EFFECTS OF COLLAGEN AND FREEZING ON THE QUALITY OF BEEF LOAVES AND BEEF PATTIES

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CHAPTER I

INTRODUCTION

The world's population is doubling every 35 years (Richardson, 1975). The increase in population has caused an increase in the number of hungry people. If the population continues to increase and the food supply is not improved drastically, "the world will be flooded with children--children with bloated bellies, misshapened limbs, and running sores--who are literally born to starve" (Tyding, 1970, p. 6). Thirty million people die of starvation and malnutrition each year (Laffin, 1966). This problem exists not only in the Third World or developing countries but also in the United States. "Fourteen million Americans go to bed each night without enough food to keep them healthy" (Citizen's Board of Inquiry-Hunger U.S.A., 1968, p. 7).

Protein malnutrition is probably more prevalent than insufficient caloric intake, and evidence suggests that it is more devasting due to permanent brain damage (Myers, 1970). The 1967 Report by the President's Advisory Committee (Tyding, 1970) states that

> The world food supply panel considered all possible food sources, including both unexploited products and unexplored methods, in estimating the food required to meet present shortages and provide for population growth in the future. Among the sources considered were new foods from the sea, certain types of bacteria, petroleum, and synthetics (p. 121).

The committee points out that no single new source is the answer but that all possibilities must be studied.

Use of supplements and fortification is being studied, but there is still need for more research to enhance the quality of the diets of human beings. Another problem in nutritive quality is making "strange" foods acceptable.

Proteins are essential nutrients in the diet, and there are many sources; however, some of the sources provide incomplete protein. Beef, a complete protein, and the main source of meat in the United States, is not considered an efficient and economical producer of protein. As Meyer (1970, p. 127) has pointed out, "the yield/acre of food energy and protein is greater from crops like soybeans and corn than it is when cycled through domestic animals." "More than three billion livestock are maintained to supply the animal protein consumed annually in the United States" (Pimental, Oltenacu, Nesheim, Krummel, Allen, and Chick (1980, p. 843). "In the United States, an estimated 37 million tons of plant protein is fed to livestock annually to produce an estimated 5.4 million tons of animal protein for human consumption" (Pimental et al., 1980, p. 844).

In addition to the food and protein shortages, the cost of food, especially of complete protein sources, is becoming a problem. The United States is a country of plenty in terms of the availability of food; but, with the increased cost of meat, people find it more difficult to "stretch the food dollar." If the retail cost of meat in 1967 is assigned the value of 100, then the average cost in 1979 is 241.9 (USDA, Summer, 1980). Based on the theory of supply and demand, the cost of meat will increase as the shortage of food increases.

Considering the food shortage, lack of protein, inefficiency of animal-produced protein, and the high cost of meat, there is a need

to find other sources of protein. Collagen is a by-product of meat production, which may be obtained from the hides of cattle. While collagen alone is an incomplete protein, its use as an extender and nutrient-enhancer would increase the amount of protein available and the amount of protein obtained per beef, and should also help decrease the cost of protein to the consumer.

Data about collagen as a food are so limited that dissemination of information to the public about its potential as an alternative source of protein is not possible. Its functional properties, as an extender, binder, filler, texturizer, and nutrient-enhancer, need to be explored. Some of the criteria to explore these functional properties of collagen are its detectability in meat products and its effect upon nutritional quality. Investigation of these criteria are the subjects of this study.

Purpose and Objectives

The purposes of this research were to determine its effects on beef loaves and beef patties. The same products, beef patties and beef loaves containing collagen, will also be frozen and held in storage for 60 days to determine if freezing has any effects on the characteristics being investigated. Specific objectives are:

1. to assess effects of collagen levels in beef patties and beef loaves on
    a. sensory attributes of color, texture, aroma, juciness, tenderness, and flavor.
    b. objective measurements of percentage vapor, percentage moisture, percentage fat, and percentage total cooking

losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat.

c. nutritional values of ash content.

2. to assess the effects of freezing beef loaves and beef patties containing added collagen by analyzing

a. sensory attributes of color, texture, aroma, juciness, tenderness, and flavor.

b. objectives measurements of percentage vapor, percentage moisture, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat.

3. to make recommendations for further research in this area.

Hypotheses

The following hypotheses were postulated for the study:

$H_{1(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation, due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(b)}$: There will be no significant differences in the mean percentage vapor, percentage moisture, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture,

percentage fat, and percentage ash of cooked meat due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(c)}$: There will be no significant differences in the mean nutrient value measured by ash content due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{2(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation of fresh and frozen beef loaves and beef patties which contain the same levels of collagen.

$H_{2(b)}$: There will be no significant differences in the mean percentage moisture, percentage vapor, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat of fresh and frozen beef loaves and beef patties which contain the same level of collagen.

Assumptions and Limitations

The assumptions impacting on the outcomes of the study are:

1. The trained panel will evaluate the beef patties and loaves as instructed (Carlin and Harrison, 1978).
2. All patties and loaves are prepared in the food research laboratory under the same controlled environmental conditions (Carlin and Harrison, 1978).

The following limitations are accepted for this study:

1. The study will use only "frozen-wet" collagen obtained from the Eastern Regional Research Center (ERRC), U.S. Department of Agriculture, Philadelphia, Pennsylvania.

2. Only ground beef prepared at the Meat Laboratory, Animal Science Department, Oklahoma State University, under controlled conditions and containing 20 percent fat, will be used.

Definition of Terms

The following terms are important to this research:

1. Aroma - "Sensations perceived by the nose when a substance is sniffed" (Civille, Dethmers, and Norat, 1978, p. 2).
2. Binder - ". . . a material that produces or promotes cohesion in loosely assembled substances" (Webster's New Collegiate Dictionary, 1972, p. 110).
3. Collagen - ". . . a protein that forms the chief constituents of connective tissue, cartilage, tendon, bone, and skin" (Guthrie, 1977, p. 492).
4. Color - The shade ranging from light to dark of the internal portion of the meat products as determined by the attribute panel and by the Hunter colorimeter (Carlin and Harrison, 1978).
5. Complete protein - A nutrient that contains all eight essential amino acids needed by animals, including man, for growth and maintenance of life (Stare and McWilliams, 1977).
6. Essential amino acid - ". . . a nutrient needed for growth and maintenance of the body and which must be supplied in the diet" (Stare and McWilliams, 1977, p. 172).
7. Extender - ". . . a substance or ingredient added to another to give more bulk or body . . . or dilute it" (Webster's New Collegiate Dictionary, 1972, p. 405).

8. Filler - ". . . a substance added to a product to increase bulk, weight, viscosity, opacity, and strength" (Webster's New Collegiate Dictionary, 1972, p. 428).

9. Fresh samples - Meat products prepared fresh from ground beef with 20 percent fat and experimental levels of collagen. The patties weigh 142 grams, are shaped into a mold (18.3 cm x 11.7 cm x 1.3 cm), cooked 10 to 12 minutes on each side, depending on the level of collagen, on a pre-heated Farberware grill, and served warm to the attribute panel. The beef loaves weigh 900 grams, are shaped into an aluminum (20 cm x 9.5 cm x 6.4 cm) pan, cooked at 176°C (350°F) in a pre-heated electric oven, to an internal temperature of 75°C, and served warm to the attribute panel (Cross and Berry, 1980; Cross, Muse, and Green, 1979).

10. Frozen sample - Prepared in the same way as the fresh samples, but the patties are cooked six minutes on each side in the initial cooking, and frozen in a blast freezer. After 60 days, they are rethermalized in a 176°C (350°F) pre-heated conventional electric oven for 10 to 12 minutes, and served warm to the attribute panel. The loaves are cooked to an internal temperature of 60°C and frozen in the blast freezer for 60 days. They are rethermalized at 176°C (350°F) in a pre-heated oven to an internal temperature of 75°C and served warm to the attribute panel.

11. Incomplete protein - A nutrient that does not contain sufficient amount of all the essential amino acids even though they do contain at least some or all of the amino acids

required for growth and maintenance (Stare and McWilliams, 1977).

12. Juiciness -

> . . . divided into two characteristics: one an impression of wetness produced by the rapid release of meat fluids during the first chews, the other is one of sustained juiciness apparently due to slow release of serum and to the stimulating effect of fat on salivary flow (Carlin and Harrison, 1978, p. 48).

13. Nutrient enhancer - A substance or ingredient added to a food product to increase its nutrient content.

14. Tenderness - ". . . softness: the sensations from tongue and cheek and the ease with which the teeth sink into the meat at the first bite" (Carlin and Harrison, 1978, p. 48).

15. Texture - A property which includes viscosity, cohesiveness, elasticity, and other physical properties of food experienced when food is touched with the fingers or felt by the mouth (Amerine, Pangborn, and Roessler, 1965).

16. Texturizer - A substance added to effect the arrangement of particles or constituent parts which effect viscosity, cohesiveness, and elasticity of a product (Webster's New Collegiate Dictionary, 1972; Amerine, Pangborn, and Roessler, 1965).

CHAPTER II

REVIEW OF LITERATURE

Literature pertinent to the study was reviewed and presented in this chapter. An understanding of protein malnutrition, protein sources, extenders in beef patties and loaves, collagen, and sensory evaluation will be discussed. Knowledge of these areas will aid in the planning of this research.

Protein and Protein Malnutrition

"The two prime concerns regarding the food supply are the availability of sufficient calories and an adequate amount of protein particularly animal protein" (Stare and McWilliams, 1977, p. 167). Two-thirds of the world population survive almost entirely on rice, corn, wheat, and cassava.

In 1830, Gerardus Mulder, a Dutch chemist proposed the term "protein," meaning "to come first" because he believed that protein was the most important of all known substances in the organic kingdom. Proteins are composed of carbon, hydrogen, oxygen, nitrogen, and sulfur. The subcomponent of protein is amino acids. When protein is digested it is broken down into amino acids, then the body uses the amino acids to build what is needed. Proteins vary in the amino acids present and in the arrangement of the amino acids. DNA requires different amounts of amino acids than hemoglobin. One-half the dry body

weight is protein, which is found in skin, bones, muscles, cartilage, tissue, and body fluids.

When protein is not eaten in sufficient amounts, a breakdown of the protein in the muscles will occur. In children, when a protein deficiency occurs, there is a lack of development of the brain and the body.

In terms of biological values, protein is divided into two subgroups--complete and incomplete. Complete sources provide the essential amino acids in proper proportions. The essential amino acids include leucine, lysine, isoleucine, methionine, phenylalanine, tryptophan, threonine, and valine. Incomplete protein sources are legumes, nuts, and grains. Incomplete proteins are low in at least one of the essential amino acids but if combined with a complete protein, the efficiency of the incomplete protein is increased because the complete protein provides more of the limiting or low amino acids. Two incomplete proteins, which are limiting in different amino acids, can be eaten together to provide a higher quality protein (Stare and McWilliams, 1977; Wilson, Fisher, and Fugua, 1975).

Evaluation of the quality of protein is done several ways. Biological value is a measure of the body's retention of the nitrogen contained in the ingested protein. It is a means of stating the efficiency of protein to meet the body's needs. Protein efficiency ratio (PER) is another way to evaluate the quality of the protein. PER is a measure of the efficiency of weight gain per gram of nitrogen in the diet. Net protein utilization (NPU) is based on the biological value and availability of nitrogen contained in dietary protein (Stare and McWilliams, 1977).

Sources of Protein

Conventional proteins have been discussed; hence, non-conventional protein will be covered in this section. As the need for protein continues to increase, it will be necessary to look at other possible sources such as fishmeal, oilseeds, nuts, leaves, single cell protein, and any other possible sources.

Fish Protein Concentrate

The ocean is an excellent source of food which many times is ignored. Fish Protein Concentrate (FPC) is a group of products produced by different methods. Most of the methods are based on solvent extraction which produces a product that is 75 to 95 percent protein. FPC is defined as a "low-cost, stable, wholesome product of high nutritive quality, hygienically prepared from fish, in which the protein and other nutrient material are more concentrated than they were in the fresh fish" (Stillings and Knobl, 1970, p. 412). FPC was developed mainly to be used as a supplement. It can be added in small quantities to food products as bread, pasta, crackers, cookies, soup, and beverages.

When FPC was added to recipes, an increase in water was required. A graying of the products was attributed to FPC. Between 5 and 10 percent were added to bread, pasta, crackers, cookies, and soup. These levels were acceptable when evaluated for texture and flavor. One acceptable product is a beverage powder which could be reconstituted and used for baby formula or a flavored drink for older children. This product contains four percent FPC (Stidwell, Stillings, and Knobl, 1970).

Oilseeds

Oilseeds include soybean, peanut, cotton, corn, sunflower, and sesame (Stare and McWilliams, 1977); coconut and safflower. The oil is pressed out of the seeds and leaves a meal with is 50 percent protein. Peanuts have been used for many years to supplement the diet.

A large amount of research has been done on using the soybean. The soybean has been used in the Orient for centuries, but the western world has been very slow to accept it. It has only been in the last 10 years that Americans have considered eating soybean products. The bean contains a high content of amino acids, expecially lysine, leucine, and isoleucine. Soy is ideal to blend with cereal which is low in lysine. Soy flour has been used in breads and soy concentrates containing 70 percent protein have been used in production of high quality meat products. Soy can be considered an emulsifying and stabilizing agent in meat products. It also helps retain moisture and fat during storage and cooking. The problem inherit to soy as a meat extender or substitute is that it imparts its own flavor (Coppock, 1974).

Kramer, King, and Westhoff (1976) evaluated products containing TSP after the products had been frozen. The results indicated that "ready-to-heat-and-eat" frozen entrees that were enriched or extended with soy at a level of eight percent would maintain a high quality for three months and up to six months if stored at a constant -20°C. When seasoning food, more than the desired amount must be added as flavor is lost during storage. The mushy quality of TSP is decreased after

storage. Fluctuation in temperature has a detrimental effect on texture and flavor, particularly when stored below 0°F.

Another oilseed is cottonseed protein, which has been used in crackers at levels of 25 and 35 percent. The 25 percent level was found to be a very acceptable product; however, the 35 percent level was noted as leaving an after taste. The interior and exterior appearance acceptability decreased as the percent of cottonseed protein increased (Staats and Tolman, 1974). Bread made using 5 and 10 percent cottonseed flour made an acceptable product and also increased the nutritive value (Aguilar, de la Fuente, and Valle, 1967). Each of the oilseeds in use today is receiving attention as a supplement and progress is being made in food industry to promote use of oilseed protein.

Kuo (1980) studied the effects of adding peanut grits and peanut flour to muffins made with all-purpose and whole wheat flour. It was found that peanut flour could be substituted up to 30 percent in all-purpose and whole wheat muffins and still be acceptable. A substitution of 10 percent with peanut flour and grits increased the protein content of muffins significantly.

Single Cell Protein

Single Cell Protein (SCP) includes algae, bacteria, fungi, and yeasts. With the rising costs of meat, the interest in SCP has increased. During the last decade, research on SPC has expanded. Some algae can be produced from as little as carbon dioxide and light. Bacteria, yeasts, and fungi require carbohydrates, alcohols, and hydrocarbons. A nitrogen source and inorganic elements (calcium, phosphorus, magnesium, and iron) are required in addition to the carbon

and energy sources for all SCP. Microbial protein is low in methionine, but supplements can be obtained at a low cost, so it is very practical to supplement the diet with SPC. The use of SPC as a source of protein has been limited due to its flavor. Bacteria have a nucleic acid content which can cause problems for the human body but this is not true of yeasts. Before SCP can be used as an extender for gorund meat and frankfurters, and as an enrichment in baked products, the non-protein nitrogen content must be reduced. The functional properties must also be improved. Some work has been done to produce a fiber from SCP (Lipinsky and Litchfield, 1974). Zabik and Garrison (1975) used baker's yeast protein in cornmeal muffins at 10, 20, and 30 percent of the cornmeal. All muffins had similar texture, tenderness, and moisture to the control. Color and flavor were evauated lower as the level of yeast increased, but the change was not great enough to cause the muffins to be non-acceptable. Zouranjian (1979) substituted yeast for flour in all-purpose and whole wheat muffins at 4, 7, and 9 percent. Boost-100 was acceptable in the all-purpose muffins up to 7 percent yeast and up to 9 percent in the whole wheat flour. Torutein-LF was acceptable in all-purpose and whole wheat muffins up to 7 percent yeast. Toruway-49 was acceptable in all-purpose and whole wheat muffins up to the 9 percent level. This was based on a sensory evalution. The addition of yeast significantly increased the protein level of muffins.

Beef Patties

Several studies have been done to determine the effect of using extenders in beef patties. Beef patties have been extended with such

substances as soy, milk precipitates, defatted peanut meal, and field pea meals.

Drake, Hinnegardt, Kluter, and Prell (1975) studied the effect of textured soy protein (TSP) and fat levels on quality and acceptability of beef patties. The fat levels used were 15, 20, 25, and 30 percent and the soy levels were 0, 15, 20, and 25 percent by raw weight. The patties were 71 grams with a 7.6 cm diameter and 1.27 cm thick. Total cooking loss, moisture content of raw and cooked patties, and fat content of raw and cooked patties were all measured. A trained sensory panel of 12 to 14 members did an evaluation of the patties for color, odor, flavor, texture, and appearance. The amount of fat and total cooking losses were reduced as the TSP level was increased. An increase in cooking loss occurred as the fat increased. The largest increase occurred between 20 and 25 percent fat. The TSP had no effect on fat losses. The moisture loss was equivalent at all levels of TSP and significantly less than patties with no TSP.

The color and texture were not effected by the fat and TSP levels. TSP had a significant effect on odor and flavor. The TSP decreased the scores for odor and flavor. The TSP extended patty resulted in a lower quality and acceptance rating, regardless of fat content.

McWatters (1977) studied the effect of defatted peanut, soybean, and field pea meals on ground beef patties. The peanut, soybean, and pea meals were substituted at 5, 10, and 15 percent of the meat. These were compared against an all beef control patty. Cooking losses, water and fat retention, protein content, specific volume, compression, tenderness by shear force, color, and sensory quality attributes were compared.

Extended patties had lower cooking loss than the control. The water retention was high and the fat retention was variable. Protein content of patties with soybean and peanut were higher than the all beef patties, but the patties with pea had a lower protein content. Less force was required to compress or shear the extended patties. Substitution higher than five percent caused decreased acceptability on sensory attributes.

Beef patties extended with co-precipitates of milk (CCP) with or without wheat flour were compared to all beef patties and soy extended patties (Thomas, McBride, Turner, and Aba, 1978). Twenty and 30 percent fat were used and 30 percent of the weight of the meat was substituted with the extender. Total loss was determined by weight differences. The methods described in Horwitz (1975) were used to evaluate the fat and moisture content, after cooking. Firmness of the patties were determined by a single-pin penetrometer, one hour after cooking. Sensory evaluation was done using an incomplete block design. The patties were evaluated by taste panelists on flavor, texture, and general acceptability.

The all beef patties have the highest yield and the patties with CCP had more cooking loss than the patties with TSP. The fat content of beef patties with 20 and 30 percent fat were the same after cooking. The addition of TSP and CCP did not decrease the firmness. The panelists ranked the extended patties above the control in appearnace and there was no significant difference in flavor, possibly because they were all seasoned. The greatest differences were in the texture, with the CCP at 5 percent and whole wheat flour at 2-1/2 percent being liked the most. All patties containing extenders were preferred to the all beef patty, based on general acceptability.

Vaisye, Tasso, McDonald, and Young (1975) evaluated the effects of fababean and field pea concentrates as ground beef extenders. Patties were prepared containing 70 percent ground beef, 10 percent legume, and 20 percent water. There were four leguminous products--fababean as flakes, fababean as flour, pea as flakes, and pea as flour. These patties were compared with beef patties with TSP and all beef patties. All treatments were replicated three times. The sensory evaluation was done by an untrained 30 member panel. The results showed that by flaking the legumes, their acceptability was greatly improved. The products made with flour were judged as unacceptable. The control patty was preferred over all the extended patties. The patties with TSP were preferred for color when compared to the other extended patties. All legume-meat patties had less cooking loss. The patties with flakes (fababean and pea) were lower in fat and higher in moisture than those prepared with flour.

Cross and Berry studied the effects of varying patty size (1980). Patty sizes included 227 grams, 114 grams, and 102 grams. The 102 grams patty received lower ratings for tenderness, juiciness, amount of detectable connective tissue, and flavor intensity than did the 144 gram and 227 gram patties. The smaller the patty, the greater the percent cooking loss.

The effects of pre-cooking beef patties was studied by Cross, Muse, and Green (1979). Patties were broiled to an internal temperature of 60°C and then were frozen to below zero. The frozen raw and frozen precooked patties were cooked for 13 minutes at 200°C. An eight member trained taste panel was used. The panel evaluated tenderness, fragmentation, juiciness, detectable connective tissue, and

flavor. Patties that were cooked from the raw state were significantly more tender than the pre-cooked, reheated patties.

Beef Loaves

Nielson and Carlin (1974) studied the eating quality, fat, moisture, and thiamin content of beef and beef-soy (SPC) loaves. The loaves were frozen raw and pre-cooked to 165°F and frozen. The loaves were frozen for zero, two, four, and six months. Volatile cooking losses were similar for fresh, all beef, beef-soy, and beef-soy with TP loaves before freezing. The volatile losses during reheating of frozen pre-cooked loaves were similar. The all beef loaves did have five times more lipid and three times more total loss than the SPC loaves.

Sensory evaluation indicated that the products were not affected by storage time. However, the addition of SPC significantly affected the beef flavor. The loaves containing soy were all evaluated as having pronounced soy flavor. Replacement of 30 percent of the ground beef with SPC decreased the juiciness of the loaves.

Carlin, Ziprin, Zabik, Kragt, Polsiri, Bowers, Rainey, Van Duyne, and Perry (1978) studied the effects of texturized soy flours on cooking losses, flavor, juiciness, fat, moisture, and thiamin content and retention in 15 or 30 percent substituted beef-soy loaves. The study included six different soy flours. Some soy flours increased cooking time, others decreased the time, while others had no effect. The total cooking losses were decreased by soy flour. The soy protein has the ability to bind both fat and water to reduce cooking losses. The level of soy had no effect on juiciness. To correspond with

decreased losses, the moisture content was higher in the soy loaves. The fat retention was also higher.

Hwang and Carpenter (1975) studied the effects of adding pork hearts to meat loaves. The pork hearts were added at 10, 20, and 30 percent of the total weight. The heart content affected shrinkage, texture (mechanically and subjectively), and preference. The increase in pork hearts increased the shrinkage and decreased the sensory scores for firmness and preference. Additives were also used in the meat loaves. Nonfat dry milk (NFDM), isolated soy protein (ISP), soy protein concentrate (SPC), peanut grits (PG), and peanut flour (PF) were used at 0, 3.5, and 7.0 percent. The additive had no effect on shrinkage or texture measured mechanically. The sensory panel rated samples with SPC as being firmer than NFDM or PF samples. The NFDM loaves were preferred to the SPC or PF loaves.

Ebro, Harris, Henrickson, and Sneed (1979) studied the effects of five types of collagen at three levels in meat loaves (with binder and seasoning). The collagen was added at 10, 20, and 30 percent of total weight. The first objective was to determine what type of collagen best suited use in beef loaves. The second objective was to determine the most acceptable level of collagen for the types preferred in the first objective. Types #1 and #4 were determined to be most acceptable in beef loaves. The level did not effect appearance (comparing 10, 20, and 30 percent). The texture of the 10 and 20 percent were preferred over the 30 percent. The flavor was not significantly different due to level of collagen. The fat content was not held constant and the peak force was done on chilled meat. The peak force (kg/g) increased (tenderness decreased) as the level of collagen increased. Meat

loaves with type four collagen were frozen for six months and compared with fresh loaves. Frozen-wet was substituted for meat at 20 percent, freeze-dried substituted for the binder, and air-dried substitution for the binder. The cooking losses were higher in the frozen-reheated loaves for all types of collagen. Appearance, aroma, and beef flavor were not affected by type of collagen added in the frozen loaves. Juiciness, off-flavor, and texture were affected by the type of collagen added in the frozen loaves. Texture was affected by the type of collagen in the fresh loaves. The frozen-wet collagen produced the juiciest, most tender loaves. The frozen-wet collagen loaves had more beef flavor than the freeze-dried or air-dried in the frozen loaves. The loaves with frozen-wet collagen were more tender by objective evaluation.

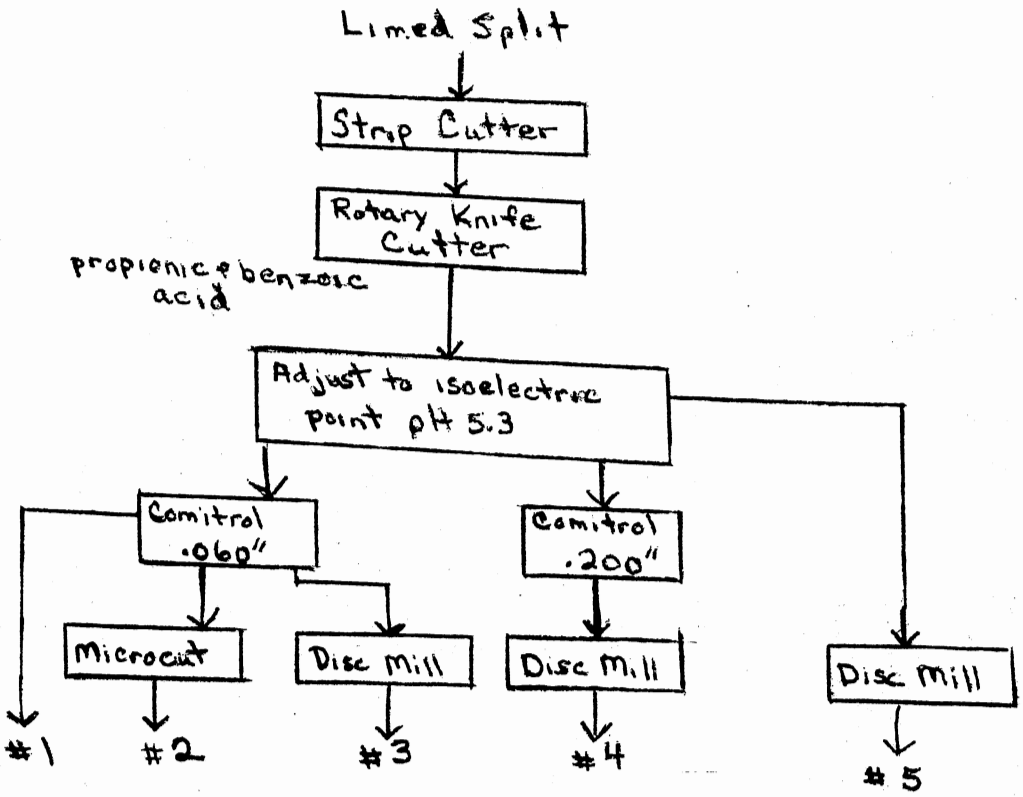
Collagen

Collagen is an insoluable fibrous protein that has a high molecular weight. It is in the hides and skins of animals. The trimmings and splits from the production of leather goods are considered waste and are usually used to make gelatin and glue. The hides are becoming a surplus commodity (Whitmore, Jones, Windus, and Naghski, 1972). Research has indicated that collagen has unique physical and chemical properties which could be utilized better than as sources of gelatin and glue. Possible uses include being added as an ingredient to food systems. The hides for this purpose are obtained from inspected beef. At the present time many different pre-processing procedures are being used. For collagen to be used as a binder and extender of meat the communited collagen must be undenatured. Denaturation is caused by pH

or heat. Care must be taken to prevent any denaturation during processing. The process is to first pre-cut the limed splits by being forced through a horizontal cutterhead with four fly knives and the splits are cut into 3/8 inch wide strips. Secondly, they are run through a rotary knife cutter after which they are acidified using propionic and benzoic acid. The pH reaches 5.3. The splits are then ground by a comitrol or a disc mill. At present there are five products that vary in particle size and shape. Figure 1 indicates the source of all five types. These five products can be canned in wet form in #10 cans and frozen. Product #1 has also been air-dried and freeze-dried (Komanowsky, Sinnamon, Elian, Heiland, and Aceto, 1974).

Collagen is an incomplete protein because it contains no tryptophan and cystine and is low in lysine, threonine, and methionine. The amino acid composition of lean beef and collagen are compared in Table I.

Moisture-free collagen is 91.5 percent protein. When collagen is combined with lean beef acceptable levels of all amino acids is produced. Collagen products are odorless and tasteless. The collagen has a bland flavor and hydrothermal shrinkage occurs at 60 to 65°C, therefore it can be incorporated into other food systems. Collagen binds and absorbs water, so its addition would produce a more moist product. Its use as an extender could vary the viscosity, consistency, mouthfeel, tenderness, and juiciness of meat and other food products. The only use of collagen in food, at the present, is in sausage casings. This use of collagen is considered acceptable and is considered generally acceptable as safe (GRAS).



Source: Turkot, Komanowsky, and Sinnamon (1978), p. 49.

Figure 1. Processing Procedure for Food-Grade Collagen

TABLE I

AMINO ACID CONTENT OF COLLAGEN AND LEAN BEEF

Amino Acid	Collagen(g)[1]	Lean Beef(g)[1]
	Essential Amino Acids	
Isoleucine	1.6	5.0
Leucine	3.0	8.3
Lysine	3.7	8.8
Methionine	0.7	2.6
Cystine	--	1.3
Phenylalanine	2.1	4.9
Tyrosine	0.9	3.9
Threonine	1.9	4.4
Tryptophan	--	1.3
Valine	2.3	5.5

[1]Grams of amino acid residue per 100 grams of total amino acid residue.

Source: Happich (1975).

In 1980, a draft of the report evaluating collagen as a food ingredient from a health point of view was released.

> There is no evidence in the available information on regenerated collagen that demonstrates, or suggests reasonable grounds to suspect a hazard to the public, when it is used in sausage casings in the manner now practiced or that might reasonably be expected in the future. There is no evidence in the available information on native collagen that demonstrates, or suggests reasonable grounds to suspect a hazard to the public, should it be used as a food ingredient (Federation of American Societies for Experimental Biology, 1980, p. 11).

VanDusen (1980) added collagen (air-dried, type #1) to carrot cake and applesauce cake. The levels used were 10, 15, and 20 percent

by weight of flour. The sensory evaluation of the applesauce and carrot cakes indicated that the collagen was detectable but that the products containing the collagen were acceptable.

Preliminary work on collagen in ground beef has been done (Ebro et al., 1979). From sensory evaluation of meat loaves, it was determined that type #1 was the preferred collagen product. Preliminary work on beef patties was limited to objective tests.

The use of vegetable extenders causes a loss of texture in meat products. Indications are that collagen would increase the desired texture. Possibly the two extenders could be combined together but first, further studies need to be done on collagen as the only extender (Happich, 1975).

Sensory Evaluation

There are basically two types of sensory evaluation. One is preference testing which is used to determine acceptability to the general public and the second is difference testing. In preference testing, a large number of individuals which represent the public were used. These people received no training.

Difference tests are used to determine differences in odor, tastes, texture, and any other characteristics which can be detected. A small panel is used for this type of evaluating. "Three to five discriminating and conscientious judges are sufficient for food difference testing panels" (Charley, 1970, p. 23).

Training of a taste panel has no one specific definition. "Training may be no more than an introduction to the scoring methods and procedures or it may be the three to four month intensive training

period required for an expert panel" (American Meat Science Association, 1978, p. 9). For data from several research projects to be comparable, the panel must have been trained following the same selection and training. Panel members are like any other instrument used to study specific parameters; they must be calibrated to measure the parameter.

Meat evaluation is very difficult because meat has many qualities or characteristics. Cross, Moen, and Stanfield (1978) describe a procedure for screening and training a panel which is specifically designed for meat, a "descriptive attribute panel."

The first step is the personal interview for pre-screening. Since there is controversial evidence about the effect of age, it is not a consideration. Only ability will be used to evaluate the perspective panelists. During the personal interview, the researcher should determine the interest of the person and any prior experiences in taste panel work.

The second step is screening. Screening should focus on the parameters that the panelists will be using in the actual evaluation. Triangle tests are excellent for screening.

The third step is training. Training should familiarize the individuals with the procedures, improve sensitivity to characteristics, and increase recognition of characteristics. Training should include tasting of the samples and discussions of the characteristics. The panelist should also practice using the evaluation form.

Sneed (1977) suggests that the use of basic taste tests could be helpful in screening possible panelists. Rank ordering three intensities of one basic taste indicates the panelists' ability to determine

different levels of the same taste. If the product will be evaluated by odor, then odor identification should be included in the screening. The actual identification of the odor is not important but the person's ability to describe the odor is.

Mineral Analysis

Morris (1978) studied the mineral content of meat. This research compared mechanically deboned meat with hand deboned meat. The meat samples were digested with acid, reconstituted, and read on an Atomic Absorption Spectrophotometer. This procedure was used to determine calcium, magnesium, manganese, zinc, iron, chromium, copper, lead, and potassium.

Mechanically processed beef was higher in calcium, iron, chromium, and lead than was the hand-deboned beef. Magnesium, zinc, and potassium levels were higher in the hand-deboned meat.

CHAPTER III

METHOD

The purpose of this research was to assess the effects of added collagen on organoleptic properties, objective properties, and nutritive values of beef loaves and beef patties. The investigation was conducted also to assess the effects of frozen storage by analyses of organoleptic and objective properties of fresh and frozen beef loaves and beef patties which contain collagen. This chapter includes the research design, product development including procedures and materials, procedures for evaluation, instrumentation, and methods which were used for statistical analysis.

Research Design

The mineral analysis was a randomized complete block with one treatment factor at four levels. The treatment factor was collagen content at 0, 10, 20, and 30 percent levels. A 2 x 4 factorial arrangement of treatment factors in a randomized complete block was used for the objective tests. "In factorial analyses of variance, two or more independent variables vary independently or interact with each other to produce variation in a dependent variable" (Kerlinger, 1973, p. 245). The two independent variables were the two production systems, "fresh" and "frozen," and the level of added collagen. The dependent variables were the objective qualities and the nutritional

values of the products. All possible combinations of independent variables were tested at the same time.

The sensory evaluation was made with a split-plot experimental design. This design was utilized when subunits of variables were used. It is an imcomplete block design (Steel and Torrie, 1960). The main-plot treatment was "fresh" vs. "frozen" or level of collagen. The split-plot treatment or subunit was the panelists'. Judges evaluated beef samples which contained all four levels of collagen at each session--a control without collagen, 10 percent collagen, 20 percent collagen, and 30 percent collagen, substituted for the lean meat only. Each of the following was tasted during three sessions--fresh patties, frozen patties, fresh loaves, and frozen loaves (Griswold, 1962). Randomization was achieved by preparing "batches" of raw ground beef from one carcass, randomly assigning numbers to the samples for tasting, and randomly assigning a piece of the sample to a judge.

Manipulation of an independent variable must occur under controlled conditions. The conditions that were controlled in preparation of the food products were ingredients, storage procedures, preparation procedures, oven or grill type, pre-heating temperature or time, and internal temperature or time of cooking. The evaluation by the taste panel was done under controlled conditions. The time of day, days of week, room, noise level, privacy during tasting, lighting, and room temperature were all controlled (Amerine, Pangborn, and Roessler, 1965; Carlin and Harrison, 1978).

All samples of ground beef for this experiment were obtained from the Meat Laboratory, Oklahoma State University, and they were prepared containing 20 percent fat. All of the collagen was from one

batch which was prepared by the U.S. Department of Agriculture, Eastern Regional Research Center (ERRC), Philadelphia, Pennsylvania. All of the ground beef used in the investigations were prepared at the same time and divided into batches so that all meat had an equal chance of being in any given sample.

Preparation of Meat

The beef was trimmed and cut into chunks which weighed six ounces or less. The chunks were tumbled by hand when they were cut to aid in even distribution of the fatty pieces. Then the meat was run through a grinder (Model BIRO 5 42 4852, serial 9741) using a coarse plate (32-1-½-DC) and mixed in a cooled Duty Master Mixer (Model 1138c) for five minutes. The ground meat was divided into four tubs containing 45 pounds, 40 pounds, 35 pounds, and 30 pounds for the 0, 10, 20, and 30 percent batches, respectively. The decrease in weight of meat was to allow for the increase in weight of collagen added. The "grab" method was used to remove approximately five pounds of meat for fat analysis, at the time meat was placed in the tubs. The meat was labelled, covered with plastic wrap, and stored overnight at 34°F while the fat analysis was done.

The five pounds of meat used for fat analysis was run through the grinder twice with a fine plate (3246-11-72-G). It was divided into 15 samples and placed in storage bags. Fat analysis was doen by the Modified Babcock Method on 10 samples and the average percentages of fat was determined (Appendix A).

The collagen ("frozen-wet," run 90ED121-.06, type #1) was removed from the blast freezer and placed in the refrigerator 24 hours

before it was needed. The collagen was removed from the cans and turned onto racks to drain for 10 minutes. The contents of one #10 can at a time were placed in a collander and squeezed by hand until no water would come out.

The meat which was stored in the refrigerator, fat (pre-ground with a coarse plate) and the collagen were combined in proper amounts. The collagen was substituted for lean mean so that 0, 10, 20, and 30 percentages of the lean meat was replaced by collagen. At this point, the different combinations (0, 10, 20, and 30 percent) were divided into three subgroups to be used for the three replications. Each subgroup, or batch, weighed 16-1/3 pounds and was prepared with the amounts of lean meat, fat, and collagen listed in Table II. All weighing was done on an ESI Scale (MK II/25 S/N). All mixes were returned to the refrigerator and brought out one at a time for grinding.

TABLE II

WEIGHTS OF COLLAGEN, FAT, AND LEAN MEAT FOR EACH BATCH

Sample (percent)	Collagen (lbs.)	Fat (lbs.)	Lean (lbs.)	Total (lbs.)
0	0	2.67	13.67	16.33
10	1.33	2.67	12.33	16.33
20	2.67	2.67	11.00	16.33
30	4.00	2.67	9.67	16.33

The batches were run through the grinder twice, first with the coarse plate then with the fine plate. Each batch was divided into three five pound packages and one 1.33 pound package. Each package was wrapped in freezer paper, labelled, and frozen in the blast freezer. The prepared meat in the five pound package was for patties and loaves and the 1.33 pound package was for determinations which might require raw meat.

Preparation of Samples

The frozen samples were prepared 60 days prior to being evaluated. All meat was removed from the freezer and stored in the refrigerator for 48 hours before it was needed. The frozen loaves and frozen patties were prepared from one five pound package. The fresh loaves and fresh patties were prepared from two separate packages.

Meat Loaf. The (20 cm x 9.5 cm x 6.4 cm) aluminum pans were labelled and weighed on a Mettler 4400 electronic top loading precision balance, and weights were recorded on the analysis sheet (Appendix B). Nine hundred grams of meat for each percentage level of collagen (0, 10, 20, and 30) were weighed and then molded into the pan, being sure the loaf was flat on top. A thermocoupler (.005 cm diameter) from a Honeywell recorder was placed in each loaf, which was placed in a pre-heated 176°C (350°F) conventional electric oven (General Electric model CN50) in random order. The loaves were cooked to an internal temperature of 60°C (Cross, Muse, and Green, 1978). When the meat reached the prescribed internal temperature, the pans were removed from the oven and weighed. The weights were recorded on the analysis sheet. The loaves were allowed to cool in the pans for 30 minutes, and then were sealed

in Zip-Loc bags and stored in a Hobart upright freezer (model H1). The meat loaves remained in the Hobart freezer for 24 hours and were then moved to a blast freezer for 60 days.

The loaves were moved from the blast freezer to a refrigerator 24 hours before they were needed for evaluation. The day they were evaluated, the loaves were weighed and the weights were recorded on the analysis sheet. A thermocoupler (.005 cm diameter) was placed in the center of each loaf and the loaves were rethermalized in a pre-heated 176°C (350°F) conventional electric oven (General Electric model CN50) to an internal temperature of 75°C. When the internal temperature was reached, the loaves were removed from the oven, weighed, and the weights recorded. The loaves were allowed to set for 10 minutes after which they were removed from the pan and the pan and drippings were weighed. The pan was placed in the refrigerator for the fat to solidify.

The samples were prepared from the loaves by cutting the end and edges off the loaves using an electric knife, and discarding them. The loaf was cut in half through the width and one-half set aside for objective tests. The remaining half was cut in half lengthwise and into five slices through the width to produce 10 pieces of approximately the same size. One of the pieces was placed on each sensory-evaluation plate. From the remaining half of the loaf, two one-centimeter-thick slices were cut through the width, one for analysis with the Hunter colorimeter and one for Instron. The sample piece for Instron was sliced in half to form two squares, which were weighed (approximately 25 grams). The remaining piece and any scraps were frozen for other objective determinations. The fat from the pan in the refrigerator

was removed by scraping it off of the top. The pan was then weighed to determine the weight of the non-fat liquid from the meat.

Fresh loaves were prepared by the procedure described previously except the loaves were cooked to an internal temperature of 75°C on the initial cooking. The samples from the fresh loaves were prepared in the same manner as the samples from frozen loaves. The analysis sheet for fresh loaves is shown in Appendix B.

Beef Patties. Six 142-gram portions of meat containing each level of collagen (0, 10, 20, and 30 percent) were weighed out. Each patty was shaped into a 18.3 cm x 11.7 cm x 1.3 cm rectangle using a modified (without grooves) plexiglass mold (American Meat Science Association, 1978). All six patties with the same percentage of collagen were cooked on one grill (Farberware "Open Hearth" Broiler and Rotisserie No. 455N) which was pre-heated for 20 minutes. The patties were placed randomly on the grill and cooked for six minutes on each side. The weights of the patties and the weight of the dripping and patties were recorded on the analysis sheet. The patties were cooled for 30 minutes, then sealed in Zip-Loc bags and stored in the Hobart upright freezer for 24 hours, then moved to the blast freezer for 60 days.

Twenty-four hours prior to evaluation the patties were removed from the blast freezer and placed in the Hobart refrigerator. They were re-thermalized by placing the patties and the juice from the patties in a preweighed aluminum pan and cooking at 176°C (350°F) in a pre-heated conventional electric oven (General Electric model CN50) for 10 minutes, 11 minutes, 11½ minutes, and 12 minutes for the 30, 20, 10, and 0 percents of collagen, respectively. These times were pre-determined from

several pre-analysis tests. Variation in cooking time allowed all patties to have the same internal temperature.

The patties and the pan were weighed. The pans were placed in the refrigerator to allow the fat to solidify. Two of the patties were picked randomly to be used by the taste panel. An electric knife was used to remove all four sides from each of the two patties. Each patty was divided into six equal pieces. Each of the samples was then placed on the evaluation plates. Two of the remaining patties and the scraps were frozen for objective tests. One patty had the face of the side removed for color analysis. The remaining patty was cut in half to form two squares. All uncut edges were trimmed off. Each square was weighed (approximately 25 grams). These samples were for measuring tenderness by Instron. The pans were removed from the refrigerator and the fat was scraped off of the top, and the pan and remaining drippings were weighed.

Cooking was the same for fresh patties as it was for frozen patties except the control was cooked for 12 minutes on each side, the patty containing 10 percent collagen was cooked for 11½ minutes on each side, and the patty containing 30 percent collagen was cooked for 10 minutes on each side. The samples for evaluation were prepared by the same procedure as the frozen patty samples.

Subjective Tests

Selection and Training of the Taste Panel

> As judges, a small panel (5-15) should be used who have earlier shown ability to detect sensory differences between samples. In addition, the panel should be trained to recognize the attributes of the profile and to score them reproducibly (Swedish Food Institute, 1978, p. 7).

Individuals were contacted by the researcher until 11 potential panelists were found. The time, dates, and their responsibilities (Appendix C) were discussed at the time of the personal contact. Only those who were interested and motivated were asked to participate. The first day of screening and training for the taste panel included identification of the four basic tastes (salty, sweet, bitter, and sour). The basic tastes were prepared from distilled water and concentrations of sodium chloride, sucrose, quinine sulfate, and citric acid just above the threshold (Sneed, 1977). The panelists were also asked to identify odors. The odors were mint, cinnamon, peppermint, onion, cloves, vanilla, vinegar, wintergreen, molasses, yeast, and a pine cleaner. These were prepared by putting the ingredients on cotton balls and placing them in small dark bottles with tight-fitting caps. The third exercise for the panelists was to identify character notes and their intensities (texture, flavor, etc.) discernible in V-8 juice (Sneed, 1977) (Appendix C).

The second day, their ability to rank intensity levels was tested. Four levels of two of the basic tastes were evaluated. Then a triangle test was performed to see if the panelists could pick out the taste that was different. A control sample of meat without collagen and a sample of meat with 30 percent collagen were used. The panelists then identified characteristics of meat and determined the intensities of these properties. After this test, characteristics of the meat samples were discussed with the panelists.

The panelists who completes Days 1 and 2 of training successfully, participated in a test run on the third day. The use of the evaluation form (Appendix C) was explained before the test run. A written

description was given to the panelists to use while completing the form. Eight panelists were qualified at the end of the three days of testing and training.

Samples

To prepare the samples for the panel, the pieces of meat were placed on a white plate with codes written on the plate to identify them. These codes were randomly selected from a table of random numbers. The numbers were in numerical order around the plate. The plate contained four samples, one with each level of collagen. The taste panel member was given the samples, the evaluation forms, and distilled water for rinsing the mouth after tasting each sample. The panelists evaluated the products in a sensory-evaluation room by following the instructions that accompanied the evaluation form (Appendix C).

Objective Tests

The tests used to evaluate the quality of beef loaves and beef patties included measures of cooking loss, tenderness, color, fat, moisture, ash, mineral, and amino acids. The losses were determined by weighing during the preparation procedure and calculating loss (Appendix B). The tenderness was measured by the Kramer shear cell of the Instron Universal Testing Instrument Model 1122 and color by Hunter colorimeter. These were done each day that the sensory panel covened. The samples were evaluated at the same time each day. The fat analysis was done by a modified version of the ether extraction process described in the AOAC Handbook (Horwitz, 1975) (Appendix A). The moisture analysis was done by heating for 24 hours in a 102°C

oven (Appendix A). The percent ash analysis was done by ashing the meat from the moisture analysis in a muffle furnace at 500°C for 24 hours (Appendix A). The mineral analysis was determined by removing the moisture at 102°C for 24 hours, ashing for 24 hours, and reconstituting the ash with 7N nitric acid (Appendix A). The samples were read on the Atmoic Absorption Spectrophotometer (Type AA-5).

Instrumentation

"The score card and number of gradations on the scale must be determined for each experiment" (Carlin and Harrison, 1978, p. 19). A score card should be developed for each experiment so that it reflects detectable variation in the factors which are being studied. The attributes in the instrument for this study have been used by other researchers (Tapp, 1978; Ebro, Morris, and Coburn, 1980).

The instrument developed is a modified magnitude estimation scale. Using magnitude estimation scaling enhances the sensitivity of the panelists and allows small and large differences to be expressed (Moskowitz, Fishken, and Ritacco, 1979). A standard with a designated value was not used in this experiment. In a magnitude estimation study by Sevens (1975) he noted that most observers (panelists) seemed reasonably well pleased with the elimination of the standard. The attribute scales on the instrument (Appendix C) are horizontal instead of the typical vertical lines. Holsinger (1980), a researcher at the USDA Eastern Regional Research Center, suggested the horizontal lines and has shown it to be reliable.

The instrument was checked for content validity and clarity by four faculty members in the Food, Nutrition and Institution

Administration Department at Oklahoma State University. The taste panel was trained in the use of the instrument and was given an explanation of the attributes to be evaluated.

Data Analysis

An analysis of variance permits comparison of any number of samples at the same time to determine whether they came from populations with identical means (Mueller, Schuessler, and Costner, 1977). It was used to determine if a significant difference existed between the production systems (fresh and frozen), level of collagen, panelists, and replications of beef loaves and beef patties. The sensory analysis, objective tests, and nutritional quality data were analyzed. A significance level of .05 was used to determine significance in all tests resulting from an analysis of variance.

Duncan's New Multiple Range Test "is a systematic procedure for comparing all possible pairs of group means (Nie, Hull, Jenkins, Steinbrenner, and Bent, 1975, p. 427). Duncan analysis was used to determine the location of significant differences found by analysis of variance. A protection level of .05 was applied. The Statistical Analysis System (SAS), developed by Barr and Goodnight (1972) was used to analyze the data.

CHAPTER IV

RESULTS AND DISCUSSION

Frozen-wet collagen was substituted for lean meat in beef loaves and beef patties. The products were served immediately after cooking or were cooked, frozen for 60 days, reheated, and served. The four products (fresh loaves, fresh patties, frozen loaves, and frozen patties) were evaluated by a trained attribute panel for color (light to dark), color (even to streaked), texture, aroma, initial juiciness, sustained juiciness, tenderness, amount of connective tissue, beefy flavor, and off-flavor. Objective tests consisted of tenderness by shear force (kg/g), area of peak (cm^2/g), moisture content, fat content, ash content, and color evaluation. The percentages of vapor, moisture, fat, and total loss from cooking were calculated. The mineral levels of zinc, copper, iron, calcium, and magnesium were analyzed. This chapter presents the data analyses to determine if there were any differences in the products with the collagen and those without the collagen. The effects of freezing on the cooked collagen and the two different cooking methods (loaves and patties) were compared.

Sensory Evaluation

Each attribute scale on the instrument (Appendix C) was 200 units in length. Each panelist's marks were measured and assigned the

appropriate value. All scales except aroma and beefy flavor were measured from top to bottom. Aroma and beefy flavor were measured from bottom to top so that a larger number would indicate a more intense level of the attribute. These values were used to calculate analysis of variance and Duncan multiple range tests.

Fresh Loaves

The analysis of variance among the fresh loaves is shown in Table III. Aroma was the only attribute which varied significantly ($p<0.05$) between replications. Significant differences ($p<0.05$) in color (even to streaked), texture, aroma, beefy flavor, and off-flavor were obtained due to collagen level. The amount of connective tissue varied at the $p<0.01$ level for the different levels of collagen. No significant variation was observed in color (light to dark), initial juiciness, and tenderness.

The results of the Duncan multiple range tests for collagen in fresh loaves are presented in Table IV for those attributes determined to have significant differences by analysis of variance. Color (even to streaked), texture, aroma, beefy flavor, and off-flavor had significant differences.

Color (even to streaked) varied significantly when evaluated by collagen level. The amount of streaking increased as the collagen level increased. There was a significant difference between the control and the loaves containing collagen but the level of collagen had no significant effect.

Texture varied significantly when evaluated by collagen level. The coarseness of the texture increased as the collagen level increased

TABLE III

ANALYSIS OF VARIANCE FOR SENSORY EVALUATION OF FRESH LOAVES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color (light to dark)	Day	2	475.00	0.51	0.6242
	Collagen	3	450.30	0.48	0.7058
	Error A	6	930.95		
	Panelist	6	4411.71	2.85	0.0224
	P x D	12	594.44	0.38	0.9606
	P x C	18	2491.73	1.61	0.1096
	Error B	36	1546.69		
Color (even to streaked)	Day	2	8534.22	1.75	0.2516
	Collagen	3	32854.76	6.74	0.0238
	Error A	6	4871.13		
	Panelist	6	1670.73	0.80	0.5794
	P x D	12	1798.12	0.86	0.5954
	P x C	18	2458.69	1.17	0.3328
	Error B	36	2099.37		
Texture	Day	2	1987.79	0.35	0.7180
	Collagen	3	33266.96	5.86	0.0324
	Error A	6	5675.89		
	Panelist	6	2006.75	1.08	0.3938
	P x D	12	1296.48	0.70	0.7440
	P x C	18	1054.23	0.57	0.9005
	Error B	36	1861.43		
Aroma	Day	2	5029.76	7.86	0.0211
	Collagen	3	13898.71	21.71	0.0013
	Error A	6	640.08		
	Panelist	6	2340.18	1.50	0.2051
	P x D	12	1659.97	1.07	0.4152
	P x C	18	2390.84	1.54	0.1340
	Error B	36	1556.86		
Initial Juiciness	Day	2	5368.75	1.21	0.3610
	Collagen	3	6522.52	1.47	0.3132
	Error A	6	4425.50		
	Panelist	6	10184.33	7.49	0.0001
	P x D	12	1637.15	1.20	0.3179
	P x C	18	2707.47	1.99	0.0388
	Error B	36	1360.10		
Sustained Juiciness	Day	2	2068.75	0.64	0.5609
	Collagen	3	8436.80	2.60	0.1473
	Error A	6	3244.54		

TABLE III (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Sustained Juiciness (cont.)	Panelist	6	10283.23	7.23	0.0001
	P x D	12	1268.06	0.89	0.5631
	P x C	18	2494.21	1.75	0.0744
	Error B	36	1422.09		
Tenderness	Day	2	1191.36	1.03	0.4119
	Collagen	3	2838.39	2.46	0.1605
	Error A	6	1154.46		
	Panelist	6	10632.24	7.47	0.0001
	P x D	12	1155.61	0.81	0.6368
	P x C	18	2241.63	1.57	0.1207
	Error B	36	1423.33		
Amount of Connective Tissue	Day	2	327.08	0.21	0.8196
	Collagen	3	7045.23	4.43	0.0576
	Error A	6	1590.18		
	Panelist	6	11020.63	6.58	0.0001
	P x D	12	4363.19	2.61	0.0131
	P x C	18	3434.13	2.05	0.0328
	Error B	36	1674.90		
Beefy Flavor	Day	2	1747.32	0.82	0.4833
	Collagen	3	40307.44	18.98	0.0018
	Error A	6	2123.51		
	Panelist	6	1367.16	1.48	0.2134
	P x D	12	1258.08	1.36	0.2298
	P x C	18	1577.35	1.71	0.0850
	Error B	36	925.02		
Off-Flavor	Day	2	44.05	0.04	0.9604
	Collagen	3	7076.49	6.53	0.0256
	Error A	6	1083.33		
	Panelist	6	4177.53	4.03	0.0034
	P x D	12	899.60	0.82	0.6289
	P x C	18	1541.99	1.49	0.1515
	Error B	36	1035.65		

TABLE IV

DUNCAN'S MULTIPLE RANGE TESTS FOR VARIABILITY DUE TO COLLAGEN OF SENSORY ANALYSIS*

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Color	60.714			
10	(even to	123.095			
20	streaked)	142.857			
30		145.714			
0	Texture	59.524	67.143	47.857	51.429
10		116.191	100.714	88.571	97.381
20		134.524	112.858	120.238	104.286
30		150.952	148.333	133.095	135.952
0	Aroma	124.29	130.24	128.81	134.52
10		97.38	108.33	105.48	87.14
20		82.08	75.71	77.62	95.71
30		63.33	77.86	76.19	59.76
0	Initial	107.143			
10	Juiciness	98.333			
20		86.190			
30		70.000			
0	Sustained	119.286			
10	Juiciness	100.476			
20		90.238			
30		77.143			

TABLE IV (Continued)

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Beefy	134.76	126.90	150.47	150.00
10	Flavor	101.90	114.29	118.57	108.10
20		58.78	70.24	61.67	79.52
30		36.91	50.00	52.62	40.48
0	Off-Flavor	15.714	27.619	13.571	16.905
10		44.286	18.571	25.476	35.000
20		46.191	40.952	48.333	35.238
30		59.286	48.095	52.857	58.810

*Means not marked by a common solid line are significantly different ($p<0.05$). Common line may not be continuous.

in the fresh loaves. The texture of the control was significantly different from the texture of the samples which contained 20 and 30 percent collagen.

Aroma varied significantly between days (Table V) and due to level of collagen (Table IV). The beefy aroma decreased as the collagen level increased. There was a wide range in the variation. The aroma of the control loaves was significantly different from the aroma of the loaves which contained 10, 20, and 30 percent collagen and the loaves that contained 10 percent collagen were different from loaves which contained 30 percent collagen.

TABLE V

DUNCAN'S MULTIPLE RANGE TESTS FOR VARIABILITY
DUE TO DAYS FOR SENSORY ANALYSIS

Product	Attribute	Day	Means
Fresh Loaf	Aroma	1	121.250
		2	94.464
		3	108.750
Frozen Loaf	Off-Flavor	1	33.393
		2	30.000
		3	38.036
Fresh Patty	Initial Juiciness	1	118.214
		2	102.679
		3	113.571

Beefy flavor varied significantly due to level of collagen. The beefy flavor decreased as the level of collagen increased. The flavor of the control and the loaves that contained 10 percent collagen were in a group that was significantly different from a second group that included the loaves which contained 20 and 30 percent collagen.

Off-flavor varied significantly due to level of collagen. The off-flavor increased as the level of collagen increased. Off-flavor was significantly different between the control and the loaves which contained 10, 20, and 30 percent collagen. All values for off-flavor were in the upper one-fourth of the scale, which is the end representing no off-flavor.

Frozen Loaves

The analysis of variance for frozen loaves is presented in Table VI. Off-flavor was the only characteristic which varied significantly ($p<0.05$) from day to day. Significant differences in texture, aroma, initial juiciness, sustained juiciness, beefy flavor, and off-flavor were obtained due to level of collagen.

The results of the Duncan multiple range tests are shown in Table IV. A significant variation was found for texture, aroma, initial juiciness, sustained juiciness, beefy flavor, and off-flavor.

Texture varied significantly due to level of collagen. The coarse texture increased as the level of collagen increased. The control loaves were significantly different from the 20 and 30 percent collagen loaves. The 10 percent collagen loaves were also significantly different from the 30 percent collagen loaves.

TABLE VI

ANALYSIS OF VARIANCE FOR SENSORY EVALUATION OF FROZEN LOAVES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color (light to dark)	Day	2	702.08	0.45	0.6591
	Collagen	3	2099.60	1.34	0.3474
	Error A	6	3139.09		
	Panelist	6	2892.66	3.75	0.0053
	P x D	12	780.90	1.01	0.4584
	P x C	18	1140.81	1.48	0.1556
	Error B	36	771.51		
Color (even to streaked)	Day	2	2937.79	2.25	0.1865
	Collagen	3	2998.71	2.30	0.1775
	Error A	6	1305.26		
	Panelist	6	2425.47	12.62	0.0001
	P x D	12	682.24	0.35	0.9710
	P x C	18	1710.98	0.89	0.5929
	Error B	36	1922.39		
Texture	Day	2	800.29	0.28	0.7686
	Collagen	3	23594.05	8.11	0.0156
	Error A	6	2909.82		
	Panelist	6	11018.65	6.25	0.0001
	P x D	12	930.85	0.53	0.8823
	P x C	18	2013.49	1.14	0.3561
	Error B	36	1763.99		
Aroma	Day	2	793.75	0.37	0.7051
	Collagen	3	14339.20	6.69	0.0242
	Error A	6	2142.16		
	Panelist	6	8630.36	13.97	0.0001
	P x D	12	915.62	1.48	0.1763
	P x C	18	2457.47	3.98	0.0002
	Error B	36	617.74		
Initial Juiciness	Day	2	2214.58	2.24	0.1877
	Collagen	3	5439.98	5.50	0.0371
	Error A	6	988.79		
	Panelist	6	17218.05	9.69	0.0001
	P x D	12	1090.97	0.61	0.8163
	P x C	18	1326.78	0.75	0.7427
	Error B	36	1777.68		
Sustained Juiciness	Day	2	2646.43	2.23	0.1892
	Collagen	3	6640.08	5.59	0.0359
	Error A	6	1188.89		

TABLE VI (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Sustained Juiciness (cont.)	Panelist	6	16726.88	11.03	0.0001
	P x D	12	1001.29	0.66	0.7766
	P x C	18	1508.36	0.99	0.4873
	Error B	36	1516.90		
Tenderness	Day	2	161.01	0.19	0.8318
	Collagen	3	1307.44	1.54	0.2978
	Error A	6	8479.17		
	Panelist	6	10322.82	10.91	0.0001
	P x D	12	376.98	0.40	0.9550
	P x C	18	5354.43	5.66	0.0001
	Error B	36	946.30		
Amount of Connective Tissue	Day	2	1123.50	1.76	0.2506
	Collagen	3	1742.86	2.73	0.1366
	Error A	6	638.99		
	Panelist	6	17901.29	21.32	0.0001
	P x D	12	894.00	1.06	0.4163
	P x C	18	5059.76	6.03	0.0001
	Error B	36	839.57		
Beefy Flavor	Day	2	2052.67	1.61	0.2758
	Collagen	3	27592.46	21.62	0.0013
	Error A	6	1276.09		
	Panelist	6	10654.46	13.68	0.0001
	P x D	12	456.85	0.59	0.8384
	P x C	18	2484.43	3.19	0.0015
	Error B	36	778.87		
Off-Flavor	Day	2	455.66	7.17	0.0257
	Collagen	3	3679.37	57.86	0.0001
	Error A	6	63.59		
	Panelist	6	19372.52	103.42	0.0001
	P x D	12	195.59	1.04	0.4325
	P x C	18	1851.36	9.88	0.0001

Aroma varied significantly due to level of collagen. The aroma decreased as the level of collagen increased. Aroma varied significantly between the control and the loaves containing 20 and 30 percent collagen.

Initial juiciness varied significantly due to the level of collagen in the frozen loaves. The juiciness increased as the level of collagen increased. For this attribute, the control and the loaves containing 10 percent collagen varied significantly from the 30 percent collagen loaves.

Sustained juiciness also varied significantly due to the level of collagen. Like initial juiciness, sustained juiciness increased as the collagen level increased. The sustained juiciness of the loaves was significantly different between the control loaves and those containing 20 and 30 percent collagen.

Beefy flavor varied significantly in the frozen loaves. The flavor decreased as the level of collagen in the loaves increased. There were two distinctive groups for the beefy flavor--the control and the 10 percent collagen loaves in one group, and the 20 and 30 percent collagen loaves in the other group.

Off-flavor varied significantly due to day (Table VI) and level of collagen (Table IV). The off-flavor increased as the level of collagen in the loaves was significantly different from each other but the order from least to most off-flavor was 10 percent collagen loaves, control loaves, 20 percent collagen loaves, and 30 percent collagen loaves.

Fresh Patties

Analysis of variance for fresh patties is shown in Table VII.

TABLE VII

ANALYSIS OF VARIANCE FOR SENSORY EVALUATION OF FRESH PATTIES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color (light to dark)	Day	2	91.96	0.03	0.9708
	Collagen	3	8941.57	2.90	0.1239
	Error A	6	3086.80		
	Panelist	6	7201.69	4.76	0.0012
	P x D	12	2267.30	1.50	0.1699
	P x C	18	1614.25	1.07	0.4192
	Error B	36	1512.62		
Color (even to streaked)	Day	2	2472.62	0.77	0.5039
	Collagen	3	10165.37	3.17	0.1068
	Error A	6	3211.51		
	Panelist	6	7827.08	4.46	0.0018
	P x D	12	862.20	0.49	0.9062
	P x C	18	2258.43	1.29	0.2524
	Error B	36	1753.87		
Texture	Day	2	919.05	1.02	0.4158
	Collagen	3	30297.12	33.60	0.0004
	Error A	6	901.58		
	Panelist	6	8107.24	6.18	0.0002
	P x D	12	1064.53	0.81	0.6370
	P x C	18	3455.92	2.64	0.0065
	Error B	36	1311.42		
Aroma	Day	2	901.19	0.44	0.6658
	Collagen	3	13246.43	6.40	0.0267
	Error A	6	2069.05		
	Panelist	6	6068.41	4.92	0.0009
	P x D	12	637.30	0.51	0.8910
	P x C	18	2673.98	2.06	0.0242
	Error B	36	1237.57		
Initial Juiciness	Day	2	1780.66	1.79	0.2451
	Collagen	3	363.79	0.81	0.5328
	Error A	6	448.91		
	Panelist	6	9953.37	9.25	0.0001
	P x D	12	1343.85	1.25	0.2903
	P x C	18	6444.11	5.99	0.0001
	Error B	36	1076.45		
Sustained Juiciness	Day	2	1675.00	1.79	0.2451
	Collagen	3	80.46	0.09	0.9651
	Error A	6	933.73		

TABLE VII (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Sustained Juiciness (cont.)	Panelist	6	14827.78	13.96	0.0001
	P x D	12	625.69	0.59	0.8364
	P x C	18	4754.76	4.48	010001
	Error B	36	1062.20		
Tenderness	Day	2	1302.69	0.51	0.6263
	Collagen	3	1821.73		
	Error A	6	2572.92		
	Panelist	6	3331.85	2.93	0.0196
	P x D	12	1034.97	0.91	0.5458
	P x C	18	2380.52	2.10	0.0290
	Error B	36	1136.23		
Amount of Connective Tissue	Day	2	863.39	0.33	0.7331
	Collagen	3	5724.90	2.17	0.1927
	Error a	6	2639.18		
	Panelist	6	11551.88	6.07	0.0002
	P x D	12	3368.25	1.77	0.0920
	P x C	18	2235.08	1.17	0.3302
	Error B	36	1902.84		
Beefy Flavor	Day	2	108.33	0.18	0.8432
	Collagen	3	45763.89		0.0001
	Error A	6	617.46		
	Panelist	6	2134.72	3.98	0.0037
	P x D	12	384.72	0.72	0.7251
	P x C	18	2677.32	4.99	0.0001
	Error B	36	536.44		
Off-Flavor	Day	2	2058.33	2.35	0.1759
	Collagen	3	7325.69	8.38	0.0145
	Error A	6	874.20		
	Panelist	6	9269.74	15.43	0.0001
	P x D	12	1152.78	1.92	0.0651
	P x C	18	1667.36	2.78	0.0045
	Error B	36	600.59		

Texture, aroma, beefy flavor, and off-flavor varied significantly among the collagen levels. The Duncan Multiple Range Results are presented in Table IV.

Texture varied significantly among the levels of collagen. The coarseness of the texture increased as the level of collagen in the fresh patties increased. There were three significantly different groups. The control patties were the first group, the patties containing 10 percent collagen were the second group, and the 20 and 30 percent collagen patties were the third group.

Aroma showed significant variation due to level of collagen. The intensity of the aroma decreased as the level of collagen in the patties increased. The aroma of the control patties was significantly different ($p<0.05$) from the aroma of the 20 and 30 percent collagen patties.

Initial juiciness varied significantly from day to day (Table V). There was no significant difference due to level of collagen in the patties.

Beefy flavor varied significantly due to the level of collagen in the patties. As the amount of collagen increased, the beefy flavor decreased. Beefy flavor had the same three groups as texture: the control, the 10 percent collagen patties, and the 20 and 30 percent collagen patties.

Off-flavor varied significantly due to the level of collagen. The off-flavor increased as the level of collagen increased but the values remained in the upper one-fourth of the scale, which is the end representing no off-flavor. The off-flavor was divided into two significantly different groups--the first was the control and 10 percent

collagen patties and the second was the 20 and 30 percent collagen patties.

Frozen Patties

The analysis of variance for frozen patties is presented in Table VIII. A significant variation ($p<0.05$) due to level of collagen was found in texture, aroma, beefy flavor, and off-flavor. The Duncan Multiple Range Tests (Table IV) showed a significant difference for collagen level in texture, aroma, beefy flavor, and off-flavor.

Texture showed a significant difference due to level of collagen. The texture became coarser as the level of collagen increased. There were three significantly different groups--the control, the 10 and 20 percent collagen patties, and the 30 percent collagen patties.

Aroma varied significantly due to the level of collagen in the frozen patties. The strength of the aroma decreased as the level of collagen increased. There were three groups based on aroma--the control patties were one group, the 10 and 20 percent collagen patties were a group, and the 30 percent collagen patties were a group.

Beefy flavor also varied due to the level of collagen. The flavor decreased as the level of collagen increased. Each level of collagen was significantly different from every other level of collagen.

Off-flavor varied significantly with the level of collagen. The off-flavor increased as the level of collagen increased but the value was never greater than one-fourth of the scale, which is the end representing no off-flavor. The control frozen patties were significantly different from the 30 percent collagen frozen patties.

TABLE VIII

ANALYSIS OF VARIANCE FOR SENSORY EVALUATION OF FROZEN PATTIES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color (light to dark)	Day	2	2472.62	2.53	0.1598
	Collagen	3	3543.95	3.62	0.0842
	Error A	6	978.17		
	Panelist	6	8920.44	5.12	0.0007
	P x D	12	905.25	0.52	0.8879
	P x C	18	1770.57	1.02	0.4662
	Error B	36	1742.29		
Color (even to streaked)	Day	2	158.33	0.10	0.9034
	Collagen	3	4765.48	3.11	0.1101
	Error A	6	1532.14		
	Panelist	6	5555.55	2.38	0.0485
	P x D	12	896.18	0.38	0.9606
	P x C	18	2219.18	0.95	0.5296
	Error B	36	2332.03		
Texture	Day	2	111.01	0.08	0.0001
	Collagen	3	25528.97	17.31	0.0023
	Error A	6	1474.50		
	Panelist	6	10658.93	7.85	0.0001
	P x D	12	2390.55	1.77	0.0025
	P x C	18	1919.25	1.41	0.1840
Aroma	Day	2	436.61	0.51	0.6245
	Collagen	3	19573.41	22.85	0.0011
	Error A	6	856.45		
	Panelist	6	12692.86	9.10	0.0001
	P x D	12	1013.69	0.73	0.7164
	P x C	18	1897.49	1.36	0.2109
Initial Juiciness	Day	2	104.46	0.15	0.8677
	Collagen	3	2318.25	3.22	0.1035
	Error A	6	719.15		
	Panelist	6	22023.91	29.71	0.0001
	P x D	12	929.13	1.88	0.0711
	P x C	18	28219.97	3.85	0.0003
	Error B	36	740.79		
Sustained Juiciness	Day	2	218.16	0.36	0.7089
	Collagen	3	2466.17	4.12	0.0662
	Error A	6	598.31		

TABLE VIII (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Sustained Juiciness (cont.)	Panelist	6	21411.61	28.49	0.0001
	P x D	12	1901.49	2.53	0.0156
	P x C	18	2741.63	3.65	0.0005
	Error B	36	751.55		
Tenderness	Day	2	314.58	0.37	0.7084
	Collagen	3	1895.24	2.20	0.1888
	Error A	6	861.01		
	Panelist	6	4013.79	4.80	0.0011
	P x D	12	825.69	0.99	0.4793
	P x C	18	2900.56	3.47	0.0007
	Error B	36	836.47		
Amount of Connective Tissue	Day	2	1771.72	2.18	0.1942
	Collagen	3	2061.41	2.54	0.1530
	Error A	6	812.60		
	Panelist	6	10951.89	5.52	0.0004
	P x D	12	1413.05	0.71	0.7298
	P x C	18	3353.09	1.69	0.0887
	Error B	36	1984.47		
Beefy Flavor	Day	2	98.51	0.07	0.9318
	Collagen	4	44855.56	32.55	0.0004
	Error A	6	1377.88		
	Panelist	6	2962.79	2.56	0.0360
	P x D	12	717.26	0.62	0.8106
	P x C	18	1528.01	1.32	0.2320
	Error B	36	1155.89		
Off-Flavor	Day	2	452.08	0.44	0.6651
	Collagen	3	6198.71	5.99	0.0309
	Error A	6	1035.02		
	Panelist	6	17468.65	22.06	0.0001
	P x D	12	326.39	0.41	0.9490
	P x C	18	1622.09	2.05	0.0330
	Error B	36	791.73		

Texture, aroma, beefy flavor, and off-flavor varied significantly ($p<0.05$) in all four products due to level of collagen. The direction (increase or decrease) was the same for all four products (Figures 2, 3, 4, and 5). The decrease in aroma and beefy flavor was due to the decrease in lean meat present. Off-flavor was perceived by the panelist as a more diminished beef flavor than as a distinguishable "off" flavor. In terms of texture (coarseness), meat particles appeared larger and more loosely bound. This could be attributed to binding capacity which is a functional property of collagen.

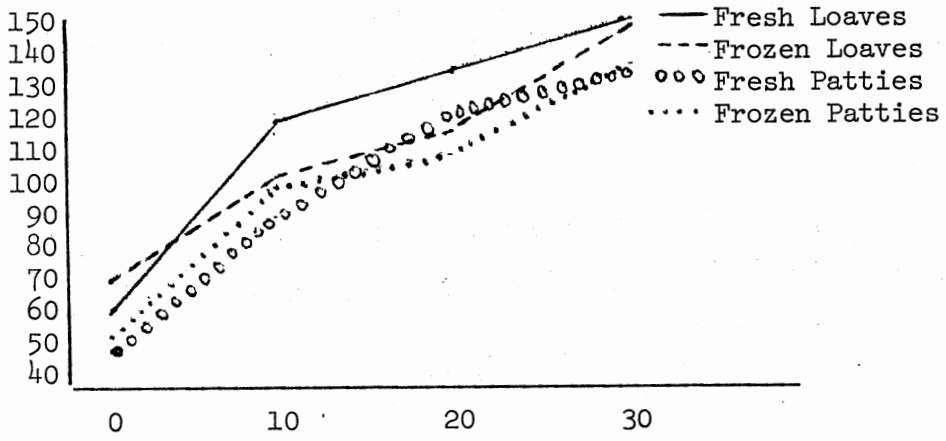


Figure 2. Mean Scores for Texture of Four Products at Four Levels of Collagen

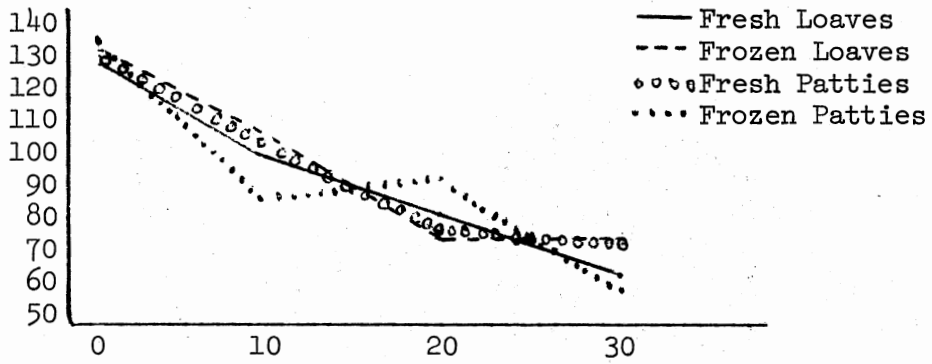


Figure 3. Mean Scores for Aroma of Four Products at Four Levels of Collagen

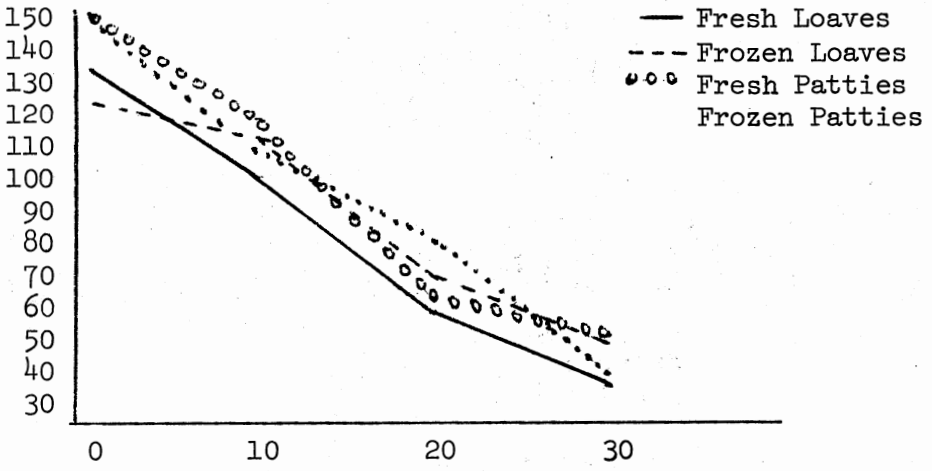


Figure 4. Mean Scores for Beefy Flavor of Four Products at Four Levels of Collagen

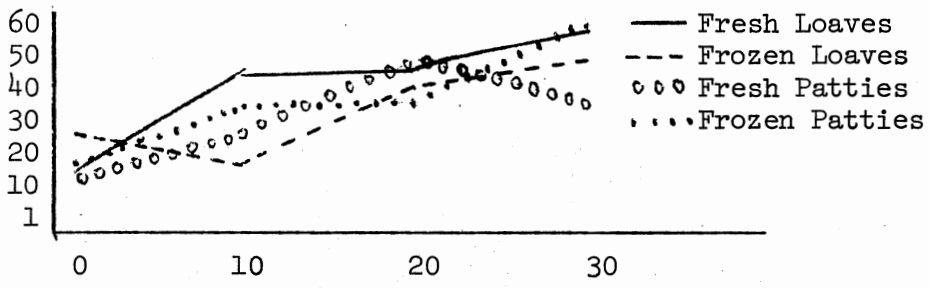


Figure 5. Mean Scores for Off-Flavor of Four Products at Four Levels of Collegen

When comparing fresh to frozen without regard to level of collagen, the amount of connective tissue varied significantly in the loaves ($p<0.05$) (Table IX). The frozen samples had less connective tissue. This is due to the increase in cooking time (rethermalizing). There was no sensory attribute significantly different between fresh and frozen patties.

TABLE IX

DUNCAN'S MULTIPLE RANGE TESTS FOR SUBJECTIVE ANALYSIS OF FRESH AND FROZEN PRODUCTS

Cooking Method	Attribute	Product	Mean
Loaf	Amount of Connective Tissue	Fresh	82.619
		Frozen	64.762

Objective Evaluation

Objective evaluation included losses (moisture, vapor, drip, fat), content percentages (moisture, fat, ash), tenderness measurement, and color measurement. The losses were determined in grams and calculated as a percent of original weight of meat. The content percentages were based on the weight of the cooked meat. Tenderness was determined by use of a Kramer shear cell on an Instron Universal Instrument. The color was determined by use of a Hunter colorimeter.

Fresh Loaves

Analysis of variance for objective evaluation of the fresh loaves is shown in Table X. Vapor loss, drip loss, moisture loss, total cooking loss, moisture content, ash content, tenderness by shear force (kg/g), and area of peak (cm^2/g) by Instron varied significantly ($p<0.05$) over the levels of collagen.

Results of the Duncan Multiple Range Tests for fresh loaves are shown in Table XI. Vapor loss, drip loss, moisture loss, total cooking loss, moisture content, ash content, shear force (kg/g), and area of peak (cm^2/g) by Instron varied.

Vapor loss varied significantly due to the level of collagen. The vapor loss decreased as the level of collagen increased. There were two significantly different ($p<0.05$) groups--the control and 10 percent collagen loaves were in one group and the 20 and 30 percent collagen loaves were in the second group.

Drip loss varied significantly ($p<0.05$) due to the level of collagen. The loss decreased as the level of collagen increased. There

TABLE X

ANALYSIS OF VARIANCE FOR OBJECTIVE EVALUATION OF FRESH LOAVES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color L	Day	2	17.91	1.15	0.0095
	Collagen	3	1.66	1.03	0.4424
	Error A	6	1.61		
Color A	Day	2	.1734	0.25	0.7868
	Collagen	3	.393	0.57	0.6573
	Error A	6	.695		
Color B	Day	2	.334	12.57	0.0072
	Collagen	3	.114	4.31	0.0608
	Error A	6	.026		
Vapor Loss	Day	2	.459	1.52	0.2927
	Collagen	3	4.28	14.17	0.0039
	Error A	6	.302		
Drip Loss	Day	2	.024	0.01	0.9861
	Collagen	3	30.37	18.10	0.0021
	Error A	6	1.678		
Fat Loss	Day	2	.5526	1.33	0.3332
	Collagen	3	.067	0.16	0.9192
	Error A	6	.416		
Moisture Loss	Day	2	.395	0.41	0.6829
	Collagen	3	27.889	28.67	0.0006
	Error A	6	.97		
Total Cooking Loss	Day	2	3.45	1.68	0.2632
	Collagen	3	47.57	23.18	0.0011
	Error A	6	2.05		
Fat Content	Day	2	1.454	0.41	0.6725
	Collagen	3	5.98	1.69	0.2225
	D x C	6	7.716	2.18	0.1185
	Error A	12	3.54		
Moisture Content	Day	2	51.58	14.41	0.0006
	Collagen	3	19.26	5.38	0.0140
	D x C	6	31.90	8.91	0.0008
	Error A	12	3.58		

TABLE X (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Ash Content	Day	2	.0395	0.83	0.4589
	Collagen	3	.484	10.17	0.0013
	D x C	6	.123	2.60	0.0752
	Error A	12	.048		
Peak Force	Day	2	.0012	2.45	0.1286
	Collagen	3	.0063	12.95	0.0005
	D x C	6	.0011	2.32	0.1014
	Error A	12	.0004		
Area	Day	2	.0005	0.35	0.7134
	Collagen	3	.0216	13.89	0.0005
	D x C	6	.002	1.56	0.2464
	Error A	11	.001		

were three significantly different groups--the control, the 20 percent collagen loaves, and the 30 percent collagen loaves.

Moisture loss, like vapor and drip loss, was significantly different due to level of collagen. The moisture loss decreased as the level of collagen increased. Every level of collagen was significantly different from every other level of collagen for moisture loss.

Total cooking loss, which includes vapor and drip (moisture and fat), was significantly different due to collagen level. The cooking loss decreased as the level of collagen increased. The cooking loss had the same pattern as the moisture loss. Every level of collagen was significantly different from every other level.

Moisture content was also significantly different due to level of collagen. The moisture content decreased as the level of collagen

TABLE XI

DUNCAN'S MULTIPLE RANGE TESTS FOR VARIABILITY DUE TO COLLAGEN OF OBJECTIVE EVALUATION

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Vapor Loss	7.726	12.00	30.14	
10		7.083	11.08	28.47	
20		5.883	10.51	22.39	
30		5.06	8.32	21.69	
0	Drip Loss	17.91	18.58	4.97	4.92
10		15.51	16.73	5.25	2.52
20		12.98	13.68	3.94	2.46
30		10.54	9.35	2.90	1.01
0	Fat Loss			4.97	4.38
10				5.26	2.52
20				3.94	2.46
30				2.90	1.01
0	Moisture Loss	12.52	11.45		
10		10.29	8.94		
20		7.93	7.15		
30		5.44	3.95		
0	Total Cook-	25.63	30.57	35.12	26.29
10	ing Loss	22.59	27.31	33.72	22.91
20		19.61	24.20	26.34	20.35
30		16.34	17.68	24.59	18.58

TABLE XI (Continued)

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Fat Content		19.10	21.62	23.24
10			16.24	19.48	19.81
20			15.44	18.61	17.79
30			14.97	19.57	23.82
0	Moisture Content	44.30			
10		42.89			
20		42.41			
30		40.04			
0	Ash Content	.494	.643	1.01	.85
10		.930	1.25	.93	1.31
20		1.045	1.19	1.31	1.24
30		1.135	1.12	1.12	1.12
0	Peak Force	.23	.25	.31	.25
10		.18	.22	.31	.24
20		.18	.22	.28	.21
30		.14	.20	.24	.23
0	Area	.476		.63	.49
10		.374		.59	.47
20		.379		.56	.42
30		.329		.48	.44

increased. There were two groups--the control and 10 percent collagen were one group and the 30 percent collagen loaves were the other group.

Tenderness by shear force (kg/g) and area of peak (cm^2/g) by Instron measured the same quality. Although the taste panel found no significant difference in tenderness, both shear force (kg/g) and area were significantly different. Both increased (decreased in the shear force value) as the level of collagen increased. The control loaves were significantly different in tenderness from those containing collagen.

Ash content varied significantly due to level of collagen. The ash content increased as the level of collagen increased. There were two significantly different groups--the control and those containing collagen.

Frozen Loaves

Analysis of variance for the objective evaluation of frozen loaves is presented in Table XII. Vapor loss, drip loss, moisture loss, total cooking loss, fat content, tenderness by shear force (kg/g), and ash content varied significantly ($p<0.05$) due to level of collagen. Vapor loss, drip loss, moisture loss, total cooking loss, fat content, tenderness by shear force (kg/g), and ash content varied in the Duncan Analysis of Variance (Table XI).

Vapor loss was significantly different due to level of collagen. The loss decreased as the level of collagen increased. The control, 10 and 20 percent collagen loaves, were in one significantly different group and the 30 percent collagen loaves were in a second significantly different group.

TABLE XII

ANALYSIS OF VARIANCE FOR OBJECTIVE EVALUATION OF FROZEN LOAVES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color L	Day	2	1.547	1.00	0.4220
	Collagen	3	3.294	2.13	0.1979
	Error A	6	1.547		
Color A	Day	2	.530	4.43	0.0659
	Collagen	3	.204	1.70	0.2659
	Error A	6	.120		
Color B	Day	2	.266	2.85	0.1350
	Collagen	3	.124	1.33	0.3492
	Error A	6	.093		
Vapor Loss	Day	2	.1605	0.25	0.7894
	Collagen	3	7.3270	11.23	0.0071
	Error A	6	.652		
Drip Loss	Day	2	3.7044	9.11	0.0152
	Collagen	3	46.75	114.98	0.0001
	Error A	6	.416		
Fat Loss	Day	2	4.02	3.31	0.1073
	Collagen	3	2.18	1.79	0.2484
	Error A	6	1.21		
Moisture Loss	Day	2	.118	0.08	0.9272
	Collagen	3	29.88	19.43	0.0017
	Error A	6	1.54		
Total Cooking Loss	Day	2	3.56	7.04	0.0267
	Collagen	3	90.61	249.34	0.0001
	Error A	6	.363		
Fat Content	Day	2	10.50	4.54	0.0339
	Collagen	3	20.91	9.05	0.0021
	D x C	6	7.09	3.07	0.0466
	Error A	12	2.31		
Moisture Content	Day	2	66.82	6.17	0.0159
	Collagen	3	18.35	1.70	0.2252
	D x C	6	41.32	3.82	0.0264
	Error A	11	10.82		

TABLE XII (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Ash Content	Day	2	.263	3.90	0.0524
	Collagen	3	.440	6.53	0.0085
	D x C	6	.204	3.03	0.0532
	Error A	11	0.67		
Peak Force	Day	2	.0064	10.97	0.0020
	Collagen	3	.0021	3.57	0.0470
	D x C	6	.0008	1.38	0.2994
	Error A	12	.0006		
Area	Day	2	.027	11.00	0.0019
	Collagen	3	.006	2.37	0.1219
	D x C	6	.004	1.58	0.2357
	Error A	12	.002		

Drip loss was also significantly different due to level of collagen. The drip loss decreased as the level of collagen increased. Each level was significantly different from every other level of collagen.

Moisture loss was significantly different due to level of collagen. The loss decreased as the level of collagen increased. There were three significantly different groups--the control, the 10 and 20 percent collagen loaves, and the 30 percent collagen loaves.

Total cooking which includes vapor and drip (fat and moisture) loss was significantly different due to level of collagen. Like all of the losses, total cooking loss decreased as the level of collagen increased. Each level of collagen was significantly different ($p<0.05$) from every other level of collagen.

Fat content varied significantly due to level of collagen. The content decreased as the level of collagen increased. The loaves without collagen were significantly different from the loaves with collagen.

Tenderness by shear force (kg/g) was, again, significantly different due to level of collagen. The area of peak, which is also a measure of tenderness, was not significantly different, but the values decreased as the level of collagen increased, as it did for shear force. The tenderness increased (decrease in shear force value) as the level of collagen increased. The control loaves were significantly different from the 30 percent collagen loaves.

The ash content was significantly different due to level of collagen. The content increased as the level of collagen increased. The control was significantly different from the 10, 20, and 30 percent collagen loaves.

Vapor loss, drip loss, moisture loss, and total cooking loss had similar patterns on fresh and frozen loaves. The addition of collagen decreased the losses, indicating its binding capacity. The products with collagen had less shrinkage. The tenderness attribute increased as the level of collagen increased in both fresh and frozen loaves. This property is due to the larger particle size in the collagen-meat mixtures and because there were less cooking losses in the collagen-containing loaves. The ash content increased as the collagen level increased in both fresh and frozen loaves. The differences in the ash content is explained in the mineral analysis section. The moisture content was only slightly significant ($p<0.05$) in the fresh loaves. The fat content was only significant ($p<0.05$) in the frozen loaves.

When fresh and frozen loaves were compared, without regard to level of collagen, vapor, moisture, and total cooking losses varied significantly ($p<0.05$) (Table XIII). All three were highest in the frozen and lowest in the fresh. This is due to the fact that the frozen meat was rethermalized or cooked twice and hence the additional loss.

TABLE XIII

DUNCAN'S MULTIPLE RANGE TESTS FOR OBJECTIVE ANALYSIS OF FRESH AND FROZEN PRODUCTS

Cooking Method	Attribute	Product	Mean
Loaf	Vapor Loss	Fresh	6.438
		Frozen	10.480
	Moisture Loss	Fresh	9.046
		Frozen	7.874
	Total Cooking Loss	Fresh	21.043
		Frozen	24.941
Patty	Fat Loss	Fresh	4.269
		Frozen	2.591
	Total Cooking Loss	Fresh	29.944
		Frozen	22.034

Fresh Patties

Table XIV presents the analysis of variance for the objective evaluation of the fresh patties. Vapor loss, drip loss, fat loss,

TABLE XIV

ANALYSIS OF VARIANCE FOR OBJECTIVE EVALUATION OF FRESH PATTIES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color L	Day	2	1.915	0.64	0.5649
	Collagen	3	4.09	1.34	0.3461
	Error A	6	3.04		
Color A	Day	2	.519	1.04	0.4094
	Collagen	3	.097	0.19	0.8967
	Error A	6	.499		
Color B	Day	2	.072	0.64	0.5596
	Collagen	3	.233	2.09	0.2030
	Error A	6	.112		
Vapor Loss	Day	2	15.975	1.39	0.3184
	Collagen	3	54.41	4.75	0.0502
	Error A	6	11.46		
Drip Loss	Day	2	.205	0.47	0.6449
	Collagen	3	3.436	7.90	0.0166
	Error A	6	.435		
Fat Loss	Day	2	.205	0.47	0.6449
	Collagen	3	3.436	7.90	0.0166
	Error A	6	.435		
Moisture Loss	Day	2	0.00		
	Collagen	3	0.00		
	Error A	6	0.00		
Total Cooking Loss	Day	2	19.12	1.64	0.2706
	Collagen	3	82.63	7.08	0.0214
	Error A	6	11.67		
Fat Content	Day	2	9.76	5.10	0.0249
	Collagen	3	9.75	5.10	0.0167
	D x C	6	7.42	3.88	0.0218
	Error A	12	1.91		
Moisture Content	Day	2	.558	0.68	0.5270
	Collagen	3	28.27	3.44	0.0556
	D x C	6	6.53	0.79	0.5934
	Error A	11	8.22		

TABLE XIV (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Ash Content	Day	2	.128	6.14	0.0182
	Collagen	3	.184	8.81	0.0037
	D x C	6	.102	4.88	0.0140
	Error	10	.021		
Peak Force	Day	2	.0038	4.68	0.0314
	Collagen	3	.0059	7.33	0.0047
	D x C	6	.0020	2.50	0.0834
	Error A	12	.0008		
Area	Day	2	.023	13.38	0.0011
	Collagen	3	.023	13.18	0.0006
	D x C	6	.003	.51	0.2616
	Error A	11	.002		

total cooking loss, fat content, ash content, and tenderness by shear force (kg/g) were significantly different ($p<0.05$) for the level of collagen. The Duncan Multiple Range Test results are in Table XI. Vapor loss, drip loss, fat loss, total cooking loss, fat content, ash content, and tenderness by shear force (kg/g) varied significantly ($p<0.05$) in the fresh patties.

Vapor loss was significantly different in the fresh patties due to level of collagen. The loss decreased as the level of collagen increased. The vapor loss of the control was significantly different ($p<0.05$) from the 20 and 30 percent collagen patties.

Drip loss was also significantly different due to level of collagen. Again, the loss decreased as the level of collagen increased.

The control and 10 percent collagen patties were significantly different from the 30 percent collagen patties for drip loss.

The fat loss was significantly different due to level of collagen. Like the others, fat loss decreased as the level of collagen increased. The control and 10 percent collagen patties were significantly different from the 30 percent collagen patties.

The total cooking loss which included vapor and drip (fat and moisture) losses was significantly different due to level of collagen. The total loss decreased as the level of collagen increased. The control and 10 percent collagen patties were significantly different from the 20 and 30 percent collagen patties.

The fat content varied significantly due to level of collagen. The fat content decreased as the level of collagen increased. The control patties were significantly different from the patties with collagen.

The tenderness by shear force (kg/g) and area of peak by Instron were both significantly different due to level of collagen. The tenderness attribute increased (the values of shear force decreased) as the level of collagen increased. The shear force (kg/g) showed that the control and 10 percent collagen patties were significantly different from the 30 percent collagen patties.

The ash content was significantly different due to level of collagen. As the level of collagen increased, the ash content increased. The control, and 10 percent collagen patties were significantly different ($p<0.05$) from the 20 and 30 percent collagen patties (Table XI).

Frozen Patties

The analysis of variance of the objective evaluations for the frozen patties are in Table XV. Drip loss, fat loss, total cooking loss, fat content, ash content, and tenderness by shear force (kg/g) and area of peak (cm^2/g) were significantly different ($p<0.05$) due to level of collagen. The Duncan Multiple Range Test results are presented in Table XI. Drip loss, fat loss, total cooking loss, fat content, ash content, and tenderness by shear force (kg/g) and area of peak (cm^2/g) varied.

Drip loss was significantly different due to level of collagen. The drip loss decreased as the level of collagen increased. The loss from the control was significantly different from the 10, 20, and 30 percent collagen patties and the 10 and 20 percent collagen patties were significantly different from the 30 percent collagen patties.

Fat loss was significantly different due to level of collagen. The loss decreased as the level of collagen increased. The fat loss was significantly different between the control and the samples containing collagen.

Total cooking loss varied significantly due to level of collagen. The total loss decreased as the level of collagen increased. The control was significantly different from the 20 and 30 percent collagen patties.

Fat content varied significantly due to level of collagen. The content decreased as the level of collagen increased except for the 30 percent collagen patty. The high fat content in the 30 percent collagen samples may have been due to uneven distribution in the

TABLE XV

ANALYSIS OF VARIANCE FOR OBJECTIVE EVALUATION OF FROZEN PATTIES

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Color L	Day	2	.894	0.19	0.8323
	Collagen	3	.774	0.16	0.9167
	Error A	6	4.72		
Color A	Day	2	.293	0.83	0.4795
	Collagen	3	.465	1.40	0.3308
	Error A	6	.323		
Color B	Day	2	.972	5.57	0.0429
	Collagen	3	.086	0.49	0.7007
	Error A	6	.175		
Vapor Loss	Day	2	12.91	3.92	0.0814
	Collagen	3	8.07	2.45	0.1614
	Error A	6	3.29		
Drip Loss	Day	2	3.24	7.55	0.0230
	Collagen	3	7.88	18.35	0.0020
	Error A	6	.43		
Fat Loss	Day	2	1.86	3.39	0.1036
	Collagen	3	5.72	10.41	0.0086
	Error A	6	.55		
Moisture Loss	Day	2	.224	1.00	0.4219
	Collagen	3	.224	1.00	0.4547
	Error A	6	.224		
Total Cooking Loss	Day	2	6.47	1.22	0.3587
	Collagen	3	33.70	6.37	0.0270
	Error A	6	5.29		
Fat Content	Day	2	20.11	7.53	0.0076
	Collagen	3	49.14	18.41	0.0001
	D x C	6	28.20	10.57	0.0003
	Error A	12	2.67		
Moisture Content	Day	2	14.39	1.81	0.2051
	Collagen	3	20.02	2.52	0.1071
	D x C	6	27.84	3.51	0.0306
	Error A	12	7.94		

TABLE XV (Continued)

Attribute	Source	df	Mean Square	F Value	Observed Sig. Difference
Ash Content	Day	2	.088	2.15	0.1589
	Collagen	3	.239	5.82	0.0108
	D x C	6	.033	0.81	0.5812
	Error A	12	.041		
Peak Force	Day	2	.0015	4.45	0.0358
	Collagen	3	.0018	5.35	0.0143
	D x C	6	.0016	4.68	0.0111
	Error A	12	.0008		
Area	Day	2	.007	26.70	0.0003
	Collagen	3	.006	14.57	0.0003
	D x C	6	.004	8.78	0.0008
	Error A	12	.0004		

portions of the patty used for the sample. The control and 30 percent collagen patties were in one group and the 10 and 20 percent collagen patties (cm^2/g) were in another significantly different group.

Tenderness by shear force (kg/g) and area of peak (cm^2/g) were significantly different due to level of collagen. The tenderness attribute increased (the value for shear force decreased) as the level of collagen increased. The control, 10 and 30 percent collagen patties, were in one group and the 20 percent collagen patties were in another significantly different group. The area of the peak had two significantly different groups--the control and 10 percent collagen patties were one group and the 20 and 30 percent collagen patties were the second group. These differences in results are due to the way the two tests evaluate tenderness. The shear force is the peak force that is

required to go through the meat. The area takes into account the force over the entire time force is being applied, hence the area results are more reliable.

The ash content varied significantly due to level of collagen. The ash content increased as the level of collagen increased. The ash content has two significantly different groups. The control is one group and the collagen containing patties are the other group.

The only test which did not vary significantly in both the fresh and frozen patties was vapor loss. It was only significantly different ($p<0.05$) in the fresh patties. This is perhaps due to the cooking procedure, where fresh patties were cooked on the grill while the frozen patties cooked on the grill and were rethermalized in the oven.

When the fresh patties and the frozen patties were compared without regard for the level of collagen (Table XIII), only fat loss and total cooking loss were significantly different ($p<0.05$). The fat loss was lower in the frozen than in the fresh. This was because the drippings were poured on the patties when they were frozen so the fat had time to be reabsorbed. The total cooking loss which includes vapor and drip (fat and moisture) was also lower in the frozen than in the fresh. There was a slight difference in the drip and vapor loss (less in the frozen), so by combining vapor, moisture, and fat losses, the total cooking loss was significantly different.

Drip loss, total cooking loss, ash content, and tenderness by shear force (kg/g) varied in all four products. The drip and total cooking losses decreased as the level of collagen increased, which agrees with findings reported in the review of literature (Happich,

1975). The tenderness by shear force (kg/g) and ash content increased as the level of collagen increased.

Moisture loss was significant ($p<0.05$) only in the loaves. The loaves cooked at 350°F while the patties cooked at a broiling temperature (500°F+) and had more moisture loss. The high loss in the patties caused the significance to disappear. Fat loss was significant only in the patties. This was because of the high temperature the patties were cooked at. The temperature caused shrinkage of meat particles and the fat could drip out. Fat content was significantly different ($p<0.05$) in the frozen loaves, fresh patties, and frozen patties. The fat content decreased as the level of collagen increased. This trend appears contradictory to fat loss, but it is not. The fat loss was based on the weight of the raw meat but the fat content was based on the weight of cooked meat. The control shrunk at a faster rate than the samples containing collagen, so the same weight of fat is distributed through a larger volume of loaf or patty at the 30 percent collagen level and would be evaluated as having a lower percent of fat content. Vapor loss was significantly different ($p<0.05$) in fresh loaves, frozen loaves, and fresh patties. It was not significant in the frozen patties since they were rethermalized in the oven in their own juices.

When fresh and frozen products were compared, without regard for level of collagen, vapor, moisture, and total cooking losses were significant in the loaves (Table XIV). The vapor and total cooking loss were highest in the frozen loaves. The rethermalizing caused the greater loss. The moisture loss was highest in the fresh loaves. The frozen loaves could reabsorb moisture from the juice during

freezing and rethermalizing. Fat loss and total cooking losses were significantly different in the patties (Table XVI). The losses were highest in the fresh patties, since the fresh patties were cooked on the grill for the full time while the frozen patties were cooked on the grill and then rethermalized in the oven in its juices.

Nutritional Evaluation

Nutritional evaluation included analysis of selected minerals (calcium, copper, iron, magnesium, and zinc). For mineral analysis, meat samples were ashed, dissolved, and read on the Atomic Absorption Spectrophotometer (Type AA-5). The micrograms of minerals per gram of meat was calculated.

Analysis of variance for the mineral content of the cooked meat samples is presented in Table XVI. Calcium, iron, magnesium, and zinc varied significantly ($p<0.05$) due to level of collagen. Results of the Duncan Multiple Range Tests for mineral content are in Table XVII. Calcium, iron, magnesium, and zinc varied significantly ($p<0.05$) due to level of collagen.

Calcium varied significantly due to level of collagen. The calcium content increased as the level of collagen increased. Each level of collagen was significantly different from every other level of collagen.

Iron varied significantly ($p<0.05$) due to level of collagen. Cooked meat with collagen had significantly less iron than the meat with no collagen. There were two significantly different groups--the control and those containing collagen.

TABLE XVI

ANALYSIS OF VARIANCE FOR MINERAL ANALYSIS

Mineral	Source	df	Mean Square	F Value	Observed Sig. Difference
Calcium	Collagen	3	11030.288	511.18	0.0001
	Run No.	3	11.128	0.52	0.6817
	Error	9	21.578		
Copper	Collagen	3	.0195	1.49	0.3083
	Run No.	2	.0149	1.15	0.3785
	Error	6	.0130		
Iron	Collagen	3	60.156	5.30	0.0222
	Run No.	3	19.107	1.68	0.2392
	Error	9	11.346		
Magnesium	Collagen	3	4878.975	519.17	0.0001
	Run No.	3	4.666	0.50	0.6937
	Error	9	9.398		

Magnesium varied significantly due to level of collagen. The magnesium content decreased as the level of collagen increased. Every level of collagen was significantly different from every other level of collagen.

The zinc content values have an unusual pattern and no conclusive statement can be made. The control, 10 percent collagen, and 20 percent collagen meat samples, had about the same zinc content but the 30 percent collagen meat sample had significantly less.

Comparing the total micrograms of mineral per gram of meat (Table XVIII), the collagen-meat mixture contained more minerals than the meat without collagen. This parallels the results for the ash

content--the ash content increased as the level of collagen increased. The increase due to level of collagen would be greater if it had been based on gram of raw meat because the collagen retains more liquid, hence a gram of meat sample containing 30 percent collagen actually had less meat and collagen combined than the control sample. The retention of liquids is probably responsible for the significant differences in iron and zinc. The difference in magnesium would be decreased and the differences in calcium would be increased.

TABLE XVII

DUNCAN'S MULTIPLE RANGE TESTS FOR VARIABILITY OF MINERAL ANALYSIS*

Mineral	Percent Collagen	Mean
Calcium	0	53.173
	10	110.173
	20	126.997
	30	180.661
Iron	0	29.932
	10	21.947
	20	21.638
	30	23.413
Magnesium	0	242.851
	10	219.856
	20	194.868
	30	161.371
Zinc	0	49.171
	10	49.460
	20	49.171
	30	40.170

*Means not marked by a common solid line are significantly different ($p<0.05$). Common line may not be continuous.

TABLE XVIII

TOTAL ASH CONTENT (CALCIUM, IRON, MAGNESIUM, AND ZINC)

Collagen Percent	Total Mineral (ug/g)
0	380.127
10	401.308
20	392.777
30	405.616

The decrease in magnesium is due to a decrease in lean meat. Magnesium is not found in as high a level in collagen as it is in meat. The increase in calcium is due to the preservation process of liming, in which the hides undergo before they are converted to food-grade collagen.

Testing the Hypotheses

The first hypothesis for this study has three parts:

$H_{1(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation, due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(b)}$: There will be no significant differences in the mean percentage vapor, percentage moisture, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture,

percentage fat, and percentage ash of cooked meat due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(c)}$: There will be no significant differences in the mean nutrient value measured by ash content due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

The second hypothesis has two parts:

$H_{2(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation of fresh and frozen beef loaves and beef patties which contain the same levels of collagen. The researcher failed to accept this hypothesis for the loaves because the amount of connective tissue was significantly higher in the fresh than in the frozen loaves. The researcher failed to reject this hypothesis for the patties because no differences were found.

$H_{2(b)}$: There will be no significant differences in the mean percentage moisture, percentage vapor, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat of fresh and frozen beef loaves and beef patties which contain the same level of collagen. The researcher failed to accept this hypothesis because vapor loss, moisture loss, and total cooking loss were significantly different in the loaves and fat loss and total cooking loss were significantly different in the patties (Table XIV).

CHAPTER V

SUMMARY, CONCLUSIONS, RECOMMENDATIONS, AND IMPLICATIONS

This research was undertaken to determine at what level collagen was detectable in beef loaves and beef patties, and the effect of freezing on pre-cooked meat-collagen mixtures. The collagen was substituted for lean meat only at the 10, 20, and 30 percent levels with a constant 20 percent fat. The beef loaves and beef patties were prepared fresh and also cooked, frozen, and rethermalized.

The need for better utilization of present protein sources and development of new sources has been well documented. Extending meat with soybeans, milk precipitates, defatted peanut meal, field pea meal, and fababean have been studied. The extent of acceptability of the products varied and the level of substitution has only been up to 20 percent. There are side effects for each extender. Uses of food collagen has been limited to sausage casings and emulsions. The need for finding uses for this protein-rich byproduct which is presently not being used to its fullest potential warranted this study.

The hypotheses for the study were:

$H_{1(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation, due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(b)}$: There will be no significant differences in the mean percentage vapor, percentage moisture, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of peak (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{1(c)}$: There will be no significant differences in the mean nutrient value measured by ash content due to the four collagen levels (0, 10, 20, 30) in beef loaves and beef patties.

$H_{2(a)}$: There will be no significant differences in the mean color, texture, aroma, juiciness, tenderness, and flavor values as determined by sensory evaluation of fresh and frozen beef loaves and beef patties which contain the same levels of collagen.

$H_{2(b)}$: There will be no significant differences in the mean percentage moisture, percentage vapor, percentage fat, and percentage total cooking losses; tenderness by shear force (kg/g) and area of pean (cm^2/g); color by Hunter colorimeter; and percentage moisture, percentage fat, and percentage ash of cooked meat of fresh and frozen beef loaves and beef patties which contain the same level of collagen.

Sensory evaluation by a trained test panel was done on all four products. Objective tests of loss, tenderness, color, and content percentages were done. The nutritional analysis included a selected mineral analysis.

Summary and Conclusions

An analysis of variance was performed on subjective (sensory), objective, and mineral analysis data using the Statistical Analysis

System (Barr and Goodnight, 1972). Duncan's Multiple Range Test was done to determine significant differences ($p<0.05$) in beef loaves and beef patties. The attribute panel detected differences in texture, aroma, beefy flavor, and off-flavor in all four products (fresh loaves, frozen loaves, fresh patties, and frozen patties (Table XIX). The coarse texture and off-flavor increased as the level of collagen increased while aroma and beefy flavor decreased as the level of collagen increased. Based on these results, the researcher failed to accept hypothesis 1(a). When the sensory data was evaluated without regard to level of collagen (Table XX), the fresh loaves had a significantly ($p<0.05$) higher level of connective tissue than the frozen loaves, hence the researcher failed to accept hypothesis 2(a) for the loaves. There were no differences between the fresh and frozen patties. Based on this result, the researcher failed to reject hypothesis 2(a) for the patties.

Drip loss, total cooking loss, ash content, and tenderness by shear force (kg/g) were significantly different in all four products (Table XIX). Drip loss and total cooking loss decreased as level of collagen increased. Ash content and tenderness by shear force (kg/g) increased as the level of collagen increased. Based on these results, the researcher failed to accept hypothesis 1(b). The comparison between fresh and frozen without regard to level of collagen (Table XX) showed significant differences in vapor loss, moisture loss, and total cooking loss in the beef loaves. All three were highest in the frozen and lowest in the fresh. In the beef patties, fat loss and total cooking loss were significantly different. The losses were highest in

TABLE XIX

DUNCAN'S MULTIPLE RANGE TESTS DUE TO COLLAGEN FOR SENSORY AND OBJECTIVE EVALUATION*

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Texture	59.524	67.143	47.857	51.429
10		116.191	100.714	88.571	97.381
20		134.524	112.858	120.238	104.286
30		150.952	148.333	133.095	135.952
0	Aroma	124.29	130.24	128.81	134.52
10		97.38	108.33	105.48	87.14
20		82.08	75.71	77.62	95.71
30		63.33	77.86	76.19	59.76
0	Beef Flavor	134.76	126.90	150.47	150.00
10		101.90	114.29	118.57	108.10
20		58.78	70.24	61.67	79.52
30		36.91	50.00	52.62	40.48
0	Off-Flavor	15.714	27.619	13.571	16.905
10		44.286	18.571	25.476	35.000
20		46.191	40.952	48.333	35.238
30		59.286	48.095	52.857	58.816
0	Drip Loss	17.91	18.58	4.97	4.92
10		15.51	16.73	5.25	2.52
20		12.98	13.68	3.94	2.46
30		10.51	9.35	2.90	1.01

TABLE XIX (Continued)

Percent Collagen	Attribute	Fresh Loaf	Frozen Loaf	Fresh Patty	Frozen Patty
0	Total Cooking Loss	25.63	30.57	35.12	26.29
10		22.59	27.31	33.71	22.91
20		19.61	24.20	26.34	20.35
30		16.34	17.68	24.59	18.58
0	Ash Content	.494	.643	1.01	.85
10		.930	1.25	.93	1.31
20		1.045	1.19	1.31	1.24
30		1.135	1.12	1.21	1.12
0	Tenderness	.23	.25	.31	.25
10		.18	.22	.31	.24
20		.18	.22	.28	.21
30		.14	.20	.24	.23

*Means not marked by a common solid line are significantly different ($p<0.05$). Common line may not be continuous.

the fresh and lowest in the frozen patties. Based on these significant differences, the researcher failed to accept hypothesis 2(b).

TABLE XX

DUNCAN'S MULTIPLE RANGE TESTS FOR VARIABILITY DUE TO PRODUCTION

Attribute	Fresh Loaves	Frozen Loaves	Fresh Patties	Frozen Patties
Amount of Connective Tissue	82.619	64.762		
Vapor Loss	6.438	10.480		
Moisture Loss	9.046	7.874		
Fat Loss			4.269	2.591
Total Cooking Loss	21.043	24.941	29.944	22.034

Mineral analysis found significant differences in the level of calcium, iron, magnesium, and zinc (Table XXI). Iron, magnesium, and zinc decreased as the level of collagen increased. Calcium content increased as the level of collagen increased. Based on these results, the researcher cannot accept hypothesis 1(c).

TABLE XXI

DUNCAN'S MULTIPLE RANGE TESTS FOR MINERAL ANALYSIS

Mineral	Calcium	Iron	Magnesium	Zinc
Collagen Level				
0	53.173	29.932	242.851	49.171
10	110.045	21.947	219.856	49.640
20	126.997	21.638	194.868	49.171
30	180.661	23.413	161.371	40.170

Recommendations

Further studies on the effect of cattlehide collagen on beef loaves, beef patties, and other food systems or meat products need to be investigated. The effects of rethermalization in other equipment such as microwave oven, air-ducted convection oven, or the grill on fresh and frozen beef loaves and beef patties could be studied.

A replication of this study should be done to reaffirm present conclusions. The number of replications used in ashing could perhaps be increased. The content percentages (fat and ash) should be based on the dry weight of the meat sample instead of the wet weight. It is not practical in industry or cooking for large groups to pour the juice back on the patties so the study could be done without pouring the juices on the patties before freezing.

Implications

The application for consumer use of collagen-supplemented products could be widespread. School foodservices, elderly feeding, and international food assistance programs are possible outlets for utilizing this protein source. Because freezing does not change the quality attributes of beef loaves and beef patties supplemented with collagen, companies that manufacture fully prepared meals, pre-cooked entrees, or other products could very well utilize collagen as a meat extender.

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APPENDIXES

APPENDIX A

PROCEDURES

BABCOCK PROCEDURE

Sample Preparation

Place 100 grams of meat in a clean waring blender jar and blend to a paste consistency. After the sample has been blended, place it into a clean screw cap glass bottle and store at -20°F until the analyses are completed. Or, if preferred, use finely ground products, well mixed, to make a uniform sample.

Procedure

Content of fat is determined by a modification of the Babcock milk and cream test. This method is designed for muscle tissue samples. All tests should be made in duplicate.

1. Weigh accurately 3 grams of meat paste and place into a 20% Paley-type bottle, or weigh 9 grams into a 50 percent Paley-type bottle. Add 30 ml of 1:1 perchloric acid-acetic acid solution. Mix contents by swirling. Insert rubber stopper.

2. Immerse in boiling water bath 30-60 minutes. Swirl occasionally in bath until meat solids are digested. Then allow another 15 minutes for complete digestion.

3. Insert rubber stoppers firmly in lower opening. Pipet additional acid mixture until fat column rises into the calibrated neck of the bottle.

4. Centrifuge the solution for 2 minutes at 900 rpm in a Babcock centrifuge. Place bottle, after centrifuging, in 70°C water bath for 15 minutes. This will provide uniform temperature when making readings.

5. Drain 1/2 ml of acid mixture down wall of calibrated neck. This momentarily separates the fat phase from the digest and permits reading the meiscus using dividers. Add more acid if fat level is not high enough.

6. From the amount of fat separated, the fat content as percent weight is directly determined. The Paley bottles are calibrated for a 9 gram sample; if 20 percent bottles are used, so multiply your reading by 3; the 50 percent reading will be the direct total fat percent.

MOISTURE ANALYSIS

1. Weigh washed, dried, and dessicated crucibles. Be sure that they are numbered.

2. Add approximately 1-1/2 grams of cooked ground beef in the crucible.

3. Place the crucible with the meat in an 102°C oven and allow to dry for 24 hours.

4. Remove from the drying oven and weigh.

This procedure was used for duplicate samples of each three days for all four products for a total of 96 samples.

ASH ANALYSIS

Using the samples which have been dried:

1. Place the crucibles in a muffle furnace and ash for 24 hours at 500°C.

2. Cool for four hours.

3. Place in dessicator until completely cool.

4. Weigh crucibles.

This procedure was used for duplicate samples of each day's samples of all four products for a total of 96 samples.

ETHER EXTRACTABLE FAT

1. Preweigh numbered beakers and number glass thimbles with cotton and keep these paired.

2. Place the beakers back into dessicator and leave there until needed.

3. Place thimbles on scale, insert about 1-1/2 grams of ground beef (cooked) down into the thimble. Weigh very accurately.

4. Place the thimble filled with meat in 102°C oven for 24 hours.

5. Remove the thimbles and place in the beakers.

6. Put the samples on the ether extractor for 24 hours.

7. Remove the breaker from the ether extractor and weigh after being sure that all of the ether has vaporized and that the beaker is dessicated.

8. To determine the percent of fat, divide the weight of the fat in the beaker by the total weight of the sample.

This procedure was followed for duplicate samples of each day at each level for a total of 96 samples (Horwitz, 1975).

MINERAL ANALYSIS

Acid wash all equipment with HCl acid. Use only teflon coated tongs and tweezers.

1. Weigh acid washed, dried, and dessicated crucibles.

2. Add approximately 1-1/2 grams (or more if necessary to get proper concentration) of cooked ground beef in the crucible.

3. Place the crucible with the meat in an 102°C oven and allow to dry for 24 hours.

4. Remove from the drying oven and place in the muffle furnace.

5. Ash for 24 hours at 500°C. Ash should be completely white.

6. Dilute the ash with 3 ml of 7N nitric acid and dilute with distilled water to a volume of 10 ml.

7. Dilute the sample to the proper level for reading on the Atomic Absorption Spectrophotometer.

8. Prepare 3-4 standards for each mineral.

9. Read samples on the Atomic Absorption Spectrophotometer.

This procedure was done on two samples of each level of collagen for a total of eight samples. Each sample was read four times (Freeland-Graves, Ebangit, and Hendrikson, 1980).

APPENDIX B

DATA SHEETS

Date _____

Type of Product Meat Loaf

Batch No. _____

Cooking Temperature _____

Time in Oven _____

Time Out of Oven _____

	0	10	20	30
Before Cooking				
1. Weight of Pan				
2. Weight of Meat				
3. Weight of Meat and Pan (1+2)				
After Cooking				
4. Weight of Meat, Pan, and Drippings				
5. Weight of Vaporized (3-4)				
6. Weight of Pan and Drippings				
7. Weight of Meat (4-6)				
8. Weight of Pan and Drippings w/Fat Removed				
9. Weight of Fat (4-8)				
10. Weight of Moisture in Pan (8-1)				

Date_____

Type of Product Frozen Meat Loaf

Batch No._____

Cooking Temperature_____

Time in Oven_____

Time Out of Oven_____

	0	10	20	30
Before Cooking				
1. Weight of Pan				
2. Weight of Meat				
3. Weight of Meat and Pan (1+2)				
After Cooking				
4. Weight of Meat, Pan, and Drippings After Freeze				
5. Weight of Vaporization on First Cooking (3-4)				
6. Weight of Meat, Pan, and Drippings After Cooking Second Time				
7. Weight of Vaporization on Second Cooking (4-6)				
8. Weight of Pan and Drippings				
9. Weight of Meat (6-8)				
10. Weight of Pan and Drippings w/Fat Removed				
11. Weight of Fat (8-10)				
12. Weight of Moisture in Pan (10-1)				

Date_____

Type of Product Beef Patties

Batch No._____

Cooking Temperature_____

Time in Oven_____

Time Out of Oven_____

	0	10	20	30
Before Cooking				
1. Weight of Meat				
After Cooking				
2. Weight of Meat and Plate				
3. Weight of plate				
4. Weight of Cooked Meat (2-3)				
5. Weight of Vapor and Drippings (1-4)				
6. Weight of Drip Pan and Drippings				
7. Weight of Drip Pan w/Fat Removed				
8. Weight of Fat (6-7)				
9. Weight of Drip Pan				
10. Weight of Moisture in Pan (7-9)				
11. Weight of Vaporization (5-(8+10))				

Date_____

Type of Product Frozen Beef Patties

Batch No._____

Cooking Temperature_____

Time in Oven_____

Time Out of Oven_____

	0	10	20	30
Before Cooking				
1. Weight of Meat				
After First Cooking				
2. Weight of Meat, Plate, and Drippings				
3. Weight of Plate				
4. Weight of Meat and Drippings				
5. Weight of Vaporization (1-4)				
After Second Cooking				
6. Weight of Meat and Plate				
7. Weight of Plate				
8. Weight of Cooked Meat (6-7)				
9. Weight of Vapor and Drippings (4-8)				
10. Weight of Drip Pan and Drippings				
11. Weight of Drip Pan w/Fat Removed				
12. Weight of Fat (9-11)				
13. Weight of Drip Pan				
14. Weight of Moisture in Pan (11-13)				
15. Weight of Vaporization (9-(12+14))				

APPENDIX C

SENSORY EVALUATION OF MEAT

October 20, 1980

TO: Taste Panel Members

FROM: Nancy Cathey

SUBJECT: Dates for Taste Panel

I would like to apologize for this late change of dates. Below is the finalized schedule.

TRAINING	November 4, 5, & 6
TASTING	November 11, 12, & 13
	November 18, 19, & 20
	December 2, 3, & 4
	December 9, 10, & 11

Each day we will start at 11:30 a.m. If you have a conflict with any of these days please contact me and see if we can work something out.

Thank you for volunteering your time for my research.

BASIC TASTE IDENTIFICATION

Name_____

Instructions: In front of your are 6 cups containing weak water solutions of chemicals representing the basic taste sensations. One or more of these may be a blank or a repeat. Your task is to identify the dominant taste in each cup.

Please rinse your mouth with water before you taste each sample. For each sample, please record on the ballot below if the sample is tasteless or has a sweet, salty, sour, or bitter taste.

Sample Code	Taste Description

ODOR IDENTIFICATION

Name_____

Instructions: Ten bottles are presented which contain a common household odor. Please sniff each sample. Record the name or description of the odor below. Wait approximately 15 seconds between samples.

Sample Code	Odor Description

SENSORY EVALUATION TOOL

Name_____

Date_____

Product_____

Character Note	Intensity	
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong
_____	_____	
	weak	strong

FOOD ATTRIBUTES BALLOT

Name_____ Product Code_____

Instructions: Please record each sensory attribute of the food as you perceive it. As you record the attribute, rate its intensity using the following 1 - 4 scale:

1 = very weak, 2 = moderate, 3 = strong, 4 = extremely strong

Attribute	Intensity Rating

TRIANGLE TEST

Name_____ Date_____

Product_____

Two of the samples are identical; one is different. Taste the samples and identify the one that is different.

Code	Check the odd sample
_____	_____
_____	_____
_____	_____

Describe the difference: _____

_____

_____

INSTRUCTIONS TO JUDGES

*HINTS - Do the visual and olfactory characteristics of all samples. Then take one sample and complete all other characteristics and continue in this manner. Be sure to use the lukewarm water provided to rinse your mouth between samples. Remember that one end of the cahracteristic scale is not bad and the other end good but they are contrasting individual characteristics between products.

You will receive coded samples of beef loaves or beef patties.

1. Look at each piece and determine color and texture (see description).

   Appearance: This includes two characteristics: color and texture. These should both be checked by looking at the interior of the sample. Evaluate color on the scale from light to dark and on the scale from even to streaked. Evaluate the texture by the size of the granules on a scale of fine to coarse.

2. Smell each piece and determine the aroma.

   Aroma: Smell the sample and determine if you detect any odor other than beef and evaluate it on a scale of extremely intense to practically none.

3. Do all other characteristics listed on the evaluation sheet. Check for one attribute at a time. For example: use one bite to check juiciness, another bite for tenderness, etc. (Please describe any off-flavors detected.)

   Juiciness: Determine the initial juiciness by takine one chew. Evaluate it on a scale from very dry to very juicy. After the eighth chew determine the sustained juiciness using the same scale of very dry to very juicy.

   Tenderness: Tenderness should be determined by first impression or bite. Think about how hard it is to sink your teeth into the meat. Evaluate it on a scale of extremely tender or extremely tough.

   Connective Tissue: After eight chews determine the amount of non-breaking up material on a scale of none to abundant.

   Flavor: Determine the intensity of the beef flavor from extremely intense to extremely bland. Please check also for presence or absence of off-flavor(s). Use a scale of none to extremely intense off-flavor. Please describe any off-flavors detected in the space provided.

4. Please make any other comments you feel would help us in our evaluation of the product.

5. Be sure to complete Name, Date, and Code. Thank you.

MEAT EVALUATION

Color —Light	Color —Even	Texture —Fine	Beef Aroma —Extremely Intense	Initial Juiciness —Extremely Juice
—Dark	—Streaked	—Coarse	—Practically None	—Extremely Dry
Sustained Juiciness —Extremely Juice	Tenderness —Extremely Tender	Amt. Connective Tissue —None	Beef Flavor —Extremely Intense	Off-Flavor —None
—Extremely Dry	—Extremely Tough	—Abundant	—Extremely Bland	—Extremely Intense

Please describe any off-flavor(s) which you can detect:_____

Comments:_____

Name_____ Date_____ Code_____

VITA[2]

Nancy Pallie Jester Cathey

Candidate for the Degree of

Master of Science

Title: EFFECTS OF COLLAGEN AND FREEZING ON THE QUALITY OF BEEF LOAVES AND BEEF PATTIES

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born in Fayetteville, Arkansas, September 9, 1956, the daughter of Dr. and Mrs. Douglas B. Jester; married in Las Cruces, New Mexico, August 13, 1978, to Robert Lee Cathey.

Education: Graduated from New Mexico State University, Las Cruces, New Mexico, in 1979 with a Bachelor of Science in Home Economics degree; completed the requirements for the Administrative Dietetic Internship, Oklahoma State University, Stillwater, Oklahoma, in 1980; Registered Dietitian status attained in October, 1980; completed requirements for the Master of Science degree at Oklahoma State University in July, 1981

Professional Experience: Graduate Research Assistant, Food, Nutrition and Institution Administration Department, Oklahoma State University, Stillwater, Oklahoma, 1980-1981.

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