Effects of Collagen Content, Fiber Alignment, Storage and Heating on Cooking Characteristics, Dimensional Changes and Microstructure of Restructured Beef

by

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ABSTRACT

Studies were conducted to determine the effects of collagen level, fiber alignment, frozen storage and oven temperature on cooking characteristics and the dimensional and structural changes of restructured beef steaks. Three replicates of high (HC) and low collagen (LC) raw materials similar in proximate composition, salt content, pH and physical measurements but different in total collagen were manufactured and stored for either 40, 85 or 130 days before cooking by broiling or roasting. HC steaks tended to require less time for heat to penetrate than LC steaks. Collagen levels did not significantly ($P>0.1$) affect cooking losses and physical measurements except the width (W1) at the narrow end. Effects of storage were not different for all cooking losses except evaporation losses nor for physical measurements except W1 and the longest axis (L1). Cooking treatments affected all cooking losses and physical measurements except the midpoint thickness of the steak. Random fiber steaks tended to need less time to reach each end-point temperature than Aligned steaks. Orientation of fibers had no effect ($P>0.1$) on cooking losses and physical measurements except L1 and the straight region (L2) on the perimeter of the steak. Broiling yielded higher evaporation and total cooking losses than roasting. Drip losses were not different between heating methods. For both collagen levels and fiber alignments, Site 1 (apex of the dome) had higher residual moisture and fat contents than Site 3 (no visible change occurred). The possible mechanism for dome formation considers that: 1)complete layers of parallel muscle fibers form heavy barriers; 2)sufficient binding exists between meat pieces to form a dome; 3)excessive protein films (coat) cover meat particles; 4)a dense protein matrix traps moisture and fat components; 5)additional denatured collagen provides strength to support the dome; and 6)"microvoids" exist within the dome with larger voids in the vicinity of the dome. A possible mechanism for channel development is based upon the following observations: 1)sufficient layers of parallel muscle fibers are lacking; 2)insufficient binding exists between meat pieces; 3)insufficient protein films (coat) cover this area; 4)poor protein matrix allows more moisture and fat components to escape into the atmosphere and meat drippings; 5)prominent channels are located between muscle strands connecting the interior and exterior portions of the steak; and 6)voids are located on the steak surface among parallel muscle fibers.

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Chapter I. Introduction

Steaks and chops will remain popular, preferred meat cuts because consumers consider them to be a good value. These retail cuts satisfy today's lifestyle needs for individual portions, rapid preparation, desirable texture and palatability and compositional control of fat and protein. Yet, the beef rib and loin, which are the source for most desirable steaks, make up only 9.6% and 17.2%, respectively, of the beef carcass. This leaves 73.2% of the carcass that is less sought-after by consumers, lower in value and generally not sold in cuts that are just exactly as they are removed from the carcass (Romans et al., 1985).

Low-cost beef cuts and trimmings have been used to produce restructured meat products having textural and compositional characteristics similar to intact muscle cuts of higher value (Huffman, 1979; Pearson and Dutson, 1987). However, some problems with the appearance and sensory characteristics of restructured meats have been noted after cooking which discourage consumers from making repeat purchases of the products (Field et al., 1977). Dimensional changes during cooking such as curling, doming and welling are a major cause of dissatisfaction with restructured beef products (Jolley and Rangeley, 1986) and with restructured pork chops (Campbell et al., 1977). A distortion in thickness during cooking gave these products a swollen and deformed appearance. Volume changes and cooking losses varied widely among samples cooked to different end-point temperatures.

Several factors may be related to the dimensional changes that occur during cooking. Manufacturing procedures are known to differ in their effects on restructured product characteristics. Flaking eliminates the cylindrical pellets of gristle and connective tissue frequently associated with grinding (Anonymous, 1973). In addition, flaked pork products were preferred over ground pork (Chesney et al., 1978) due to their greater cohesiveness. Mixing for 8 to 10 minutes optimized water-holding capacity (WHC), emulsion stability and textural characteristics of restructured products(Mandigo, 1975).

Research studies utilizing salt in restructured products show that binding is enhanced, cooking losses are decreased, textural properties are improved and aroma, flavor and juiciness are more desirable (Schwartz and Mandigo, 1976). However, salt may adversely affect the raw color (Huffman, 1979; Huffman and Cordray, 1979; Huffman et al., 1981; Marriott et al., 1983), cause off-flavors and reduce the shelf life of frozen, stored products (Schwartz, 1975; Huffman et al., 1981).

Phosphates, in combination with NaCl, increase the WHC of meat myofibrillar proteins through hydration and solubilization effects (Rosinski et al., 1989). The polyphosphates break bonds between actin and myosin allowing interfilament spaces to form. Also, they change the ionic strength of the sarcoplasm and increase electrostatic repulsions between filaments and further increase the amount of space available for water binding (Ellinger, 1972).

Because raw materials of choice for restructuring are those deemed to be of relatively limited value, various combinations of grade and carcass parts have been used. However, most underutilized beef raw materials contain heavy concentrations of connective tissue embedded in the muscle mass which may be related to the frequency, magnitude and mechanism of dimensional distortions in restructured beef products.

Collagen, the major component of connective tissue, is rigid and inextensible in the native state. However, it shrinks at specific temperatures dependent upon amino acid composition and sequence (from 60°C to 70°C in meat). Also, shrinkage varies according to the types of cross-links formed among collagen molecules (Bailey and Light, 1989). Collagen from younger animals has fewer stable cross-links and upon heating denatures to gelatin while that from older animals shrinks at a higher temperature and may squeeze moisture out of the system and compress the heat-coagulated myofibrillar material. Subsequent heating in the presence of water causes hydrolysis of some of the peptide bonds present in the shrunken collagen leading to the formation of soluble gelatins and decreased toughness (Ledward, 1984). This solubilization is relatively slow below 90°C and may be related to dimensional distortions in steaks and chops. Bailey and Light (1989) indicated that the quality of these cooked steaks can be undesirably affected by the orientation of the connective tissue in the product. Poor orientation results in excessive shrinkage and dimensional changes during cooking.

According to Bernal and Stanley (1986), general microstructural characteristics of restructured beef products are different from the distinctive elements in intact beef muscle (muscle fiber surrounded by connective tissue and grouped into fasciculi). Restructured particles are covered by a protein film. In addition, fat globules which appears as a crystalline structure resembling glyceride crystals and occasional cavities probably caused by the presence and melting of ice crystals were observed in the raw product. Cooking resulted in severely damaged fibrous structures and the release of droplets from within the protein exudate.

Progress made in this study to characterize the cause of distortion induced by heating would be expected to lead to the development of improved processes to prevent this product defect. Without the negative impact of an undesirable appearance, restructured beef steaks should gain a significantly larger share of the fresh meat market.

Our objectives were to:

(1) investigate the effects of collagen content, frozen storage and heating at different rates to three end-point temperatures on the cooking losses and dimensional changes in restructured beef steaks;

(2) study progressive structural changes in heated restructured beef steaks using phase contrast microscopy (PCM) and scanning electron microscopy (SEM);

(3) determine the effects of fiber alignment and heating on cooking characteristics and on dimensional and structural changes in cooked restructured beef steaks.

Chapter II. Literature Review

The concept of restructuring meat and poultry products has been studied extensively for more than two decades. Procedures to transform secondary carcass parts into reasonably priced high-value products that have eating characteristics similar to those of steaks and chops have been developed (Secrist, 1987).

Restructured meat products are manufactured by first reducing the particle size of low value and/or less tender meat cuts and then recombining the small pieces into a new product of uniform size, shape, and texture (Seideman and Durland, 1983). The products are marketed either frozen or precooked to retain structural integrity (Schmidt, 1987b).

2.1 Meat Raw Materials for Restructured Products

The raw materials for restructured products are primarily skeletal muscles which contain approximately 76% water, 18% protein, 3% fat and 1% glycogen (Lawrie, 1966). The muscle proteins are subdivided into three groups:

1) myofibrillar (10%),
2) sarcoplasmic (6%), and
3) connective tissue (2%).

Myofibrillar proteins are an integral part of the structure of filaments in muscle and significantly influence the integrity of restructured products. They contain the thick filament (myosin, C-protein), thin filament (actin, tropomyosin, troponin), M-line (creatine kinase, M-protein), Z-line (α-actinin, desmin) and N-line (nebulin).

Sarcoplasmic proteins make up the intracellular fluid (sarcoplasm), are soluble at low ionic strength at neutral pH and contribute to cellular metabolism. They are made up of myosin, globulins, myoglobin, hemoglobin and enzymes.

Muscle proteins that are not included in either the sarcoplasmic or myofibrillar classes are termed connective tissues. Collagen is the main connective tissue, but this class also includes elastin, reticulum and lipoproteins of the cell membrane which are present in small amounts (King and MacFarlane, 1987). Collagen is associated with skeletal muscle in three designated tunics: the whole muscle (epimysium), fiber bundles (perimysium) and individual fibers (endomysium). Several different genetic variants of collagen (known as Types I, III, IV, etc.) are present in muscle with the epimysium containing predominantly Type I, the perimysium Types I and III, and the endomysium Types IV and V (Sims and Bailey, 1981; Bailey, 1984).

Collagen fibers are colorless but look white in quantity and are of variable lengths (Cummings, 1983). Veis (1960) indicated that collagen is one of the most interesting natural polymers. From the viewpoint of the basic scientist, collagen offers the possibility of examining direct relationships between molecular architecture, chemical composition and molecular function. A major biological characteristic of the collagen family of extracellular proteins is extraordinary strength. Collagen is able to form long, semi-rigid, rod-like molecules, composed of three polypeptide chains. These molecules are capable of binding together to form larger, insoluble structures outside the cell masses of the body (Bailey and Light, 1989). Kastelic (1955) indicated that collagen and elastin are members of the fibrous protein group. The fibrillar components of collagen and elastin are composed of elongated chains of amino acids joined together by peptide linkages. These fibers are aggregated into larger masses of connective tissue held together by a mucous-like matrix, termed "ground substance." This substance is a complex of mucopolysaccharides and mucoproteins (Pearson, 1986).

Some components of ground substance (laminin, fibronectin and heparan sulfate proteoglycan) emerge into basement membranes composed of Type IV collagen which has a well-known "chicken-wire" network and may have a structurally important role in contributing to meat toughness (Pearson, 1986). Laurie et al. (1986) used rotary shadowing electron microscopy to show that Type IV collagen had binding sites for laminin, fibronectin and heparan sulfate proteoglycan. They suggested that the basement membranes consisted of a lattice of Type IV collagen coated with laminin, fibronectin and heparan sulfate proteoglycan and indicated that the ground substance matrix may prevent the breakdown of Type IV collagen breakdown by enzymatic hydrolysis. Therefore, ground substances and collagen binding may maintain basement membrane and contribute to the toughness of meat (Pearson, 1986). There is some evidence suggesting a transmembrane association of intracellular actin with extracellular fibronectin which would account for the observed effects of added fibronectin on cell shape and an improved adhesion to the substrate (Fawcett, 1986).

Collagen is able to act as a major supporting framework of the body because of the strength of collagen fibers (Bailey et al., 1974). This strength is due to collagen's unique molecular configuration, highly specific alignment of molecules during extracellular aggregation and the formation of covalent cross-links (Light, 1987; Bailey and Light, 1989). These cross-links or chemical bridges are covalent in nature and result from specific structural features of the collagen α-chains and the way molecules overlap each other in a highly organized manner. Without cross-links, the collagen matrix has little or no strength (Bailey and Light, 1989) and meat with too few cross-links comes apart upon cooking. Conversely, too many cross-links makes meat tough (Bailey, 1972).

Thus, collagen must have the optimum number of cross-links to give meat an acceptable texture.

Bailey et al. (1974) indicated that as collagenous tissues advance in age they become more stable to external influences. Also, the turnover rate of mature collagen proteins in living animals is slow. Bailey and Light (1989) and Judge et al. (1989) reported that collagen fibers become progressively stronger and more rigid. The molecular changes which invoke these alterations in mechanical properties also lead to a decreased susceptibility to attack by proteolytic enzymes, a reduction in the extent of swelling of fibers in acid solution and an increase in actual fiber size.

2.1.1 Meat Proteins: Role of Specific Meat Proteins in Binding

Of the three main groups of proteins found in muscle, myofibrillar proteins (myosin in prerigor and actomyosin in postrigor muscle) are the most important binders for processed meat products (Forrest et al., 1975). Myosin and actin make up 50-55% and 20-25%, respectively, of the total protein content of muscle. Other myofibrillar proteins constitute the remaining 20-25% of the salt-soluble fraction and may affect the function of actin and myosin (Acton et al., 1983; Asghar et al., 1985; King and MacFarlane, 1987).

Actin alone exhibited poor binding characteristics

(Samejima et al., 1969; 1980) but strongly associated with myosin to form actomyosin. Actomyosin and myosin in model gelation systems produced excellent gel strengths (Fukazawa et al., 1961a, b; Samejima et al., 1969; Nakayama and Sato, 1971a, b). In most cases, these workers found that actomyosin was a more effective binding agent than myosin. In contrast, MacFarlane et al. (1977), Ford et al. (1978) and Turner et al. (1979) showed that myosin was superior to actomyosin in binding meat pieces together in both a model binding system and in a reformed beef product. Then, Yasui et al. (1980; 1982), using a model gelation system, revealed that myosin and actomyosin together made much stronger gels than either myosin or actomyosin alone.

There is some evidence that sarcoplasmic proteins and connective tissues also contribute to the binding characteristics of meat (Stanley, 1983; Asghar and Henrickson, 1982; Sikorski et al., 1984; Gillett, 1987a, b). But, sarcoplasmic proteins which are water-soluble are not important binding agents. Acton and McCaskill (1972) concluded that despite their ability to increase binding strength, the water-soluble proteins available in meat appeared less important as binders than salt-soluble proteins. MacFarlane et al. (1977) and Ford et al. (1978) found that the binding ability of the sarcoplasmic protein fraction was slight.

However, sarcoplasmic proteins may influence the characteristics of other proteins present by coagulating onto them when heating occurs. Also, sarcoplasmic proteins affect binding in meat systems by changing their ionic strength and pH and by interacting with other meat proteins (Trout, 1984).

2.2 The Binding Mechanism

A critical part of the meat restructuring process is the binding that occurs from a heat-set mechanism involving the salt-extracted myofibrillar proteins (Vadehra and Baker, 1970). A tight protein matrix develops and, upon heating, acts as a stable binder between meat chunks (Schnell et al., 1970).

Hamm (1966) indicated that high temperature cooking causes the helical portions of protein molecules in meat to unravel into randomly organized chains which randomly link together by both hydrogen and ionic bonds. This protein molecule cross-linking may be primarily responsible for the binding of chopped, ground or sectioned pieces of meat during denaturation by heat.

Siegel and Schmidt (1979a, b) showed that the mechanism for binding meat pieces dtogether involves the interaction of thick synthetic filaments from extracted intact myosin with heavy myofilaments located within muscle cells on or near the surfaces of the meat pieces. Salt is required to solubilize the myosin during mixing of raw meat. Heating produces thick filaments at high temperatures (MacFarlane et al., 1977). Ishioroshi et al. (1980) pointed out that the binding of myosin to actin could reinforce the thermal gelation of myosin. Upon heating (from 30°C to 60-70°C), an aggregation and micro-network formation resulted which was not present in the raw state of myosin. Furthermore, water mobility in the gel system was restricted (Yasui et al., 1979; Ishioroshi et al., 1981; Samejima et al., 1981; Foegeding and Lanler, 1987).

2.2.1 Excessive Binding, Insufficient Binding

Binding meat particles together is an essential step in producing restructured meats. Both meat and non-meat ingredients play important roles in the manufacturing of these products. Either excessive or insufficient binding impact on the quality of restructured product (Berry, 1987). Schmidt (1987a) indicated that excessive salt and phosphate levels along with prolonged mixing of meat can produce too much binding and result in a cooked product with a rubbery texture. Such a product will be hard to cut or chew. The juices of this cooked product will be tightly bound resulting in the meat becoming dry in the mouth. In contrast, a lack of binding causes crumbling and failure of large sectioned muscle pieces to stick together (Berry, 1987).

2.3 Distortion During Cooking

Two of the most common complaints from beefburger manufacturers about flaked and formed steaks in the U.K. pertain to the appearance of the cooked meat. First, changes in planar dimensions as evidenced by increases in height were associated with a condition termed "doming" (Campbell et al., 1977; Field, 1982; Jolley and Rangeley, 1986). Secondly, upon piercing the cooked meat, copious fluid was released (Jolley and Rangeley, 1986). This condition was called "welling."

In other work (Berry et al., 1986; Jolley and Rangeley, 1986), some comminuted meat products shrank and curled up during cooking depending on the connective tissue orientation within the products. Berry (1988) reported that excessive connective tissue can cause textural problems in restructured products. Unfortunately, placing flat surface weights on the steaks during cooking or a spiking procedures proved useless in preventing distortion (Berry, 1987).

Pockets (i.e. mini-explosions) or separation of meat particles sometimes can be seen in flaked and formed restructured beef steaks after cooking. Furthermore, restructured steaks formed from small-sized meat pieces often develop crusts, distort and separate into layers. Perhaps, these incidents are related. When a crust forms, distortion may occur due largely to the inability of steam and juices to escape. In turn, pockets may form and release the pressure inside. If these relationships are realistic, cooking losses should be less for flaked and formed meats in small-sized meat particles (Jolley and Rangeley, 1986; Berry, 1987). The placing of flat surface stresses on steaks while cooking them may reduce layering to some extent (Berry, 1987).

2.4 Factors That Influence Properties of Restructured Steaks

2.4.1 Raw Material Composition

2.4.1.1 Content, Type and Solubility of Connective Tissue

Excessive connective tissue would obviously be a problem in restructured products. Light (1987) indicated that the total collagen content of muscle affects eating quality. Restructured steaks containing high levels of collagen showed greater distortion following cooking (Berry et al., 1986; Mandigo, 1988). Acceptability of these products depend on the total collagen content and collagen solubility which influence texture (Berry, 1987).

Bailey (1985) stated that it is the quality of collagen in muscle rather than the quantity which is most likely to determine variations in textural quality. The solubility of collagen in meat, as extracted by salt, acid and alkali solutions has been positively correlated with textural quality (Light, 1987). Experiments show that higher amounts of soluble collagen may be obtained from more tender muscles. In fact, collagen solubility is influenced only by its cross-linking and physical integrity. Thus, reasons for differences in collagen solubility may be complex and might not reflect true variations in textural qualities.

2.4.1.2 Fat Content

Fat contributes to desirable quality in many foods. In meat, tenderness and flavor of steak may be influenced by fat. Therefore, customers prefer well-marbled prime beef to less desirable and low-fat meat (Drewnowski, 1987).

Restructured beef steaks containing 15, 20 or 25% fat were compared by Costello et al. (1985). Steaks with 25% fat had higher cooking losses than the steaks with 15 or 20% fat. But, the steaks containing 25% fat were softer to tooth pressure than the ones containing 15% fat.

Similar research from Berry et al. (1985) evaluated restructured beef steaks containing 10, 14, 18, or 22% fat. Steaks with higher fat (18 and 22%) were more juicy and moist. Also, they had greater mouth coating than lower fat content steaks (10 and 14%). Steaks containing 22% fat were rated as having more gristle and webbed tissue. However, cooking losses were higher for the 22% fat treatment. According to

Marriott et al. (1988), fat content of 20% or less had a minimal effect on the appearance traits of restructured beef steaks. But, restructured steaks with higher fat levels had more visual distortion and configurational changes after cooking (Berry et al., 1985). Glover (1964) determined that consumers discriminate against ground beef with high fat content because of excessive shrinkage and splattering during cooking.

2.4.2 Non-Meat Ingredients

Various processes in the manufacture of meat products require the addition of ingredients such as common salt (NaCl) and/or polyphosphates.

2.4.2.1 Effect of Salts

NaCl has been recognized to possess both desirable and undesirable effects on the acceptability of restructured meats (Marriott et al., 1986a). It is evident that NaCl improves taste and exerts beneficial flavor and protein solubilizing effects. As measured by higher TBA values and/or color deterioration, salt also can increase oxidation when used at high levels. Schwartz and Mandigo (1976) and Huffman and Cordray (1979) have noticed a drop in color desirability with increased salt levels. Ockerman and Organisciak (1979) suggested that the oxidation could be reduced by adding phosphates or antioxidants to these products.

The addition of salt to meat enhances the solubilization of myosin and other cytoplasmic proteins, giving rise to a sticky exudate which acts to bind and glue meat particles. Overall, the presence of salt greatly enhances the WHC of meat. The WHC of collagen is greatly affected by NaCl. Below pH 5 or above pH 8, NaCl decreases the water content of collagen while between pH 6 and 8 there is a small increase. High concentrations of NaCl decrease the hydration of collagen and the normal swelling of collagen in acids can be inhibited by the addition of salt (Bailey and Light, 1989).

Polyphosphates are widely used in meat products to enhance binding properties and are declared to have anti-microbial effects (Madril and Sofos, 1985). The presence of pyrophosphate can affect myosin's capability to bind protein, increase solubility and influence WHC (Regenstein et al., 1979). Yasui et al. (1964) found that pyrophosphate ion is the key phosphate ion in the dissociation of actomyosin. Other higher molecular weight phosphates hydrolyze into pyrophosphate in meat dependent upon the pH, ionic strength of solution and presence of divalent metal ions. The function of phosphates used in detergents is to assist dispersion by forming soluble complexes. Conceivably, phosphates play a similar role in dispersing sarcoplasmic proteins (Lewis et al., 1986).

Offer and Trinick (1983) indicated that phosphates can act two ways on meat structure. First, they can boost the common dispersion of myofibrillar proteins by salt. Secondly, they can promote dissociation of actomyosin and therefore encourage the selective dispersion of myosin by salt. In the presence of phosphate ions, myosin disperses into the pieces of meat or, more specifically, to adjoining pieces of whole meat or muscle tissue.

Schmidt (1979) indicated that the binding junctions in cooked ham rolls receiving low salt treatment were filled with fat and cellular fragments. But,junctions from rolls with sufficient salt and phosphate had a high degree of junction alignment and optimal binding properties(Theno et al.,1978).

Moore et al. (1976) reported that binding strength increased as salt content increased from 1% to 3%. Treatments that contained 2% salt and 0.25% sodium tripolyphosphate were the optimal choice for binding strength and cooking yield of beef rolls. Also, Siegel et al. (1978) and Ockerman and Organisciak (1979) indicated that 2 % salt appeared to be optimal to obtain the appropriate amount of protein in the exudate.

2.4.3 Effect of pH

The pH of meat is very important since it affects the color, WHC, texture and tenderness of meat (Dutson, 1983). Rao et al. (1989) reported a positive linear relationship between acidic muscle pH (below ultimate pH) and cooked meat tenderness.

Offer and Trinick (1983) revealed a relationship between WHC and ultrastructure in meat. Gains or losses of water in meat are due to the swelling or shrinkage of myofibrils produced by the expansion or shrinkage of the constituent myofilament lattices. Maximum swelling occurred when a physical part of the A-band had been removed. AT pH 5.5, there was no extraction of A-bands in a salt solution alone. But, an incomplete extraction of A-bands occurred in a salt and pyrophosphate solution (Rao et al., 1989). AT pH 8.0, a complete extraction of the A-bands occurred in a salt and pyrophosphate solution (Voyle et al., 1984). Wu (1978) found that muscle blocks incubated at pH 5.0 produced higher quantities of neutral salt-soluble collagen than those at pH 7.0. He theorized that this might be due to releasing more collagen monomers or increasing the collagen fractions degraded by protease.

2.4.4 Interrelationships of Ingredients

2.4.4.1 Water and Connective Tissue

The effects of water content on connective tissue are moderately well known for individual components (collagen and elastin). The general effect of water on connective tissue is to plasticize (Aberle and Mills, 1983). The processes in the hydration of collagen are complex since there are hydrophilic groups in the molecule. The mechanical effects of hydration are far less well known (Jeronimidis and Vincent, 1984).

2.4.4.2 Use of Salt and/or Polyphosphate in Muscle Tissue

The effect of salt is to break up the sarcomere, particularly in the myosin rich A-band region, and disperse protein. This enables the meat to retain more moisture. When electron micrographs of 24hr post-mortem meat are compared with similar preparations of freshly excised muscle, the meat generally appears with less clearly defined features (Lewis, 1981). After treating the meat with polyphosphate, the myofibrillar proteins within the sarcomeres become more clearly defined. When salt and polyphosphate are used in combination much more disruption is produced than with salt or polyphosphate alone (Lewis, 1981).

2.4.5 Manufacturing Factors Affecting Quality of Restructured Steaks

When contrasting similar particle sizes, flaked meats are more cohesive, have less drip and cooking losses, and are more tender and desirable in flavor than ground meats. Therefore, flaking becomes an essential procedure for manufacturing restructured meat products (Mandigo, 1988).

2.4.5.1 Particle Size

Flaking and grinding decrease particle sizes, increase surface area for binding lean to lean and increase available binding proteins (Acton, 1972). Particle size influences the texture of restructured meats and the physical appearance of the restructured steaks (Durland et al., 1982). Also, particle size has minimal effects on muscle cut resemblance, shear values, cooking losses and all sensory traits (Marriott et al., 1986b).

Fine flakes produced a more acceptable appearance, improved cohesiveness and increased subjective tenderness (Chesney et al., 1978; Durland et al., 1982; Marriott et al., 1986b; Mandigo, 1988). If the flaked size is too small, the connective tissue is not noticeable and since texture is a function of the particle size and binding this product would not have a steak-like texture. However, restructured steaks with larger flaked size have a better texture during the first bite, but on successive chewing an unacceptable bolus of connective tissue collects in the mouth (Whiting et al., 1987). Durland et al. (1982) determined that restructured beef steaks made from coarse particles always had large flakes of fat and were assessed too "fatty" in physical appearance. Chesney et al. (1978) found that tenderness and juiciness increased in smaller flake size (3.0 mm) and decreased with increased flake size (6.9 mm and 12.7 mm). Marriott et al. (1986b) suggested that small flaked (3.0 mm) prerigor beef possessed more desirable color, cohesiveness and overall appearance than medium (6.1 mm) and large (9.9 mm) particles. Seideman (1980) examined flaked particle size and found that restructured steaks ought to be made less than ½ inch in diameter.

Generally, flake size did not significantly affect moisture losses of restructured logs upon cooking. Smaller particle size steaks were more tender than steaks made from larger size particles (Durland et al., 1982). When particle size increased, the fat percentage lost during cooking increased. According to Acton (1972), there is increasing surface area with particle size reduction to help form a moist surface-to-surface exudate which is salt-soluble and hence forms bonds between similar surfaces.

2.4.5.2 Mixing Time

Mixing increases surface area, breaks the structure of muscle fibers, induces release of intracellular components and then exposes a greater amount of protein and non-protein moieties (Mandigo, 1988). A mixing time study in two phases by Belohlavy and Mandigo (1974) considered beef, pork, and lamb. As mixing time increased to 14 minutes, WHC decreased to its lowest value. Further mixing resulted in increased WHC. These workers declared that the initial breakdown of the cell walls released more free water from the cell. However, the additional agitation extracted abundant amounts of proteins and bound the additional water. Prolonged agitation may also loosen the muscle structure and open the intermolecular space available for water.

Longer mixing time increased cooked yields with beef rolls (Pepper and Schmidt, 1975) but had little effect on binding strength. Durland et al. (1982) found that restructured steaks mixed 10 or 15 minutes had finer textures than steaks mixed 0 or 5 minutes. However, it has been reported that restructured steaks mixed for 15 minutes were less tender and juicy than those mixed for less than 10 minutes (Seideman, 1980; Durland et al., 1982; Mandigo, 1988). Mixing time did not significantly influence fat loss and moisture content after cooking and did not significantly affect binding strength or cooking losses (Durland et al., 1982).

There was a slight increase in stress or deformation values as mixing time increased. This suggests that increased mixing time resulted in better cohesiveness between flakes (Durland et al., 1982). Booren et al. (1981a, b) showed that mixing for more than 12 minutes increased the deterioration of the desired color.

2.4.6 Frozen Storage

Miller et al. (1980) indicated that frozen storage significantly affected beef and pork lean and fat and as storage time increased the comminuted products lost their ability to retain moisture and fat ($P<0.05$). Protein denaturation and aggregation increases with increasing time in frozen storage(Colmenero and Borderias, 1983; Stoyachenko et al., 1988). The impact of storage has been shown to influence gel properties in protein from chicken (Smith, 1987), beef (Wagner and Anon, 1986), and fish (Kim et al., 1986). Also, rancidity affects the quality of frozen meat (Miller et al., 1980). Nilsson (1971) indicated that drying seriously damages meat during frozen storage. However, there was little weight loss from the meat sample vacuum packed. Schwartz and Mandigo (1976) reported that texture desirability scores, determined at monthly intervals up to 6 months, gradually declined for flaked and formed pork chops.

2.4.7 Effect of Cooking on Meat Properties

Cooking alters myofibrillar and connective tissue proteins and the interaction between these proteins. Further, it releases water immobilized in the protein network of the meat and causes the water to leave in the form of liquid and vapor (Gillett, 1987a).

Heat denaturation causes several changes in functional properties of meat proteins including a reduction in protein solubility and WHC, an increase in pH, and an increase in the rigidity of the protein network (Hamm, 1966; Hanson, 1988). The reduced WHC at these temperatures results in considerable fluid loss (Hamm and Deatherage, 1960).

Foegeding et al. (1986) observed that myosin fibers continuously formed a heat-induced gel network between 55°C to 70°C. These workers suggested that a slow heating rate may allow for more helpful protein-protein interactions, thus decreasing moisture loss. Samejima et al. (1985) also found that gel strength was a linear function of the dissolved protein concentration of beef myofibrils prior to heating.

2.4.7.1 Thermal Properties of Connective Tissue and Fiber Orientation

Bailey (1976) indicated that the physical properties of collagen, both in the native and heat-denatured condition, play a major role in developing the texture of meat. The high tensile strength of collagen fibers and their physical properties change with age. Bailey and Sims (1977), Duance et al.

(1977), Allain et al. (1978), Kopp and Valin (1979) and Light et al. (1984) showed that mature collagen cross-links are more thermally stable, thus providing a rationale for toughness in cooked meat from older animals. Ledward (1984) proposed that these more stable cross-links are not only more prevalent in older animals, but that the insoluble shrunken collagen may increase toughness by squeezing out moisture from the system and compressing the heat-coagulated myofibrillar matrix. The denatured collagen still has a great tensile strength and contributes to the toughness of cooked meat.

Shimokomaki et al. (1972) revealed that both peri- and endomysium had a higher ratio of thermally stable keto-imine cross-links than labile aldimine cross-links. Therefore, the endomysium generally is heat-stable but the matrix denatures and shrinks to its less crystalline organization upon heating to temperatures of 55°C to 80°C(Light and Bailey, 1983). As compression induced by endomysial shrinkage occurs, the tension formed is enough to cause further cooking losses from the meat.

Judge and Aberle (1982) signified that the shrinkage temperature (T_S) of intramuscular collagen from cattle of different ages was set at various times postmortem. Intramuscular collagen from older animals shrinks at slightly higher temperatures than from younger ones. Kruggel and Field (1971) and Pfeiffer et al. (1972) suggested that postmortem aging of bovine muscle lowered T_S by degrading the molecular structure of collagen. Namely, collagen cross-linking was diminished. Dransfield and Rhodes (1976) stated that the texture of cooked meat was dependent on the interaction between actomyosin and connective tissue components of muscle. On heating, there was an increase in strength of the coagulated myofibrillar proteins, a decrease in the strength of the connective tissue and shrinkage caused by heating the collagen above the T_S of 63°C. The shrunken connective tissue network was bound tightly around the heat denatured myofibrillar and sarcoplasmic proteins which produced a structure having a greater resistance to deformation.

A characteristic property of the highly crystalline collagen fiber is to shrink to one-quarter of its length at about 65°C. Under isometric conditions, the denatured fiber creates a tension (Allain et al., 1978) which results in an abundant loss of fluid and a sequential shrinkage of the meat. Contraction of the collagen can press excess fluid from the meat. Yet, the residual strength of the denatured collagen fibers still keeps the adhesion between the muscle bundles (Bailey, 1985).

Thermal conductivity is influenced by the direction of heat flow relative to the direction of the fibers of the muscle (Godsalve et al., 1977b). Lentz (1961) and Hill et al. (1967) indicated that the thermal conductivity of frozen meat was greater for heat flow parallel to the muscle fibers than for heat flow perpendicular to the fibers.

Godsalve et al. (1977b) proposed a theory of varying fiber orientation in a controlled environment oven (Figure 2.1). The muscle fibers run along the length of the muscle in parallel bundles. If a cylindrical sample is cut along the length of the muscle and stood on end in the oven, the upward air flow moves in a direction that is parallel with the orientation of the muscle fiber bundles (Figure 2.1a). If a cylindrical sample is cut across the length of the muscle and stood on end in the oven, the upward air flow moves in a direction that is perpendicular to the orientation of the muscle fiber bundles (Figure 2.1b).

In the perpendicular sample heated to 149°C, there appears to be no initial boiling front entering the sample. Instead, the moisture loss rate rises to the level attained for surface evaporation from a fully denatured muscle sample. Therefore, water released through protein denaturation flows rapidly to the surface in a relatively straightforward path and soon establishes maximum moisture loss. The high flux of moisture to the surface early in the cooking process prevents the formation of the inwardly travelling boiling front that is

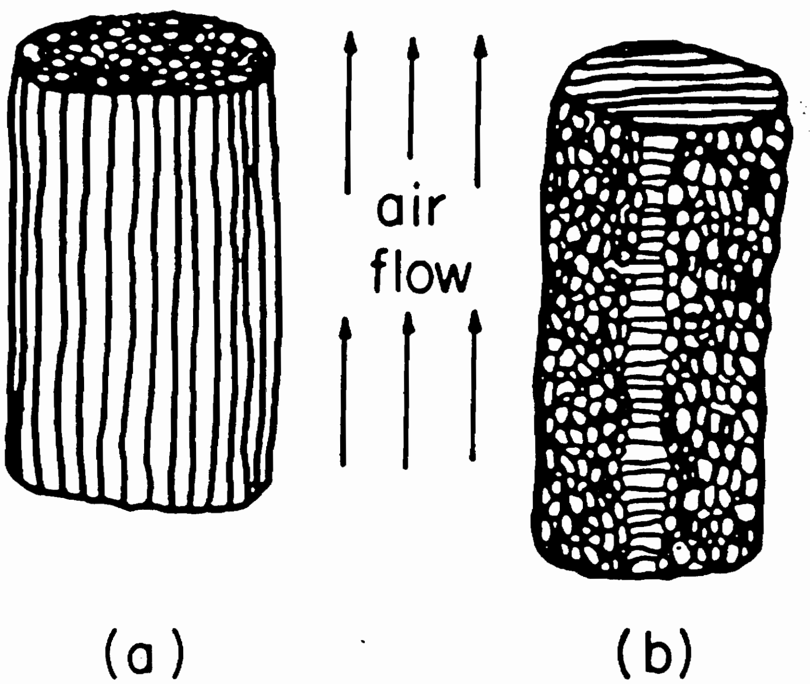


Figure 2.1. Different orientation of a bovine semitendinosus muscle in the controlled environment oven (a) parallel; (b) perpendicular (Godsalve et al., 1977b).

characteristic of the parallel case. Therefore, the amount of water lost over a fixed time period would be greater in the perpendicular orientated sample than in the parallel sample(Godsalve et al., 1977b).

The fibers and fiber bundles are surrounded by a layer of connective tissue. It is this tissue that creates the difference in moisture and heat flow in the parallel and perpendicular orientation. At the beginning of the denaturation temperature range (55 - 90°C) the connective tissue starts to shrink and is destroyed at the higher temperatures by chemical transformation to gelatin. Thus, differences in moisture flow resistance exist primarily in the early stages of cooking (Godsalve et al., 1977b).

Bouton et al. (1976) reported a greater moisture loss in shorter fiber samples than in those with longer fiber lengths. Also, Locker and Daines (1974) suggested that the sarcolemma acts as a barrier to the sideways movement of moisture perpendicular to muscle fibers.

2.4.7.2 Heat-induced Alterations in the Structure of Muscle Tissue

Histologically, heating causes collagen fibers to stretch, melt in some areas, and lose their distinctiveness. The tissue softens, decreases in weight, and becomes shorter, narrower, and thicker. On heating, the collagen structure changes from fibrous to granular. At 60°C, the collagen fibers retain much of their fibrous appearance but at 80°C the collagen fibers appear mostly coagulated (Davey and Gilbert, 1974; Locker and Carse, 1976; Wu et al., 1985). Therefore, the muscle fibers are generally closer together after heating (Landmann, 1967).

The variations in muscle microstructure produce significant changes in the overall textural properties of meat and meat products (Lewis, 1979; Voyle, 1979; Lewis, 1981; Stanley, 1983). Microscopy showed that myofibrillar proteins were compressed and sarcomeres were shortened at 50°C. Heating to 60°C caused a loss of the M-line structure, an initiation of disintegration and coagulation of thin and thick filaments, and further myofibrillar protein shrinkage. Heating to 70°C and 80°C caused more disintegration of thin filaments and coagulation of thick filaments. At 90°C an amorphous structure resulted but principal banding features of the sarcomere could be identified regardless of the internal temperature (Schmidt and Parrish, 1971). SEM studies of cooked bovine muscle (Cheng and Parrish, 1976; Jones et al., 1977) revealed that gradual changes occur in myofibril fragmentation at the Z-disc and in collagen denaturation with increased temperatures in the range of 50-90°C.

Cheng and Parrish (1976) and Leander et al. (1980) mentioned that the A-band region was more thermally stable than the I-band region. They further suggested that the loss of WHC was more severe in the I-band region than in the A-band region.

2.4.7.3 Moisture Transport Properties

Skjöldebrand and Hallström (1980) indicated that the migration of water in meat products during heating depends upon the product's WHC or water partial-pressure gradients (Kester and Fennema, 1989). Altomare (1988), who designed the scale-up in the cooking of bacon analog, showed that significant water transport occurred during cooking.

Increased product moisture concentrations were shown to cause raised diffusivity values. Moisture diffusivity has been revealed to increase with a decreasing fat-protein ratio because of the hydrophobic properties of fat. In addition, a decreased fat content results in an increase in moisture concentration which causes increased moisture diffusivity (Hanson, 1988).

2.4.7.4 Cooking Losses

Wierbicki et al. (1956) stated that cooking losses in meat products has always been important to meat manufacturers because of yield characteristics. Moreover, oven temperatures, humidities, air velocities, and product end-point temperatures have all been shown to have an effect on product cooking losses.

Chapter III. Materials And Methods

3.1 Raw Material Source and Preparation

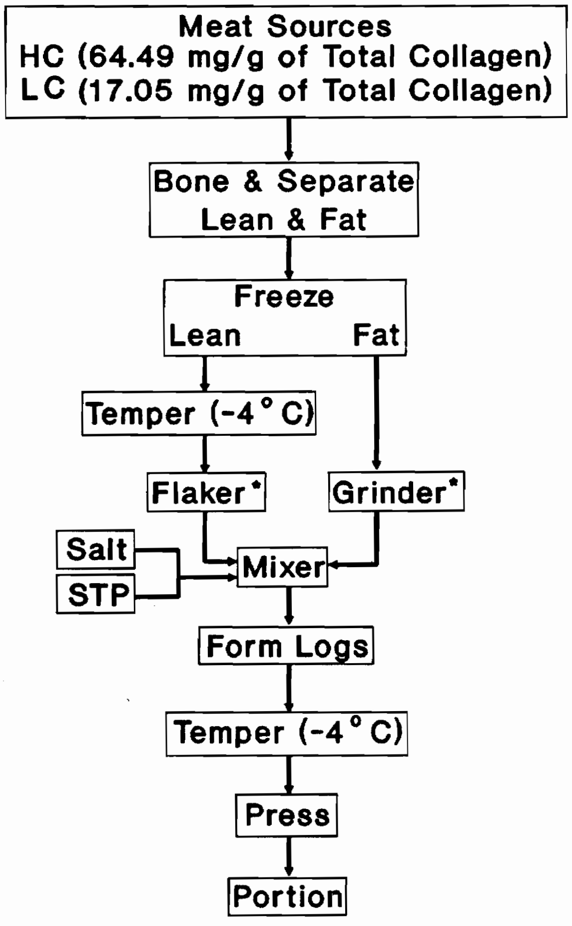
Meat raw materials having characteristics of the USDA Standard grade were obtained from three intact male bovine animals slaughtered at the VPI&SU Meat Laboratory. Longissimus thoracis and lumborum muscle samples were removed from carcasses chilled for 48 hours at 2°C and used to manufacture low collagen (LC) content (Biro, 1969; Rantsios and Papavasileiou, 1983; Abustam et al., 1987; Elkhalifa et al., 1988; Johnson et al., 1988) steaks. The extensor carpi radialis, flexor carpi radialis, flexor carpi ulnaris, superficial digital flexor, deep digital flexor (Tauber, 1968; Elkhalifa et al., 1988) and Trapezius (Rantsios and Papavasileiou, 1983; Johnson et al., 1988) muscles were used to make a high collagen (HC) content product. The epimysium was not removed from any muscles and they were cut into 3.5 cm x 3.5 cm pieces, frozen at -20°C and then tempered to -4°C overnight before flaking (Graham and Marriott, 1986). The tempered raw material was flaked using a Ross Unicom 1000 (Ross Industries, Inc., Midland, VA) containing a head opening size of 7 mm x 4 mm x 30 mm at a rotational speed of 5700 rpm.

3.1.1 Phase I - Conventional Product Manufacture

Three replicate batches of restructured beef steaks were produced from the two sources of raw materials described previously from the same carcass to yield high collagen (HC) and low collagen (LC) products (Figure 3.1). Then, the meat was frozen, tempered and flaked.

Flaked raw materials from each animal were analyzed for fat content using the modified Babcock Method (Ockerman, 1969). Beef fat trim was added (10-13°C) to adjust the fat content to approximately 20%. Non-meat ingredients consisted of 2% salt (NaCl) and 0.25% sodium tripolyphosphate (STP) based on the meat weight. Final mixing (10-13°C) occurred in a Hobart (Model C-100) Mixer for 10 minutes. The mixed formulation was stuffed into 11 cm diameter fibrous casings to preform the products prior to freezing. The stuffed logs were frozen at -20°C overnight and then tempered to -4°C in Bacteriological Incubator (The Fischman Co., Philadelphia, PA) over a 16 hour period. All products were formed into the shape of boneless beef steaks (ribeyes) with a Ross Superform 720 Press at a setting of 37 kg/cm^2 with 2 sec dwell time.

After chilling (1.5 hours, 4-5°C), steaks were sliced 13.0 mm thick with a Hobart (Model 1712-R) Slicer (The Hobart Manufacturing Co., Troy, Ohio). Individual steaks were vacuum packaged into clear, 17.5 cm x 30 cm, B550T Cryovac® bags



* indicates Babcock fat analysis done at this point.

Figure 3.1. Restructured steaks processing flow diagram.

(Longview Fiber Co., Cedar Rapids, Iowa) and stored at -20°C until evaluated. Steaks from each collagen level were randomly selected and evaluated within a 6-day period after 40, 85, and 130 days.

3.1.2 Phase II - Aligned-oriented Manufacture

Two replicate batches of restructured steaks were manufactured from high collagen (HC) raw materials. Random flaked products were manufactured similar to phase I. The aligned flaked restructured products were manually formed after raw materials were mixed. Pressing, handling, storage, sampling and evaluations of the manually formed steaks were similar to the random oriented flaked products.

3.2 Chemical / Physical Examinations

3.2.1 Proximate and Other Chemical Analyses

Samples from each treatment were analyzed for composition, such as moisture, fat, and protein of both the raw and the cooked products by AOAC methods (AOAC, 1990). Residual salt levels were determined by the modified AOAC (1990) procedure (QUANTAB, chloride titrators, Enviroment Test Systems, Inc., Elkhart, IN). The measurement of pH ($[H^+]$) was estimated by a technique reported by Ockerman (1969).

Insoluble, soluble, and total collagen were determined according to procedures described by Bergman and Loxley (1963) and Hill (1966) for raw and cooked products. Soluble (heat-labile) and insoluble hydroxyprolines were converted to collagen by using conversion factors 7.52 and 7.25, respectively, as described by Cross et al. (1973).

In this study, we used total collagen content = soluble collagen content + insoluble collagen content.

The solubility percentage was calculated according to the following expression:

$$\text{SOLUBILITY(\%)} = \frac{\text{SOLUBLE COLLAGEN}}{\text{TOTAL COLLAGEN}} \times 100$$

3.2.2 Cooking Treatments and Cooking Procedures

Randomly selected samples of restructured beef steaks from each treatment were cooked from the frozen state. The steaks were measured for length, width, and height at the appropriate locations (Jolley and Rangeley, 1986) and were marked by crayon (Figure 3.2) before cooking. A Chromel/Alumel thermocouple (Campbell Micrologger 21X) introduced into the geometric center of the frozen steak from the central side of the base (L2) monitored internal temperature during cooking. Another thermocouple for monitoring oven temperature

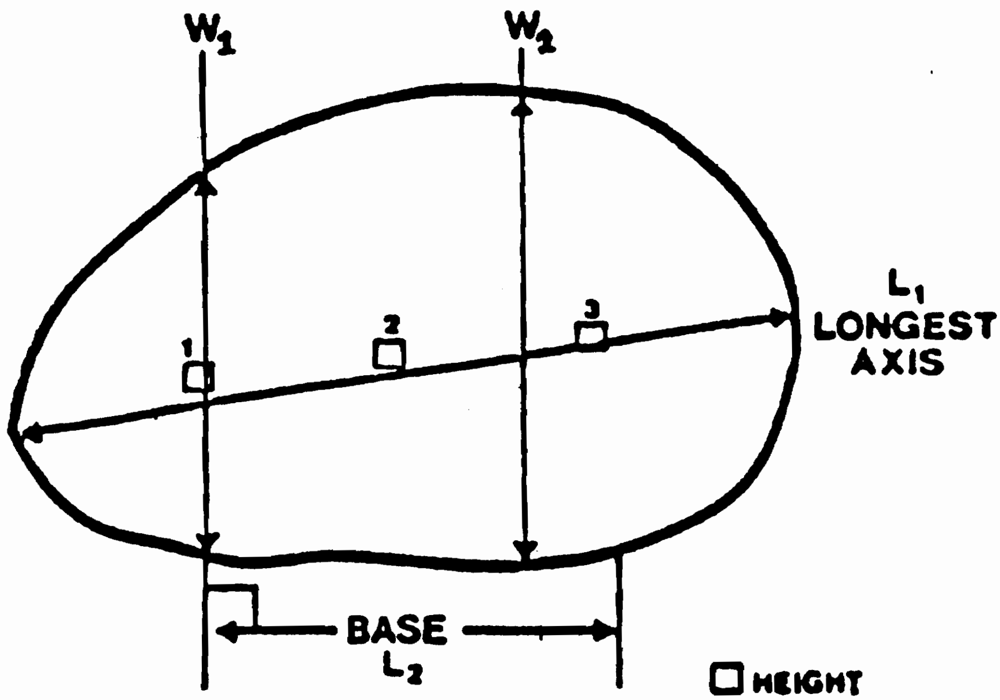


Figure 3.2. Shape of uncooked patties,and sites used for measurement. Heights 1, 2, and 3 were equidistant along an imaginary midline. The base (L2) is defined as the length of the comparatively straight region on the perimeter of the patties, and Width 1 (W1) was taken perpendicular to the base at the narrow end of the patties. Width 2 (W2) was taken parallel to W1 at the longest axis (L1) was actually about 15° off being parallel to the base (modification of Jolley and Rangeley, 1986).

was placed at almost the same height adjacent to the steak. During broiling, the cooking steak was turned at their mid-temperature. During roasting, the oven temperature was constantly monitored and the temperature was controlled to within ± 5°C (Cross et al., 1976).

According to Jolley and Rangeley (1986), each steak was weighed before and after cooking so as to determine cooking losses. The cooked steaks at equilibrium to room temperature (20°C) (Egbert et al., 1991) were vacuum packaged into B550T Cryovac® bags and stored at -20°C until further evaluation.

3.2.2.1 Fast Cook - the Method of Heat Conduction (Broiling)

Each frozen steak was placed 5.0 cm from the upper heat source and broiled to an internal temperature of 50°C (fast cook to low temperature), 70°C (fast cook to intermediate temperature) or 90°C (fast cook to high temperature). The oven was preheated at the "broil" setting for 10 minutes prior to use. Steak were turned once during broiling at its mid-temperature (modification of Cross et al., 1978).

3.2.2.2 Slow Cook - the Method of Heat Convection (Roasting)

Frozen steaks in an oven preheated to 177°C were placed 17.0 cm from the upper heat source and cooked slowly to either 50°C, 70°C or 90°C end-point temperatures (modification of

Cross et al., 1978 and Leander et al., 1980).

3.2.3 Measurements of Cooking Losses

Total cooking losses (including evaporation and drip losses) was according to the following formula:

$$\text{TOTAL COOKING LOSSES} = 100 - \text{MEAN} \left(\frac{\text{COOKED WEIGHT}}{\text{INITIAL WEIGHT}} \times 100 \right)$$

(Evaporation Losses = Total Cooking Losses - Drip Losses)

3.2.4 Measurements of Dimensional Changes (Distortions)

The physical measurements of restructured beef steaks respresented lengths, widths, heights, Shrinkage (volume) and Doming (average of hights) changes.

The physical measurements (%) of lengths, widths and hights of restructured beef steaks were respresented as dimensional changes (distortions) and calculated as following:

$$\text{DIMENSIONAL CHANGES} = \frac{\text{INITIAL DIMENSION} - \text{COOKED DIMENSION}}{\text{INITIAL DIMENSION}} \text{X100}$$

Shrinkage (volume changes) was measured as:

$$\text{SHRINKAGE} = 100 - \text{MEAN} \left(\frac{\text{COOKED VOLUME}}{\text{INITIAL VOLUME}} \times 100 \right)$$

$$(\text{VOLUME} = [(L1+L2)\div2]\text{x}[(W1+W2)\div2]\text{x}[(H1+H2+H3)\div3])$$

The doming was calculated as:

$$\text{DOMING} = \text{MEAN} \left(\frac{\text{COOKED HEIGHT}}{\text{INITIAL HEIGHT}} \times 100 \right) - 100$$

$$(\ \text{AVERAGE OF HEIGHTS} = (H1+H2+H3) \div 3\ )$$

3.3 Phase Contrast Microscopic (PCM) Observations

Meat samples (approximately 3 - 4 mm thickness) randomly selected from each contrast site of treatment were fixed at 25°C in a 10% neutral buffered formalin for lipid using Oil Red O method and in a Bouins solution for muscle fibers and collagen using Hematoxylin and Eosin (H & E) and Masson's Trichrome Stains for at least 48 hours. The dehydration and infiltration of samples were carried out, in turn, with ethanol gradients of 70%, 80%, 95% and 100%, xylene, and paraffin (Tissue-Tek® Vacuum Infiltration Processor, Vertical Model, Miles Inc., Diagnostics Division, Elkhart, IN). Samples were also embedded in paraffin (Surgipath EM 400). Samples were sectioned (5 μm) and stained with the H & E and Masson's Trichrome Stains and then mounted with coverslipping film (unique cellulose triacetate film, Tissue-Tek® Coverslipper, Miles Inc.). For the Oil Red O Stain, samples were frozen-sectioned and coverslipped in a water soluble commercial mountant (Crystal Mount, Biomeda Corp.). Slides were observed at 100X, 400X and 1000X magnification with an Olympus

(BH-2) Phase Contrast Microscope (Opical Elements Co., Dulles Int'l Airport, D.C.). Photographs were taken with a camera on the top of the microscope (a modification of Hearne et al., 1978).

3.4 Scanning Electron Microscopic (SEM) Observations

Both the distorted sites (if present) and non-defective sites of the same cooked steaks were removed. Approximately 1 cm^3 particles of samples were cut from each sampling site and fixed in a buffered (0.1 M sodium phosphate buffer, pH 7.2) 2.5% glutaraldehyde solution for 30 minutes on a ice bath to inhibit the artificial. Samples were diced into smaller (0.5 cm^3) pieces and fixed in fresh buffered glutaraldehyde solution for overnight at 4°C. Then, rinsed three times (30 minutes each) in sodium phosphate buffer. The diced samples were post-fixed in 2% osmium tetroxide in the same buffer overnight. Samples were, then, rinsed three times (30 minutes each) in sodium phosphate buffer. Dehydration of diced samples was carried out in ethanol gradients of 15, 25, 40, 50, 70, 80, 90, 95, 100, 100 and 100% series (30 minutes each). Using 100% ethanol as a transition fluid, the diced samples were critical point dried by $CO_{2(\ell)}$ in a Ladd critical point drier. The dried samples were frozen in $N_{2(\ell)}$ and fractured with the edge of a razor blade. The fractured samples were mounted on aluminum stabs with conductive silver paint, sputter coated with 20 nm gold/palladium in an Anatech Hummer X and examined in a Philips 505 Scanning Electron Microscope operated at 15 or 20 kV. Micrographs were recorded on Polaroid-Type 55 P/N film, (a modification of Cheng and Parrish, 1976, Leander et al., 1980 and Wu et al., 1985).

3.5 Statistical Analysis

Data analysis was performed using the General Linear Model (GLM) procedure of SAS® (1989). In phase I (Steel and Torrie, 1980), a 2 x 3 x 2 x 3 factorial experiment (Table 3.1) was used to determine the effects of collagen level, storage day, cooking method and end-point temperature. Phase II contrasted particle alignment, cooking method and end-point temperature (Table 3.2) in a 2 x 2 x 3 factorial experiment (Figure 3.3).

Table 3.1. Experiment (Phase I) : Factors Assessed.

Factors	Levels	Values
Collagen Level	2	high, low
Storage Day	3	40, 85, 130
Cooking Method	2	fast, slow
End-point Temperature	3	50°C, 70°C, 90°C

Table 3.2. Experiment (Phase II) : Factors Assessed.

Factors	Levels	Values
Orientation of Flakes	2	aligned, random
Cooking Method	2	fast, slow
End-point Temperature	3	50°C, 70°C, 90°C

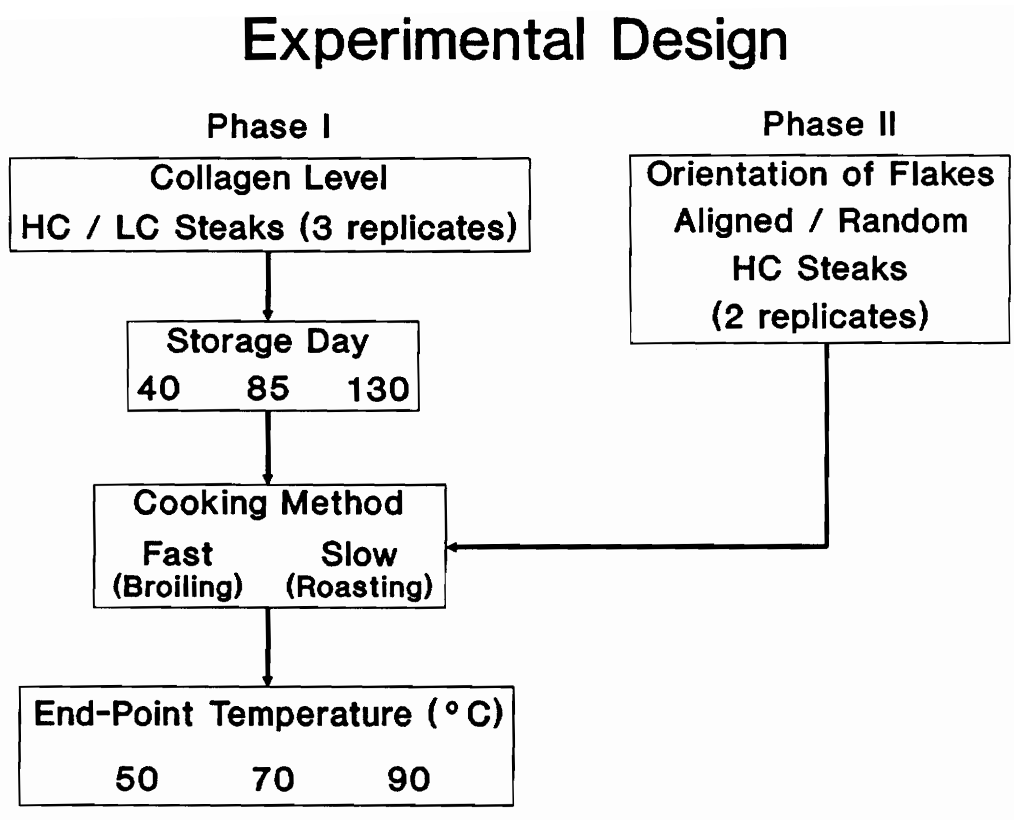


Figure 3.3. Experimental design - Phase I and Phase II (HC = high collagen; LC = low collagen)

Chapter IV. Results And Discussion

Phase I

4.1 Raw Products

Raw high collagen (HC) and low collagen (LC) restructured beef steaks were similar ($P>0.05$) in proximate composition, salt content and pH but different ($P<0.05$) in total collagen content and in the percentage of soluble collagen present (Table 4.1). Mean moisture, fat, and protein values for HC versus LC steaks were 64.92% vs. 65.20%, 17.73% vs. 17.84%, and 14.27% vs. 13.47%, respectively. Salt contents were 2.21% and 2.22% for HC and LC steaks while pH values were 6.01 and 6.23, respectively. There were no differences ($P>0.1$) in compositional, salt or pH data among replicates.

Total collagen content of HC (64.49 mg/g) steaks was higher ($P<0.05$) than that of LC (17.05 mg/g) steaks. However, the HC steaks contained a lower percentage ($P<0.05$) of soluble collagen (20.11%) than LC (41.88%) steaks. The solubility of collagen is known to vary in meat raw materials from different locations in the carcass. In fact, Light (1987) indicated that different regions of the same beef muscle (longissimus)

Table 4.1. Mean initial proximate composition, salt content, pH and collagen analyses of raw products[1].

Composition		Collagen Levels	
		HC*	LC**
% Moisture		64.92[a]	65.20[a]
% Fat		17.73[a]	17.84[a]
% Protein		14.27[a]	13.47[a]
% Salt		2.21[a]	2.22[a]
pH		6.01[a]	6.23[a]
Soluble Collagen	(mg/g)	12.97[a]	7.14[b]
Insoluble Collagen	(mg/g)	51.52[a]	9.91[b]
Total Collagen	(mg/g)	64.49[a]	17.05[b]
Collagen Solubility	(%)	20.11[b]	41.88[a]

[1] Mean values were based on 3 replications of triplicate samples.

* HC = High Collagen; ** LC = Low Collagen.

[ab] Means with different superscripts in the same row were significantly different ($P<0.05$).

muscle (longissimus dorsi) having the same total collagen content and tenderness were different in the percent of soluble collagen present. Therefore, differences in the quantity of soluble collagen present may be responsible, in part, for any dimensional changes that occur during cooking.

Mean physical measurements previously described in Figure 3.2 were similar for raw restructured beef steaks (Table 4.2). The initial L1 values, the longest axis of the steak, were approximately 14.90 cm for HC and 14.96 cm for LC. The length (L2) of the relatively straight region on the perimeter of the raw steaks was 9.00 cm for both treatment groups. Initial W1 widths taken perpendicular to the base at the narrow end of the steaks were 6.61 cm for HC and 6.55 cm for LC. A second width (W2) was taken parallel to W1 at the widest dimension of the raw steaks and averaged 8.24 cm for HC and 8.19 cm for LC. Mean thickness values (H1, H2 and H3) taken equidistant along an imaginary midline ranged from 1.30 to 1.33 cm. There were no differences ($P>0.1$) in raw physical dimensions among replicates and storage days.

4.2 Cooked Products

Figure 4.1 shows the typical heat penetration patterns of fast (broiled, Figure 4.1a) and slow (roasted, Figure 4.1b) cooked steaks to end-point temperatures of 50, 70 and 90°C.

Table 4.2. Mean initial physical measurements of raw products[1].

Physical Measurements (cm)	Collagen Levels	
	HC	LC
Length 1 (L1)	14.90[a]	14.96[a]
Length 2 (L2)	9.00[a]	9.00[a]
Width 1 (W1)	6.61[a]	6.55[a]
Width 2 (W2)	8.24[a]	8.19[a]
Height 1 (H1)	1.32[a]	1.30[a]
Height 2 (H2)	1.30[a]	1.30[a]
Height 3 (H3)	1.33[a]	1.32[a]

[1] Mean values were based on 3 replications of triplicate samples.

[a] Means with same superscripts in the same row were not significantly different ($P>0.1$).

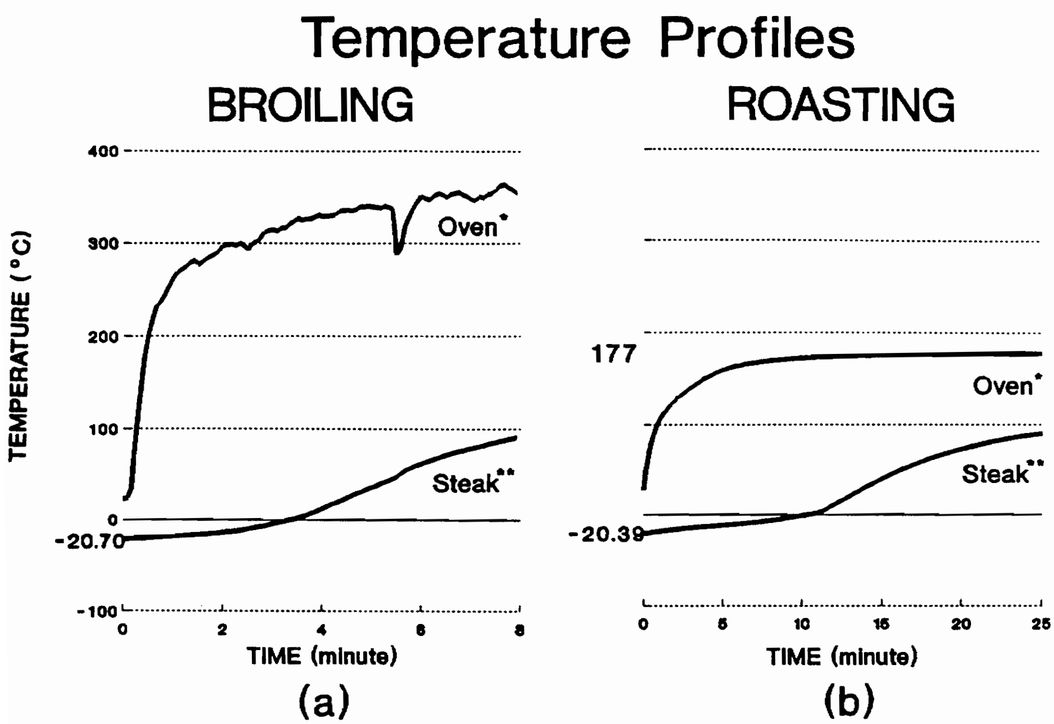


Figure 4.1. Temperature profiles of (a) fast cooking (broiling) versus (b) slow cooking (roasting).

(*: Oven temperatures adjacent to steak surface;
**: Internal steak temperatures.)

4.2.1 Temperature Profiles

Under conditions of this study for fast cooking (broiling), the oven temperatures adjacent to the steak surface (Table 4.3) reached 300°C after 2.8 and 2.53 minutes, respectively, during cooking HC and LC steaks. The variation in time required to raise the oven temperature to 300°C may have been due to differences in initial temperatures of the HC (-16.42°C) and LC (-18.43°C) products placed in it. Broiler oven temperatures increased to > 320°C after 3.64 minutes for HC and after 3.03 minutes for LC treatments.

After 2.0 and 3.6 minutes exposure to broiling condition (Table 4.3), internal HC and LC steak temperatures were at 0°C with corresponding temperatures adjacent to the steak surface at 282.76 and 319.24°C, respectively. The internal temperature increases to 50°C, 70°C and 90°C occurred at 4.13, 5.23 and 6.28 minutes for HC steaks and at corresponding oven temperatures of 326.06, 340.18 and 351.46°C, respectively. This compared to times of 5.39, 6.22 and 7.29 minutes for LC steaks which were at corresponding oven temperatures of 343.82, 352.24 and 360.66°C, respectively.

During the roasting (slow cooking) procedure, oven temperatures reached 177°C at approximately 9-11 minutes for both the HC and LC steaks (Table 4.3) and then remained steady (177±5°C). From initial internal temperatures of -20.82°C and

Table 4.3. Mean[1] times and oven temperatures at critical points in cooking temperature profiles.

Steak End-Point Temperature (°C)	Broiling				Roasting[3]			
	HC		LC		HC		LC	
	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)
Initial	0.00	(-16.42)[2]	0.00	(-18.43)[2]	0.00	(-20.82)[2]	0.00	(-21.84)[2]
-	-	-	2.53	300.00	-	-	-	-
-	-	-	3.03	320.00	-	-	-	-
0	2.00	282.76	3.60	319.24	9.00	167.73	10.68	170.87
-	2.80	300.00	-	-	-	-	-	-
-	3.64	320.00	-	-	-	-	-	-
50	4.13	326.06	5.39	343.82	14.19	174.90	16.82	175.61
70	5.23	340.18	6.22	352.24	17.37	176.74	20.26	176.20
90	6.28	351.46	7.29	360.66	22.74	179.27	26.86	178.93

[1] Mean values were based on 3 replications of triplicate samples at 90°C of end-point temperature.

[2] indicates initial internal steak temperature just before cooking and at room temperature.

[3] the oven temperature reached 177°C at about 9-11 minutes

-21.84°C for the HC and LC steaks, respective temperatures increased to 0°C after 9.0 and 10.68 minutes exposure to roasting conditions. The corresponding oven temperatures adjacent to the 0°C HC and LC steaks were 167.73 and 170.87°C, respectively. Internal temperature increases to 50°C, 70°C and 90°C occurred at 14.19, 17.37 and 22.74 minutes for HC steaks with corresponding oven temperatures of 174.9, 176.74 and 179.27°C, respectively. In comparison, LC steaks reached 50°C, 70°C and 90°C in 16.82, 20.26 and 26.86 minutes with corresponding oven temperatures of 175.61, 176.2 and 178.93°C, respectively. Thus, higher collagen containing products tended to require less time for heat to penetrate than LC steaks.

4.3 Cooking Losses (Evaporation, Drip and Total Cooking Losses)

4.3.1 Effects of Collagen Level on Cooking Losses

No differences (P>0.1) in evaporation (12.02 % vs. 10.68 %; S.E. = 0.63), drip (6.10 % vs. 5.24 %; S.E. = 0.57) and total (18.12 % vs. 15.92 %; S.E. = 1.16) cooking losses were observed between HC and LC steaks. These results agree with Berry et al. (1986) who showed that cooking properties were unaffected (P>0.05) by connective tissue level.

4.3.2 Effects of Storage

Although evaporation and total cooking losses (Table 4.4) were lower ($P<0.05$) for steaks stored 85 days (10.04% and 15.54%, respectively), than for those stored 40 days (13% and 17.79%, respectively), this reduction was attributed, in part, to a lower broiler oven temperature (fast cooking > 320°C) than the 340°C attained during broiling of steaks stored 40 days and not to moisture lost during frozen storage. Steaks stored 130 days were not different in evaporation and total cooking losses (11.01% and 16.73%, respectively) from those stored 40d although the numerical values for evaporation losses tended to reduce with storage. There were no interactions ($P>0.1$) between collagen level and storage for all cooking losses.

4.3.3 Effects of Cooking Method (Fast vs. Slow)

The effects of heating method on cooking losses ($P<0.0001$) of steaks are manifested in Table 4.5. As expected, cooking losses were different ($P<0.0001$) between fast (broiling) and slow (roasting) cooking. Broiling evaporation (16.28%), drip (6.47%) and total (22.75%) cooking losses were higher ($P<0.0001$) than roasting (6.42%, 4.88% and 11.3%, respectively). Thus, broiling leads to larger cooking losses due to the rate at which moisture arrives at the steak surface

Table 4.4. Effects of storage on mean cooking losses[1].

Storage (Day)	Cooking Losses (%) Evaporation Losses	Drip Losses	Total Cooking Losses
40*	13.00[a]	5.79[a]	18.79[a]
85**	10.04[b]	5.50[a]	15.54[b]
130*	11.01[ab]	5.72[a]	16.73[ab]
SE[2]	0.72	0.20	0.90
Probability			
Storage	0.0498	0.60	0.086
Collagen Level x Storage	0.51	0.34	0.48

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[ab] Means with different superscripts in the same column were significantly different ($P<0.05$).

* Fast cook, oven temperature > 340°C.

** Fast cook, oven temperature > 320°C.

Table 4.5. Effects of cooking method on mean cooking losses[1].

Cooking Method	Cooking Losses (%) Evaporation Losses	Drip Losses	Total Cooking Losses
Fast (broiling)	16.28[a]	6.47[a]	22.75[a]
Slow (roasting)	6.42[b]	4.88[b]	11.30[b]
SE[2]	0.37	0.18	0.46
Probability			
Cooking Method	0.0001	0.0001	0.0001
Collagen Level x Cooking Method	0.046	0.25	0.043
Storage x Cooking Method	0.0308	0.0092	0.0094

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS

[ab] Means with different superscripts in the same column were significantly different (P<0.0001).

from the interior and due to the shrinkage of muscle which further forces moisture from the steak during rapid cooking. Compared to broiling, roasting had a slower rate of heat penetration and a longer cooking time. Roasted products had a higher residual moisture than the broiled steaks. These result agree with Wei et al. (1981) who reported that a dry surface layer forms a heat and mass transfer barrier which results in a low rate of moisture loss and a stabilization of the shape of the products.

Interactions between collagen level and cooking method were significant ($P<0.05$) for evaporation and total cooking losses but not significant ($P>0.2$) for drip losses. For the total cooking losses (Figure 4.2), there were highly significant ($P<0.01$) differences between HC and LC steaks due to broiling but no differences ($P>0.1$) between the two collagen levels due to roasting.

Interactions between storage and cooking method (Figure 4.3) were significant ($P<0.05$) for cooking losses largely due to the lower broiling temperatures of the 85 day products than the 40 and 130 day products. There were no differences ($P>0.05$) among storage days due to roasting.

4.3.4 Effects of End-Point Temperature (50, 70, and 90°C)

The effects of end-point temperature on cooking losses

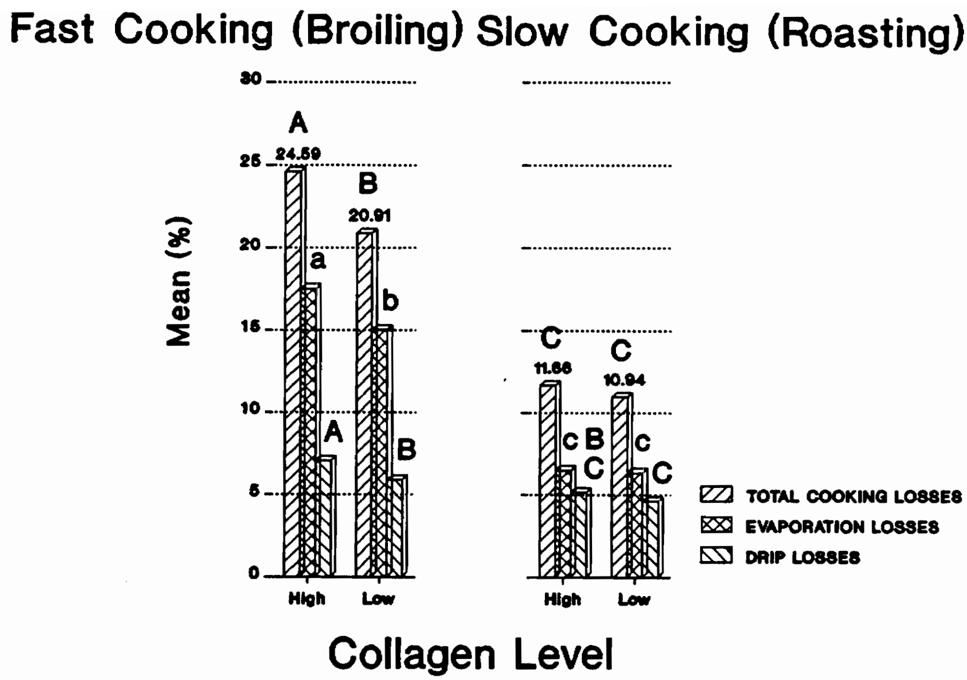


Figure 4.2. Interactions between collagen level and cooking method on cooking losses.
[ABC-abc Means with different letters in the same legend were significantly different ($P < 0.05$).]

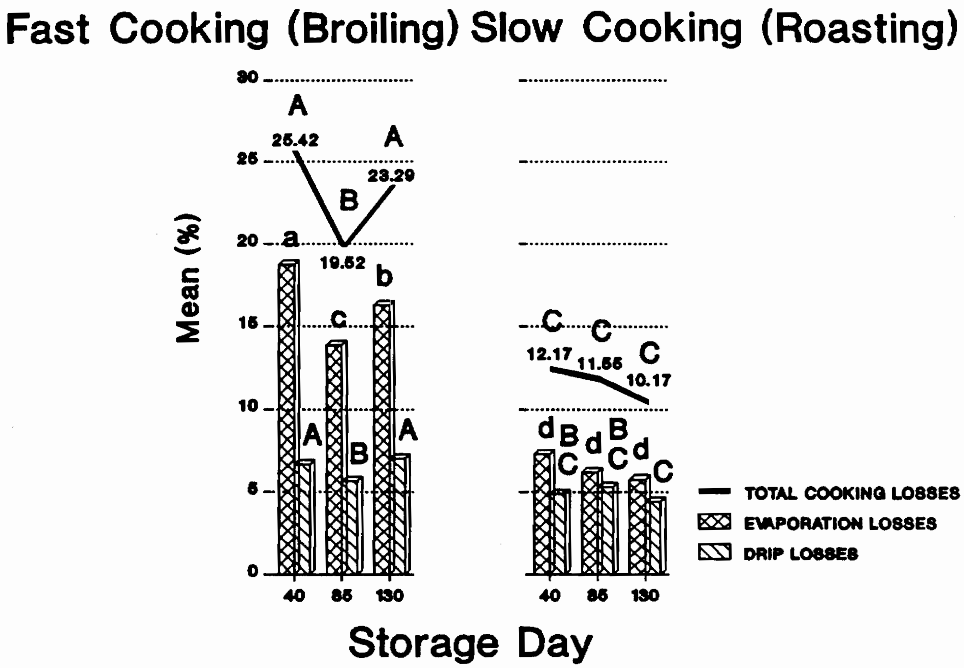


Figure 4.3. Interactions between storage day and cooking method on cooking losses.

[ABC-abc Means with different letters in the same legend were significantly different (P < 0.05).]

(P<0.0001) of steaks are displayed in Table 4.6. Evaporation, drip and total cooking losses (8.46%, 4.11% and 12.57%, respectively) at 50°C were less (P<0.0001) than those at a 70°C (10.75%, 5.58% and 16.33%, respectively), end-point temperature. Similarly, those at 70°C were less (P<0.0001) than those at 90°C (14.84%, 7.32% and 22.16%, respectively). Greater evaporation and total cooking losses are due to increased temperatures which are accompanied by a larger loss of moisture. Yet, increasing drip losses at higher temperatures could be due to variations in the melting point of fat from different sources. Within the beef carcass, the melting point of fat ranges from 40 to 48°C (Jones, 1984).

Interactions between collagen level and end-point temperature were not significant (P>0.1) for all cooking losses. Also, there were no differences (P>0.1) in interactions between storage and end-point temperature for all cooking losses or between cooking method and end-point temperature (Figure 4.4) for evaporation losses (P>0.05). However, the interactions between cooking method and end-point temperature for drip and total cooking losses were highly significant (P<0.01). Within the broiled treatment, drip losses at 70°C were not different (P>0.3) from those at 90°C. Drip was not different (P>0.05) between steaks broiled or roasted to 90°C. Broiling resulted in higher (P<0.0001) total

Table 4.6. Effects of end-point temperature on mean cooking losses[1].

End-Point Temperature (°C)	Cooking Losses (%) Evaporation Losses	Drip Losses	Total Cooking Losses
50	8.46[c]	4.11[c]	12.57[c]
70	10.75[b]	5.58[b]	16.33[b]
90	14.84[a]	7.32[a]	22.16[a]
SE[2]	0.34	0.16	0.41
Probability			
End-Point Temperature	0.0001	0.0001	0.0001
Collagen Level x End-Point Temperature	0.5	0.21	0.26
Storage x End-Point Temperature	0.67	0.65	0.61
Cooking Method x End-Point Temperature	0.076	0.0001	0.004

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[abc] Means with different superscripts in the same column were significantly different ($P<0.05$).

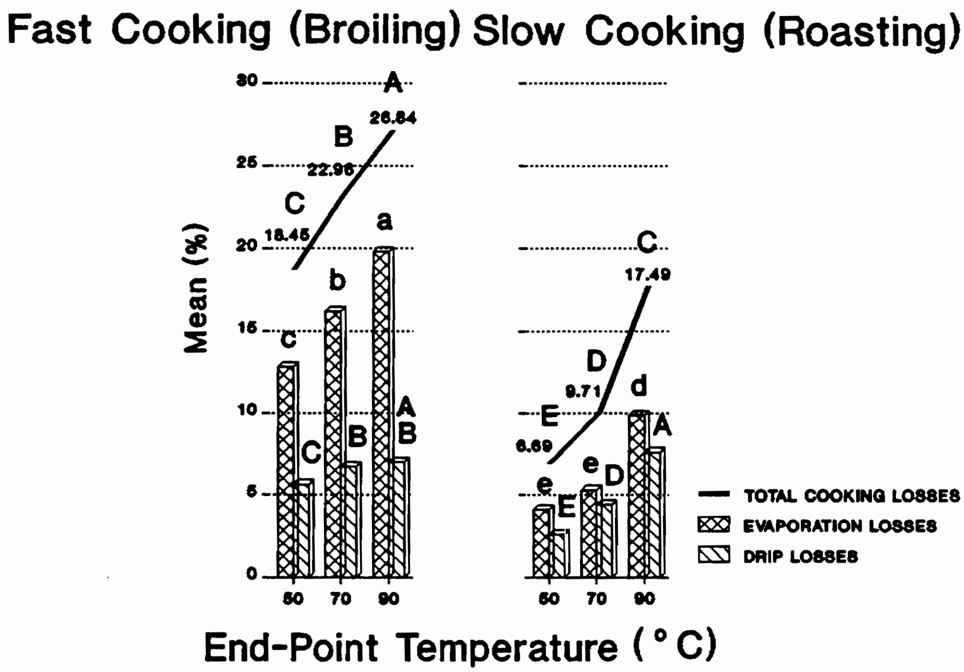


Figure 4.4. Interactions between cooking method and end-point temperature on cooking losses.

[ABC-abc Means with different letters in the same legend were significantly different ($P < 0.05$).]

cooking losses than roasting at each end-point temperature.

4.4. Chemical/Physical Examinations of Raw and Cooked Products

As stated previously (Table 4.1), differences (P>0.1) in moisture, fat, salt contents and pH of HC and LC steaks in the raw state were relatively small. Also, there were no differences in physical dimensions among the raw steaks. But, changes in certain physicochemical values were noted after cooking at selected sampling locations. These sampling locations (Figure 4.5) represented: the point of greatest dimensional change (Site 1 = apex of the dome); the point where a change in dimension began (Site 2 = valley); and a representative area of the steak where no change was seen (Site 3 = non-defective area). The residual moisture contents (Table 4.7) of Sites 1, 2 and 3 (62.38%, 62.18% and 60.51%, respectively) in HC steaks were higher (P<0.05) than similar sites (60.45%, 61.05% and 59.41%, respectively) in LC steaks. In contrast, the residual fat contents of Sites 1, 2 and 3 (14.43%, 14.33% and 13.94%, respectively) for LC steaks were higher (P<0.0005) than those (12.86%, 11.81% and 11.57%, respectively) for HC steaks.

Correlation coefficients for cooking attributes of the steaks are presented in Table 4.8. Higher % moisture in the different sites was significantly (P<0.0001) correlated

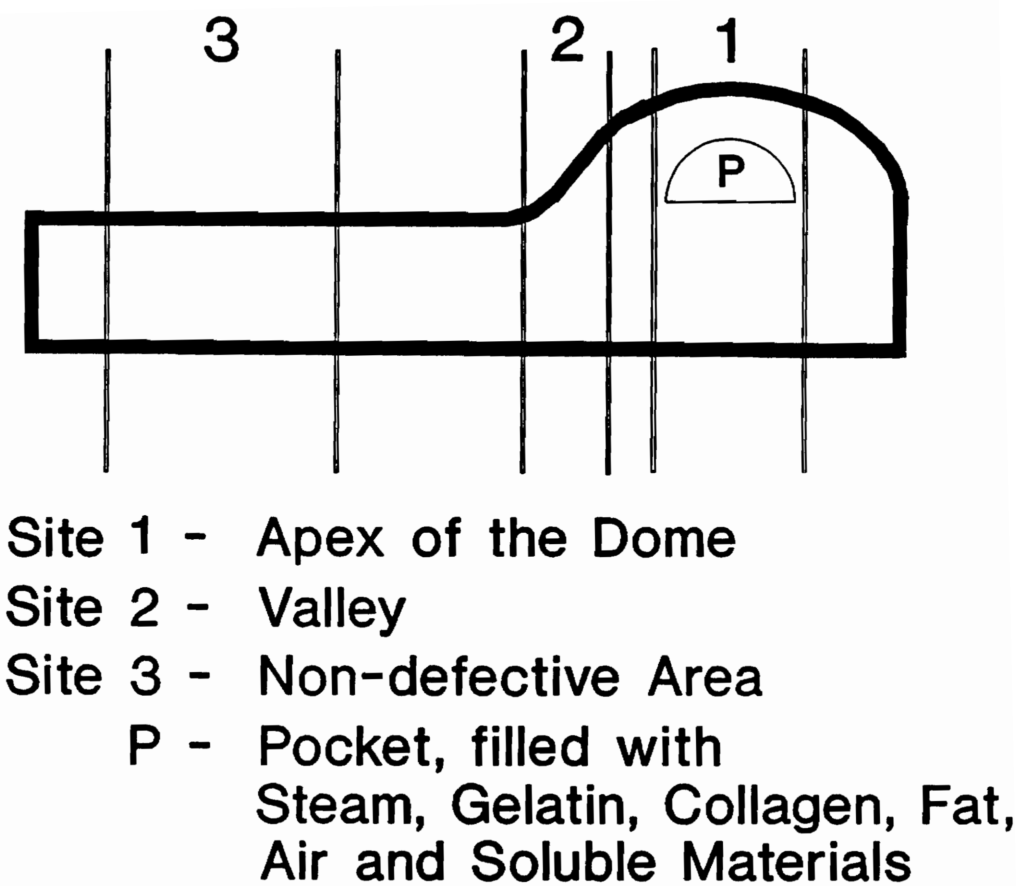


Figure 4.5. Schematic drawing of a cooked steak Sites 1, 2 and 3.

Table 4.7. Proximate composition, salt content, pH and collagen values of raw and cooked products.

Composition	Collagen Levels							
	HC				LC			
	Raw	Cooked			Raw	Cooked		
		Site 1	Site 2	Site 3		Site 1	Site 2	Site 3
% Moisture[1]	64.92[A]	62.38[a]	62.18[a]	60.51[a]	65.20[A]	60.45[b]	61.05[b]	59.41[a]
% Fat[1]	17.73[A]	12.86[b]	11.81[b]	11.57[b]	17.84[A]	14.43[a]	14.33[a]	13.94[a]
% Protein[1]	14.27	21.08	22.06	23.31	13.47	20.71	20.64	22.18
% Salt[2]	2.21	2.65	2.65	2.65	2.22	2.53	2.53	2.53
pH[2]	6.01	6.17	6.17	6.17	6.23	6.00	6.00	6.00
Soluble Collagen[3] (mg/g)	12.97	13.44	-	12.97	7.14	7.33	-	8.62
Insoluble Collagen[3] (mg/g)	51.52	39.32	-	39.28	9.91	12.29	-	12.08
Total Collagen[3] (mg/g)	64.49	52.76	-	52.64	17.05	19.63	-	20.70
Collagen Solubility[3] (%)	20.11	28.96	-	24.88	41.88	37.00	-	41.39

[1] Mean values were based on 3 replications of triplicate samples.
[2] Mean Values were based on treatments, no sites different.
[3] Mean values were based on raw steaks or on particular areas (site 1 and 3) of cooked steaks.
[Aab] Means with different superscripts in the same Site and row were highly significantly different ($P<0.0005$).

Table 4.8. Correlation coefficients with significances[1] for evaporation loss and drip loss related to the composition of cooked restructured beef steaks.

Composition[2] (%)	Evaporation Losses	Drip Losses
M (1)	-0.70^{***}	-0.24^{**}
M (2)	-0.69^{***}	-0.18^{*}
M (3)	-0.76^{***}	-0.29^{***}
F (1)	-0.31^{***}	-0.53^{***}
F (2)	-0.39^{***}	-0.59^{***}
F (3)	-0.27^{***}	-0.55^{***}

[1] Significance of correlation indicated as follows:
* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

[2] Composition:
M (X) = Residual % moisture in Site (X) area.
F (X) = Residual % fat in Site (X) area.

(r = -0.69 to -0.76) with lower evaporation losses. In addition, higher % fat in these sites was significantly ($P<0.0001$) correlated (r = -0.53 to -0.59) with lower drip losses. This result agrees with Goihl et al. (1990) who indicated that drip losses were associated with % residual fat in the cooked meat.

As expected, total collagen contents (Table 4.7) of Sites 1 (52.76 mg/g) and 3 (52.64 mg/g) for cooked HC steaks were still higher than those (19.63 and 20.7 mg/g, respectively) for cooked LC steaks. On the other hand, the collagen solubility of Sites 1 (28.96%) and 3 (24.88%) for cooked HC steaks were still lower than those (37% vs. 41.39%) for cooked LC steaks. The cooked LC steaks had higher total collagen contents at both Sites 1 or 3 than raw ones (17.05 mg/g). This could be due to an uneven collagen distribution within raw products and possibly to gelatin migration during cooking.

The physical measurements of lengths (L1, L2), widths (W1, W2) and heights (H1, H2, H3) of raw and cooked steaks are displayed in Table 4.9. Raw products were not different ($P>0.1$) in thickness at any site. For all cooked steaks observed, lengths and widths were shortened by cooking while heights were generally increased which indicated the formation of a dome. For purposes of this study, two types of domes (typical and slight) were identified. Typical domes had a height (thickness of apex of the dome) and diameter approximately $\geq$ 2 cm and $\geq$ 1.5 cm, respectively, while slight domes

Table 4.9. Effects of collagen levels on mean physical measurements[1] of raw and cooked products.

Physical Measurements (cm)	Collagen Level			
	HC		LC	
	Raw	Cooked	Raw	Cooked
Length 1 (L1)	14.90	12.60	14.96	13.36
Length 2 (L2)	9.00	7.74	9.00	8.28
Width 1 (W1)	6.61	5.54	6.55	5.68
Width 2 (W2)	8.24	7.09	8.19	7.16
Height 1 (H1)	1.32	1.74	1.30	1.50
Height 2 (H2)	1.30	1.35	1.30	1.30
Height 3 (H3)	1.33	1.75	1.32	1.61

[1] Mean values were based on 3 replications of triplicate samples.

were < 2 cm high and < 1.5 cm in diameter. Overall, the cooked HC steaks (99 steaks) had a much higher number of typical domes (158) than the cooked LC steaks (25 in 93 steaks). On the other hand, there were more (133) slight domes on cooked LC steaks than on cooked HC steaks (66).

The correlation coefficients of cooking losses and physical measurements (%) are shown in Table 4.10. Evaporation, drip and total cooking losses (%) were significantly ($P<0.05$) correlated to all the physical measurements (%) except H2 was not highly correlated to drip ($P>0.6$) and total ($0.05<P<0.1$) cooking losses.

Correlation coefficients of physical measurements (%) and proximate composition are shown in Table 4.11. Physical measurements (%) of lengths (L1 and L2), widths (W1 and W2) and H3 were significantly ($P<0.01$) correlated to the residual moisture and fat contents at the different sites. This result agrees with Irmiter et al. (1967) who reported that percent height change of ground beef cylinders caused by cooking was negatively correlated with the fat content of the cylinders.

4.5 Dimensional Changes (Distortions)

4.5.1 Effects of Collagen Level on Physical Measurements

The effects of collagen level on changes in physical

Table 4.10. Correlation coefficients with significances[1] of cooking losses on physical measurements (%) of cooked steaks.

Physical Measurements (%)	Cooking Losses (%)		
	Evaporation Losses	Drip Losses	Total Cooking Losses
Length 1 (L1)	-0.76***	-0.54***	-0.76***
Length 2 (L2)	-0.76***	-0.59***	-0.77***
Width 1 (W1)	-0.60***	-0.41***	-0.60***
Width 2 (W2)	-0.84***	-0.59***	-0.84***
Height 1 (H1)	-0.34***	-0.26***	-0.34***
Height 2 (H2)	-0.14**	-0.03	-0.12*
Height 3 (H3)	-0.56***	-0.45***	-0.58***
Shrinkage	0.64***	0.42***	0.63***
Doming	0.50***	0.37***	0.51***

[1] Significance of correlation indicated as follows:

*: 0.05<P<0.1; **: P<0.05; ***: P<0.0001

Table 4.11. Correlation coefficients with significances[1] of physical measurements (%) on proximate composition of cooked products.

(%) Composition	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
M (1)[2]	0.42***	0.34***	0.32***	0.61***	-0.04	-0.03	-0.21***	-0.51***	-0.14*
M (2)[2]	0.39***	0.36***	0.31***	0.60***	-0.07	-0.08	-0.27***	-0.46***	-0.19***
M (3)[2]	0.50***	0.46***	0.40***	0.68***	-0.16**	-0.10	-0.40***	-0.48***	-0.32***
F (1)[2]	0.43***	0.47***	0.23***	0.31***	-0.24***	-0.18**	-0.38***	-0.15*	-0.38***
F (2)[2]	0.55***	0.58***	0.36***	0.40***	-0.37***	-0.10	-0.46***	-0.24***	-0.45***
F (3)[2]	0.46***	0.51***	0.34***	0.30***	-0.32***	-0.07	-0.34***	-0.22***	-0.36***

[1] Significance of correlation indicated as follows:
*: $0.05 < P < 0.1$; **: $P<0.05$; ***: $P< 0.01$, $P<0.001$ or $P<0.0001$

[2] Based on 3 replications of triplicate samples
M (X) = % Moisture in Site X area; F (X) = % Fat in Site X area

measurements (%) of steaks are shown in Table 4.12. L1 and Doming were slightly different ($0.05<P<0.1$) between HC (-15.42% and 22.71%, respectively) and LC (-11.69% and 12. 1%, respectively) steaks. Only W1 increased more ($P<0.05$) in HC (-16.29%) than in LC (-13.19%) steaks. The other measurements were not ($P>0.1$) different between collagen levels. These results confirm the findings of Berry et al. (1986) which showed that LC steaks resulted in less distortion than HC steaks as evidenced by steak thickness and diameter changes from raw to cooked (%). The HC steaks decreased more in both length and width and increased more in height upon cooking than the LC steaks. The cooked HC steaks tended to increase in all three height measurements and in the number of domes compared to the LC steaks. There was no difference ($P>0.9$) in shrinkage between HC and LC steaks.

4.5.2 Effects of Storage

The effects of storage on physical measurements (%) of steaks are shown in Table 4.13. Due to lower broiler conditions (>320°C) for steaks stored 85 days, there was variation in results among storage days. Steaks changed more in L1 ($P<0.005$) and W2 ($P<0.05$) after 40d storage (-15.16% and -15.69%, respectively) than after 85 (-12.18% and -12.06%, respectively) or 130 (-11.82% and -12.18%, respectively) days.

Table 4.12. Effects of collagen levels on mean[1] percent changes of physical measurements.

Collagen Levels	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
HC	-15.42*	-13.96[a]	-16.29[a]	-13.93[a]	32.16[a]	3.57[a]	32.37[a]	11.74[a]	22.71*
LC	-11.69*	- 8.02[a]	-13.19[b]	-12.68[a]	15.61[a]	0.41[a]	22.02[a]	11.58[a]	12.71*
SE[2]	0.93	1.47	0.49	0.92	4.58	3.86	3.1	0.98	2.27
Probability									
Collagen Levels	0.0694	0.104	0.047	0.43	0.13	0.62	0.14	0.92	0.09

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS

[ab] Means with different superscripts in the same column were significantly different (P<0.05).

* Means in the same column were slightly significantly (0.05 < P < 0.1) different.

Table 4.13. Effects of storage on mean[1] percent changes of physical measurements.

Storage Times	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
40d*	-15.16[a]	-11.75[a]	-15.45[a]	-15.69[a]	24.31[a]	5.90[a]	29.16[a]	13.54[a]	19.83[a]
85d**	-12.18[b]	-10.71[a]	-14.77[ab]	-12.06[b]	27.06[a]	1.42[ab]	24.56[a]	10.26[a]	17.67[a]
130d*	-11.82[b]	-10.51[a]	-14.00[b]	-12.18[b]	20.29[a]	-1.34[b]	27.85[a]	11.18[a]	15.62[a]
SE[2]	0.56	0.53	0.43	0.76	2.27	1.82	2.77	1.05	1.38
Probability									
Storage Times	0.005	0.26	0.12	0.015	0.17	0.06	0.51	0.14	0.16
Collagen Levels x Storage Times	0.35	0.78	0.15	0.49	0.99	0.88	0.57	0.76	0.82

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[ab] Means with different superscripts in the same column were significantly different ($P<0.05$).

* Fast cook, oven temperature > 340°C.

** Fast cook, oven temperature > 320°C.

There were no differences (P>0.1) in these measurements between steaks stored for 85 and 130 days. For L2 (P>0.2), H1 (P>0.1), H3 (P>0.5), Shrinkage (P>0.1) and Doming (P>0.1), there were no differences due to storage. Mean measurements of W1 (P>0.1) and H2 (P>0.05) for steaks stored 40 days were different (P<0.05) from those stored 130d but not (P>0.05) 85d. Furthermore, there were no interactions (P>0.1) between collagen level and storage for all physical measurements. Conclusively, the real causative agent for dimensional changes was temperature and not storage time.

4.5.3 Effects of Cooking Method (Fast vs. Slow)

The effects of cooking method on physical measurements of steaks are exhibited in Table 4.14. Mean physical measurements (%) of fast cooked(broiled) steaks changed (P<0.0001) greater than those of slow cooked(roasted) steaks for all physical measurements except H2 (P>0.05). Interactions between collagen level and cooking method were not significant (P>0.05) for any physical measurement except L2 (P<0.01). However, interactions between storage day and cooking method were significant (P<0.05) for L1, W2 and H2 but not (P>0.1) for the other measurements.

4.5.4 Effects of End-Point Temperature (50, 70, and 90oC)

The effects of end-point temperature on physical measure-

Table 4.14. Mean[1] percent changes of physical measurements on cooking method.

Cooking Methods	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
Fast	-16.90[a]	-14.94[a]	-17.63[a]	-18.76[a]	27.82[a]	4.02[a]	34.91[a]	16.71[a]	22.27[a]
Slow	- 9.21[b]	- 7.04[b]	-11.85[b]	- 7.85[b]	19.95[b]	-0.03[a]	19.48[b]	6.61[b]	13.15[b]
SE[2]	0.31	0.48	0.52	0.37	1.2	1.53	2.33	0.87	1.26
Probability									
Cooking Methods	0.0001	0.0001	0.0001	0.0001	0.0006	0.0848	0.0005	0.0001	0.0003
Collagen Levels x Cooking Methods	0.052	0.0015	0.61	0.12	0.51	0.99	0.36	0.15	0.46
Storage Times x Cooking Methods	0.013	0.17	0.7	0.0104	0.2823	0.018	0.17	0.67	0.27

[1] Mean values were based on 3 replications of triplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[ab] Means with different superscripts in the same column were significantly different ($P<0.05$).

ments (%) are presented in Table 4.15. Changes in the L1, L2, W1, W2, and H3 dimensions and the number of domes on steaks ($P<0.0001$) cooked to a 50°C end-point temperature were less ($P<0.0001$) than for those cooked to 70°C. Also, changes for all physical measurements except H1 and H2 in steaks cooked to 70°C were much less than those of steaks cooked to 90°C.

These results basically verify the work of Campbell et al. (1977) which indicated that area change increased linearly as the cooking temperature increased. Ritchey and Hostetler (1964) indicated that the dimensional changes (shortening of muscle fibers) in two muscles, longissimus dorsi and biceps femoris, during cooking to 74 - 80°C were accompanied by a larger loss of water. Dube et al. (1972) found that when increasing the temperature of cooking a myofibrillar extract resulted in gradual shortening of sarcomeres with a maximum change taking place in the 70-80°C range.

There were significant interactions ($P<0.05$) between collagen level and end-point temperature for L1, L2, H2, H3, Shrinkage and Doming but not for the other measurements. Also, there were significant interactions ($P<0.05$) between storage day and end-point temperature for H1, H2, H3, Shrinkage and Doming but not the other measurements. There were no interactions ($P>0.07$) between cooking method and end-point temperatures for L1, L2, W1, W2, H1, H2, shrinkage or doming. However, a cooking method by end-point temperature inter-

Table 4.15. Mean[1] percent changes of physical measurements on end-point temperature.

End-Point Temperature	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
50°C	-11.06[c]	- 8.97[c]	-12.72[c]	-10.57[c]	20.85[b]	1.33[a]	19.31[c]	10.14[b]	13.83[c]
70°C	-12.62[b]	-10.82[b]	-14.91[b]	-12.86[b]	23.54[ab]	3.96[a]	24.71[b]	11.32[b]	17.44[b]
90°C	-15.48[a]	-13.19[a]	-16.54[a]	-16.49[a]	27.27[a]	0.69[a]	37.56[a]	13.53[a]	21.85[a]
SE[2]	0.43	0.36	0.57	0.49	1.61	1.41	1.41	0.7	0.93
Probability									
End-Point Temperature	0.0001	0.0001	0.0001	0.0001	0.0199	0.22	0.0001	0.0029	0.0001
Collagen Level x End-Point Temperature	0.0478	0.0491	0.56	0.14	0.23	0.007	0.0171	0.0013	0.0019
Storage x End-Point Temperature	0.42	0.6	0.37	0.17	0.016	0.017	0.01	0.005	0.0001
Cooking Method x End-Point Temperature	0.16	0.075	0.69	0.12	0.17	0.58	0.033	0.15	0.07

[1] Mean values were based on 3 replications of triplicate samples.
[2] SE = Standard Error, based on LS MEANS.
[abc] Means with different superscripts in the same column were significantly different ($P<0.05$).

action ($P<0.05$) for H3 existed.

Distortions are caused by a variety of factors. This explains why the problems are not easy to control in practice since the variable interactive effects would be difficult to identify under the conditions of commercial processing. A reduction in volume affects distortion and generally larger volumes influence doming. Contrary to expectation, there was a decrease in length and width accompanied by an increased thickness due to the typical or slight dome formations. However, these domes may not occur at either H1, H2 or H3 locations or near the sites where L1, W1 or W2 were measured. These results generally agree with those of Jolley and Rangeley (1986) except the physical measurements of L2, W2 and H2 differ due to manufacturing.

4.6 Mechanism of Distortions

The phenomena of doming may be explained by contrasting the characteristics of Site 1 (apex of the dome) and Site 3 (non-distorted control) in the same cooked steaks. At Site 1, the surface of the dome (pocket) was firmly sealed so that the steam and melted fat mixture was entrapped. As pressure increased a dome was formed and residual moisture and fat contents increased. Conversely,the release of vapor from the porous surface of the non-distorted areas (Site 3) prevented an increase in internal pressure and yielded lower residual moisture and fat contents in the meat tissues.

The sarcolemma of muscle fibers is the major barrier against mass transfer (Locker and Daines, 1974; Davey and Gilbert, 1975; Hunt et al., 1978) and although damaged in a flaked product it may remain an effective barrier to fluid transfer. It is claimed that complete layers of parallel muscle fibers primarily seal domes (Figure 4.6) against moisture loss during cooking even though increasing vapor pressure and temperature accelerate collagen shrinkage and solubilization as well as gelatin migration. To examine this claim, Site 1 (dome) and Site 3 (control) in cooked steaks were used as the contrast points. Therefore, this study was intended to investigate potential mechanisms for dome formation and channel development.

4.6.1 PCM Observations

Phase contrast micrographs of manufactured raw HC steaks are exhibited in Figure 4.7-4.8. Sections stained with Oil Red O (lipids, red color), H & E (standard staining) and Masson's Trichrome (myofibrillar components, red color; collagen, blue color) Stains provided confirmation of the nature of these components. Muscle strands with less inter-fiber space were randomly crossing and overlapping each other; intact or broken muscle fibers (MF) were due largely to agita-

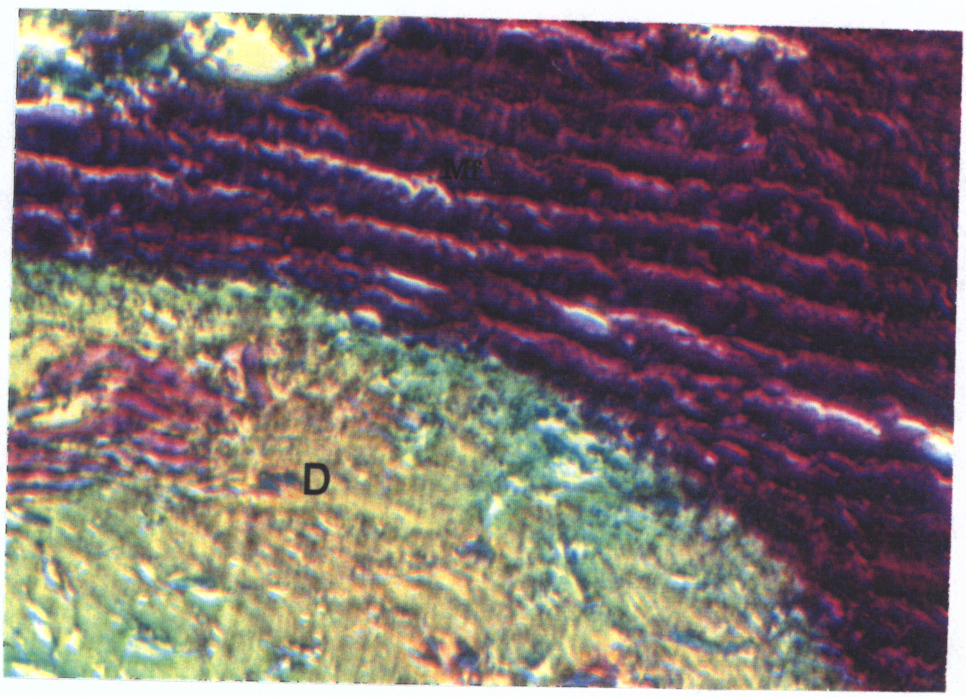


Figure 4.6. Phase contrast micrograph of the distorted site of cooked (fast cook, 50°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 400X. Complete parallel muscle fibers (dark red, Mf) sealed the growing dome (D) in Site 1.

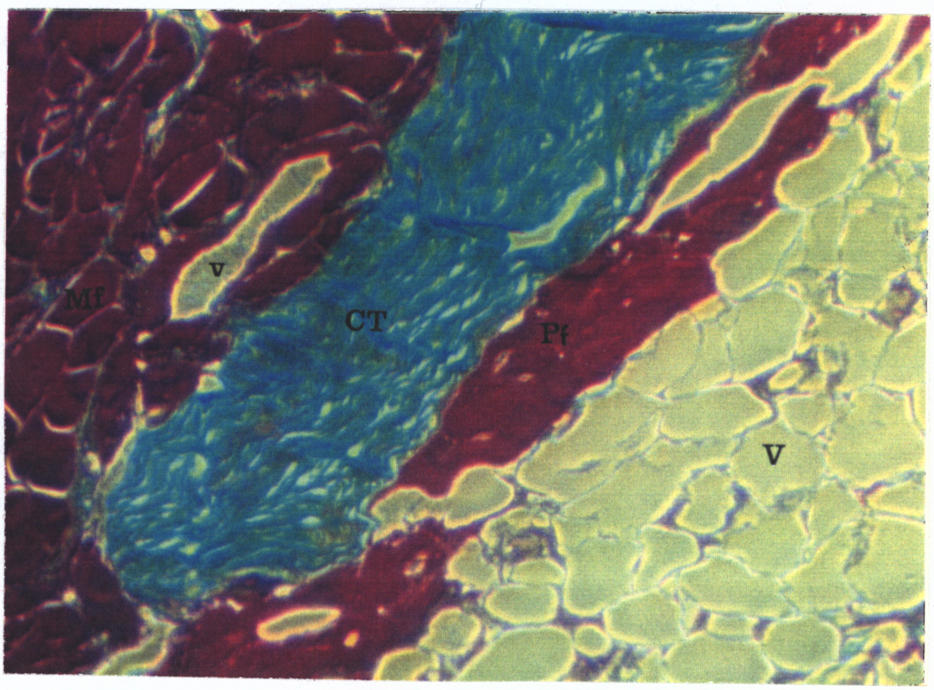


Figure 4.7. Phase contrast micrograph of a HC raw product. Bouins solution, Masson's Trichrome Stain, 100X. Cross section of this product shows muscle fibers (dark red, Mf), dense connective tissue (blue, CT), protein films (Pf) and voids (V) which could be adipose tissues or hallow areas.

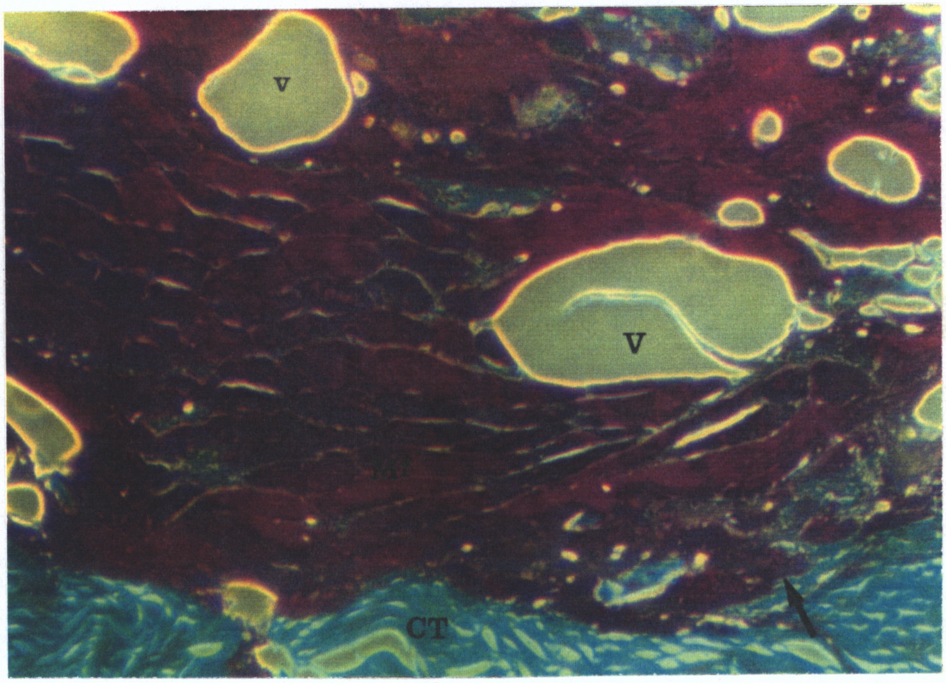


Figure 4.8. Phase contrast micrograph of a HC raw product. Bouins solution, Masson's Trichrome Stain, 400X. It shows muscle fibers (Mf), dense connective tissue (CT), soluble proteins (arrow) and voids (V) which could be air, water and/or fat components.

tion. Connective tissue fibers (CT) comprising the intact or broken epi-, peri- and/or endomysium were observed to be more abundant and denser in the HC steaks than in the LC steaks. Clouds of salt-soluble proteins and fragments from fiber disruption and protein films (PF) existed to bind and cover meat pieces and to hold fat components and some voids (V). Collagen membranes generate and stabilize adipose tissue (Lewis, 1979). Collagen fibers (i.e. mostly perimysium) are arranged in the usual sheet-like array and angular orientation of crimped structure with very little space between fibers. Some voids resulting from trapped air (by mixing) or water (by melting larger ice crystalline during initial slow freezing and/or frozen storage) were observed. Some of these voids probably resulted from loss of fat components during staining or sectioning in H & E and Masson's Trichrome Stains.

In the cooked steaks, Sites 1 and 3 (Figure 4.9) shows the typical pocket (P) possibly created by steam, air, denatured collagen, gelatin, fat components, salt-soluble proteins and dissolved materials. Layers of parallel muscle fibers, protein films (coats) and some voids (V) sealed and surrounded the pocket.

Typical pockets existing in Site 3 were small or dried out (Figure 4.10) when fast cooked at a higher temperature. In the majority of cases, there was lack of sufficient layers of parallel muscle fibers in Site 3 to act as barriers to

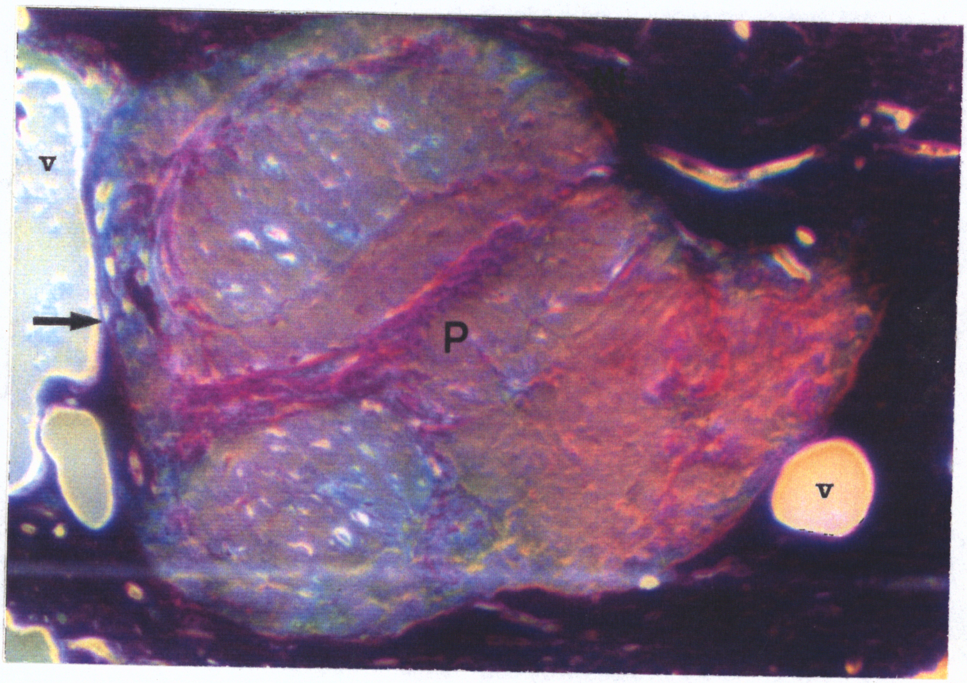


Figure 4.9. Phase contrast micrograph of the distorted site of cooked (fast cook, 50°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 100X. A typical pocket (P) which created possibly by steam, air, denatured collagen, gelatin, fat components, thin protein films and dissolved materials was surrounded and sealed by parallel muscle fibers (Mf), protein films (arrow) and some voids (V) in the vicinity of the pocket.

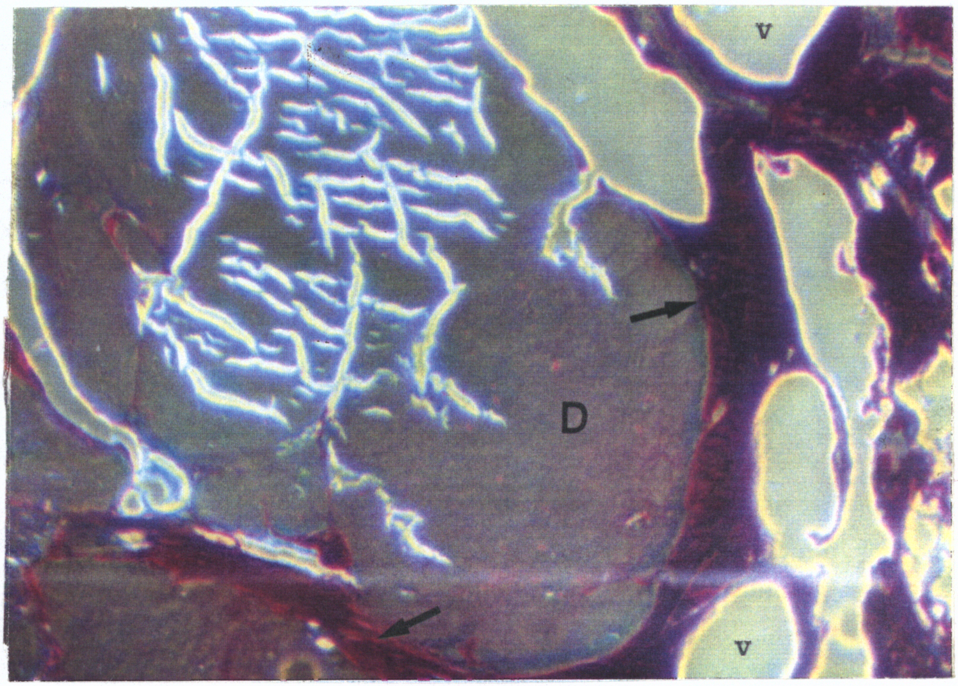


Figure 4.10. Phase contrast micrograph of the non-distorted site of cooked (fast cook, 90°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 100X. A dried dome (D) with some voids (V) happened in Site 3, indicating that there was insufficient protein films (arrows) even though there was a chance to form a dome.

effectively seal and to inhibit the growing domes. Moreover, promiment channels were located between muscle strands connecting the interior and exterior portions of the steak (Figure 4.11). Water transport through the surface of meat sample occurs as vapor diffusion in a capillary-porous system (Skjöldebrand and Hallström, 1980). Voids within cooked steaks increased in number and size and even formed channels due to the poor protein matrix (Figure 4.12) which allowed more moisture and fat components to escape into the atmosphere and/or meat drippings. Therefore, the examination of sections cut (Figure 4.13) from the surface of cooked steak shows the structure of Site 3 to be no more closed than that of Site 1 of similarly cooked steak.

Areas of coagulation (Figure 4.14) as well as intact collagen fibers suggest that smaller collagen fibers are affected first by heat treatments which probably initiate other components to contribute to the enlarging dome (D, Figure 4.15). This mixture of components was divided by some soluble proteins or emulsions which indicates the mixture migrated within the dome. Tornberg et al. (1990) indicated that myosin can make gels and also hold gelatin. Furthermore, because gelatin is a protein, it has a good surface activity and helps to stabilize other phases that may be gaseous, liquid or fibrous (Stainsby, 1987). According to the findings of Hamm and Deatherage (1960) and Harrington and Patestos (1987), the

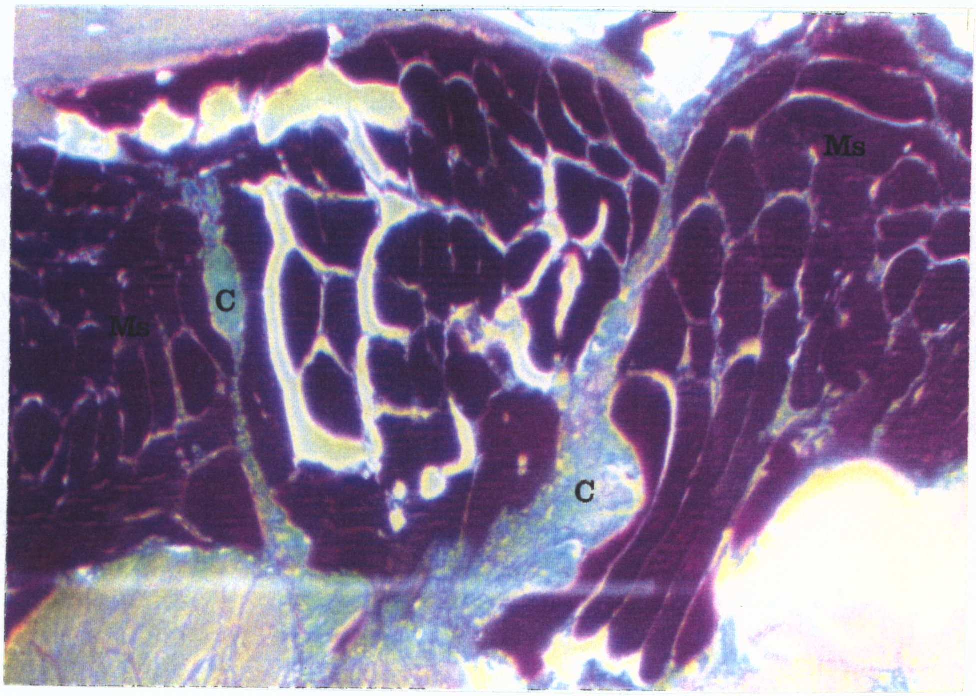


Figure 4.11. Phase contrast micrograph of the non-distorted site of cooked (fast cook, 90°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 100X. Cross section of interior of Site 3 shows prominent channels (C) were located between muscle strands (Ms) connecting the interior with exterior portion of the steak.

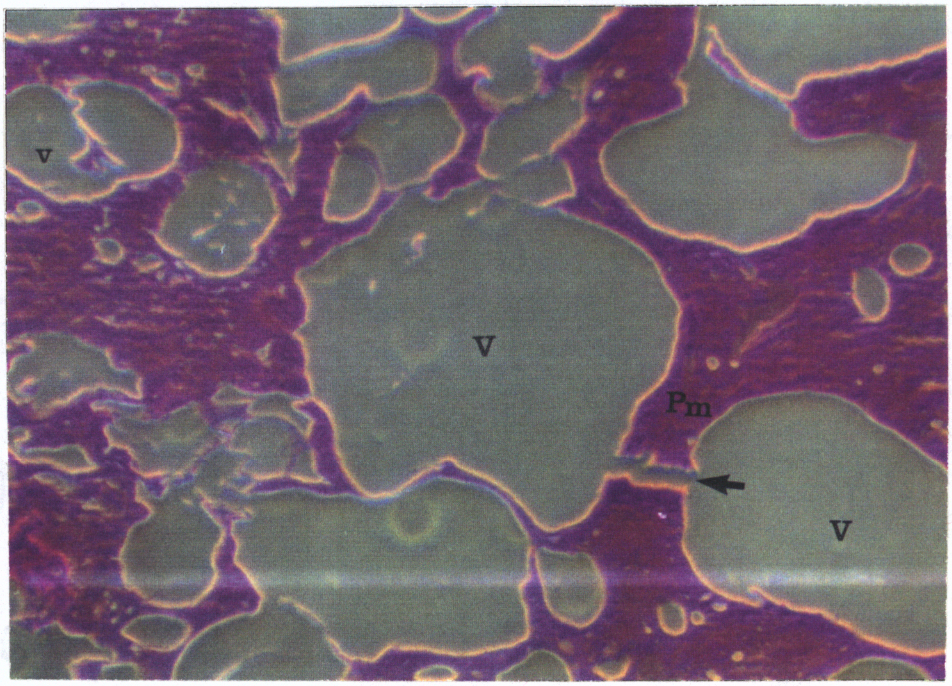


Figure 4.12. Phase contrast micrograph of the non-distorted site of cooked (slow cook, 90°C end-point) LC steaks. Bouins solution, H & E, 100X. Voids (V) within cooked steak increased in size and formed channels (arrow) within the protein matrix (Pm).

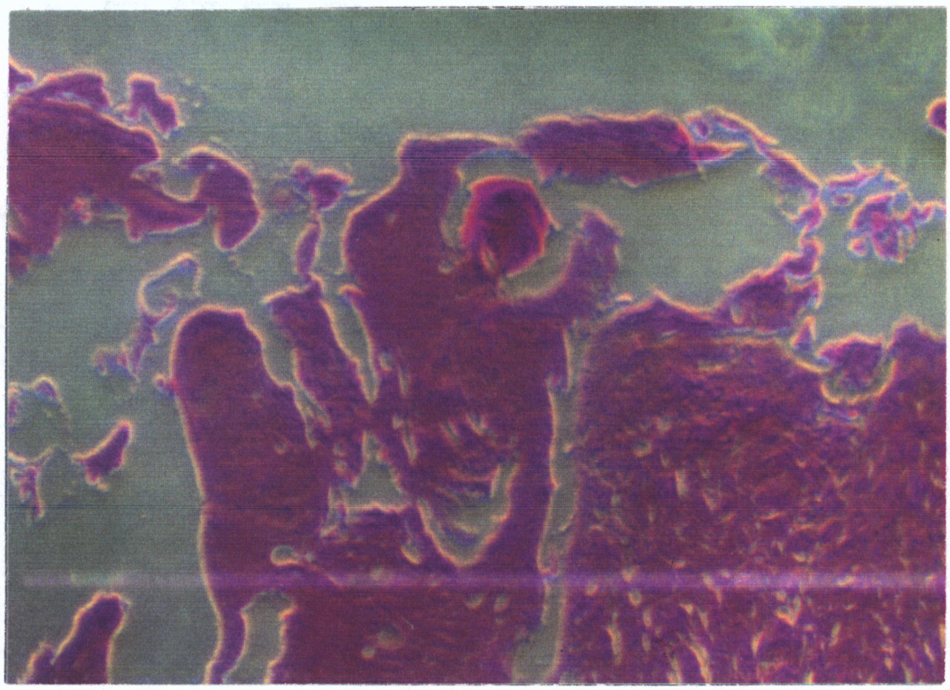


Figure 4.13. Phase contrast micrograph of the non-distorted site of cooked (slow cook, 90°C end-point) HC steak. Bouins solution, H & E, 100X. The structure of Site 3 was no more closed than that of Site 1 of similarly cooked steak.

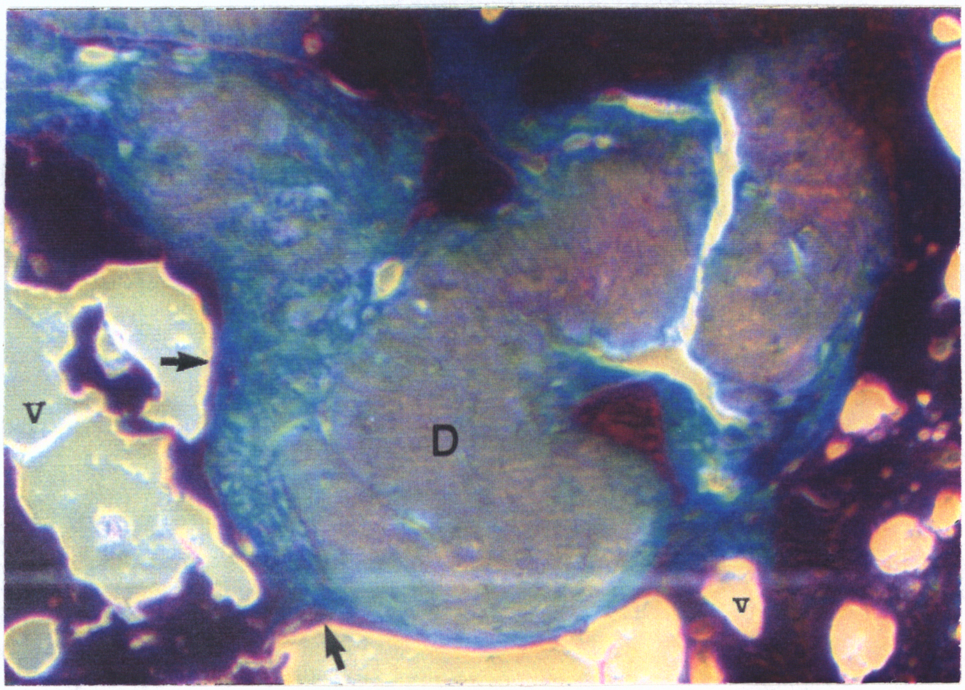


Figure 4.14. Phase contrast micrograph of a distorted site in fast cooked 50°C end-point HC steak. Bouins solution, Masson's Trichrome Stain, 100X. An initial collagen coagulation and the potential forming of a dome (D) with visible protein films (arrows) and voids (V) happened in Site 1 at a lower temperature. Some denatured collagen (blue) still could be seen and the other already turned to gelatin (pink).

proteins not found in extracts from uncooked muscle were detected both in extracts from cooked muscle and in meat drippings during cooking. Closer observation of the clusters of connective tissue (Figure 4.15) reveals the process of collagen coagulation to be more extensive than observed from 50^{o}C-heated samples (Figure 4.14).Many "minivoids" exist within the dome with larger voids in the vicinity of the dome(Fig. 4.15). Site 1 (Figure 4.16) also shows the extreme damage and more twisting along muscle fibers when fast cooked to a higher temperature; moreover, the denatured parallel muscle fibers, coagulated soluble proteins (protein films or emulsions), cavities and/or voids seal and enclose the domes (Figure 4.17).

Fat components were gradually rendered out during cooking at increased temperatures. When slow cooked to a lower temperature, Site 3 (Figure 4.18) still had locations that clumps of fat components (fat globules) existed inside the steaks. Yet, closer to the surface of the steak, there appeared to be agglomerated fat globules due to the movement of the particles toward the higher temperature on the steak surface. When fast cooked at a higher temperature, Site 1 exhibits randomly distributed fat droplets which could result from original fat globules being exuded. Fat droplets can be easily seen without protein films covering them. According to Table 4.8, Site 1 had higher

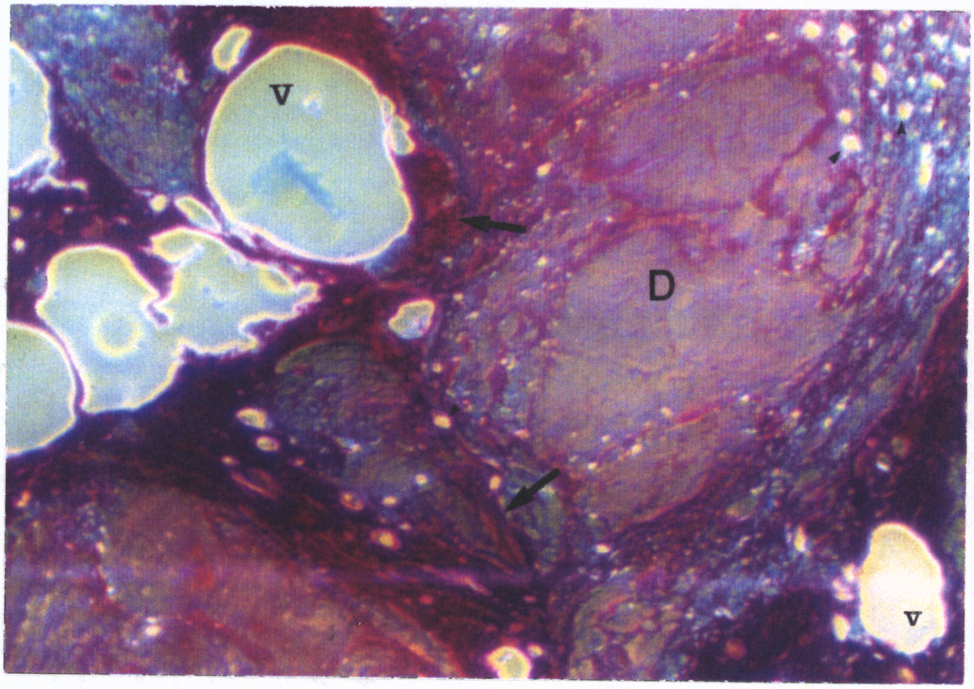


Figure 4.15. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 100X. At a higher temperature, the formation of dome (D) could come from fluid migration and be stabilized by sufficient binding of protein films (arrows) and the process of collagen coagulation was more extensive than observed for 50°C-heated sample. Some microvoids (arrowheads) and larger voids (V) could also be seen.

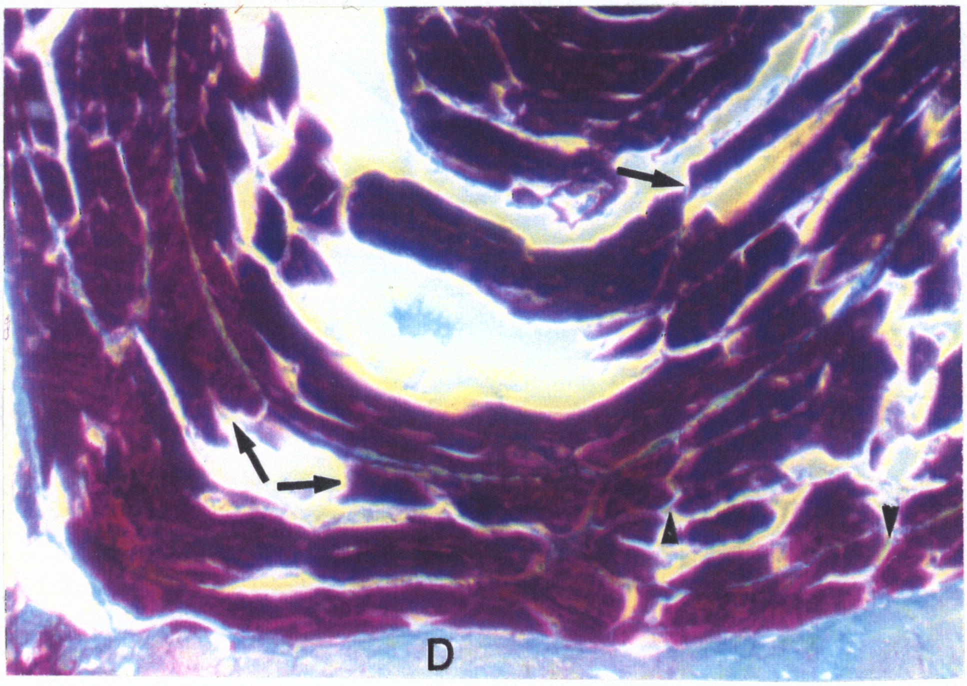


Figure 4.16. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 400X. At a higher temperature, severe damage was along muscle fibers, such as cracks (arrowheads) and breaks (arrows), and a dome (D) nearby.

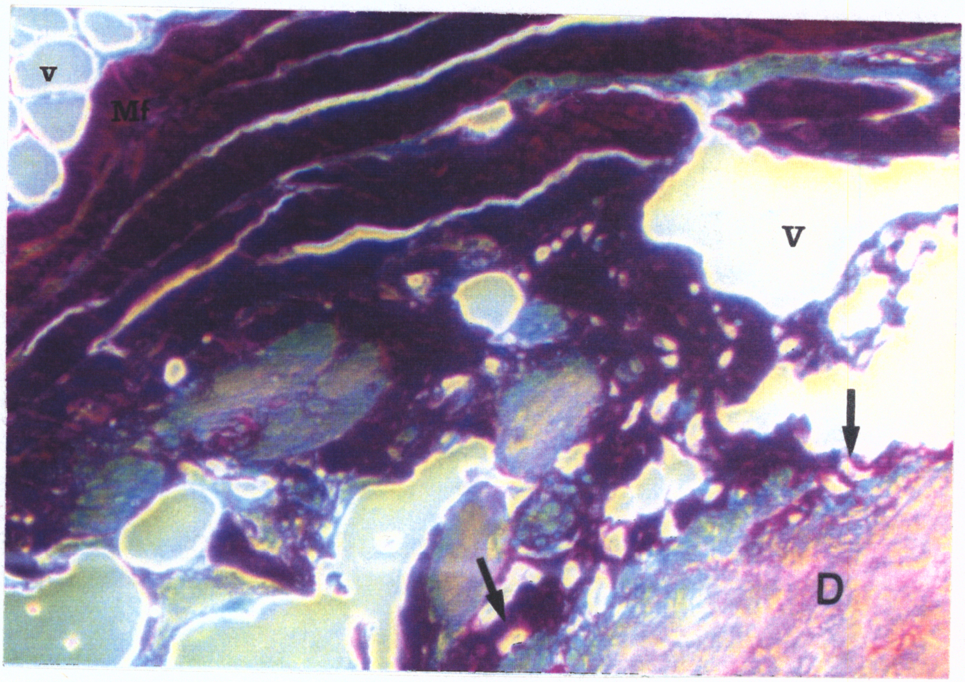


Figure 4.17. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) HC steak. Bouins solution, Masson's Trichrome Stain, 400X. Parallel wavy muscle fibers (Mf), protein films (arrows) and voids (V) were observed to seal and enclose the dome (D).

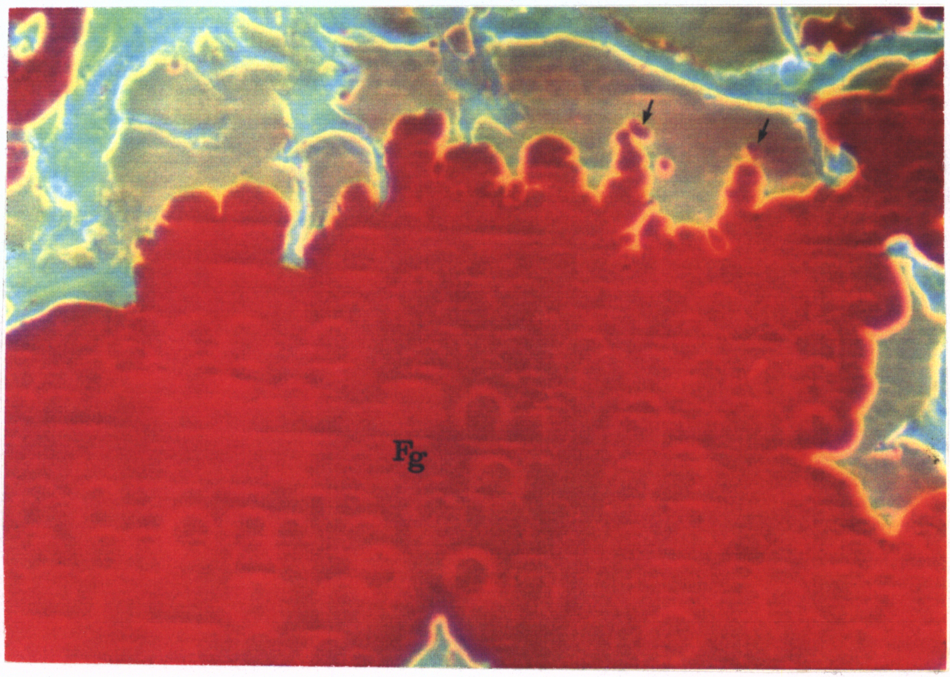


Figure 4.18. Phase contrast micrograph of the non-distorted site of cooked (slow cook, 50°C end-point) LC steak. 10% buffered formalin, Oil Red O, 100X. At slow cooking to a lower temperature, the fat globules (red, Fg) initially melt and exude droplets (arrows).

highest temperature on the steak surface. When fast cooked at a higher temperature, Site 1 exhibits randomly distributed fat droplets which could result from original fat globules being exuded. Fat droplets can be easily seen without protein films covering them. According to Table 4.8, Site 1 had higher residual fat than Site 3 at both collagen levels. Therefore, these fat components rendered totally into smaller droplets (Figure 4.19) instead of draining out of the steaks.

4.6.2 SEM Observations

The first part of this study was designed to further examine the typical domes in Site 1 vs. the reference point in Site 3 by SEM which corresponds to surface topography. The characteristics of connective tissue and muscle fibers arrangement found in meat was not visible in the raw restructured product (Figure 4.20), indicating that restructuring had a greater influence on the microstructure of the raw or even cooked products. A film-type protein exudate composed of mostly myofibrillar proteins extracted by salt during mixing, mostly covered the raw product surfaces and fat components. Raw LC steak (Figure 4.21) have protein films and a protein matrix with numerous fat droplets on their surfaces due to a fine dispersion of the fat (Koolmees et al., 1989). Possibly, these droplets with protein films are the initial or potential sites of "pores" during cooking. The air cavities apparent in

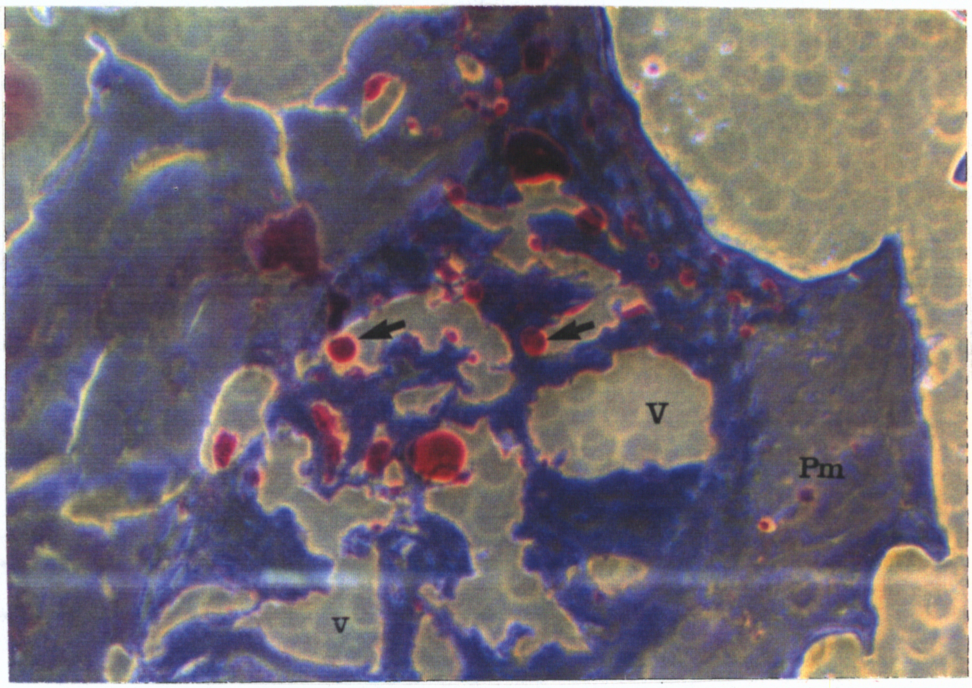


Figure 4.19. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) HC steak. 10% buffered formalin, Oil Red O, 100X. At fast cooking to a higher temperature fat globules did not exist instead of rendering excessive fat droplets (arrows). Some voids (V) existed.

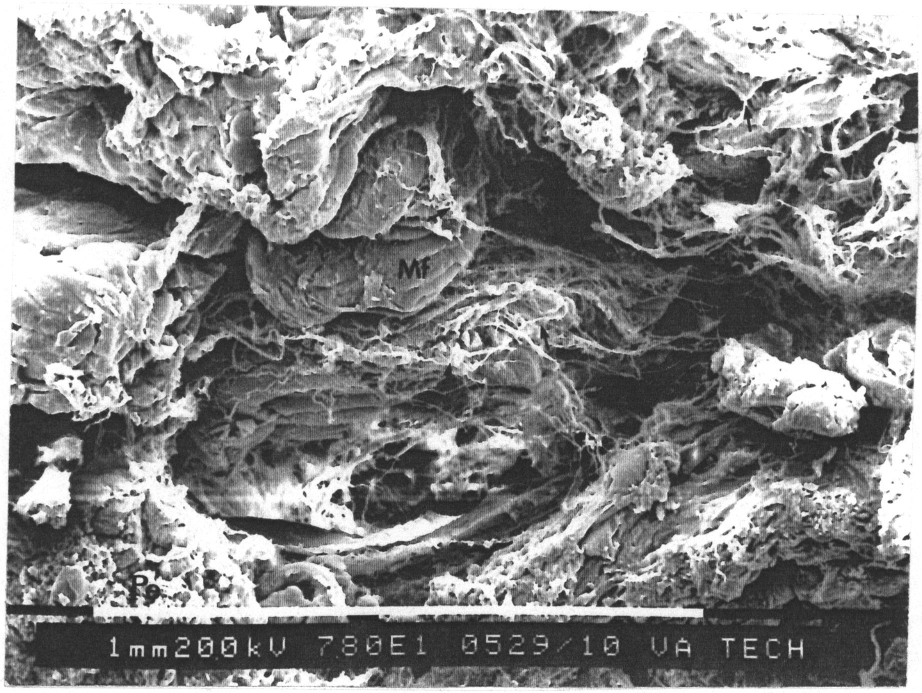


Figure 4.20. Scanning electron micrograph of a HC raw product. The broken muscle fibers (Mf) were covered by raw collagen fibers (arrows) and protein exudate (Pe). Bar represents 1 mm. Magnification is 78X.

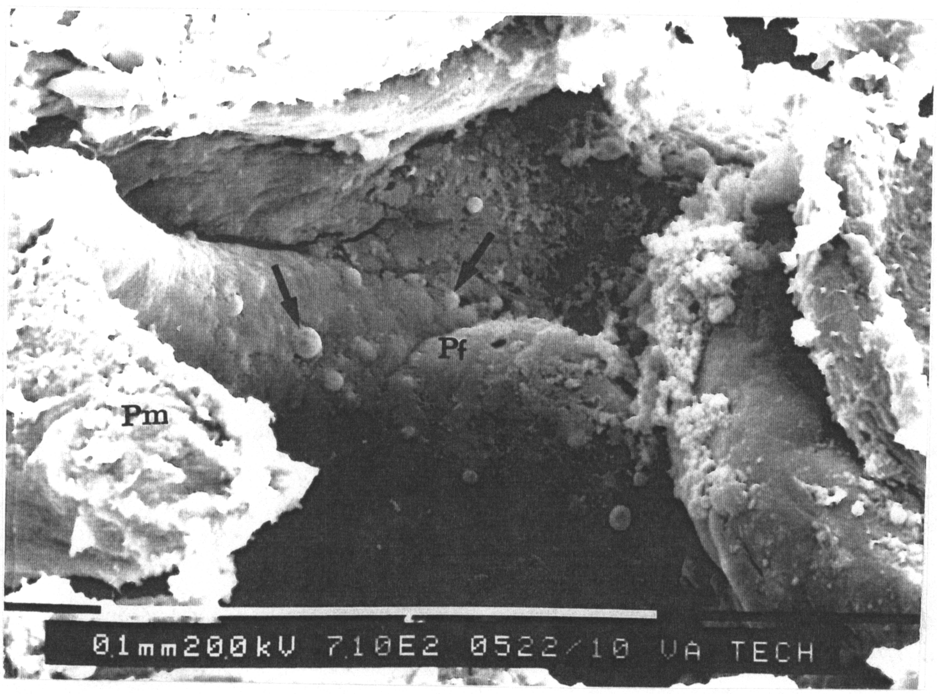


Figure 4.21. Scanning electron micrograph of a LC raw product. Protein films (Pf) and matrix (Pm) had numerous fat droplets (arrows) on their circumference; possibly, these droplets were the initial, potential sites of "pores" for further exudation during cooking. Bar represents 0.1 mm. Magnification is 710X.

the raw products suggest that a mixture of ice, air and perhaps fat rather than air alone may be responsible for this porosity. This would happen if the product undergoes a slow freezing process or repeated freezing-thawing cycles during storage (Bernal and Stanley, 1986).

Examinations after heat treatments show that the protein exudate covering the surfaces of the meat pieces or their products was denatured during cooking. Heat treatments revealed a rather damaged fibrous structure laying underneath this exudate. Fat components without protein films were observed in the cooked products in agreement to previous PCM observations (Figure 4.19). Perhaps the amount of protein exudate was not sufficient for optimal fat binding. Jones and Mandigo (1982) proposed that fat was exuded through pores which developed at weak points in the denatured protein films during cooking in response to internal pressure build-up. Therefore, shrunken fat globules (Figure 4.22) were shown using slow cooking to a higher temperature with collagen membranes also being denatured. When fast cooked to a higher temperature, great gaps (Figure 4.23) led to more extensive fat and moisture losses due to insufficient protein binding existing between meat pieces allowing vapor and juices to flow from the interior to the exterior. Again, due to insufficient protein binding between meat pieces, protein films (coats) cover this area in Site 3. There was a breakage of muscle

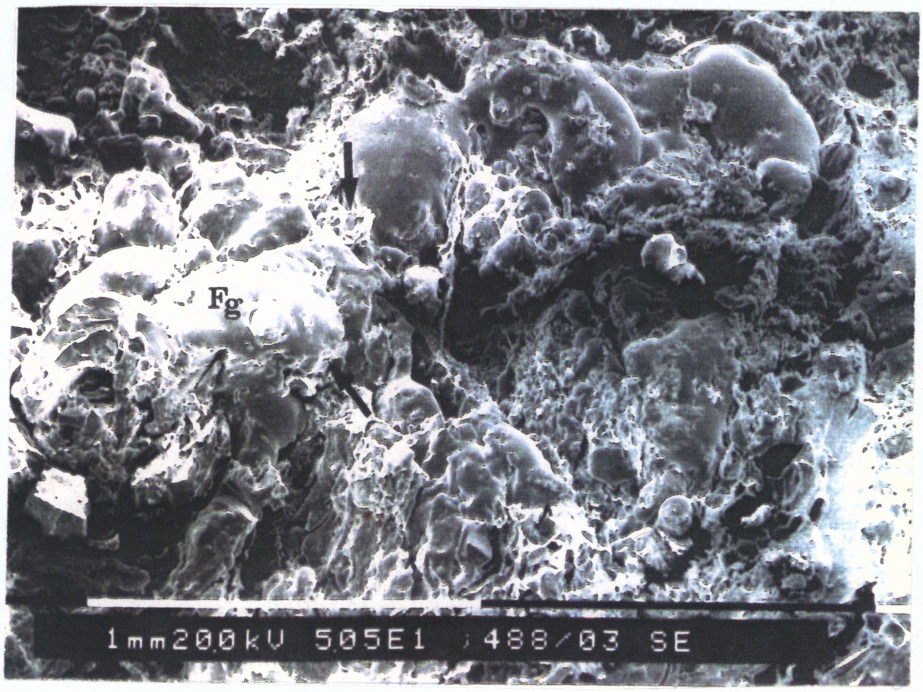


Figure 4.22. Scanning electron micrograph of the non-distorted site of cooked (slow cook, 90°C end point) HC steak. Shrunken fat globules (Fg) were covered by denatured connective tissue membranes (arrows). Bar represents 1 mm. Magnification is 50.5X.

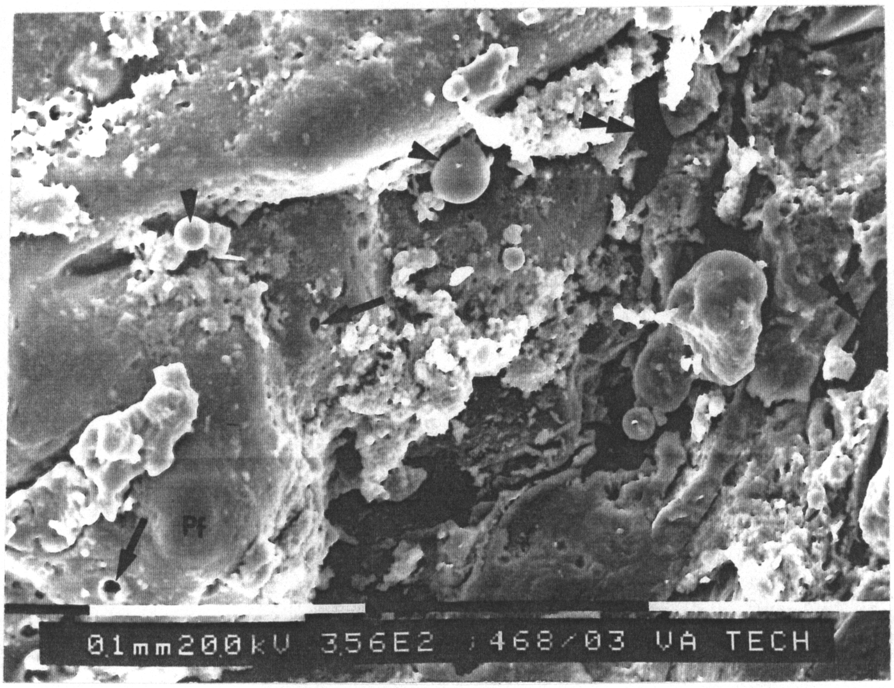


Figure 4.23. Scanning electron micrograph of the non-distorted site of cooked (fast cook, 90°C end point) HC steak. Fat droplets (arrowheads) rendered through the "pores" (arrows) of denatured protein films (Pf) covering the exhausted fat globules. There was the breakage (double arrowheads) of the muscle fibers. Bar represents 0.1 mm. Magnification is 356X.

fiber and some gaps or voids on the protein films (Figure 4.24) when cooking to a higher temperature fat exudation was extended, so that some pores were carrying exuded fat droplets and others were sealed by protein films or remained open due to insufficient soluble proteins and protein denaturation.

Site 1 (dome apex) had higher residual moisture and fat. This indicated that Site 1 had dense protein matrix to trap moisture and fat. Moreover, Figure 4.25 shows that excessive protein film (PF) cover and seal this area due to part of the protein films still on the top of exuding droplets. This implies that a resistance was involved in Site 1 to fluid migration from the interior to the exterior under the same cooking method compared to Site 3 (Figure 4.24). Protein films covered and sealed a growing dome in Figure 4.26 at slow cooking to a higher temperature, and also, additional denatured collagen provided more strength to support this dome. During fast cooking to a higher temperature there was a rapid extrusion of fluid (Figure 4.27) from the interior.

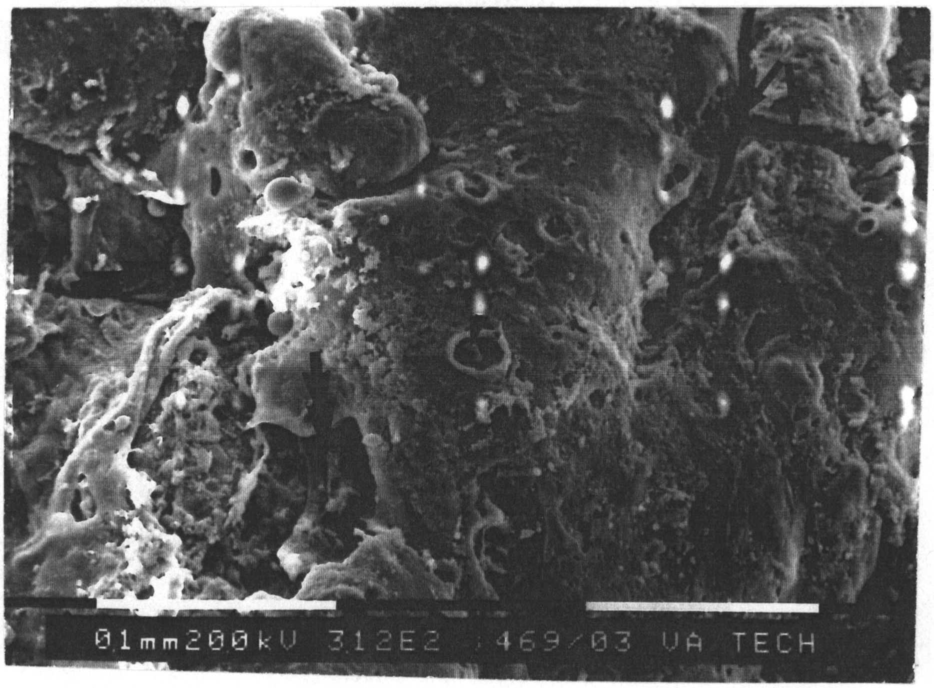


Figure 4.24. Scanning electron micrograph of the non-distorted site of cooked (slow cook, 90°C end-point) HC steak. Due to insufficient protein films (Pf) occurred in Site 3. There were more moisture losses through the breakage (arrows) of muscle fiber and the gap (double arrows) of protein films. Bar represents 0.1 mm. Magnification is 312X.

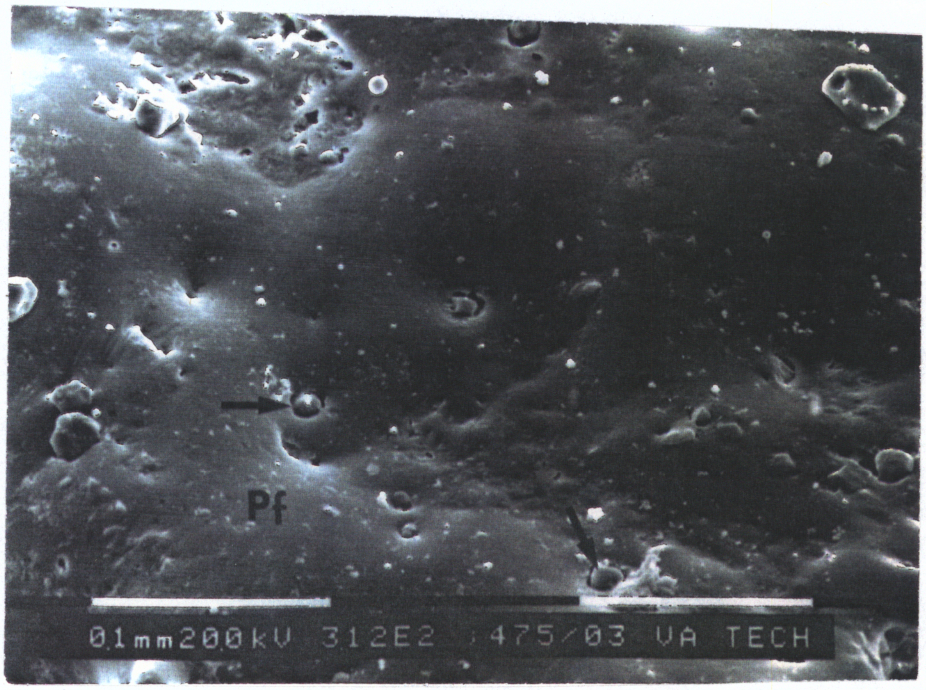


Figure 4.25. Scanning electron micrograph of the distorted site of cooked (slow cook, 90°C endpoint) HC steak. Due to sufficient protein films (Pf), the exuded components (arrows) with part of the protein film on top were trying to come out from interior. Bar represents 0.1 mm. Magnification is 312X.

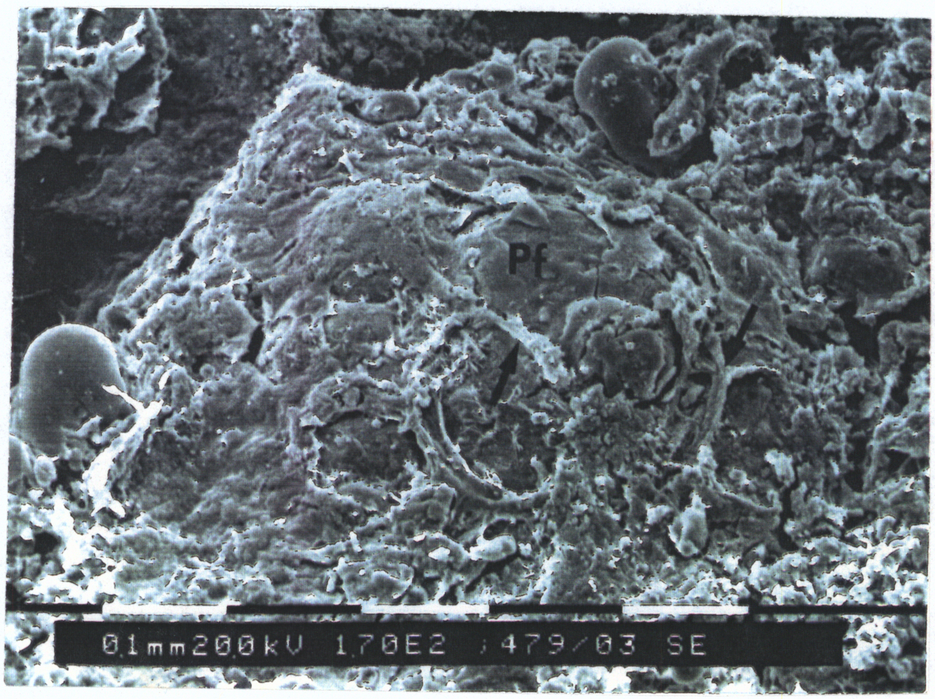


Figure 4.26. Scanning electron micrograph of the distorted site of cooked (slow cook, 90°C endpoint) HC steak. Due to sufficient protein films (Pf) existed, additional denatured collagen (arrows) provided more strength to support the dome. Bar represents 0.1 mm. Magnification is 170X.

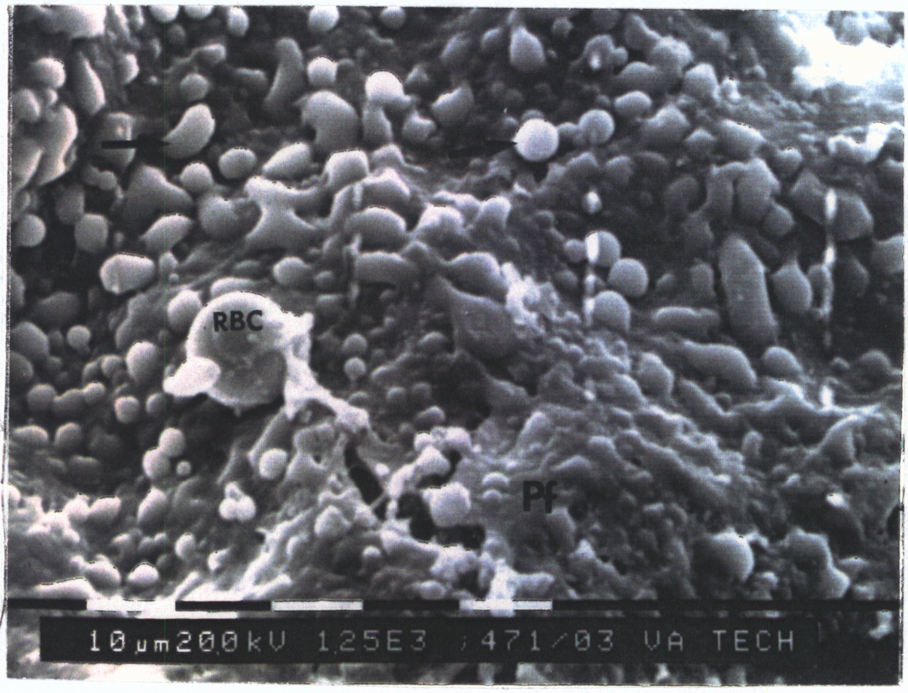


Figure 4.27. Scanning electron micrograph of the distorted site of cooked (fast cook, 90°C endpoint) HC steak. Due to the rapid cooking at 90°C, these exuded fluids (arrows) tended to migrate in a new area, indicating that there was a dome under this broken protein film (Pf) which was no longer to seal the dome. (RBC - Red Blood Cell). Bar represents 10 μm. Magnification is 1,250X.

Phase II

The results of Phase I indicated that dimensional distortions occurred when high levels of total collagen were present in restructured beef steaks. These high collagen steaks contained a high percentage of insoluble collagen. Also, moisture was lost at a faster rate and less denaturation and more twisting occured in the non-distorted than in distorted areas. Therefore, for Phase II, flakes (muscle strands) were aligned because temperature changes affect moisture removal of transverse (perpendicular) muscle strands more than longitudinal (parallel) fibers in terms of the direction of heated air flow (Figure 2.1).

4.7 Raw Products

Raw Aligned and Random high collagen restructured beef steaks were similar in proximate composition, salt content, pH, collagen content (Table 4.1) and mean physical measurements (Table 4.16). The initial L1 values were approximately 13.85 cm for Aligned and 13.78 cm for Random steaks. The L2 and W1 values were 9.00 cm and 6.28 cm, respectively, for both aligned and random steaks. The W2 values were 8.28 cm for Aligned and 8.38 cm for Random steaks. Mean Heights (H1, H2 and H3) ranged from 1.30 cm to 1.34 cm.

Table 4.16. Mean initial physical measurements[1] of raw products.

Physical Measurements (cm)	Orientation of Flakes	
	Aligned	Random
Length 1 (L1)	13.85[a]	13.76[a]
Length 2 (L2)	9.00[a]	9.00[a]
Width 1 (W1)	6.28[a]	6.28[a]
Width 2 (W2)	8.28[a]	8.38[a]
Height 1 (H1)	1.33[a]	1.30[a]
Height 2 (H2)	1.34[a]	1.32[a]
Height 3 (H3)	1.30[a]	1.32[a]

[1] Mean values were based on 1 replication of duplicate samples.

[a] Means with same superscripts in the same row were not significantly different ($P>0.1$).

4.8 Cooked Products

As designed in this study, cooking conditions (Figure 4.28a, b) were similar to Phase I.

4.8.1 Temperature Profiles

Under conditions of this study for fast cooking (broiling), the corresponding oven temperatures for the aligned and Random steaks reached 300°C after 1.9 and 2.4 minutes, respectively. The variation in time required to raise the oven temperature to 300°C may have been due to differences in initial temperatures (Table 4.17) of the Aligned (-21.77°C) and Random (-18.34°C) products placed in it. Broiler oven temperatures increased to > 320°C after 2.33 minutes for the Aligned and after 3.31 minutes for the Random treatment.

After 3.31 and 3.65 minutes exposure to broiling condition, internal temperatures of the Aligned and Random steaks were at 0°C with corresponding oven temperatures at 339.5 and 337.25°C, respectively. The internal temperature increases to 50°C, 70°C and 90°C occurred at 6.0, 6.97 and 8.15 minutes for Aligned steaks and at corresponding oven temperatures of 350.85, 367.95 and 372.95°C, respectively. This compared to 5.75, 6.7 and 7.95 minutes for Random steaks and at corresponding oven temperatures of 356.8, 365.5 and 369.8°C, respectively.

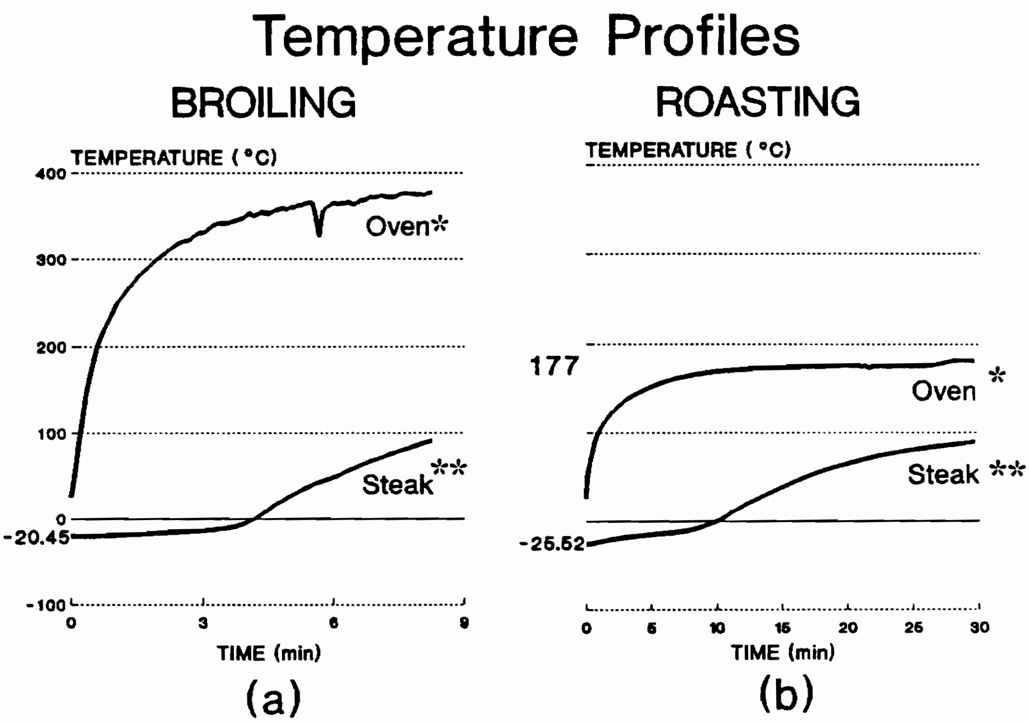


Figure 4.28. Temperature profiles of (a) fast cooking (broiling) versus (b) slow cooking (roasting).

(*: Oven temperatures adjacent to steak surface;
**: Internal steak temperatures)

Table 4.17. Mean[1] times and temperatures of critical points in cooking temperature profiles of Phase II.

Steak End-Point Temperature (°C)	Broiling				Roasting[3]			
	Aligned		Random		Aligned		Random	
	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)	Time (minute)	Temperature (°C)
Initial	0.00	(-21.77)[2]	0.00	(-18.34)[2]	0.00	(-24.68)[2]	0.00	(-24.30)[2]
-	1.90	300.00	2.40	300.00	-	-	-	-
-	2.33	320.00	3.31	320.00	-	-	-	-
0	3.31	339.50	3.65	337.25	13.10	179.40	9.95	167.85
50	6.00	350.85	5.75	356.80	19.50	181.50	17.15	176.10
70	6.97	367.95	6.70	365.50	24.00	181.20	21.10	177.50
90	8.15	372.95	7.95	369.80	33.50	181.10	28.20	181.55

[1] Mean values were based on 1 replication of duplicate samples at 90°C of end-point temperature.

[2] indicates initial internal steak temperature just before cooking and at room temperature.

[3] the oven temperature reached 177°C at about 9-11 minutes

During the roasting (slow cooking) procedure, oven temperatures reached 177°C at approximately 9-11 minutes for both the Aligned and Random steaks and then remained steady (177±5°C). From initial internal temperatures of the Aligned (-24.68°C) and Random (-24.3°C) steaks set in it. Temperature increased to 0°C after 13.1 and 9.95 minutes exposure to roasting conditions. The corresponding oven temperatures adjacent to the surface of the Aligned and Random steak were 179.4 and 167.85°C, respectively. Internal temperature increases to 50°C, 70°C and 90°C occurred at 19.5, 24 and 33.5 minutes for Aligned steaks and the corresponding oven temperatures were 181.5, 181.2 and 181.1°C, respectively. In comparison, Random steaks reached 50°C, 70°C and 90°C in 17.15, 21.1 and 28.2 minutes with the corresponding oven temperatures of 176.1, 177.5 and 181.55°C, respectively. Therefore, Random steaks tended to need less time to reach the respective end-point temperatures than Aligned steaks.

4.9 Cooking Losses (Evaporation, Drip, and Total Cooking Losses)

4.9.1 Effects of Orientation of Flakes on Cooking Losses

No differences (P>0.1) in evaporation (11.80 % vs. 12.67 %), drip (10.03 % vs. 8.38 %), and total (21.84 % vs. 21.05 %) cooking losses were observed between Aligned and Random steaks

(Table 4.18). Yet, Aligned steaks tended to (P= 0.19)have higher drip losses than Random steaks. Perhaps this indicates that pores or gaps between aligned flakes might be lined up and release melted fat during cooking.

4.9.2 Effects of Cooking Method (Fast vs. Slow)

The effects of heating method on cooking losses from steaks are manifested in Table 4.19. Broiling evaporation (17.81%) and total (28.01%) cooking losses were higher (P<0.05) than those for roasting (6.66% and 14.88%, respectively). Drip losses during broiling (10.19%) tended to be higher (P=0.16) than those during roasting (8.22%). Therefore, broiling leads to larger cooking losses, mainly due to additional evaporation of moisture from the steak surfaces. The interactions between orientation of flakes and cooking method were not significant (P>0.1) for all cooking losses.

4.9.3 Effects of End-Point Temperature (50, 70, and 90°C)

The effects of end-point temperature on cooking losses (P<0.0001) of steaks are displayed in Table 4.20. Evaporation and total cooking losses at 50°C (8.74% and 15.13%, respectively) less (P<0.0016) than those at 70°C (11.56% and 21.86%, respectively) which were less (P<0.0001) than those of 90°C (16.41% and 27.33%, respectively). However, drip losses at

Table 4.18. Effects of orientation of flakes on mean cooking losses[1].

Orientation of Flakes	Cooking Losses (%)		
	Evaporation Losses	Drip Losses	Total Cooking Losses
Aligned	11.80[a]	10.03[a]	21.84[a]
Random	12.67[a]	8.38[a]	21.05[a]
SE[2]	0.35	0.35	0.7
Probability			
Orientation of Flakes	0.33	0.19	0.57

[1] Mean values were based on 1 replication of duplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[a] Means with same superscripts in the same column were not significantly different (P>0.1).

Table 4.19. Effects of cooking method on mean cooking losses[1].

Cooking Method	Cooking Losses (%)		
	Evaporation Losses	Drip Losses	Total Cooking Losses
Fast (broiling)	17.81[a]	10.19[a]	28.01[a]
Slow (roasting)	6.66[b]	8.22[a]	14.88[b]
SE[2]	0.35	0.35	0.7
Probability			
Cooking Method	0.028	0.16	0.048
Orientation of Flakes x Cooking Method	0.67	0.81	0.43

[1] Mean values were based on 1 replication of duplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[ab] Means with different superscripts in the same column were significantly different ($P<0.05$).

Table 4.20. Effects of end-point temperature on cooking losses[1].

	Cooking Losses (%)		
End-Point Temperature (°C)	Evaporation Losses	Drip Losses	Total Cooking Losses
50	8.74[c]	6.39[b]	15.13[c]
70	11.56[b]	10.30[a]	21.86[b]
90	16.41[a]	10.93[a]	27.34[a]
SE[2]	0.51	0.42	0.67
Probability			
End-Point Temperature	0.0001	0.0001	0.0001
Orientation of Flakes x End-Point Temperature	0.62	0.98	0.75
Cooking Method x End-Point Temperature	0.15	0.0004	0.21

[1] Mean values were based on 1 replication of triplicate samples.

[2] SE = Standard Error, based on LS MEANS.

[abc] Means with different superscripts in the same column were highly significantly different ($P<0.001$).

50°C (6.39%) were less ($P<0.0001$) than those at 70°C (10.30%) and 90°C (10.93%).

Interactions (Table 4.20) between orientation of flakes and end-point temperature were not significant different ($P>0.1$) for all cooking losses. The interactions between cooking method and end-point temperature were not significant ($P>0.1$) for evaporation and total cooking losses but were highly significant ($P<0.01$) for drip losses (Figure 4.29).

4.10 Chemical/Physical Examinations of Raw and Cooked Products

As in Phase I, three sites on each steak were sampled. Two types of doming (Figure 4.5) occurred in the cooked steaks of Phase II. Compositional, pH and collagen data for raw and cooked steaks are presented in Table 4.21.

The physical measurements of lengths (L1, L2), widths (W1, W2), heights (H1, H2, H3) of both raw and cooked steaks are displayed in Table 4.22. The raw products were not different ($P>0.1$) in physical dimensions among treatments. For all cooked steaks, lengths and widths were shortened by cooking and heights were generally increased. The cooked Aligned steaks (12 steaks) had a higher number of typical domes (10) than cooked Random steaks (7 in 12 steaks). On the other hand, there were more (7) slight domes on cooked Random

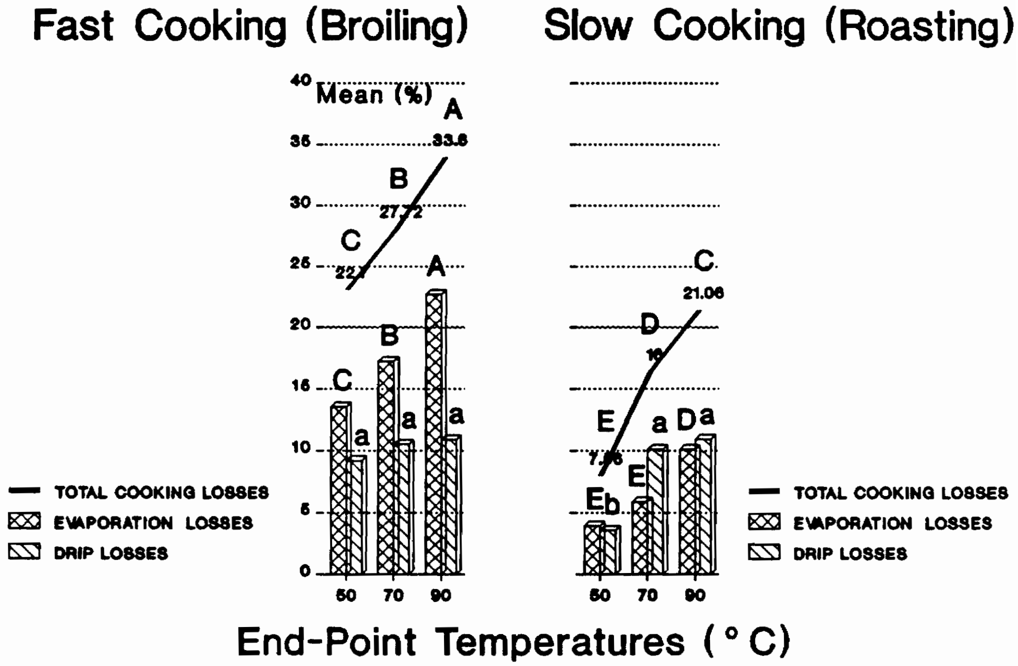


Figure 4.29. The interactions between cooking methods and end-point temperatures on cooking losses.

Table 4.21. Proximate composition, salt content, pH and collagen values of raw and cooked products.

Composition	Orientation of Flakes						
		Aligned			Random		
		Cooked			Cooked		
	Raw	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
% Moisture[1]	64.92	61.72	61.63	60.67	62.06	61.65	61.02
% Fat[1]	17.73	12.01	11.84	11.10	12.04	11.29	10.72
% Protein[1]	14.27	22.82	23.03	24.89	21.95	23.76	24.56
% Salt[1]	2.21	2.68	2.68	2.68	2.60	2.60	2.60
pH[1]	6.01	6.06	6.06	6.06	6.04	6.04	6.04

[1] Mean values were based on 1 replication of duplicate samples.

[ab] Means with different superscripts in the same site row were highly significantly different ($P<0.0005$).

Table 4.22. Effects of flake orientation on mean physical measurements (%) of flakes of raw and cooked products[1].

Physical Measurements (cm)	Orientation of Flakes: Aligned Raw	Aligned Cooked	Random Raw	Random Cooked
Length 1 (L1)	13.85	11.23	13.78	10.77
Length 2 (L2)	9.00	7.14	9.00	6.99
Width 1 (W1)	6.28	5.53	6.28	5.52
Width 2 (W2)	8.28	7.85	8.38	7.94
Height 1 (H1)	1.33	1.54	1.30	1.43
Height 2 (H2)	1.34	1.40	1.32	1.48
Height 3 (H3)	1.30	1.50	1.32	1.56

[1] Mean values were based on 1 replication of duplicate samples.

steaks than on cooked Aligned steaks (3).

4.11 Dimensional Changes (Distortions)

4.11.1 Effects of Orientation of Flakes on Physical Measurements

The effects of orientation of flakes on physical measurements of steaks are shown in Table 4.25. Both L1 and L2 were shorter($P < 0.05$) in Aligned (-18.95% and -20.65%, respectively) than in Random (-21.97% and -22.31%, respectively) steaks. Also, H2 of the Aligned steaks(4.92%) was slightly different ($0.05 < P < 0.1$) from H2 of the Random (12.17%) steaks. All other measurements were not ($P > 0.1$) different between treatments due to orientation of flakes.

4.11.2 Effects of Cooking Method (Fast vs. Slow)

The effects of cooking methods on physical measurements of steaks are exhibited in Table 4.25. Both L1 and L2 were higher for fast cooking(broiling) (L1, $P < 0.05$; L2, $P < 0.0001$). Shrinkage also tended to be greater($0.05 < P < 0.1$) for broiling than roasting. The other measurements were not ($P > 0.1$) different between cooking methods. The interactions between orientation of flakes and cooking method were significant ($P < 0.01$) for L1, L2, W2 and Shrinkage but all other physical measurements were not different ($P > 0.1$).

Table 4.23. Mean[1] percent changes of physical measurements on orientation of flakes.

Orientation of Flakes	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
Aligned	-18.95^{b}	-20.65^{b}	-12.26^{a}	-5.14^{a}	16.05^{a}	4.92^{*}	15.19^{a}	17.78^{a}	12.01^{a}
Random	-21.97^{a}	-22.31^{a}	-12.30^{a}	-5.22^{a}	10.33^{a}	12.17^{*}	18.21^{a}	18.99^{a}	13.57^{a}
SE[2]	0.14	0.00	2.74	1.50	5.07	0.63	2.34	1.10	1.07
Probability									
Orientation of Flakes	0.042	0.0001	0.99	0.97	0.57	0.078	0.53	0.58	0.49

[1] Mean values were based on 1 replication of duplicate samples.

[2] SE = Standard Error, based on LS MEANS

[ab] Means with different superscripts in the same column were significantly different (P<0.05).

[*] Means in the same column were slightly significantly (0.05 < P <0.1) different.

Table 4.24. Mean[1] percent changes of physical measurements on cooking methods.

Cooking Methods	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
Fast	-25.00[a]	-27.13[a]	-14.67[a]	-8.96[a]	17.99[a]	7.46[a]	21.18[a]	24.09[*]	15.55[a]
Slow	-15.91[b]	-15.83[b]	- 9.89[a]	-1.40[a]	8.39[a]	9.64[a]	12.22[a]	12.68[*]	10.03[a]
SE[2]	0.14	0.00	2.74	1.50	5.07	0.63	2.34	1.10	1.07
Probability									
Cooking Methods	0.014	0.0001	0.43	0.17	0.41	0.25	0.23	0.086	0.17
Orientation of Flakes x Cooking Methods	0.0001	0.0001	0.18	0.0001	0.11	0.64	0.3	0.0014	0.63

[1] Mean values were based on 1 replication of duplicate samples.

[2] SE = Standard Error, based on LS MEANS

[ab] Means with different superscripts in the same column were significantly different ($P<0.05$).

[*] Means in the same column were slightly significantly ($0.05 < P<0.1$) different.

4.11.3 Effects of End-Point Temperature (50, 70, and 90°C)

The effects of end-point temperature on physical measurements are presented in Table 4.25. Both L1 and L2 increased ($P<0.0001$) as end-point temperature (-17.5 vs. -17.64) were less ($P<0.003$) than those of 70°C (-19.99 vs. -21.67). Similarly, 70°C were less ($P<0.0006$) than 90°C (-23.87 vs. -25.14). For Shrinkage ($P<0.0008$), 50°C (13.35) was less ($P<0.001$) than either 70°C (20.6) or 90°C (21.2), but 70°C was not different ($P>0.7$) from 90°C. For H1 ($P<0.012$), there were lower ($P<0.02$) mean physical measurements (%) of cooked steaks from 70°C (4.9) than steaks from either 50°C (15.63) or 90°C (19.04), but there were not different ($P>0.4$) from either 50°C or 90°C. The other physical measurements were not different ($P>0.05$) from either end-point temperature. These results partly agree with Phase I on significant physical measurements.

There were significant ($P<0.05$) interactions between orientation of flakes and end-point temperature in H1 and H3. Also, interactions between cooking method and end-point temperature were significant ($P<0.05$) in L1, L2, H1, H2, H3, Shrinkage and Doming.

4.12 Specifics of Distortion (Magnitude)

Table 4.25. Mean[1] percent changes of physical measurements on end-point temperatures.

End-Point Temperatures	Physical Measurements (%)								
	L1	L2	W1	W2	H1	H2	H3	Shrinkage	Doming
50°C	-17.50[c]	-17.64[c]	- 8.72[b]	-5.20[a]	15.63[a]	9.27[a]	16.83[a]	13.35[b]	13.87[a]
70°C	-19.99[b]	-21.67[b]	-12.53[ab]	-4.59[a]	4.90[b]	7.11[a]	15.63[a]	20.60[a]	9.13[a]
90°C	-23.87[a]	-25.14[a]	-15.59[a]	-5.76[a]	19.04[a]	9.27[a]	17.64[a]	21.20[a]	15.38[a]
SE[2]	0.48	0.56	1.9	1.03	2.95	3.55	2.90	1.25	2.43
Probability									
End-Point Temperatures	0.0001	0.0001	0.068	0.73	0.012	0.88	0.89	0.0008	0.2
Orientation of Flakes x End-Point Temperatures	0.24	0.47	0.2	0.52	0.023	0.18	0.039	0.35	0.12
Cooking Methods x End-Point Temperatures	0.0001	0.0003	0.10	0.66	0.03	0.0012	0.0012	0.0051	0.0004

[1] Mean values were based on 1 replications of duplicate samples.

[2] SE = Standard Error, based on LS MEANS

[abc] Means with different superscripts in the same column were significantly different ($P<0.05$).

4.12.1 PCM Observations

Phase contrast micrographs of cooked Aligned steaks are exhibited in Figures 4.30-4.33. In Phase II, Random steaks were the control groups for the Aligned steaks. Random steaks were similar to the HC steaks of Phase I with the exception of having more extensive spaces and/or voids. Also, Site 3 of the Aligned steaks was the reference for Site 1 (dome) observations. Aligned steaks had the muscle fibers and connective tissues manually arranged in the same direction even after the products were cooked (Figure 4.32). Parallel muscle fibers were heavy barriers to interior moisture movement which had a rather sinuous path to freedom. The cumulative vapor pressure broke through the weak points (gaps) between parallel muscle fibers. Therefore, there are more severely damaged parallel muscle fibers within Site 1 of Aligned steaks (Figures 4.30-4.32) than in undistorted tissue.

Site 3 of Aligned steaks (Figures 4.33) had a distinctive outlet as evidenced by the voids located on the steak surface among parallel muscle fibers. It was easier for moisture to escape or juices to be released in Site 3. Therefore, Aligned steaks tended to have higher drip losses than Random steaks.

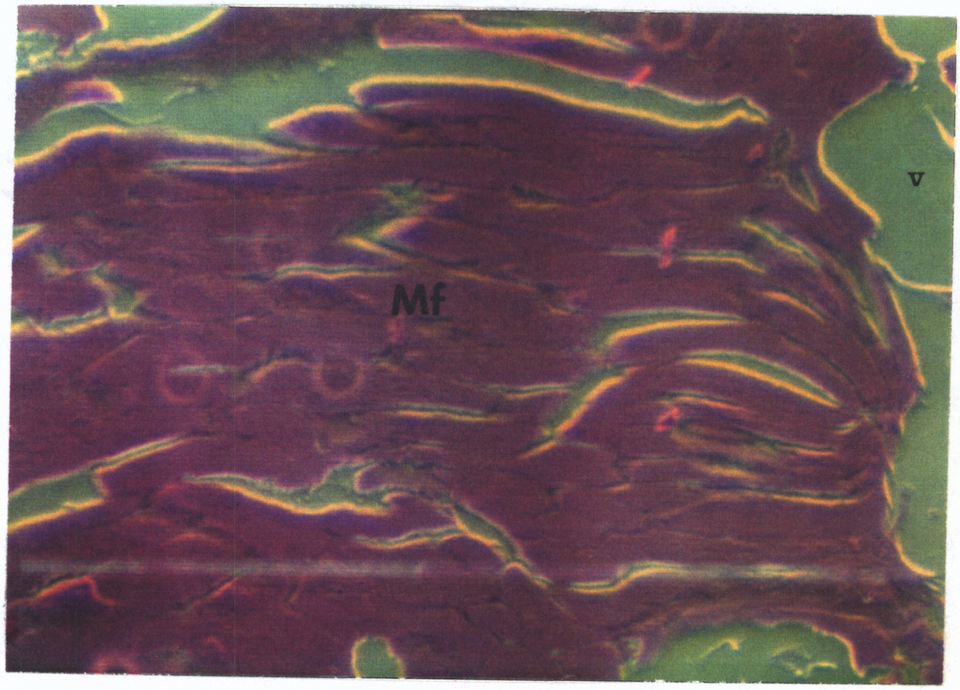


Figure 4.30. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) Aligned steak. Bouins solution, H & E, 100X. Aligned steaks mostly presented the orientation of parallel muscle fibers (Mf) even in the cooked state. Some voids (V) existed.

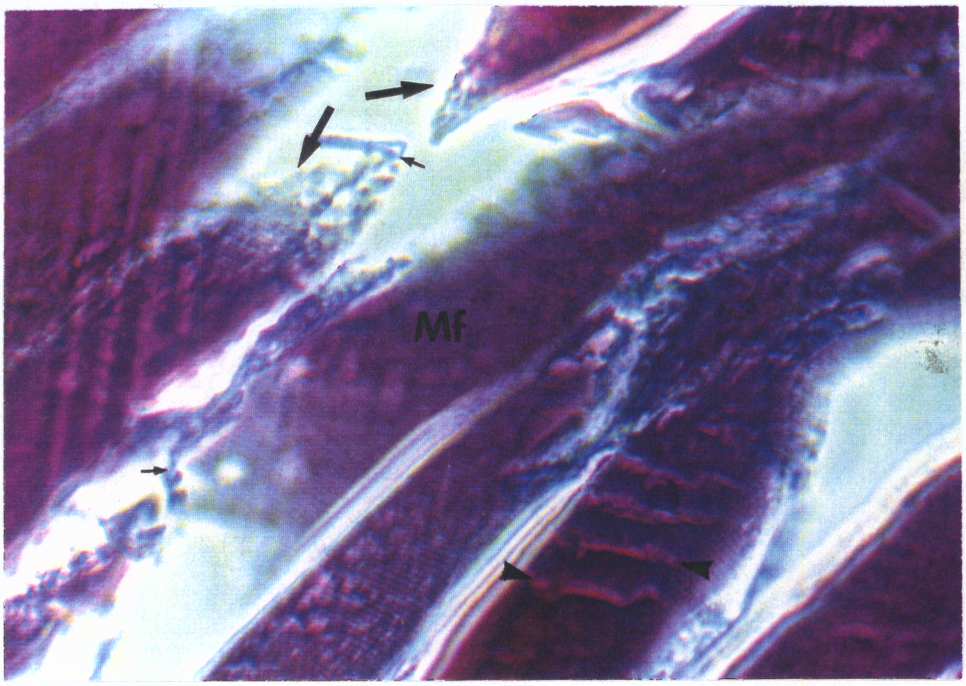


Figure 4.31. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) Aligned steak. Bouins solution, Masson's Trichrome Stain, 1,000X. The greater fibers (Mf) damage existed in this cooking treatment; banding patterns were clear and distinct, heavy granulation existed in muscle fibers, numerous cracks (arrowheads) happened in muscle fibers, severe breaks (arrows) interrupted muscle fibers and collagen fragments (thin arrow) existed.

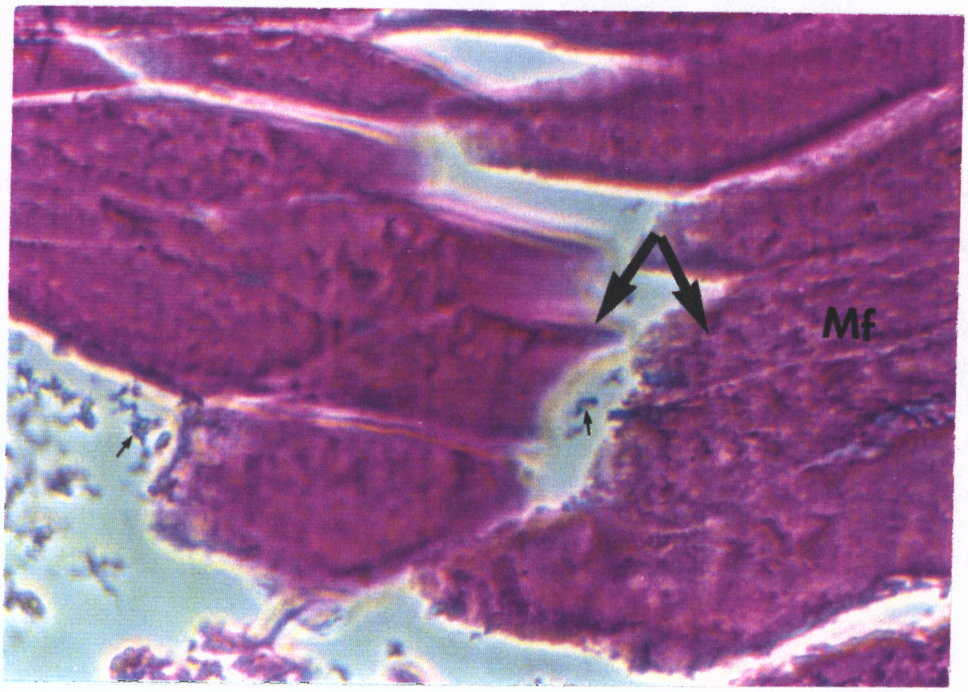


Figure 4.32. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) Aligned steak. Bouins solution, H & E, 1,000X. The severe breaks (arrows) interrupted muscle fibers (Mf). There was different in height. Collagen fragments (thin arrows) existed.

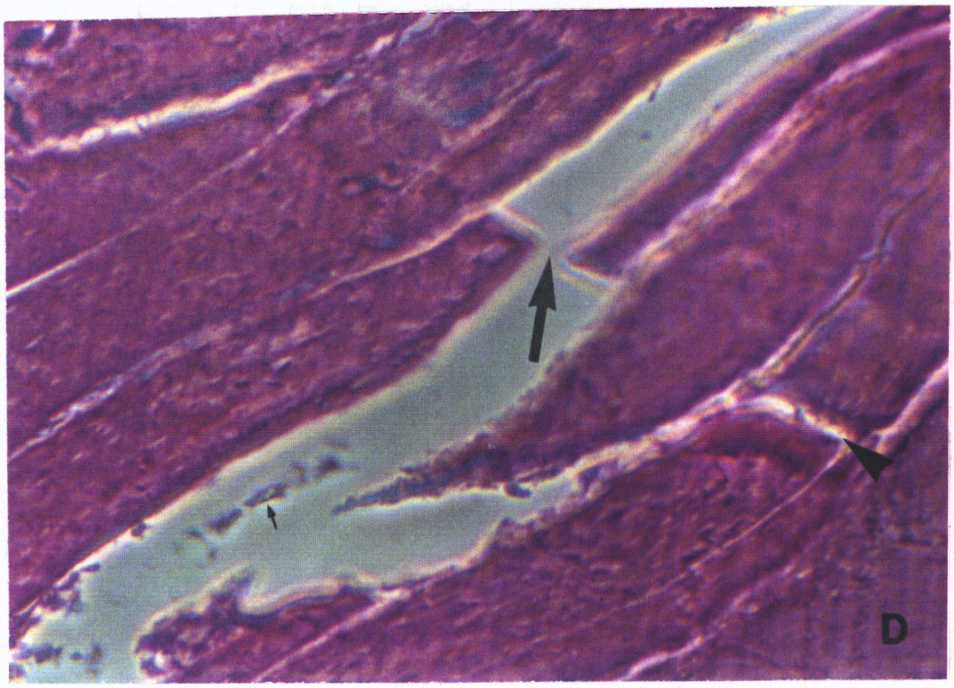


Figure 4.33. Phase contrast micrograph of the distorted site of cooked (fast cook, 90°C end-point) Aligned steak. Bouins solution, H & E, 1,000X. The severe break (arrow) and creak (arrowhead) existed beside the dome (D), indicating that parallel muscle fibers could be as heavy barriers to seal the dome even though they were damaged.

Chapter V. Summary And Conclusions

Phase I

Restructured beef steaks high in connective tissue exhibited dimensional distortions (curling, doming and welling) during cooking. They had variations in residual moisture and fat contents at different sites. Three sites existed in the cooked steaks, namely, Site 1 (apex of dome), Site 2 (valley) and Site 3 (non-distorted area). In both high (HC) and low (LC) collagen steaks, Site 1 had higher residual moisture and fat contents than Site 3. Collagen levels did not have any significant effects on cooking losses and physical measurements except W1. However, cooked HC steaks tended to increase dimensional distortions during cooking and had higher cooking losses than LC steaks. Storage times did not affect cooking losses or dimensional distortions. However, L1 and W2 measurements were lower ($P<.05$) for restructured steaks stored 85d than 0d and a lower broiler oven temperature. Heat penetration patterns (fast vs. slow cooking) and end-point temperatures (50, 70 and 90°C) were the principal causes of cooking losses and dimensional distortions. Residual moisture (%) was negatively correlated to evaporation losses and residual fat (%) to drip losses. In addition, highly significant negative relationships existed between cooking losses and dimensional distortions. Thus, dimensional distortions apparently allowed more moisture (mostly evaporation) and fat (drip) to be lost in Site 3 than in Site 1 during cooking. In contrast, Site 1 had more moisture and fat remaining, excessive twisting and less denaturation than Site 3. Additional information from PCM and/or SEM evaluations indicate that the formation of domes (Site 1) were due to the presence of multiple layers of parallel muscle fibers or bundles covered by endomysium which serve as heavy barriers to seal fluid in the growing dome. Also, denatured solubilized proteins and some voids which act as cohesive pastes and function as a buffer, respectively, were frequently observed around the dome and layers of muscle fibers. On the other hand, the microscopic sections of Site 3 from the same cooked steaks exhibited insufficient parallel muscle fibers, wrong direction (perpendicular) muscle fibers and plenty of inner or surface open areas (i.e. cavities and channels) existing which inhibit dome formation.

Phase II

Flake orientation did not have any significant effects on cooking losses and dimensional distortions of HC steaks with the exception of L1 and L2 measurements. Steaks containing random oriented flakes shrink in lengths more than Aligned steaks upon heating. Similar changes in widths occurred for both Random and Aligned steaks during cooking. PCM micrographs of Site 3 on the Aligned steaks had more parallel muscle fibers side by side than did Site 1 (same as Phase I). Within Site 1 of the Aligned steaks, there was more severe and more wide spread denaturation of muscle fibers than in Site 3. This suggests that Site 1 of Aligned steaks had more layers of heavy barriers to compress the dome which results in an increase in internal vapor pressure and the formation of a bigger dome in Aligned steaks.

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