels had significantly lower TBA numbers ($P<0.05$) than the sausages replaced at the 5% level and the control. In the case of the fat tissue-replaced sausages, the decrease in TBA numbers with increasing levels of collagen might be expected since the total amount of unsaturated fatty acids would be less than normally found in the sausage. On the other hand, a decrease in the TBA numbers with increasing levels of collagen was not expected for the lean tissue-replaced sausages since the fat content of the collagen was very similar. It is possible that collagen may be acting alone or with the other components present in the sausages to inhibit the oxidation of the unsaturated fatty acids.

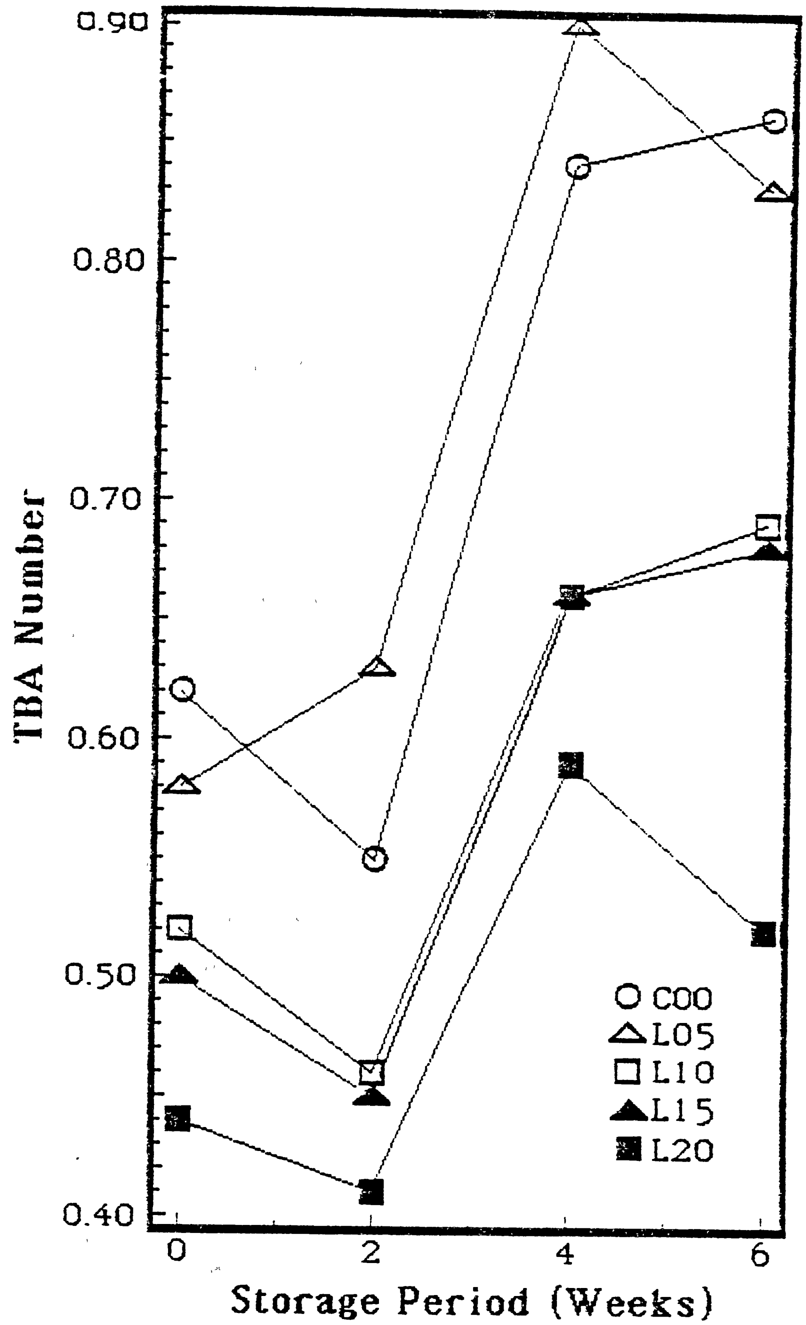

Figure 10. Effect of Storage Period on the TBA Numbers of Pork Sausages when Collagen Replaced Portions of the Lean Tissue

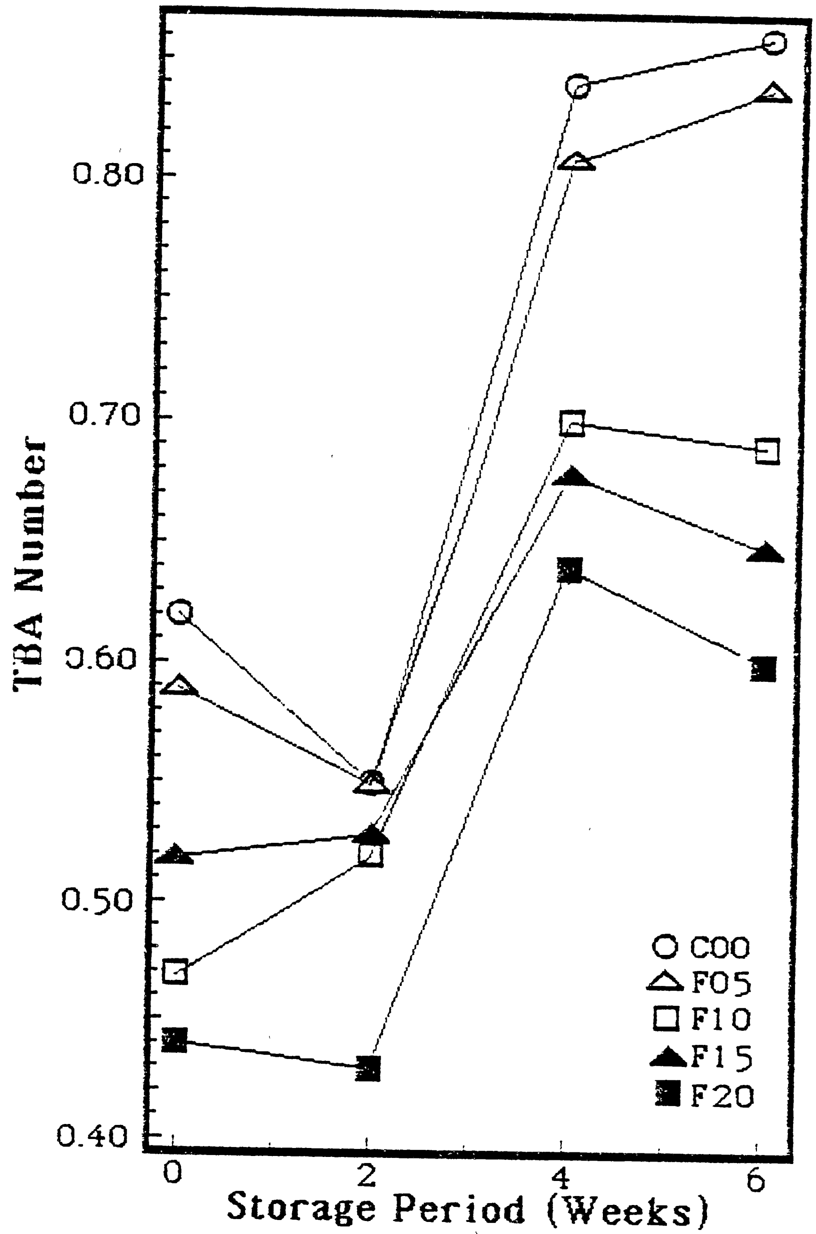

Figure 11. Effect of Storage Period on the TBA Numbers of Pork Sausages when Collagen Replaced Portions of the Fat Tissue

The storage periods significantly increased ($P<0.05$) the TBA numbers of the sausages involving both replacement types (Appendix Table XIII). Figures 10 and 11 again show that the TBA numbers for both the lean tissue and the fat tissue replaced sausages had similar values for Week 0 and Week 2 indicating that the sausages were stable during the first two weeks of storage probably due to the antioxidant activity of the spices such as sage. There was a significant increase ($P<0.05$) in TBA numbers between Week 2 and Week 4 in both instances. Evidently, it was only after this storage period that the oxidation reaction had already proceeded to a measurable degree. Between Week 4 and Week 6, the TBA numbers for both types of sausages seem to have levelled off. It is unlikely that all the unsaturated fatty acids had already been oxidized. It would be more probable that the oxygen originally present within the sausage had already been utilized. Assuming that the type of casing used in this study is permeable to oxygen in particular, it would still take a considerable length of time for any additional oxygen to diffuse into the sausage to cause any significant increase in the TBA numbers.

Cook Yield

Replacing the lean tissue at the 20% level significantly decreased ($P<0.05$) the cook yield (Appendix Table XII). At lower levels of replacement, there were no significant differences ($P>0.05$) in the mean cook yield.

These results showed that at lower levels, food grade collagen was as good as the other myofibrillar proteins in terms of their water-holding capacity in pork sausage. For the fat tissue-replaced sausage, the cook yield was significantly greater (P<0.05) for those sausages replaced at the 10, 15, and 20% levels with collagen when compared with the control (Appendix Table XII). These results indicated that food grade collagen may supplement the water-holding capacity of the myofibrillar proteins. Also by replacing the fat tissue in the sausage, there would be less fat to be lost during cooking.

The storage period signficantly affected (P<0.05) the cook yield of both the lean tissue and the fat tissue replaced sausages (Appendix Table XIII). Figures 12 and 13 show a significant decline in the cook yield between Week 0 and Week 2 and between Week 2 and Week 4 with the cook yield levelling off between Week 4 and Week 6. This decline in the cook yield may be due to protein denaturation which would lead to a lower water-holding capacity. This decreased water-holding capacity would eventually lead to a greater loss of water upon cooking.

Instron Shear Force

Shear force, measured in kg/g of sample, did not vary (P>0.05) with increasing levels of collagen as a replacement for lean tissue (Table VI). These results are not in agreement with those obtained by Chavez (1983) who found

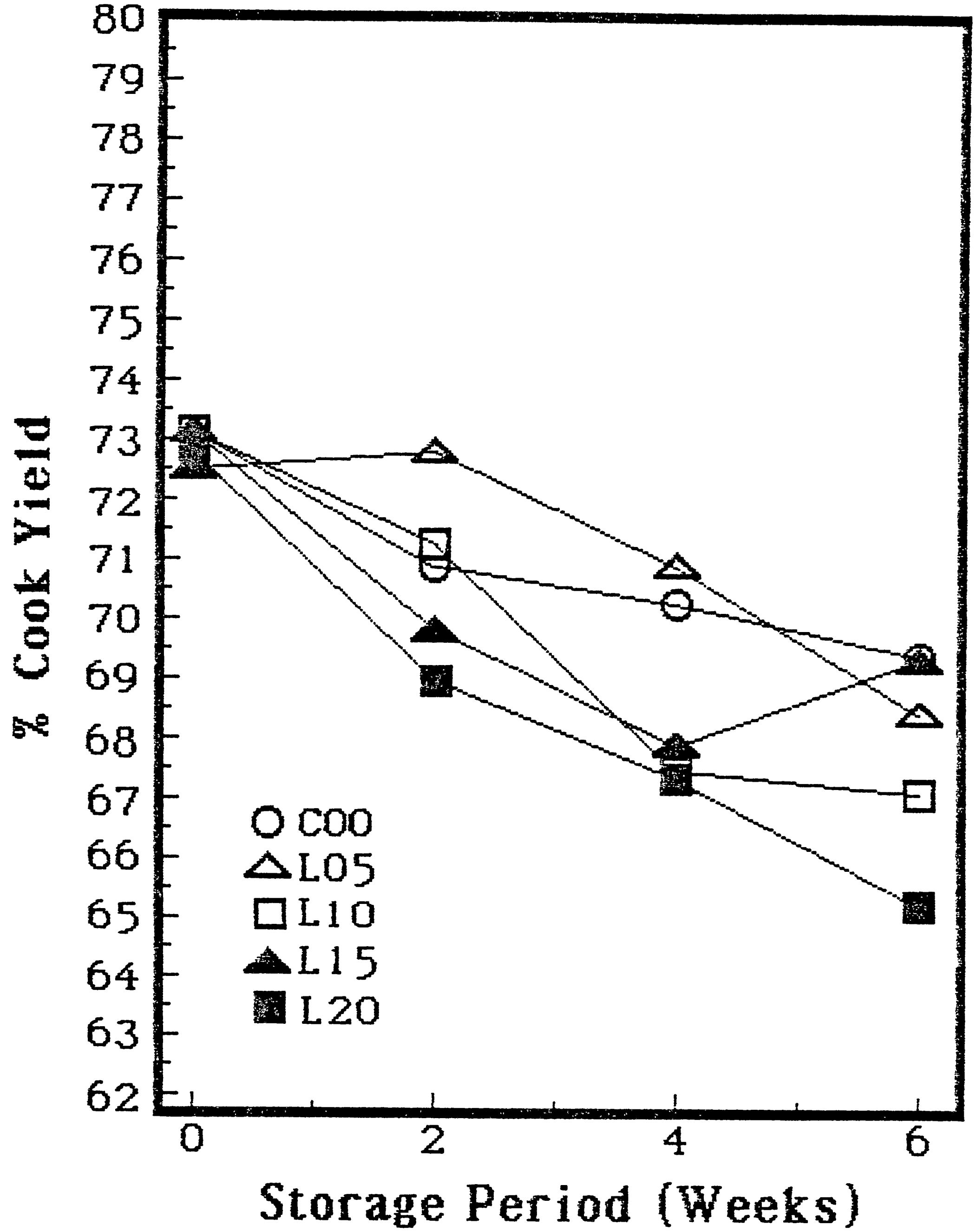

Figure 12. Effect of Storage Period on the Cook Yield of Pork Sausages when Collagen Replaced Portions of the Lean Tissue

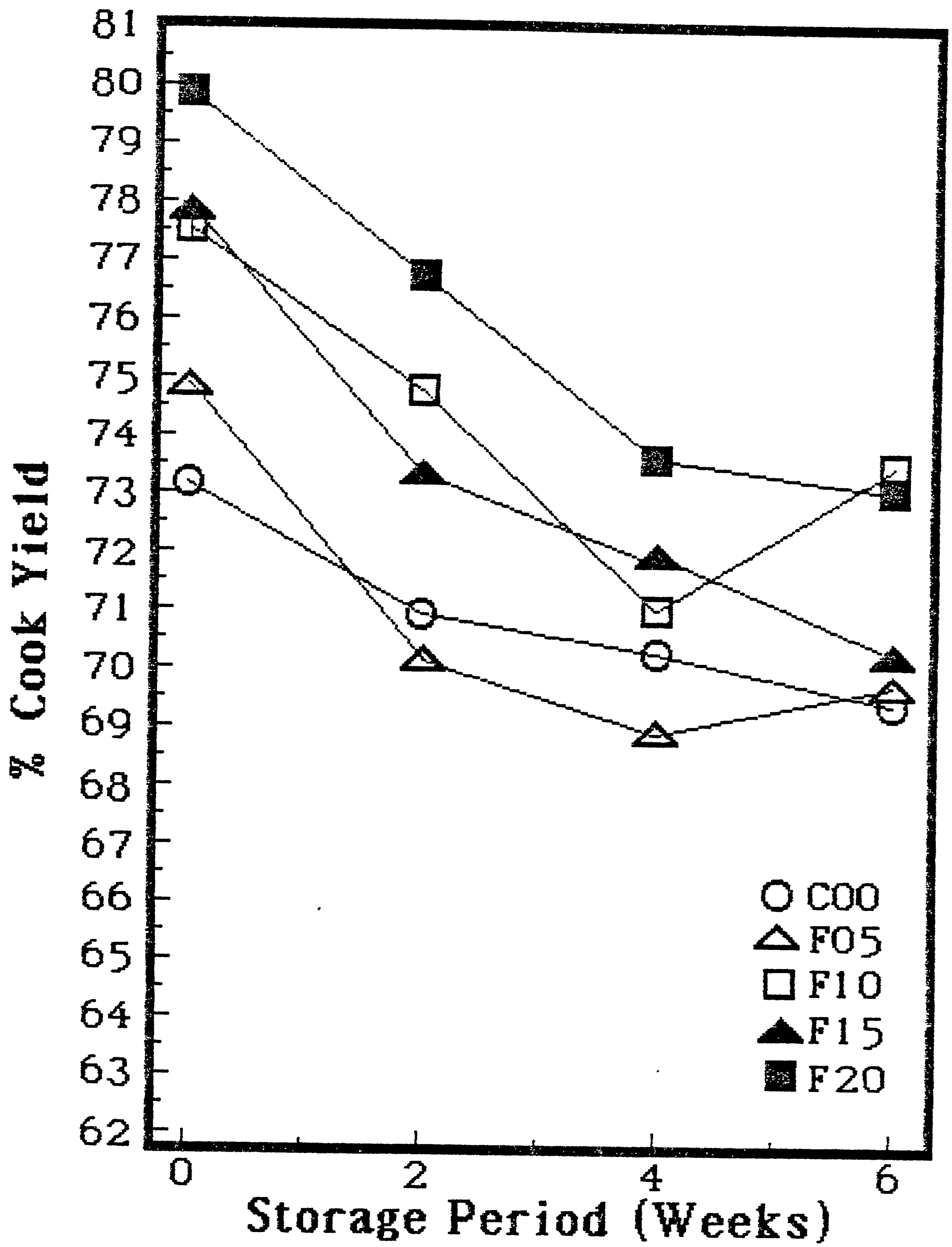

Figure 13. Effect of Storage Period on the Cook Yield of Pork Sausages when Collagen Replaced Portions of the Fat Tissue

TABLE VI

SUMMARY OF MEAN SHEAR FORCE VALUES FOR EACH STORAGE PERIOD FOR COOKED PORK SAUSAGE WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT (1)

Treatment	Weeks of Storage 0	2	4	6
C00	9.10(2.85)	9.05(3.36)	8.65(3.25)-	7.68(2.56)
L05	7.96(2.56)	8.54(1.87)	9.36(4.16)	6.84(2.47)
L10	8.63(2.56)	9.37(2.93)	7.43(3.22)	7.76(3.15)
L15	8.97(2.60)	8.40(2.86)-	8.74(4.30)	8.69(4.23)
L20	7.78(2.91)	9.17(3.56)	8.69(4.73)	7.30(2.64)
C00	9.10(2.85)	9.05(3.36)	8.65(3.25)-	7.68(2.56)
F05	8.65(3.34)	7.95(1.37)	8.79(4.13)	8.04(3.21)
F10	7.15(2.56)	7.72(2.35)	7.44(3.58)	7.16(4.66)-
F15	7.42(2.78)	9.06(9.30)	7.31(3.19)	7.26(3.27)
F20	5.07(1.69)	5.74(1.76)	6.73(3.22)	5.80(2.44)

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
(-) One missing observation.

that increasing collagen levels in hamburger patties decreased the mean shear force. Some of the factors that may account for these differences are: differences in the types of collagen used, differences in the types of meat and differences in the cooking method employed. The sausage involving the replacement of fat tissue at the 20% level (Table VI) yielded a significantly lower ($P<0.05$) mean shear value compared to the four other treatments.

Storage period did not have an effect ($P>0.05$) on the mean shear force of the sausages involving both types of replacement (Tables VII). Chavez (1983) and Schalk (1981) found that the mean shear force of hamburger patties and coarse bologna, respectively increased with storage. Again, their results are in contradiction with the results obtained in this study. These differences may be accounted for by the reasons previously cited.

Objective Color

The Hunter 'L' value has a standard of 0 for black and 100 for white. Increasing the collagen level and reducing the lean tissue produced significantly whiter ($P<0.05$) uncooked pork sausage patties with lean tissue replacement (Appendix Table XIV). The patties replaced with collagen at the 10, 15 and 20% levels were significantly whiter ($P<0.05$) than the control. Replacement of a portion of the lean tissue results in a reduction in the total amount of myoglobin pigments available for oxygenation.

Replacing the fat tissue portion of pork sausage with collagen did not significantly affect (P>0.05) the whiteness or darkness of the uncooked patties (Appendix Table XIV). Food grade collagen and pork fat are similar in appearance in terms of their degree of whiteness. Consequently, one would not expect to see a color difference.

The uncooked pork sausage patties involving both lean tissue and fat tissue replacement produced significantly darker patties (P<0.05) upon storage (Figures 14 and 15). At Week 2, the patties were significantly darker (P<0.05) compared to the patties at Week 0. At this stage, the darkening effect may be attributed to the red color contributed by the production of oxymyoglobin from the reaction of myoglobin with oxygen. Patties at Week 4 and 6 did not become significantly darker (P>0.05) compared to the patties at Week 2. Any further discoloration would have been due to the color contributed by metmyoglobin, an oxidized form of oxymyoglobin which is brown in color. Further discoloration did not occur probably because the oxygen originally present in the sausage had already been utilized.

In a similar study on pork sausage, Reagan et al. (1983) found progressively increasing Hunter 'L' values of the internal surfaces of pork sausage upon storage. This increase in values was attributed to the rapid formation of metmyoglobin pigments from the myoglobin or oxymyoglobin pigments. The results obtained from this experiment were

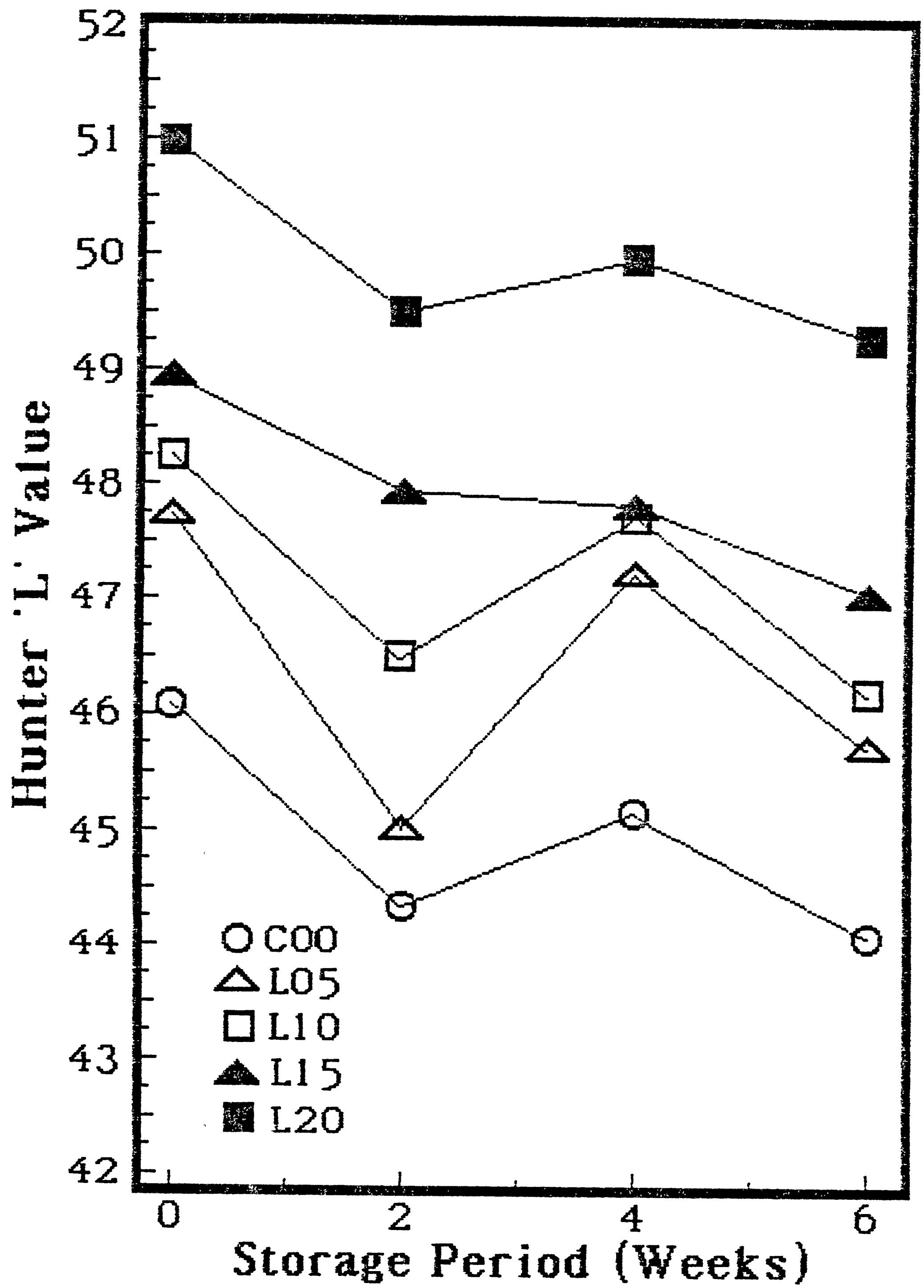

Figure 14. Effect of Storage Period on the Hunter 'L' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of Lean Tissue

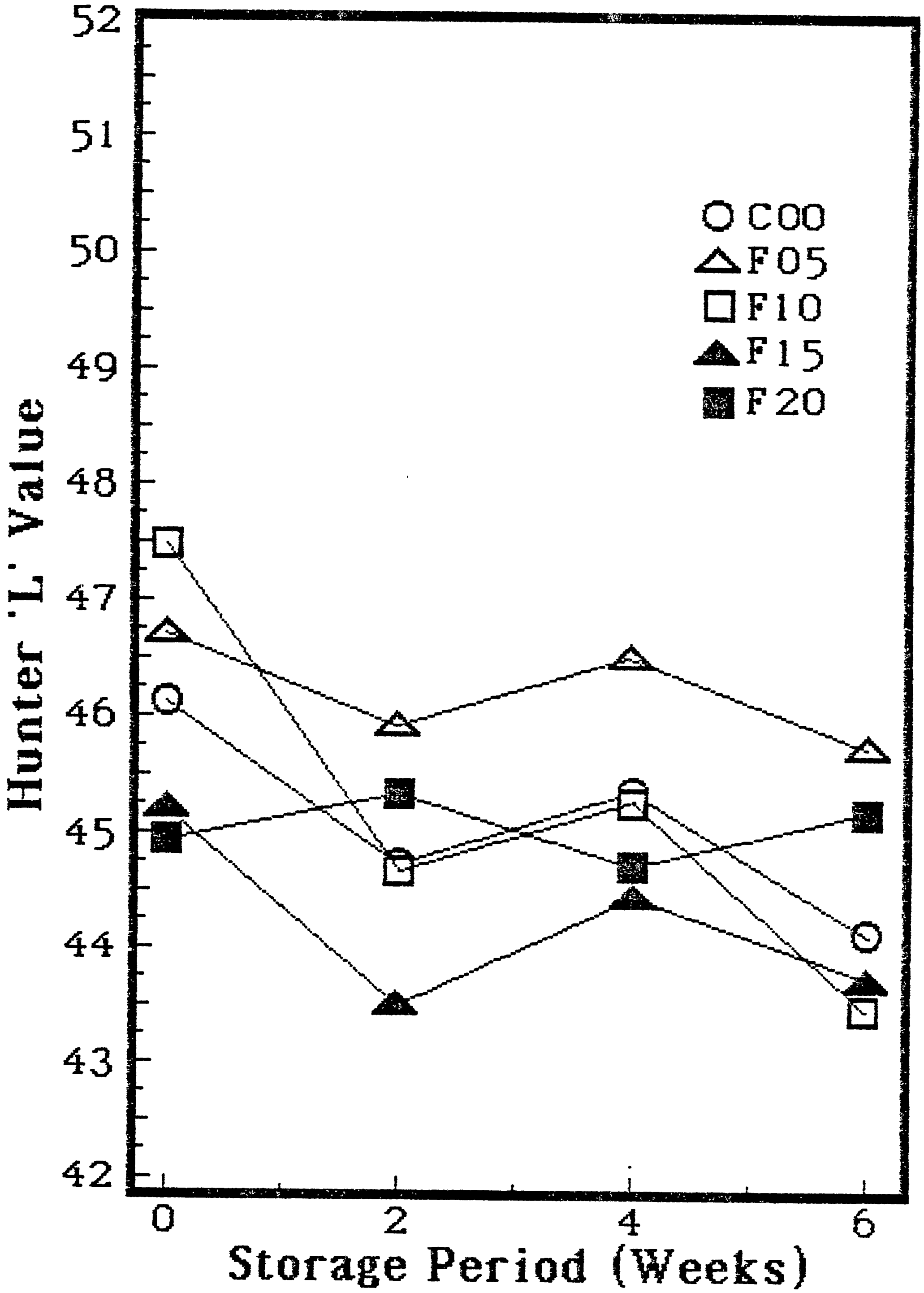

Figure 15. Effect of Storage Period on the Hunter 'L' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

quite different probably due to a slower rate of formation of the metmyoglobin pigments.

The Hunter 'a' value has a standard of -80 for green and +100 for red. Patties wherein 10, 15, and 20% of the lean tissue portion were replaced with collagen were found to be significantly less red (P<0.05) than the patties of the control or the sausage replaced with collagen at the 5% level (Appendix Table XIV). Lesser amounts of myoglobin, as a result of the replacement of some of the lean tissue, result into a decreased level of redness. Consequently, less myoglobin would be available for oxygenation to produce oxygmyoglobin when exposed to air before taking color readings. Oxymyoglobin is the compound that contributes to the bright red color of meat. On the other hand, the redness - greenness of the uncooked patties involving fat tissue replacement were not significantly affected (P>0.05) by an increased level of collagen (Appendix Table XIV).

The patties involving lean tissue replacement were significantly more red (P<0.05) only for Week 2 (Figure 16). The graph shows an initial increase in the Hunter 'a' value at Week 2. This increase may attributed to the production of the oxymyoglobin as has been mentioned earlier in relation to the changes occurring with the Hunter 'L' values. It appears that a decrease in the Hunter 'L' value at Week 2 is accompanied by an increase in the Hunter 'a' value. The patties at Week 4 and Week 6, however, were not significantly different (P>0.05) than the patties at Week 0. This decline in the Hunter 'a' after Week 2 can be

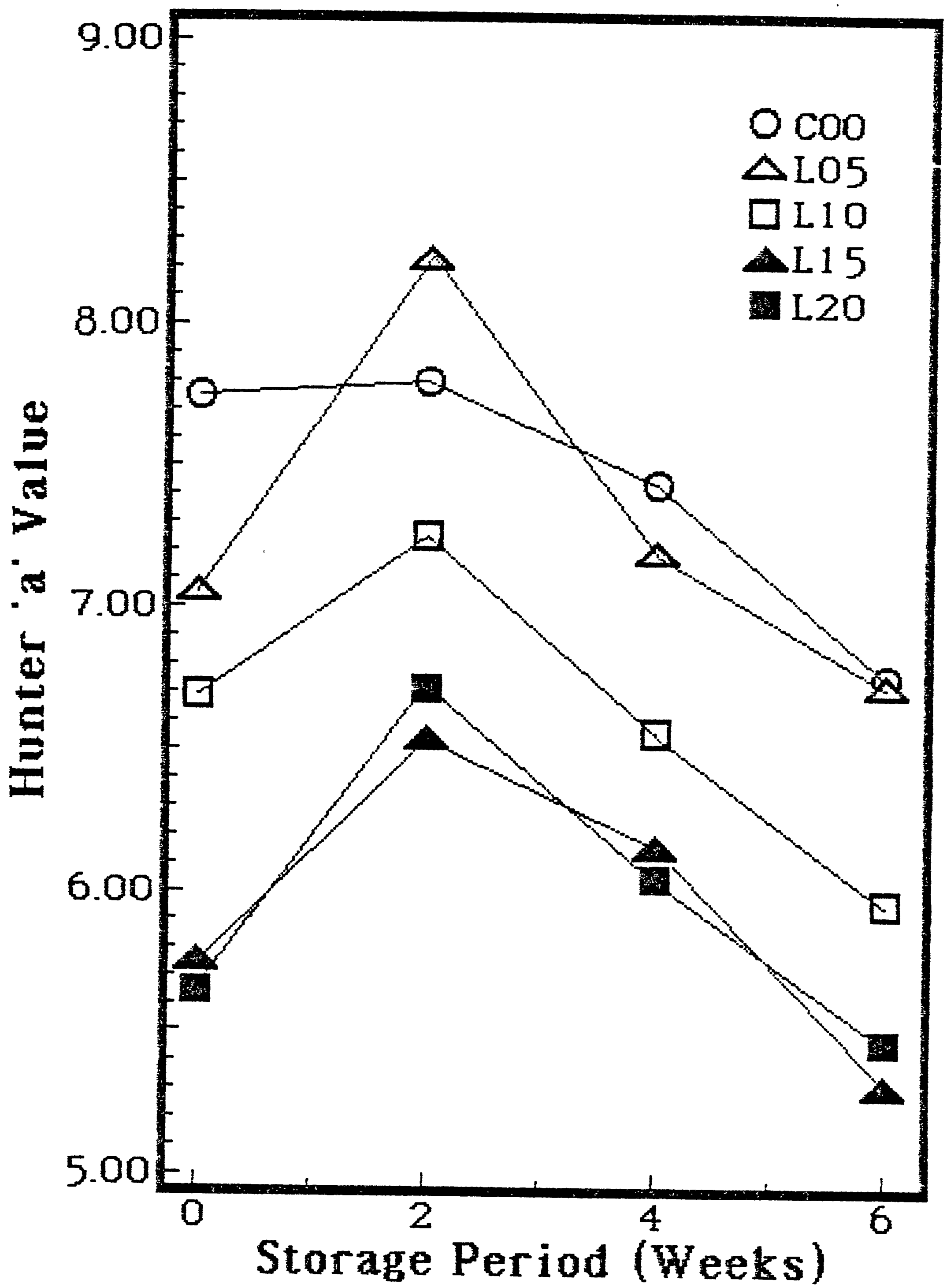

Figure 16. Effect of Storage Period on the Hunter 'a' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of the Lean Tissue

attributed to the discoloration as has been previously discussed.

The patties involving fat tissue replacement (Figure 17) followed the same trend as the patties involving lean tissue replacement except that the patties were significantly less red ($P<0.05$) only after six weeks of storage.

The Hunter 'b' value has a standard of -70 for blue and +70 for yellow. Replacing either the lean tissue or the fat tissue with collagen at 15 and 20% level produced patties that were significantly more blue ($P<0.05$) than the patties of the control (Appendix Table XIV). Figures 18 and 19 illustrate the changes in the Hunter 'b' values of the patties involving both types of replacement. The patties involving both types of replacement were significantly more blue ($P<0.05$) only after six weeks of storage (Appendix Table XIV).

Increasing collagen levels in pork sausage did not significantly affect ($P>0.05$) the color attributes of the cooked patties involving the replacement of lean tissue (Appendix Table XVI). For those sausages involving the replacement of fat tissue, only the patties at the 20% replacement level were found to be significantly whiter ($P<0.05$) than the control (Appendix Table XVI).

Storage periods produced significantly whiter ($P<0.05$) cooked patties involving both types of replacement (Figures 20 and 21). For the lean tissue-replaced sausages, the cooked patties were significantly whiter ($P<0.05$) in color

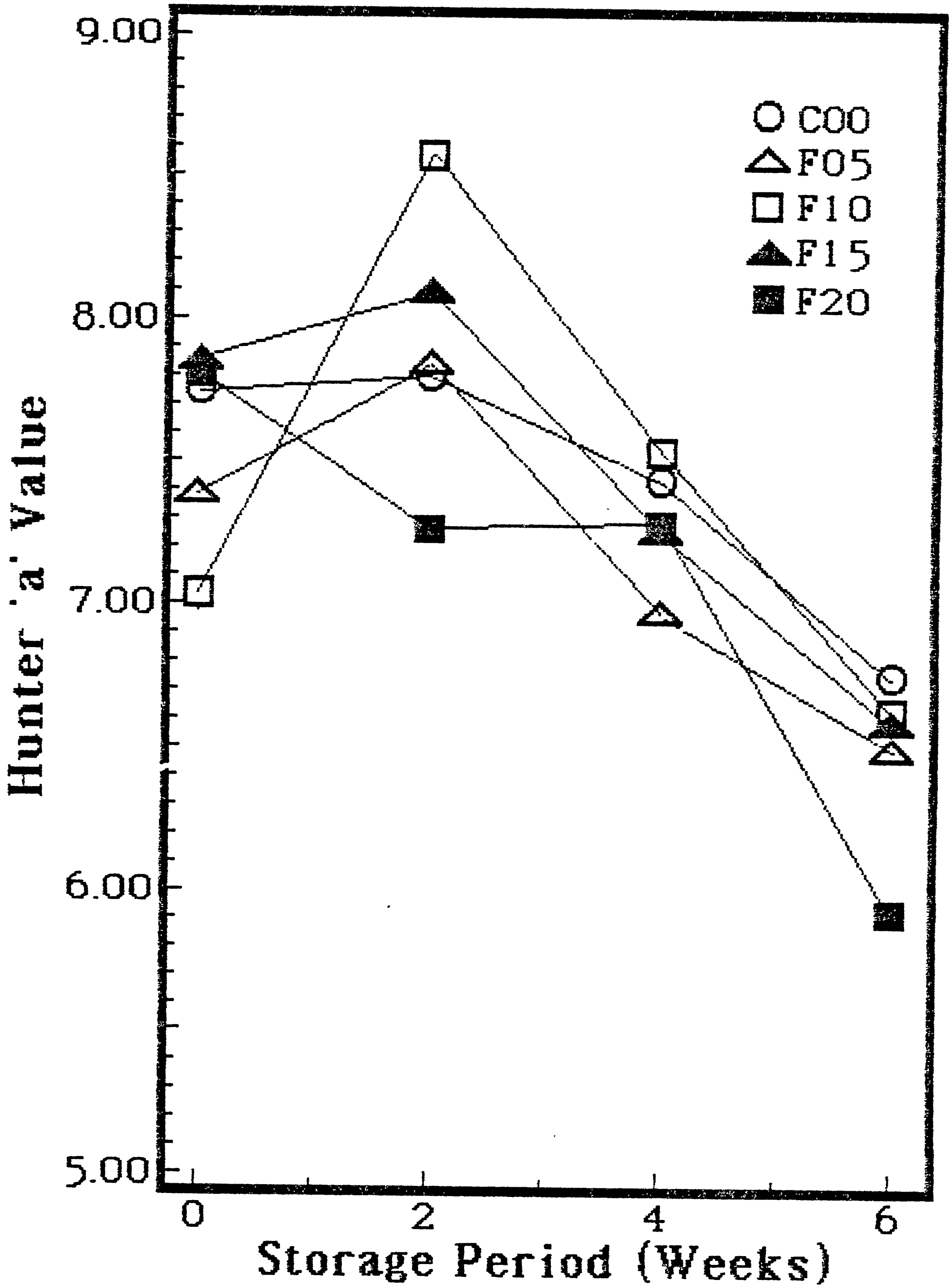

Figure 17. Effect of Storage Period on the Hunter 'a' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

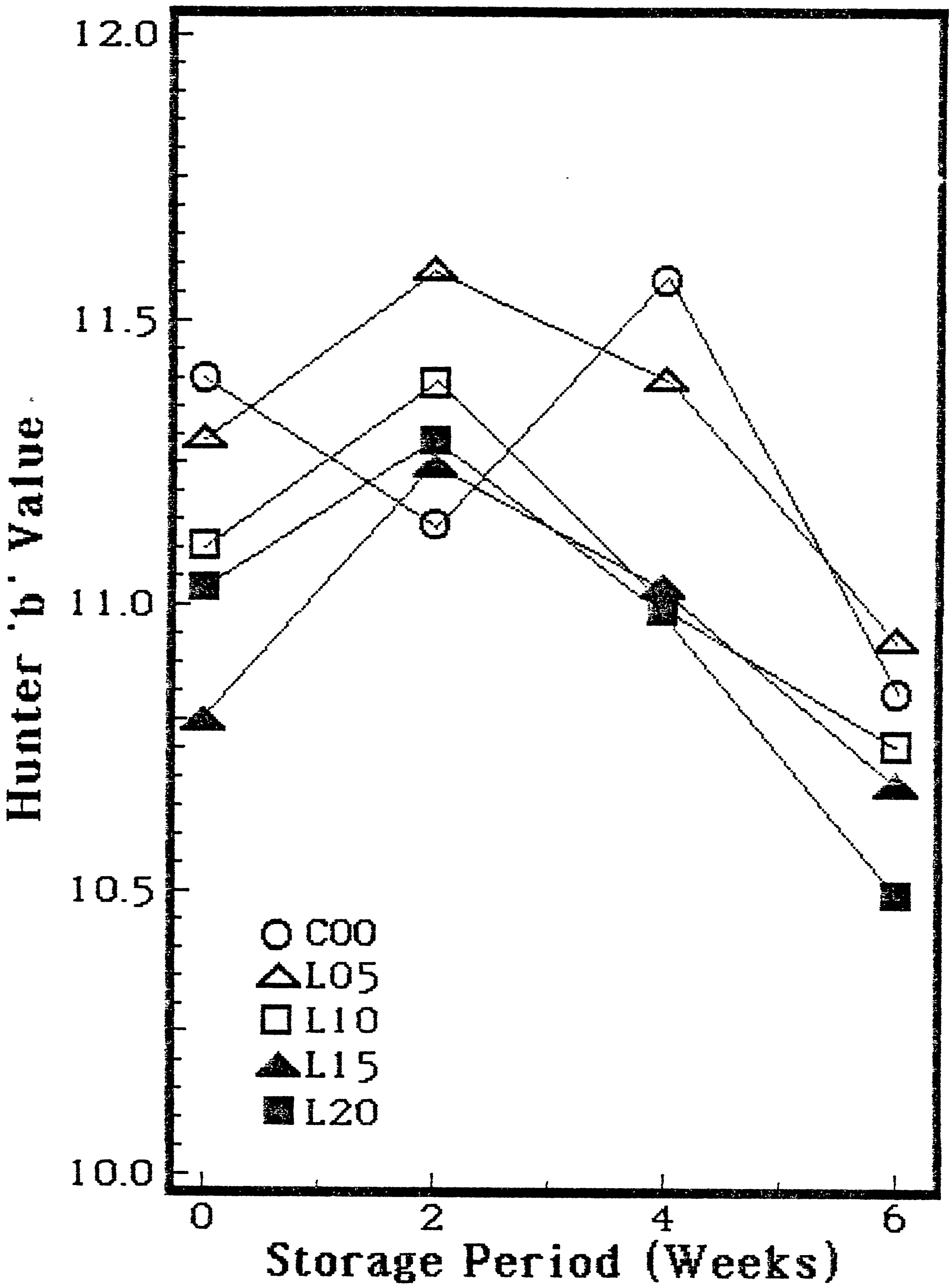

Figure 18. Effect of Storage Period on the Hunter 'b' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of the Lean Tissue

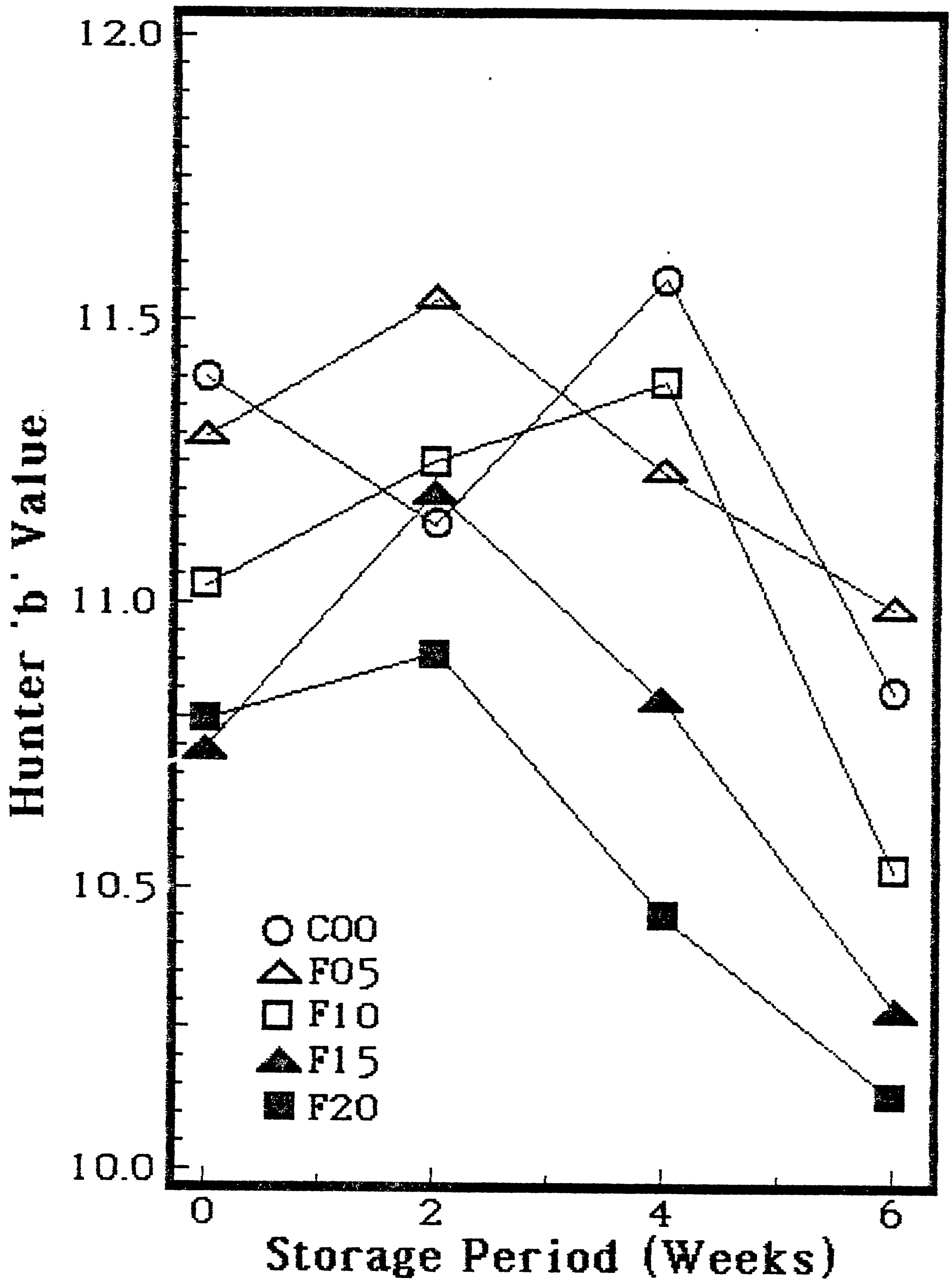

Figure 19. Effect of Storage Period on the Hunter 'b' Values of Uncooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

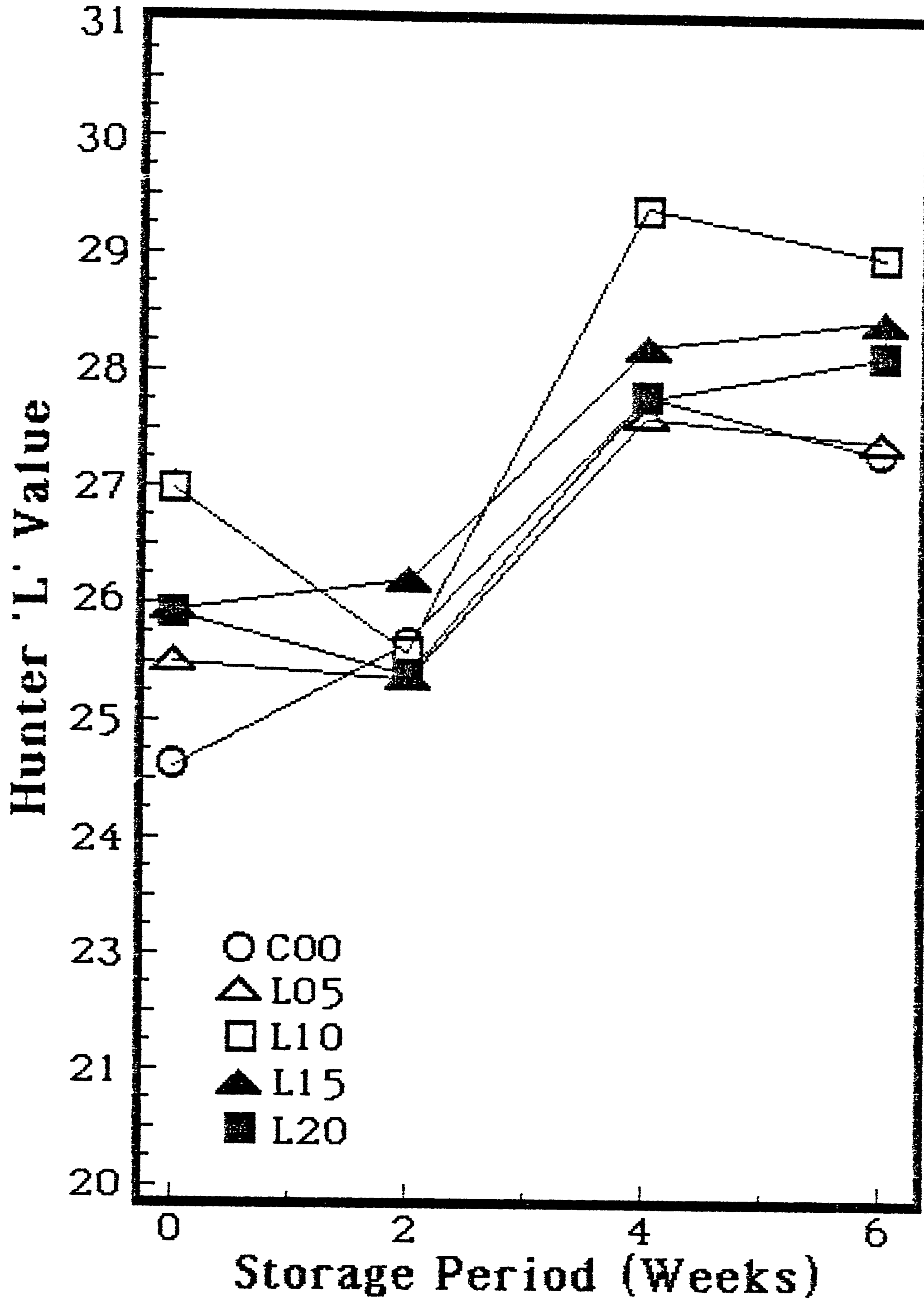

Figure 20. Effect of Storage Period on the Hunter 'L' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Lean Tissue

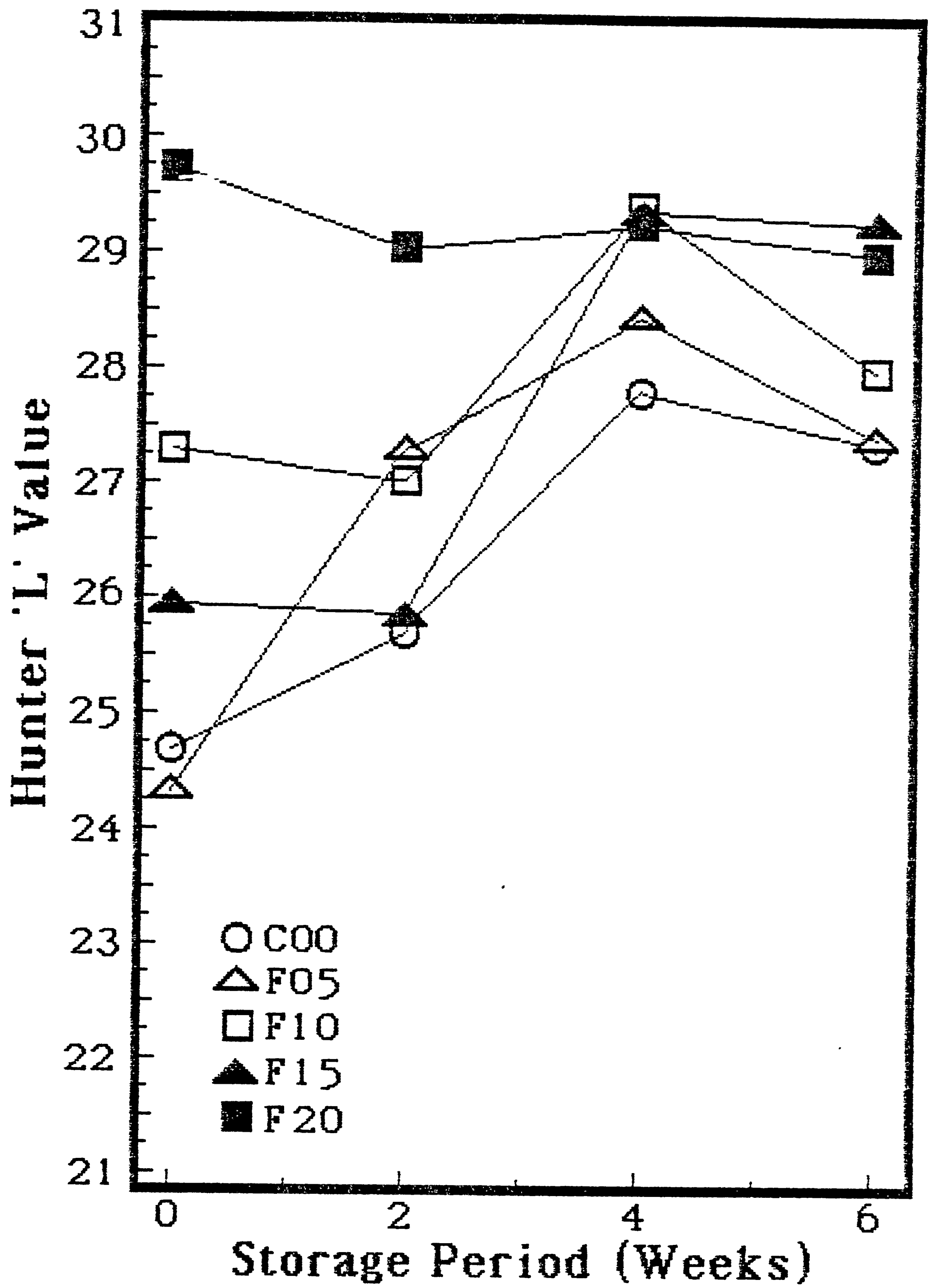

Figure 21. Effect of Storage Period on the Hunter 'L' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

at Week 4 compared to the patties obtained at Week 0 or Week 2. At Week 6, there was no significant difference (P>0.05) in the whiteness of the patties compared to the patties at Week 4. It is possible that between the second and the fourth weeks of storage, denaturation in the globin portion of myoglobin or in the amino groups of the proteins may have occurred. The denaturation of the globin moiety of the myoglobin may possibly affect the extent to which heat would denature the myoglobin molecules during heating. Alteration of the amino groups, meanwhile, may affect the degree to which the Maillard reaction proceeds. Heating and the Maillard reaction are both responsible for the alterations in cooked meat color during heating or cooking. For the fat tissue-replaced sausages, the cooked patties were significantly whiter (P<0.05) at the sixth week of storage (Table XI).

There were no significant differences (P>0.05) in the redness - greenness of the cooked patties involving both types of replacement (Appendix Table XVI). Figures 22 and 23 demonstrate the decrease in the Hunter 'a' values at Week 2. There were no significant differences (P>0.05), however, in the Hunter 'a' values at Weeks 4 and 6 when compared to the initial values at Week 0.

Hunter 'b' values of the cooked patties involving both types of replacement were not significantly affected (P>0.05) by increasing collagen levels. Significantly more yellow (P<0.05) patties were produced due to storage periods (Figures 24 and 25). For the sausages involving the

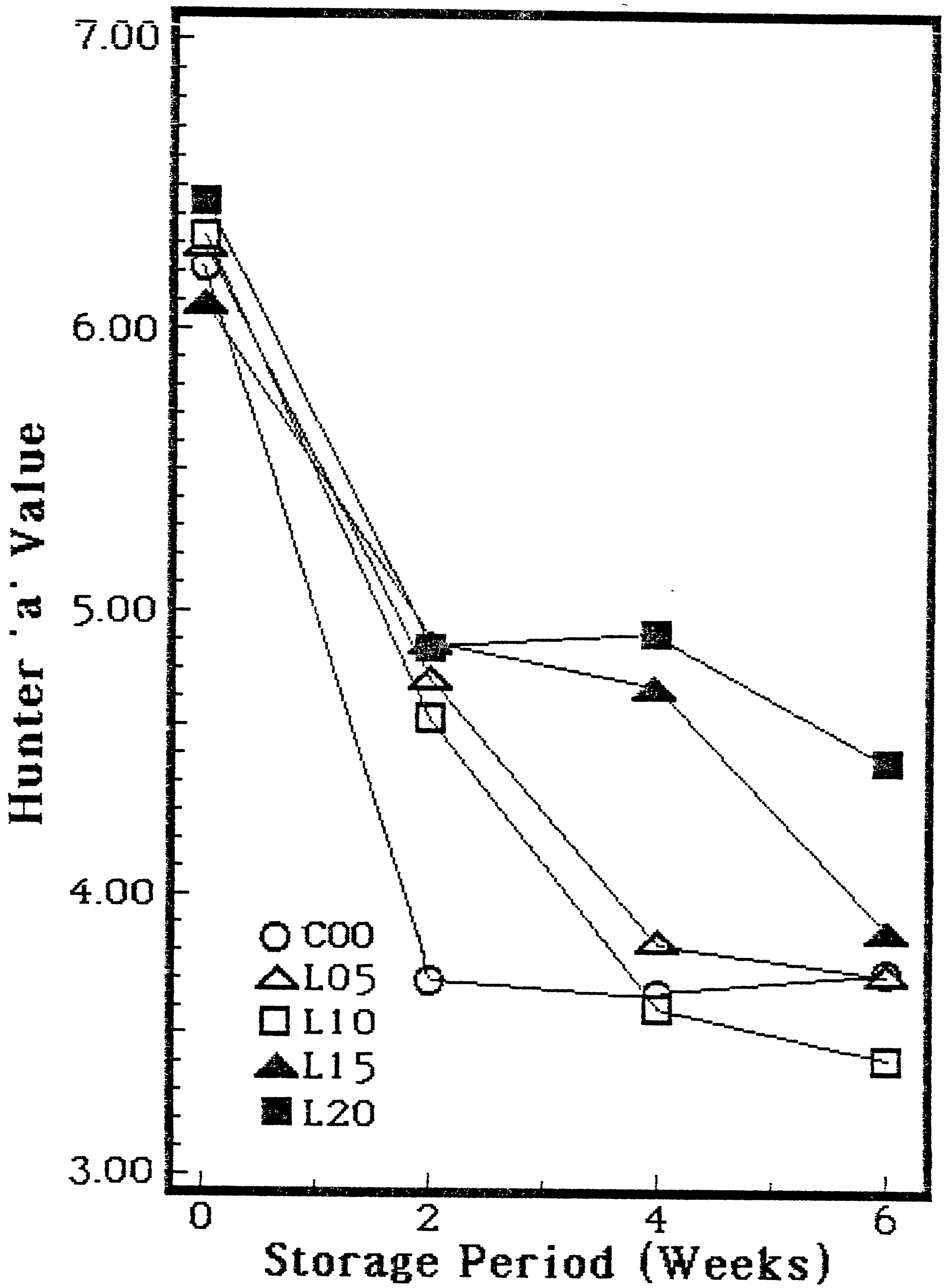

Figure 22. Effect of Storage Period on the Hunter 'a' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Lean Tissue

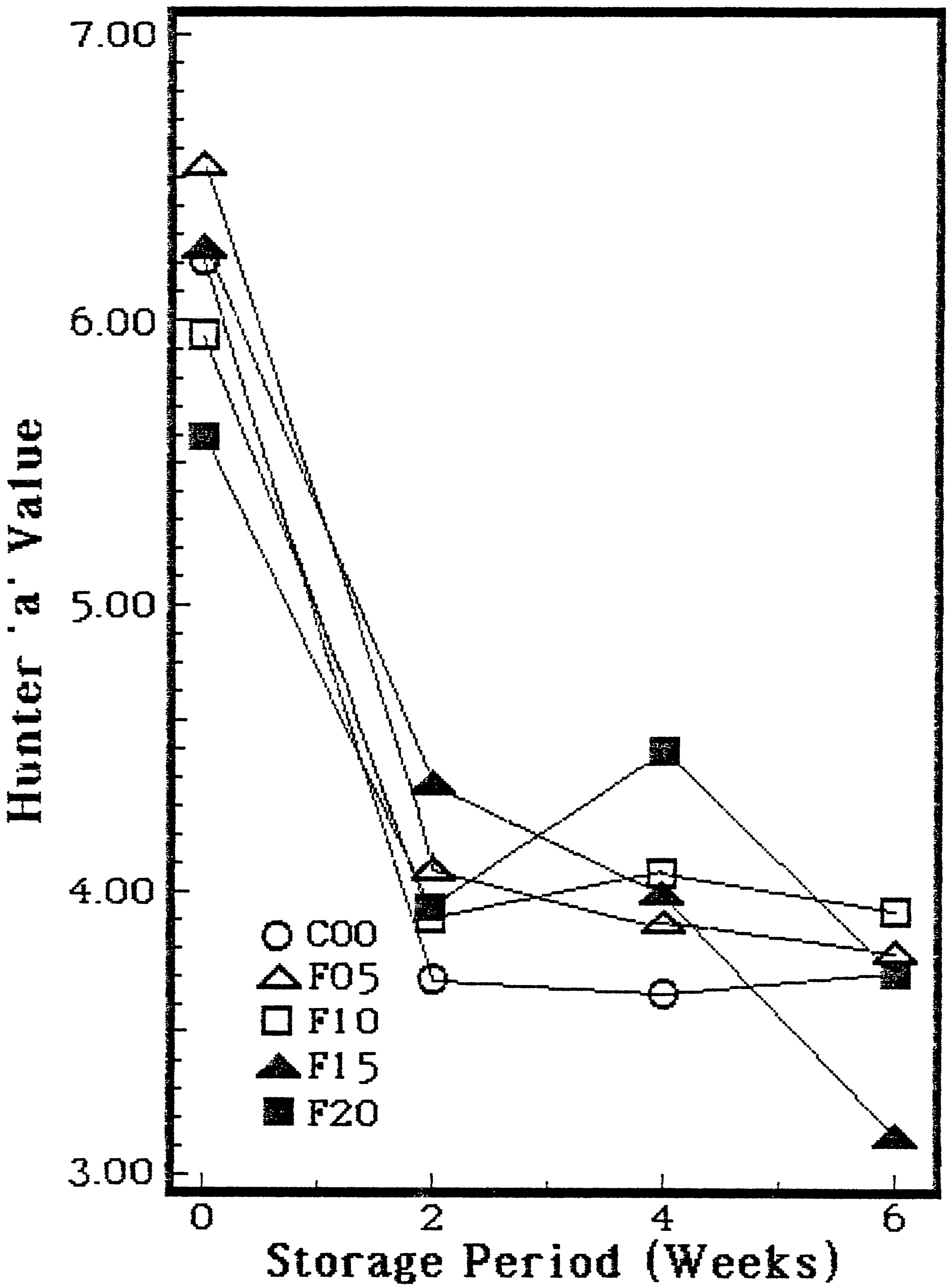

Figure 23. Effect of Storage Period on the Hunter 'a' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

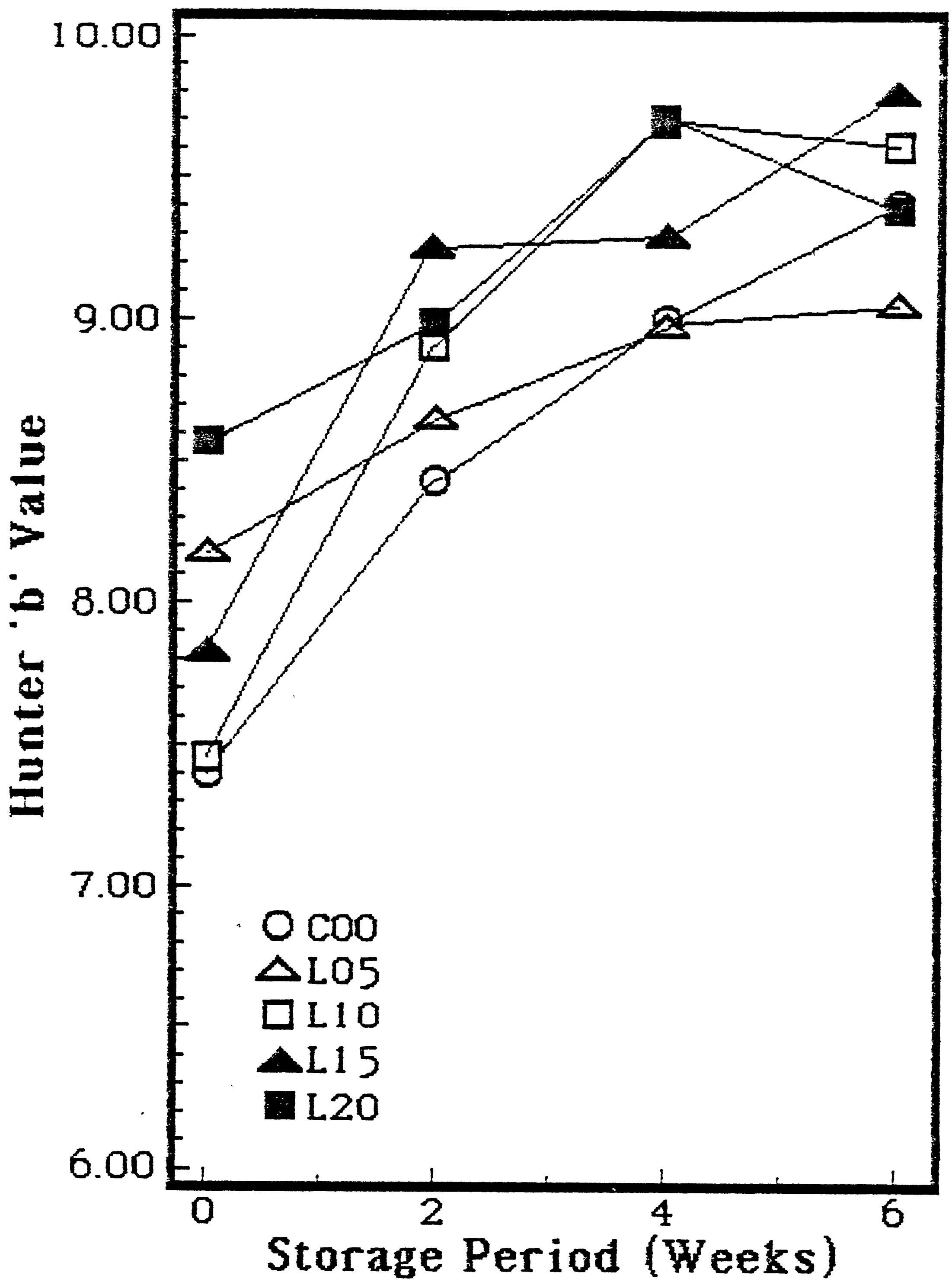

Figure 24. Effect of Storage Period on the Hunter 'b' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Lean Tissue

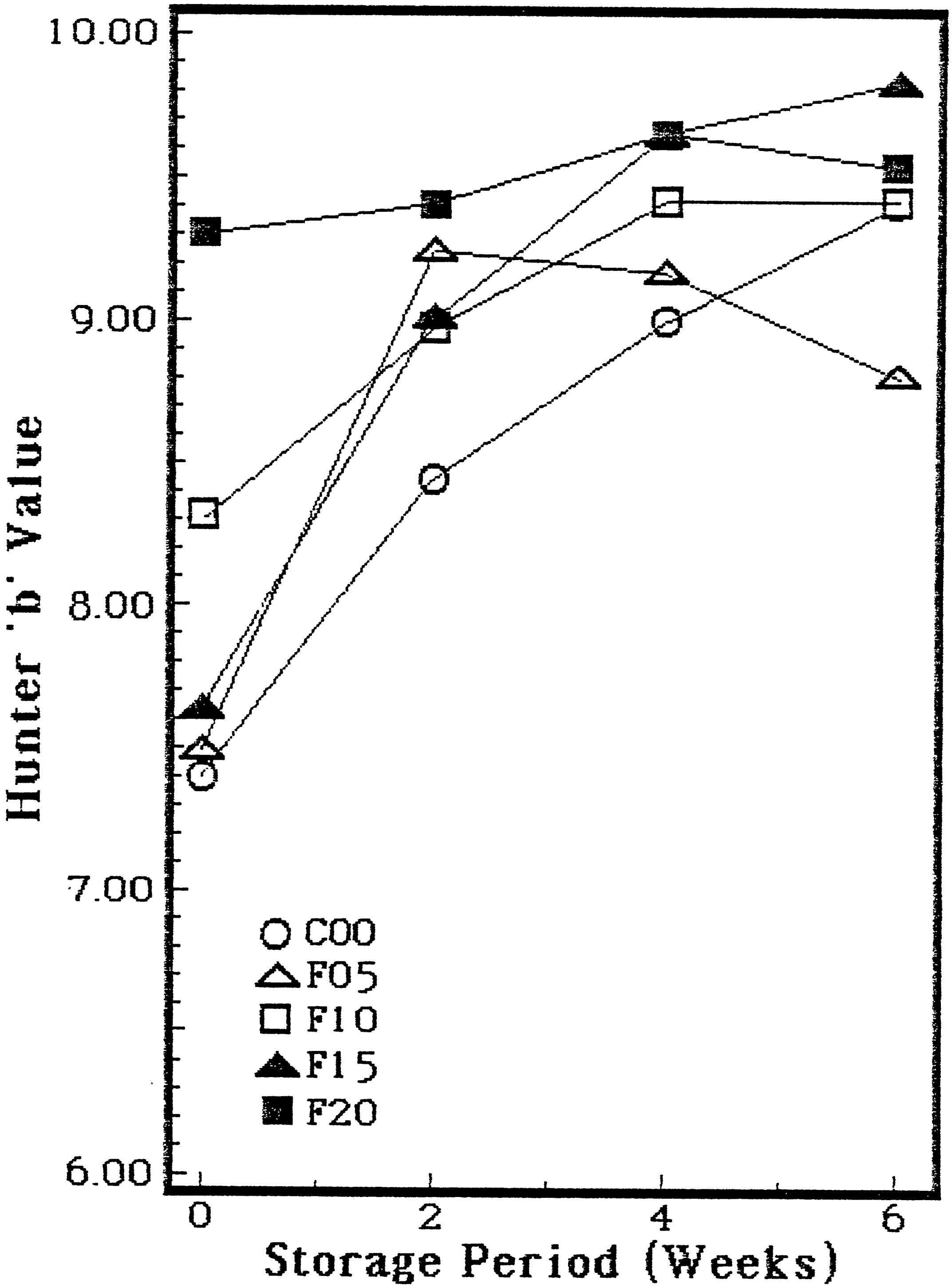

Figure 25. Effect of Storage Period on the Hunter 'b' Values of Cooked Pork Sausage Patties when Collagen Replaced Portions of the Fat Tissue

replacement of lean tissue, the cooked patties were more yellow (P<0.05) at Week 2 compared to the patties at Week 0. There were no further significant increases (P>0.05) in the yellowness of the patties at Weeks 4 and 6. In the case of the sausages involving the replacement of fat tissue, the cooked patties were significantly more yellow (P<0.05) only after the sixth week of storage.

Sensory Evaluation

Sensory evaluation was conducted to determine if the taste panelists could discriminate any differences in the quality attributes of the pork sausage such as color, juiciness, texture, flavor, and overall acceptability. The semi-trained panelists found no significant differences (P>0.05) in the quality attributes of the cooked pork sausage patties involving both types of replacement (Tables VII-XI, Appendix Table XVIII) except for the patties involving the replacement of fat tissue at 15% which had a significantly lower taste panel score for flavor. This difference may primarily be attributed to differences among the panelists (Appendix Table LIV). There were also no significant differences (P>0.05) in these quality attributes due to storage periods of the sausages involving the replacement of lean tissue (Tables VII-XI, Appendix Table XIX). With the fat tissue-replaced sausages (Appendix Table XIX), no significant differences (P>0.05) in the color and flavor were found due to storage period. These sausages, however, were found to be less juicy (P<0.05) and less

TABLE VII

SUMMARY OF MEAN TASTE PANEL SCORES FOR EACH STORAGE PERIOD FOR COLOR OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT (1)

Treatment	Weeks of Storage 0	2	4	6
C00	4.76(0.95)-	4.09(1.36)	4.00(1.30)=	4.12(1.08)
L05	4.45(1.14)-	4.25(1.29)	4.76(1.06)-	4.16(1.19)
L10	4.42(1.19)-	4.50(1.24)-	3.92(1.31)-	3.97(1.25)*
L15	4.15(1.25)	4.25(1.34)	4.08(1.06)-	4.00(1.31)
L20	4.22(1.22)=	3.81(1.51)	4.58(1.33)=	3.89(1.39)*
C00	4.76(0.95)-	4.09(1.36)	4.00(1.30)=	4.12(1.08)
F05	4.48(1.24)-	4.58(0.90)-	4.18(1.47)-	4.16(1.08)-
F10	4.79(0.86)+	4.14(1.28)	4.11(1.56)-	3.73(1.25)*
F15	4.79(0.98)+	3.84(1.17)-	3.90(1.13)=	4.21(1.13)-
F20	4.20(1.36)-	4.14(1.41)-	4.17(1.21)=	4.28(1.19)-

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
(-) One missing observation.
(=) Two missing observations.
(*) Three missing observations.
(+) One additional observation.

TABLE VIII

SUMMARY OF MEAN TASTE PANEL SCORES FOR EACH STORAGE PERIOD FOR JUICINESS OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT (1)

	Weeks of Storage			
Treatment	0	2	4	6
C00	4.40(1.25)-	3.75(1.27)	3.40(1.20)=	3.17(0.90)
L05	4.15(0.92)-	3.87(1.28)	4.18(1.20)-	3.56(0.99)=
L10	3.97(1.19)-	3.84(1.34)-	3.79(1.39)-	3.71(1.22)*
L15	3.83(1.10)	3.65(1.38)	4.20(0.81)-	3.44(0.91)
L20	4.18(1.25)=	3.95(1.12)	4.25(1.00)=	3.44(1.02)*
C00	4.40(1.25)-	3.75(1.27)	3.40(1.20)=	3.17(0.90)
F05	4.22(1.22)-	3.54(1.28)-	3.59(1.46)-	3.18(1.26)-
F10	4.11(1.19)+	4.18(1.38)	3.74(1.09)-	3.34(1.02)*
F15	3.98(1.18)	3.43(1.17)-	3.11(1.21)=	3.04(0.98)-
F20	3.96(1.24)-	3.59(1.16)-	3.17(1.57)=	3.26(1.25)-

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
(-) One missing observation.
(=) Two missing observations.
(*) Three missing observations.
(+) One additional observation.

TABLE IX

SUMMARY OF MEAN TASTE PANEL SCORES FOR EACH STORAGE PERIOD FOR TEXTURE OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT

Treatment	Weeks of Storage 0	2	4	6
C00	3.76(1.13)-	3.44(1.37)	3.39(1.11)=	2.99(1.06)
L05	3.81(1.20)=	3.73(1.13)	3.95(1.18)-	3.29(1.14)=
L10	3.77(1.29)-	3.53(1.39)-	3.57(1.51)-	3.66(1.09)*
L15	3.91(1.19)	3.21(1.32)-	3.19(1.17)-	3.47(1.04)
L20	3.63(1.56)=	3.78(1.23)	3.82(1.36)=	3.05(1.04)*
C00	3.76(1.13)-	3.44(1.37)	3.39(1.11)=	2.99(1.06)
F05	4.04(1.26)-	3.81(1.30)=	3.61(1.32)-	3.46(1.01)-
F10	3.86(1.34)+	3.99(1.45)	3.46(1.25)-	3.22(1.13)*
F15	3.91(1.22)*	3.34(1.34)=	3.36(1.04)=	3.20(1.15)-
F20	3.96(1.31)-	3.54(1.30)-	3.89(1.14)=	3.43(1.18)-

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
- (-) One missing observation.
- (=) Two missing observations.
- (*) Three missing observations.
- (+) One additional observation.

TABLE X

SUMMARY OF MEAN TASTE PANEL SCORES FOR EACH STORAGE PERIOD FOR FLAVOR OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT (1)

Treatment	Weeks of Storage 0	2	4	6
C00	4.72(0.98)-	4.24(1.20)	3.72(1.50)=	3.83(1.15)
L05	4.40(0.99)=	4.13(1.03)	4.34(1.37)-	4.03(0.73)=
L10	4.02(0.98)-	4.17(1.49)-	4.05(1.25)=	3.95(1.19)*
L15	4.18(0.72)	4.16(1.22)	4.38(0.87)-	3.86(0.81)
L20	4.02(1.21)=	3.62(1.36)	4.08(1.27)=	3.96(0.84)*
C00	4.72(0.98)-	4.24(1.20)	3.72(1.50)=	3.83(1.15)
F05	4.19(1.39)-	4.17(1.09)-	4.05(1.20)-	3.78(0.74)-
F10	4.17(1.14)+	4.35(1.21)	4.21(1.01)-	3.93(0.98)*
F15	4.01(1.04)+	3.11(1.12)-	3.80(0.85)=	3.65(0.99)-
F20	3.85(1.33)-	4.18(1.24)-	3.82(1.23)=	3.66(1.38)-

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
(-) One missing observation.
(=) Two missing observations.
(*) Three missing observations.
(+) One additional observation.

TABLE XI

SUMMARY OF MEAN TASTE PANEL SCORES FOR EACH STORAGE PERIOD FOR OVERALL ACCEPTABILITY OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT (1)

Treatment	Weeks of Storage 0	2	4	6
C00	4.76(1.14)-	3.97(1.46)	3.74(1.32)=	3.40(1.04)
L05	4.45(1.01)=	3.98(1.00)	4.39(1.36)-	3.80(0.93)=
L10	4.02(1.02)-	4.26(1.20)-	4.01(1.28)-	4.04(1.10)=
L15	4.16(1.19)	3.87(1.14)	4.22(1.10)-	3.54(1.03)
L20	4.08(1.44)=	3.49(1.24)	4.14(1.51)=	3.63(1.15)*
C00	4.76(1.14)-	3.97(1.46)	3.74(1.32)=	3.40(1.04)
F05	4.30(1.31)-	4.24(1.10)-	3.83(1.53)-	3.59(1.14)-
F10	4.47(1.17)+	4.53(1.36)	3.72(1.29)-	3.86(1.43)*
F15	4.25(1.23)+	3.02(1.25)-	3.61(1.07)=	3.53(1.06)-
F20	3.92(1.59)-	3.88(1.38)-	3.77(1.60)=	3.29(1.55)-

(1) Means from 24 observations unless indicated otherwise. Values in parentheses indicate the standard deviation.
- (-) One missing observation.
- (=) Two missing observations.
- (*) Three missing observations.
- (+) One additional observation.

acceptable at Weeks 4 and 6 compared to Week 0. The patties at Week 6 were also found to have a less desirable ($P<0.05$) texture compared to the patties at Week 0.

The results obtained from the objective measurements of color and texture do not follow the same trend as the results obtained from the subjective measurements. This difference may attributed to the variation among the panelists (Appendix Tables XLVI and XLVIII).

Differences in the juiciness, flavor, and overall acceptability of the cooked patties were probably not detected because of the differences among the panelists (Appendix Tables XLVII, XLIX, and L). It is also possible that the spices used in this formulation may have shielded the effect that collagen may have had upon the juiciness and flavor of the patties.

CHAPTER V

SUMMARY AND CONCLUSIONS

Food grade collagen was used to replace either the lean tissue or fat tissue of pork sausage at 5, 10, 15, and 20% levels. These sausages were stored at 0 C for up to six weeks to determine the effect of collagen levels and storage period on the quality characteristics of pork sausage. Objective measurements were done for: total aerobic plate count, color, texture, cook yield and TBA. Subjective measurements were made by a semitrained panel to evaluate color, juiciness, texture, flavor, and overall acceptability.

The total microbial counts of both the lean tissue and the fat tissue replaced sausages were not affected by increasing levels of collagen. The storage periods increased the total microbial counts in the sausages involving both types of replacement.

Increasing levels of collagen decreased the TBA numbers of both the lean tissue and fat tissue replaced sausages. The TBA numbers increased with storage time in the sausages involving both types of replacement.

Increasing levels of collagen decreased the cook yield of the lean tissue-replaced sausages but increased the cook yield of the fat tissue-replaced sausages. These cook yields decreased with storage period in both lean tissue and fat tissue replaced sausages.

Increasing levels of collagen did not affect the mean Instron shear force of either the lean tissue or fat tissue replaced sausages except for the sausages involving the replacement of the fat tissue at 20% which yielded a significantly lower Instron shear force value.

Increasing collagen levels produced darker uncooked patties involving lean tissue replacement. Only the patties involving the replacement of lean tissue were less red compared to the control. For both the lean tissue and fat tissue replaced sausages, the patties became less yellow with increasing levels of collagen. Storage periods decreased the color attributes of the uncooked patties of both the lean tissue and fat tissue replaced sausages except for the redness - greenness of the uncooked patties involving lean tissue replacement. Increasing collagen levels did not affect the color attributes of the cooked patties involving both types of replacement except for the sausage involving the 20% replacement of fat which produced patties that were whiter in color. Patties involving both the replacement of lean tissue and fat tissue were whiter, less red and more yellow due to storage periods.

The sensory evaluation showed no significant differences in color, juiciness, texture, flavor, and overall acceptability in both the lean tissue and fat tissue replaced sausages as affected by increasing levels of collagen except for the fat tissue-replaced sausages at 15% which had a significantly less acceptable flavor. These quality attributes were not significantly affected by storage in the lean tissue-replaced samples but for the fat tissue-replaced sausages, the juiciness and flavor were less acceptable at the sixth week of storage. The overall acceptability decreased at the fourth week of storage.

Food grade collagen can be used as a suitable substitute for either the lean tissue or fat tissue of pork sausage to form products that are equal to or even better than the pork sausage readily available at the supermarket counter.

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APPENDIXES

APPENDIX A

TABLES OF MEANS

TABLE XII

TOTAL AEROBIC PLATE COUNT, TBA NUMBER, COOK YIELD AND TEXTURE OF PORK SAUSAGE WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY COLLAGEN LEVEL (1)

Collagen Level	Log_{10} Count per g (2)	TBA No. (3,4)	Cook Yield %(3)	Texture (kg/g)(5)
C00	7.26a	0.72a	70.93a	8.62a-
L05	6.93a	0.74a	71.15a	8.18a
L10	7.06a	0.58b-	69.76a,b	8.30a
L15	6.89a	0.57b	70.04a,b	8.70a-
L20	6.91a	0.49b	68.58b	8.24a
C00	7.26a	0.72a	70.93a	8.62a-
F05	7.09a-	0.70a	70.90a	8.36a
F10	7.18a	0.60b=	74.16b,c=	7.37a-
F15	7.04a-	0.59b	73.27b-	7.76a
F20	7.23a	0.52b-	75.84c	5.83b

(1) Means for each collagen level represent the average across all storage periods.
(2) Means from 32 observations unless indicated otherwise.
(3) Means from 48 observations unless indicated otherwise
(4) Concentration of malonaldehyde (mg/kg of sample).
(5) Means from 96 observations unless indicated otherwise.
Means in the same column which are not followed by the same letter are significantly different ($P<0.05$).
(-) One missing observation.
(=) Two missing observations.

TABLE XIII

TOTAL AEROBIC PLATE COUNT, TBA NUMBER, COOK YIELD AND TEXTURE OF PORK SAUSAGE WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY STORAGE (1)

Storage Time (Week)	Log_{10} Count per g (2)	TBA No. (3,4)	Cook Yield %(3)	Texture (kg/g)(5)
LEAN TISSUE REPLACEMENT				
0	5.36a	0.53a	72.94a	8.49a
2	7.17b	0.50a-	70.71b	8.91a-
4	7.72c	0.73b	68.79c	8.57a-
6	7.78c	0.72b	67.92c	7.66a
FAT TISSUE REPLACEMENT				
0	5.38a-	0.53a-	76.73a=	7.48a
2	7.43b	0.52a	73.20b	7.90a
4	7.90c	0.74b=	71.14c	7.77a-
6	7.92c-	0.73b	71.15c-	7.19a-

(1) Means for each storage period represent the average across all collagen levels.
(2) Means from 40 observations unless indicated otherwise.
(3) Means from 60 observations unless indicated otherwise.
(4) Concentration of malonaldehyde (mg/kg of sample).
(5) Means from 120 observations unless indicated otherwise.
Means in the same column which are not followed by the same letter are significantly different ($P<0.05$).
(-) One missing observation.
(=) Two missing observations.

TABLE XIV

COLOR OF UNCOOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY COLLAGEN LEVEL (1)

Collagen Level	L(3)	Color(2) a(4)	b(5)
C00	45.08a	7.43a	11.24ab
L05	46.47a,b	7.30a	11.31a
L10	47.30b,c	6.61b	11.05bc
L15	47.97c	5.95c	10.91c
L20	49.91d	5.96c	10.94c-
C00	45.08a	7.43a	11.24a
F05	46.25a	7.18a	11.27a
F10	45.18a	7.44a	11.04a
F15	44.23a-	7.46a-	10.74b-
F20	45.03a	7.06a	10.55b

(1) Means for each collagen level represent the average across all storage periods.
(2) Means from 192 observations unless indicated otherwise. Means in a column which are not followed by the same letter are significantly different ($P<0.05$).
(-) One missing observation.
(3) Standard of 0 for black and 100 for white.
(4) Standard of -80 for green and +100 for red.
(5) Standard of -70 for blue and +70 for yellow.

TABLE XV

COLOR OF UNCOOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY STORAGE (1)

STORAGE Time (Week)	L(3)	Color(2) a(4)	b(5)
LEAN TISSUE REPLACEMENT			
0	48.52a	6.57a	11.12a-
2	46.74b	7.30b	11.32a
4	47.60a,b	6.68a	11.20a
6	46.52b	6.04a	10.72b
FAT TISSUE REPLACEMENT			
0	46.10a	7.56a	11.04a
2	44.84b	7.92a	11.20a
4	45.25a,b-	7.29a-	11.08a-
6	44.44b	6.48b	10.56b

(1) Means for each storage period represent the average across all collagen levels.
(2) Means from 240 unless indicated otherwise.
Means in a column which are not followed by the same letter are significantly different ($P<0.05$).
(-) One missing observation.
(3) Standard of 0 for black and 100 for white.
(4) Standard of -80 for green and +100 for red.
(5) Standard of -70 for blue and +70 for yellow.

TABLE XVI

COLOR OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY COLLAGEN LEVEL (1)

Collagen Level	Color(2) L(3)	a(4)	b(5)
C00	26.37a	4.32a	8.56a
L05	26.49a-	4.64a	8.71a
L10	27.72a	4.47a	8.92a
L15	27.21a	4.90a	9.06a
L20	26.82a	5.15a	9.16a
C00	26.37a	4.32a	8.56a
F05	27.15a	4.61a	8.69a
F10	27.93a,b	4.46a	9.04a
F15	27.61a,b	4.47a	9.03a
F20	29.25b	4.41a	9.47a

(1) Means for each collagen level represent the average across all storage periods.
(2) Means from 192 observations.
Means in a column which are not followed by the same letter are significantly different (P<0.05).
(-) One missing observation.
(3) Standard of 0 for black and 100 for white.
(4) Standard of -80 for green and +100 for red.
(5) Standard of -70 for blue and +70 for yellow.

TABLE XVII

COLOR OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY STORAGE (1)

Storage Time (Week)	Color (2) L(3)	a(4)	b(5)
LEAN TISSUE REPLACEMENT			
0	25.81a-	6.28a	7.88a
2	25.66a	4.54b	8.85b
4	28.16b	4.14b,c	9.34b
6	28.05b	3.82c	9.45b
FAT TISSUE REPLACEMENT			
0	26.59a	6.11a	8.03a
2	27.00a	4.00b	9.01a
4	28.85a,b	4.02b	9.38a,b
6	28.20b	3.68b	9.41b

(1) Means for each storage period represent the average across all collagen levels. Means in a column which are not followed by the same letter are significantly different (P<0.05).

(2) Means from 240 observations.
Means in a column which are not followed by the same letter are significantly different (P<0.05).
(-) One missing observation.

(3) Standard of 0 for black and 100 for white.

(4) Standard of -80 for green and +100 for red.

(5) Standard of -70 for blue and +70 for yellow.

TABLE XVIII

TASTE PANEL EVALUATION OF PORK SAUSAGE WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY COLLAGEN LEVEL (1,2)

Collagen Level	N	Color	Juiciness	Texture	Flavor	Overall Acceptability
C00	93	4.24a	3.68a	3.39a	4.13a	3.96a
L05	92	4.41a	3.94a	3.70a-	4.22a	4.15a
L10	90	4.21a	3.83a	3.64a	4.05a-	4.08a
L15	95	4.12a	3.78a	3.45a-	4.14a	3.94a
L20	89	4.12a	3.96a	3.58a	3.91a	3.83a
C00	93	4.24a	3.68a	3.39a	4.13a	3.96a,c
F05	92	4.35a	3.63a	3.73a-	4.05a	3.99a,c
F10	93	4.21a	3.86a	3.65a	4.17a	4.16a,c
F15	93	4.20a	3.40a-	3.47a-	3.65b	3.62b,c
F20	91	4.20a	3.50a	3.70a	3.88a	3.71b,c

(1) Means for each collagen level represent the average across all storage periods. Means in a column which are not followed by the same letter are significantly different ($P<0.05$).
(-) N - 1 observations.

(2) Scores range from 0.00 to 7.00.

TABLE XIX

TASTE PANEL EVALUATION OF PORK SAUSAGE WITH LEAN TISSUE OR FAT TISSUE REPLACEMENT AS AFFECTED BY STORAGE (1,2)

Storage Time (Week)	N	Color	Juiciness	Texture	Flavor	Overall Acceptability
LEAN TISSUE REPLACEMENT						
0	115	4.40a	4.10a	3.78a-	4.27a-	4.29a-
2	119	4.18a	3.81a	3.54a-	4.06a	3.91a
4	113	4.27a	3.97a	3.58a	4.12a-	4.10a
6	112	4.03a	3.46a	3.29a	3.92a	3.67a
FAT TISSUE REPLACEMENT						
0	119	4.61a-	4.13a	3.91a	4.18a	4.34a
2	117	4.16a	3.70a,c	3.63a,c=	4.01a	3.93a,b
4	112	4.07a	3.40b,c	3.54a,c	3.92a	3.73b
6	114	4.10a	3.19b,c	3.26b,c	3.77a	3.52b

(1) Means for each storage period represent the average across all collagen levels. Means in a column which are not followed by the same letter are significantly different ($P<0.05$).
(-) N - 1 observations.
(=) N - 2 observations.
(2) Scores range from 0.00 to 7.00.

APPENDIX B

ANALYSIS OF VARIATION TABLES

TABLE XX

ANALYSIS OF VARIATION FOR PERCENT FAT IN PORK SAUSAGE

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	107	2201.1717			
Block	3	5.3350	1.7783	0.71	0.5558
Treatment	8	2074.0838	259.2605	103.44	0.0001
Error (a)	24	60.1554	2.5065	2.93	
Error (b)	72	61.5975	0.8555		

Note: Error (a) = Block x Treatment
Error (b) = Sampling Error

TABLE XXI

ANALYSIS OF VARIATION FOR PERCENT MOISTURE IN PORK SAUSAGE

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	107	2308.4944			
Block	3	101.8721	33.9574	6.29	0.0026
Treatment	8	1714.2218	214.2777	39.71	0.0001
Error (a)	24	129.5093	5.3962	1.07	
Error (b)	72	362.4944	5.0402		

Note: Error (a) = Block x Treatment
Error (b) = Sampling Error

TABLE XXII

ANALYSIS OF VARIATION FOR PERCENT PROTEIN IN PORK SAUSAGE

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	107	200.6437			
Block	3	39.4035	13.1345	6.64	0.0020
Treatment	8	49.9683	6.2460	3.16	0.0138
Error (a)	24	47.4914	1.9788	2.23	
Error (b)	72	63.7804	0.8858		

Note: Error (a) = Block x Treatment
Error (b) = Sampling Error

TABLE XXIII

ANALYSIS OF VARIATION FOR PERCENT FAT IN COLLAGEN

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	11	0.3718			
Block	3	0.0152	0.0051	0.11	0.9496
Error	8	0.3718			

TABLE XXIV

ANALYSIS OF VARIATION FOR PERCENT MOISTURE IN COLLAGEN

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	11	5.2781			
Block	3	1.14664	0.4888	1.03	0.4311
Error	8	3.8117			

TABLE XXV

ANALYSIS OF VARIATION FOR PERCENT PROTEIN IN COLLAGEN

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	11	23.8801			
Block	3	12.2323	4.0774	2.80	0.1086
Error	8	11.6477			

TABLE XXVI

ANALYSIS OF VARIATION FOR THE LOGARITHM OF THE TOTAL AEROBIC PLATE COUNT OF THE PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	159	196.5226			
Block	3	8.1738	2.7246	5.45	0.0024
Storage	3	154.1752	51.3917	102.77	0.0001
Treatment	4	3.1404	0.7851	1.57	0.1947
S x T	12	2.1279	0.1773	0.35	0.9738
Error (a)	57	28.5036	0.5001	99.60	0.0001
Error (b)	80	0.4016	0.0050		

Note: B = Block; S = Storage Time; T = Treatment
Error (a) = Experimental error.
Error (b) = Sampling error.

TABLE XXVII

ANALYSIS OF VARIATION FOR THE LOGARITHM OF THE TOTAL AEROBIC PLATE COUNT OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	157	209.3551			
Block	3	12.6093	4.2031	11.31	0.0001
Storage	3	172.0497	57.3499	154.27	0.0001
Treatment	4	1.0712	0.2678	0.72	0.5816
S x T	12	2.0376	0.1698	0.46	0.9313
Error (a)	57	21.1900	0.3718	72.97	0.0001
Error (b)	78	0.3974	0.0051		

Note: B = Block; S = Storage Time; T = Treatment
Error (a) = Experimental error
Error (b) = Sampling error

TABLE XXVIII

ANALYSIS OF VARIATION FOR TBA NUMBER OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	238	46.9029			
Block	3	37.3972	12.4657	159.09	0.0001
Storage	3	2.5842	0.8614	10.99	0.0001
Treatment	4	2.0982	0.5246	6.69	0.0002
S x T	12	0.2769	0.0231	0.29	0.9879
Error (a)	57	4.4664	0.0784	155.89	0.0001
Error (b)	159	0.0799	0.0005		

TABLE XXIX

ANALYSIS OF VARIATION FOR TBA NUMBER OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	236	48.2452			
Block	3	41.2282	13.7427	268.50	0.0001
Storage	3	2.6118	0.8706	17.01	0.0001
Treatment	4	1.2125	0.3031	5.92	0.0005
S x T	12	0.1783	0.0149	0.29	0.9887
Error (a)	57	2.9174	0.0512	82.96	0.0001
Error (b)	157	0.0969	0.0006		

Note: B = Block; S = Storage Time; T = Treatment
Error (a) = Experimental error
Error (b) = Sampling error

TABLE XXX

ANALYSIS OF VARIATION FOR COOK YIELD OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	239	8499.5022			
Block	3	5838.8335	1912.9445	96.91	0.0001
Storage	3	895.6300	298.5433	15.12	0.0001
Treatment	4	202.5212	50.6303	2.57	0.0478
S x T	12	183.4785	15.2899	0.77	0.6734
Error (a)	57	1125.1132	19.7388	8.92	0.0001
Error (b)	160	353.5022	2.2120		

TABLE XXXI

ANALYSIS OF VARIATION FOR COOK YIELD OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	236	9323.9123			
Block	3	5081.2411	1693.7470	64.11	0.0001
Storage	3	1177.5388	392.5129	14.86	0.0001
Treatment	4	868.9145	217.2286	8.22	0.0001
S x T	12	153.7756	12.8146	0.49	0.9153
Error (a)	57	1505.9808	26.4207	7.73	0.0001
Error (b)	157	536.9123	3.4170		

Note: B = Block; S = Storage Time; T = Treatment
Error (a) = Experimental error
Error (b) = Sampling error

TABLE XXXII

ANALYSIS OF VARIATION FOR TEXTURE OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	477	4993.5290			
Block	3	1600.4190	533.4730	29.78	0.0001
Storage	3	101.9066	33.9689	1.90	0.1389
Treatment	4	21.4946	5.3736	0.30	0.8768
S x T	12	120.6563	10.0547	0.56	0.8636
Error (a)	57	1021.0709	17.9135	3.81	0.0001
Error (b)	160	1007.7555	6.2985	1.34	0.0208
Error (c)	238	1120.2260	4.7068		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XXXIII

ANALYSIS OF VARIATION FOR TEXTURE OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	477	6571.6333			
Block	3	1183.1703	394.3901	17.67	0.0001
Storage	3	36.6892	12.2298	0.55	0.6555
Treatment	4	460.5540	115.1385	5.16	0.0013
S x T	12	100.2407	8.3534	0.37	0.9675
Error (a)	57	1272.1708	22.3188	2.74	0.0001
Error (b)	160	1580.1297	9.8758	1.21	0.0889
Error (c)	238	1938.6785	8.1457		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XXXIV

ANALYSIS OF VARIATION FOR HUNTER L VALUES OF UNCOOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	9354.7733			
Block	3	605.2516	201.7505	4.32	0.0083
Storage	3	597.8606	199.2869	4.27	0.0088
Treatment	4	2462.9946	615.7486	13.18	0.0001
S x T	12	86.0619	7.1718	0.15	0.9995
Error (a)	57	2662.0385	46.7024	20.92	0.0001
Error (b)	160	1333.1762	8.8824	3.73	0.0001
Error (c)	720	1607.3899	2.2325		

TABLE XXXV

ANALYSIS OF VARIATION FOR HUNTER a VALUES
OF UNCOOKED PORK SAUSAGE PATTIES
WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	1610.4304			
Block	3	93.2316	31.0772	3.46	0.0219
Storage	3	192.2229	64.0743	7.13	0.0004
Treatment	4	383.6210	95.9052	10.67	0.0001
S x T	12	25.3661	2.1138	0.24	0.9956
Error (a)	57	512.1262	8.9847	29.60	0.0001
Error (b)	160	185.3235	1.1583	3.82	0.0001
Error (c)	720	218.5388	0.3035		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XXXVI

ANALYSIS OF VARIATION FOR HUNTER b VALUES
OF UNCOOKED PORK SAUSAGE PATTIES
WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	958	299.7175			
Block	3	7.1537	2.3846	1.66	0.1846
Storage	3	48.7433	16.2478	11.30	0.0001
Treatment	4	23.7572	5.9393	4.13	0.0052
S x T	12	13.9736	1.1645	0.81	0.6389
Error (a)	57	81.9274	1.4373	18.01	0.0001
Error (b)	160	66.7706	0.4173	5.23	0.0001
Error (c)	719	57.3916	0.0798		

TABLE XXXVII

ANALYSIS OF VARIATION FOR HUNTER L VALUES OF UNCOOKED PORK SAUSAGE PATTIES WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	958	6359.7382			
Block	3	1209.4650	403.1550	9.73	0.0001
Storage	3	365.5209	121.8403	2.94	0.0402
Treatment	4	397.1960	99.2990	2.40	0.0609
S x T	12	283.9399	23.6616	0.57	0.8563
Error (a)	57	2362.7004	41.4509	53.50	0.0001
Error (b)	160	1183.8772	7.3992	9.55	0.0001
Error (c)	719	557.0388	0.7747		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XXXVIII

ANALYSIS OF VARIATION FOR HUNTER a VALUES OF UNCOOKED PORK SAUSAGE PATTIES WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	958	1793.5983			
Block	3	324.5465	108.1822	8.37	0.0001
Storage	3	268.7141	89.5714	6.93	0.0005
Treatment	4	25.6112	6.4028	0.50	0.7390
S x T	12	71.2155	5.9346	0.46	0.9300
Error (a)	57	736.5468	12.9219	55.96	0.0001
Error (b)	160	200.9419	1.2559	5.44	0.0001
Error (c)	719	166.0222	0.2309		

TABLE XXXIX

ANALYSIS OF VARIATION FOR HUNTER b VALUES
OF UNCOOKED PORK SAUSAGE PATTIES
WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	958	577.1934			
Block	3	35.6111	11.8704	5.48	0.0023
Storage	3	57.7168	19.2389	8.89	0.0001
Treatment	4	75.9462	18.9865	8.77	0.0001
S x T	12	19.8550	1.6546	0.76	0.6835
Error (a)	57	123.4144	2.1652	10.62	0.0001
Error (b)	160	118.0178	0.7376	3.62	0.0001
Error (c)	719	146.6321	0.2039		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XL

ANALYSIS OF VARIATION FOR HUNTER L VALUES
OF COOKED PORK SAUSAGE PATTIES
WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	958	11429.6128			
Block	3	4348.8767	1449.6256	28.78	0.0001
Storage	3	1350.3204	450.1068	8.32	0.0001
Treatment	4	233.2097	58.3024	1.08	0.3763
S x T	12	104.8379	8.7365	0.16	0.9993
Error (a)	57	3084.8964	54.1210	84.53	0.0001
Error (b)	160	1847.1206	11.5445	18.03	0.0001
Error (c)	719	460.3511	0.6403		

TABLE XLI

ANALYSIS OF VARIATION FOR HUNTER a VALUES OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	2356.9329			
Block	3	284.4227	94.8976	7.92	0.0002
Storage	3	866.4054	288.8018	24.12	0.0001
Treatment	4	86.8237	21.7059	1.81	0.1389
S x T	12	64.7966	5.3997	0.45	0.9349
Error (a)	57	682.4161	11.9722	55.16	0.0001
Error (b)	160	285.8012	1.3488	6.21	0.0001
Error (c)	720	156.2671	0.2170		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XLII

ANALYSIS OF VARIATION FOR HUNTER b VALUES OF COOKED PORK SAUSAGE PATTIES WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	2070.3067			
Block	3	364.7647	121.5882	9.53	0.0001
Storage	3	368.6000	122.8666	9.63	0.0001
Treatment	4	46.2681	11.5670	0.91	0.4664
S x T	12	62.9285	5.2440	0.41	0.9534
Error (a)	57	727.3838	12.7611	42.70	0.0001
Error (b)	160	285.1650	1.7823	5.96	0.0001
Error (c)	720	215.1968	0.2989		

TABLE XLIII

ANALYSIS OF VARIATION FOR HUNTER L VALUES
OF COOKED PORK SAUSAGE PATTIES
WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	15052.7581			
Block	3	5622.5819	1874.1940	23.95	0.0001
Storage	3	789.8982	263.2994	3.36	0.0245
Treatment	4	869.0693	217.2673	2.78	0.0354
S x T	12	499.2351	41.6029	0.53	0.8851
Error (a)	57	4460.5384	78.2550	105.81	0.0001
Error (b)	160	2278.9357	14.2433	19.26	0.0001
Error (c)	720	532.4996	0.7396		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XLIV

ANALYSIS OF VARIATION FOR HUNTER a VALUES
OF COOKED PORK SAUSAGE PATTIES
WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	2377.2359			
Block	3	269.2100	89.7366	7.75	0.0002
Storage	3	896.7470	298.9157	25.82	0.0001
Treatment	4	8.7974	2.1993	0.19	0.9427
S x T	12	63.2694	5.2724	0.46	0.9320
Error (a)	57	659.7762	11.5750	35.95	0.0001
Error (b)	160	247.6054	1.5475	4.81	0.0001
Error (c)	720	231.8303	0.3220		

TABLE XLV

ANALYSIS OF VARIATION FOR HUNTER b VALUES OF COOKED PORK SAUSAGE PATTIES WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	959	2149.6068			
Block	3	317.1918	105.7306	7.56	0.0003
Storage	3	298.4267	99.4756	7.11	0.0004
Treatment	4	96.7276	24.1819	1.73	0.1561
S x T	12	90.1885	7.5157	0.54	0.8810
Error (a)	57	797.0945	13.9841	81.44	0.0001
Error (b)	160	426.3463	2.6647	15.52	0.0001
Error (c)	720	123.6314	0.1717		

Note: B = Block; S = Storage Time; T = Treatment
P = Patty
Error (a) = Experimental error
Error (b) = Sampling error
Error (c) = Subsampling error

TABLE XLVI

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR COLOR OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	458	714.0347			
Block	3	19.3000	6.4333	3.44	0.0653
Storage	3	7.5834	2.5278	1.35	0.3187
Error (a)	9	16.8239	1.8693	1.41	
Treatment	4	4.9906	1.2477	0.94	0.4406
S x T	12	20.7873	1.7323	1.31	0.2133
Panelist	6	47.7986	7.9664	6.01	0.0001
T x P	22	13.0645	0.5938	0.45	0.9863
S x P	16	61.6968	3.8560	2.91	0.0002
S x T x P	60	93.5645	1.5594	1.18	0.1912
Error (b)	323	428.4249			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE XLVII

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR JUICINESS OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	458	625.2104			
Block	3	42.7797	14.2599	4.60	0.0324
Storage	3	25.3139	8.4380	2.72	0.1069
Error (a)	9	27.8786	3.0976	3.01	
Treatment	4	4.5539	1.1385	1.11	0.3536
S x T	12	17.4206	1.4517	1.41	0.1592
Panelist	6	61.4115	10.2352	9.94	0.0001
T x P	22	15.4656	0.7030	0.68	0.8562
S x P	16	29.4071	1.8379	1.79	0.0319
S x T x P	60	68.5495	1.1425	1.11	0.2822
Error (b)	323	332.4299			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE XLVIII

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR TEXTURE OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	456	700.4975			
Block	3	33.8995	11.2998	4.19	0.0410
Storage	3	12.6203	4.2068	1.56	0.2657
Error (a)	9	24.2776	2.6975	2.22	
Treatment	4	5.3512	1.3378	1.10	0.3561
S x T	12	16.2436	1.3536	1.11	0.3477
Panelist	6	81.8210	3.6368	11.22	0.0001
T x P	22	24.6118	1.1187	0.92	0.5677
S x P	16	42.8992	2.6812	2.21	0.0051
S x T x P	60	68.7104	1.1452	0.94	0.5939
Error (b)	321	390.0627			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
Note: The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE XLIX

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR FLAVOR OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	456	587.9662			
Block	3	2.7581	0.9194	0.43	0.7365
Storage	3	6.7104	2.2368	1.04	0.4207
Error (a)	9	19.4191	2.1577	1.90	
Treatment	4	4.9955	1.2489	1.10	0.3572
S x T	12	16.0662	1.3388	1.18	0.2977
Panelist	6	63.3959	10.5660	9.30	0.0001
T x P	22	24.1471	1.0067	0.89	0.6143
S x P	16	25.8369	1.6148	1.42	0.1296
S x T x P	60	61.7798	1.0297	0.91	0.6719
Error (b)	321	364.8571			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
Note: The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE L

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR OVERALL ACCEPTABILITY OF PORK SAUSAGE WITH LEAN TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	457	674.6248			
Block	3	2.7236	0.9078	0.32	0.8109
Storage	3	23.9422	7.9807	2.84	0.0981
Error (a)	9	25.3310	2.8146	2.19	
Treatment	4	5.7864	1.4466	1.13	0.3446
S x T	12	21.3802	1.7817	1.39	0.1710
Panelist	6	62.9899	10.4983	8.16	0.0001
T x P	22	19.6758	0.8944	0.70	0.8441
S x P	16	25.4024	1.5876	1.23	0.2395
S x T x P	60	73.3445	1.2224	0.95	0.5821
Error (b)	322	674.6248			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
Note: The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE LI

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR COLOR OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	461	684.6031			
Block	3	11.8356	3.9452	1.14	0.3842
Storage	3	22.3545	7.4515	2.16	0.1627
Error (a)	9	30.9910	3.4434	2.38	
Treatment	4	1.8144	0.4536	0.31	0.8687
S x T	12	17.0636	1.4220	0.98	0.4640
Panelist	6	9.7366	1.6228	1.12	0.3486
T x P	24	25.5493	1.0646	0.74	0.8131
S x P	15	29.7064	1.9804	1.37	0.1594
S x T x P	60	65.8408	1.0973	0.76	0.9023
Error (b)	325	469.7109			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE LII

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR JUICINESS OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	460	736.9616			
Block	3	8.2329	2.7443	0.70	0.5753
Storage	3	58.2800	19.4267	4.94	0.0269
Error (a)	9	35.4165	3.9352	2.91	
Treatment	4	11.5019	2.8755	2.13	0.0769
S x T	12	8.2932	0.6911	0.51	0.9068
Panelist	6	59.4464	9.9077	7.34	0.0001
T x P	24	17.2738	0.7197	0.53	0.9666
S x P	15	21.3523	1.4235	1.05	0.3990
S x T x P	60	79.6875	1.3281	0.98	0.5149
Error (b)	324	437.4769			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE LIII

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR TEXTURE OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	459	705.2505			
Block	3	24.0110	8.0037	4.66	0.0314
Storage	3	23.7442	7.9147	4.61	0.0324
Error (a)	9	15.4452	1.7161	1.27	
Treatment	4	8.4197	2.1049	1.55	0.1864
S x T	12	6.8618	0.5718	0.42	0.9544
Panelist	6	50.8497	8.4750	6.26	0.0001
T x P	24	25.7718	1.0738	0.79	0.7461
S x P	15	42.7146	2.8476	2.10	0.0097
S x T x P	60	69.9191	1.1653	0.86	0.7572
Error (b)	323	437.5135			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE LIV

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR FLAVOR OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	461	636.9136			
Block	3	12.3134	4.1045	1.99	0.1861
Storage	3	10.0246	3.3415	1.62	0.2525
Error (a)	9	18.5593	2.0621	1.63	
Treatment	4	17.2546	4.3137	3.42	0.0094
S x T	12	21.7617	1.8135	1.44	0.1478
Panelist	6	37.7195	6.2866	4.98	0.0001
T x P	24	20.2085	0.8420	0.67	0.8828
S x P	15	18.5908	1.2394	0.98	0.4746
S x T x P	60	70.0895	1.1682	0.93	0.6339
Error (b)	325	410.3916			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

TABLE LV

ANALYSIS OF VARIATION FOR TASTE PANEL SCORES FOR OVERALL ACCEPTABILITY OF PORK SAUSAGE WITH FAT TISSUE REPLACEMENT

Source	DF	Sum of Squares	Mean Square	F	PR > F
Total	461	845.7473			
Block	3	9.7928	3.2643	1.16	0.3774
Storage	3	42.2111	14.0704	4.98	0.0236
Error (a)	9	25.4027	2.8225	1.67	
Treatment	4	18.2523	4.5631	2.70	0.0306
S x T	12	25.3260	2.1105	1.25	0.2478
Panelist	6	43.3330	7.2222	4.28	0.0004
T x P	24	22.8873	0.9536	0.56	0.9527
S x P	15	26.1668	1.7444	1.03	0.4205
S x T x P	60	83.5219	1.3920	0.82	0.8170
Error (b)	325	548.7473			

Note: Error (a) = Block x Storage Time
Error (b) = Error
P = Panelist; S = Storage Time; T = Treatment
The sum of squares for each effect is adjusted for all sum of squares above it.

VITA

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