EFFECT OF WHEY PROTEIN CONCENTRATE ON BIND AND OTHER QUALITY CHARACTERISTICS OF STRUCTURED BEEF

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CHAPTER I

INTRODUCTION

Structuring of meat is an alternative for the meat industry's need to better utilization of lower-cost meat and for the consumer in search of less expensive products of good quality, palatability, and appearance. These desires of industry and the consumer continue to motivate meat processors and researchers to look for new forms of structured meat products.

Chunking, grinding, and/or flaking then forming meat to produce a structured item that resembles steak from whole muscle are relatively recent manufacturing processes (Mandigo, 1974; Schmidt, 1978; Booren, et al., 1981a,b; Mandigo, 1982a). The success of new structured products is limited, however, by problems such as amount and distribution of connective tissue, discoloration, rancidity, and binding. Some of these problems are associated with the use of salt (NaCl) as an additive during manufacture (Field, 1982; Mandigo, 1982b).

Salt is added to structured meat to promote and enhance the extraction of intracellular proteins, in particular myosin, which act as binding agents to facilitate adhesion of the recombined meat pieces. However, due to the adverse

effects of salt, the search for binders of superior binding efficiency has been identified as a research priority for the meat industry (Breidenstein, 1982).

The mechanism of binding among the meat pieces is a complex phenomenom not completely understood which, so far, has been attributed to the heat induced gelation of myofibrillar proteins (Fukazawa et al., 1961; Siegel and Schmidt, 1979a) and seems to involve head-head aggregation of myosin filaments (Ishioroshi et al., 1983).

If non-meat proteins are to be used as meat binders in structured meat products they must assist in maintaining the textural properties of the meat system. Chemical or physical effects, or both, could be involved in such phenomenon. In this regard, some studies have reported formation of a protein complex between soy protein and myosin (King, 1977; Peng et al., 1982a,b; Kurth and Rogers, 1984), fibrinogen and albumin with myosin (Foegeding et al., 1986), and casein or gluten with myosin (Kurth, 1983; Kurth and Rogers, 1984), suggesting further investigation to determine the role of such interactions in the more complex systems of manufactured meat products.

Whey proteins have good gel forming characteristics (Kalab et al., 1973; Hillier et al., 1980) which make them potential binders in structured meat products. Since they have been shown to interact with themselves and with casein (Doi et al., 1983a,b), interaction with myosin may also be

possible when whey proteins are incorporated in a meat system.

This research intended 1) To evaluate the effect of two commercially manufactured whey protein concentrates upon a) bind, tenderness, cook loss, and color of structured beef steaks shortly after manufacture and after three months of frozen storage at -13^{o}C. b) bind in a meat model system. 2) To demonstrate, in a model system, the interaction between each of the major whey proteins and myosin by high pressure liquid chromatography (HPLC).

CHAPTER II

REVIEW OF LITERATURE

Processed meat products vary greatly in their composition and method of preparation; however they all depend on the ability of the meat pieces to bind together. Structured steaks have not overcome this need. Effective bind is essential if the product is to retain its structural integrity during subsequent handling and slicing (Schmidt and Trout, 1982). In addition to bind; tenderness, cook loss and color are important factors, evaluated by consumers, which contribute to the success of structured meat products. Bind, tenderness and cook loss are mainly dependent on the behavior of the myofibrillar proteins during processing, while color is affected not only by the processing methods but also by other factors such as light, oxygen, and temperature during handling and storage.

Numerous studies have been carried out in trying to establish optimum conditions for processing of structured meat while others have searched for suitable processing alternatives or ingredients. Furthermore, several studies have assessed the potential role of meat and non-meat proteins in food model systems or in model systems employing protein solutions.

Structured Steaks

Binding

Binding of the raw meat pieces in structured steaks is usually achieved by freezing. However, heat induced gelation of the meat protein is held responsible for meat binding during cooking. Within this general concept, the degree of binding among the meat pieces has been evaluated using different processing schemes and different types and concentrations of additives, particularly salt and tripolyphosphate. Although differences found may reflect actual variations in product characteristics, they may also be a reflection of different processing techniques and methods of evaluation.

Cross and Stanfield (1976) prepared structured steaks from beef forequarters and asked consumer panelists to evaluate the "ease of cutting" the steaks with a knife. Measurement of this trait would give an indication of the degree of bind among the meat pieces forming the steaks. They found that increased amount of fat in the formulation significantly increased the ease of cutting. However, a number (not reported) of panelists indicated that the sample "tore" apart rather than being cut as a whole muscle steak.

Booren et al. (1981a) found that sectioned and formed beef steaks processed by vacuum mixing and evaluated by a trained sensory panel had more bind than steaks processed with no vacuum during mixing. Instron analysis, however,

indicated no differences in bind strenght due to vacuum. This difference between sensory and Instron analysis was explained in terms of Instron measurements being more sensitive than sensory measurements. In another study by the same authors (Booren et al., 1981b) whose title suggested an assessment of the effect of blade tenderization, vacuum mixing, salt, and mixing time on binding of structured steaks, only the effect of mixing time on binding was reported. Bind increased 60% when the mixing time was increased from 8 minutes to 16 minutes. There was no difference in bind when mixing was increased from 16 to 24 minutes. In the former study steaks were produced from choice chucks and formulated to contain 12% fat, while in the latter standard grade rounds formulated to less than 2% fat were used.

Wiebe and Schmidt (1982) studied the effect of vacuum mixing and precooking on the binding strength and cook yield of structured steaks. They reported an increase in binding as a result of vacuum mixing. Steaks produced from treatments in which vacuum was included during mixing had stronger binding than steaks made from treatments mixed without vacuum. However, mixing for 3 minutes without vacuum followed by only 1 minute of vacuum produced steaks with greater bind strength than steaks in which the whole mixing step was done for 4 minutes under vacuum. In this regard, these authors speculated that 3 minutes mixing in the absence of vacuum increased availability of muscle cells

to be swelled by vacuum, resulting in an increased surface area for interaction with the extraction solution, therefore increasing protein extraction and consequently increasing bind. Steaks obtained from beef rolls cooked to 68°C in a smokehouse had similar bind strength to steaks broiled from the frozen state. However, subsequent broiling of the former steaks significantly increased bind strength.

Tensile strength of meat strips (1.25 cm x 4.5 cm) obtained from cooked structured steaks manufactured from hot boned or cold boned beef rounds was determined by Huffman et al. (1984). Intact meat strips obtained from short loin (longissimus dorsi) steaks were used as controls. They reported that meat strips from structured steaks showed less tensile strength (less cohesivity or less bind) than control meat strips, and meat strips from salted structured steaks were slightly, but not significantly, more cohesive than those of unsalted structured steaks. Since no reference was made to the effect of rigor state, it is assumed that these results were similar for steaks from both hot and cold boned meat.

The use of myosin, actomyosin and sarcoplasmic proteins extracted from beef to bind chunks of meat in structured steaks or in model systems has been investigated (Macfarlane et al., 1977; Ford et al., 1978; Siegel and Schmidt, 1979a,b; Turner et al., 1979). In general, these authors have indicated that "crude" myosin or a mixture of it with sarcoplasmic proteins, with little or no added salt,

had potential for binding pieces of meat to produce a cohesive structured product of low salt content.

The influence of some non-meat proteins on binding of structured meat has also been studied. Hand et al. (1981) evaluated the properties of flaked and formed steaks containing wheat gluten, soy isolate and/or flavorings. All products made with flavorings (44% NaCl, 25% sodium tripolyphosphate, and 31% hydrolyzed vegetable protein) were more cohesive and required more effort to fragment than those made without flavorings. Siegel et al. (1979) ranked the meat binding ability of several non-meat proteins in decreasing order as follows: wheat gluten, egg white, corn gluten, calcium-reduced dry skim milk, bovine blood plasma, isolated soy protein, and sodium caseinate. The high ability of wheat gluten to bind meat pieces was attributed to its ability to interact with myosin, while the inability of sodium caseinate as a meat binder was attributed to its inhibitory effect on interactions between myosin molecules. Terrell et al. (1982) found, however, that animal proteins (plasma protein and egg albumen) were superior meat binders than plant proteins (vital wheat gluten and isolated soy protein). These authors suggested that metal ions and/or insoluble materials in the plant proteins may interfere with hydration mechanisms of both plant and myofibrillar proteins at the meat surfaces and thus decrease binding. Means and Schmidt (1986) evaluated subjectively the effectiveness of an algin/calcium gel system as a binder in raw and cooked

structured beef steaks. Their findings indicated that the system could be used to produce structured steaks which bind well not only in the cooked state, but also in the raw, refrigerated state. Optimal ingredient levels used were 0.8-1.2% sodium alginate and 0.144-0.216% calcium carbonate, with 500 ppm of sodium erythorbate.

Color

Booren et al. (1981c) subjectively compared steaks made from standard beef rounds and choice chucks and found no differences in color between the two. In addition reflectance spectrophotometry indicated no difference in color between steaks made from chuck or round when evaluated as percent reflectance at 630 nm minus percent reflectance at 580 nm or as K/S (K= light absorbed, S= light scattered) ratios. However, a look at the reported values showed that subjective color scores and K/S ratios (% metmyoglobin) were notoriously higher (less desirable) for chuck than for round. The inconsistencies found were attributed to the fact that spectrophotometric values reflect the contributions of single pigments examined over a small surface area and are not affected by the presence of fat while visual appraisal involves the entire steak surface and is affected not only by the presence of fat but also by the contribution of all color pigments. Despite the above mentioned inconsistencies, the subjective and objective

color values indicated a highly acceptable color for beef steaks.

Color of raw structured steaks prepared from hot boned beef was significantly more stable (less discoloration) than in steaks prepared from cold boned beef, as evaluated by sensory panel scores (Huffman et al., 1984). Regardless of rigor state, addition of salt in the formulations undesirably affected color. The presence of salt was reported to lower the pH of the meat and increase myoglobin oxidation by four or five times.

Means and Schmidt (1986) used a sensory panel to assess changes in color of structured steaks containing various concentrations of a mixture of sodium alginate and calcium carbonate. Structured steaks containing no additives, steaks with salt and polyphosphate, and intact muscle steaks were also evaluated. They found that all treatments scored equal for percentage discoloration of raw steaks except those with salt, which were significantly more discolored. Salt-induced discoloration was related to increased mixing, frozen storage, and refrigerated storage. Similar findings had been previously reported by Ockerman and Organisciak (1979), Booren et al. (1981a), and Chastain et al. (1982).

Tenderness

Tenderness in structured steaks is usually measured by subjective evaluation or objectively by Kramer shear values using an Instron or similar machine. Cross and Stanfield

(1976) carried out a study on consumer evaluation of structured beef steaks prepared from utility grade beef forequarters. Steaks were formulated with or without salt and with either 20% or 30% fat. Consumer panelists considered the steaks containing salt plus 30% fat the more tender and desirable of all products tested.

Booren et al. (1981c) reported that steaks prepared from beef chuck were less tender than steaks from beef round when evaluated by either a trained sensory panel or by Instron measurements. Tenderness was also found to be increased by 8% and 20% after 8 or 16 minutes of mixing respectively. In another study (Booren et al., 1981a), increased tenderness was observed up to 24 minutes of mixing. Whether mixing was accomplished with or without vacuum had no effect on tenderness of the structured steaks (Booren et al., 1981b).

Trained panelists used by Huffman et al. (1984) could not differentiate between structured steaks made from hot boned or cold boned meat on the basis of texture and overall acceptability. However, they scored texture to be more desirable in salted than in unsalted steaks. These sensory results were corroborated by Kramer shear values.

Cook Yield or Cook Loss

Cooking yields of structured steaks evaluated by Booren et al. (1981b,c) were found to increase linearly when the

time of mixing was increased up to 18 minutes regardless of the presence or absence of vacuum during the mixing step.

Wiebe and Schmidt (1982) also found no beneficial effect of vacuum mixing on cook yield of structured beef steaks when comparing with mixing in the absence of vacuum. Precooked steaks that were further broiled had lower cook yield than steaks broiled from the frozen raw state. This result is obvious since in the first case there was a double heating process.

Cook losses determined by Huffman et al. (1984) in structured steaks from cold or hot boned meat, with or without added salt, indicated that regardless of the rigor state of the raw materials the addition of salt (0.75%) reduced cooking loss.

Myofibrillar Proteins

Myofibrillar proteins are the proteins (1 to 2 μm in diameter) which compose the muscle fibers. They constitute about 55 to 60% of the total muscle protein, or 10% of the weight of the vertebrate skeletal muscle. Based on their physiological role in muscle, myofibrillar proteins can be further classified as contractile proteins and regulatory proteins (Asghar et al., 1985). The contractile proteins are myosin and actin, which combine themselves to form the contractile actomyosin of active pre-rigor muscle or the inextensible actomyosin of muscle in rigor mortis (Lawrie, 1979). The major regulatory proteins are tropomyosin and

the troponins (C, I, T), which impart calcium sensitivity to the contractile protein system (actomyosin) in muscle.

Myosin

Myosin is the most abundant (about 43%) of the myofibrillar proteins. There are about 300 myosin molecules per thick filament, each being about 160 nm long, 10 to 13 nm in diameter, and with a molecular weight of 480,000 to 500,000 daltons. The molecule is composed of two large polypeptide chains, called heavy chains, each with a molecular weight of about 200,000, and three to four small chains, called light chains, with molecular weights of about 17,000 to 25,000. The heavy chains have α-helix conformation and coil together to form a superhelix. On one side each heavy chain forms a globular structure called "head". The remaining portion of the heavy chains is called "rod" or "tail" (Asghar et al., 1985).

Myosin contains a large amount of aspartic and glutamic acid residues and a fair amount of the basic residues histidine, lysine and arginine (Schmidt and Trout, 1982). The isoelectric point of myosin is approximately 5.4 in KCl solution, but in the presence of magnesium or calcium ions it rises to 9.3, showing strong preferential bonding of the two divalent cations over the monovalent cations sodium and potassium (Bendall, 1964). Over 40 sulfhydryl residues are located mainly in the globular "head" portion of myosin.

Heat-Gelation Properties of Myosin

At present it is widely accepted that binding among meat pieces in structured meat products is dependent mainly upon heat induced myosin-myosin interaction. Actin has been shown to have no binding property in model system studies (Samejima et al., 1969). However, actin and the regulatory proteins may have a significant influence on the gel formability of myosin (Nakayama and Sato, 1971).

Yasui et al. (1979) found that during gelation of myosin the thermal transition from sol to gel begins at 30°C and reaches a maximum at 60-70°C. Optimal development of myosin or actomyosin gels seems to occur at pH values between 5.0 and 6.3 (Ishioroshi et al., 1979).

Ishioroshi et al. (1979) reported that during gelation of myosin by heat two transition temperatures (Tm) are mainly reponsible for the conformational changes in the molecule, one at 43°C (Tm1) and other at 55°C (Tm2).

Cheng and Parrish (1979) used gel electrophoresis to determine the heat induced changes in myofibrillar proteins in at-death and post-mortem samples of bovine muscle heated at 45, 50, 55, 60, 70, and 80°C. They found that alpha actinin was the most heat labile myofibrillar protein, becoming insoluble at 50°C (evidenced by disapearance from the electrophoretic profile). The heavy and light chains of myosin became insoluble at 55°C, while actin, tropomyosin and troponins resisted temperatures of up to 80°C (actin) or more (tropomyosin and troponins).

In studies to determine the mechanism of heat induced gelation of muscle myosin, Samejima et al. (1981) cleaved the molecule to assess the contribution of the resulting rod and globular fragments to the heat-gelling properties of myosin. They found evidence that the globular (head) fraction simply aggregates upon heating while the rod portion arranges itself to form a three-dimensional network. Based on the fact that dithiothreitol (DTT) inhibited association of the head portions but not crosslinking of the rod portions these authors concluded that the heat induced gelation of myosin can be represented by two reactions: a) aggregation of the globular portion, which is complementary to and closely associated with the oxidation of -SH groups, and b) network formation as a result of thermal unfolding of the helical rod portion. These reactions closely correlate respectively with the Tm1 and Tm2 observed by Ishioroshi et al. (1979). In further studies, however, Ishioroshi et al. (1983) reported that the gelation behavior of myosin at low concentration of salt (0.2 M KCl) is clearly different from that at high salt concentration (0.6 M KCl), where the molecule is mainly in monomeric form. At low salt concentration, the gel forming ability of myosin ocurred only through head-head aggregation of the myosin filaments without involving the tail portion of the molecule.

Whey Proteins

The major protein components of bovine whey are β-lactoglobulin, α-lactalbumin, and bovine serum albumin. Of these, β-lactoglobulin and α-lactalbumin are present in the highest concentration and are probably of primary importance in the physico-chemical properties of whey protein products (Schmidt and Morris, 1984).

β-Lactoglobulin is the most abundant protein in whey. At pH values near its isoelectric point (4.2 - 4.5) and up to the pH of milk (6.7) and at room temperature (25°C) the molecule exists as a stable dimer with a molecular weight of about 36,700 daltons (Swaisgood, 1982). The monomer moiety is made of 162 aminoacid residues as follows: 10 Asp, 5 Asn, 8 Thr, 7 Ser, 16 Glu, 9 Gln, 8 Pro, 4 Gly, 15 Ala, 5 Cys, 9 Val, 4 Met, 10 Ile, 22 Leu, 4 Tyr, 4 Phe, 15 Lys, 2 His, 2 Trp, and 3 Arg (Eigel et al., 1984). Two disulphide bridges and one free thiol group exist per monomer. The thiol group can react with other thiol groups to form new disulphide bonds (de Wit and Klarenbeek, 1984).

α-Lactalbumin is the most heat resistant and the second quantitatively most important protein in whey. It is a nearly spherical, very compact globular protein with a molecular weight of 14,175 daltons, composed of 123 aminoacid residues as follows: 9 Asp, 12 Asn, 7 Thr, 7 Ser, 8 Glu, 5 Gln, 2 Pro, 6 Gly, 3 Ala, 8 Cys, 6 Val, 1 Met, 8 Ile, 13 Leu, 4 Tyr, 4 Phe, 12 Lys, 3 His, 4 Trp, and 1 Arg (Eigel et al., 1984). It also has four disulphide bridges

but no free -SH groups (Gordon, 1971; de Wit and Klarenbeek, 1984). α-Lactalbumin has an important biological function. As part of the enzyme lactose synthetase it has a regulatory role in the biosynthesis of lactose (Brodbeck et al., 1967; Hambraeus, 1982). In addition, it is interesting that the primary structures of α-lactalbumin and egg white lysozyme are very similar, which suggests that they evolved from a recent common ancestor (Gordon, 1971).

Effect of Heat on Whey Proteins

Whey proteins are recognized by their high nutritional value and excellent functional properties in undenatured state. However, the heat treatments commonly used during food processing and preparation can cause denaturation of whey proteins. Thus, an understanding of the mechanisms of the heat induced effect is needed to assess the behavior of whey proteins in complex food systems.

Larson and Rolleri (1955) studied the effect of heat on serum proteins of milk (whey proteins) and observed that heating at 70°C for 30 minutes denatured 32% of the β-lactoglobulin and only 6% of the α-lactalbumin. Since α-lactalbumin is the most heat resistant whey protein, over 50% of the original amount remained after heating whey at 77°C for 30 minutes, its relative concentration in whey increased up to about 80°C, and was completely denatured by a heat treatment of 96°C for 30 minutes

Chromatographic evidence of heat induced interaction of β-lactoglobulin with α-lactalbumin was reported by Hunziker and Tarassuk (1965). After heating at 75°C for 30 minutes in phosphate buffer at pH 6.7, α-lactalbumin decreased 14% when heated alone but 84% when heated in the presence of β-lactoglobulin. Interaction of α-lactalbumin with β-lactoglobulin has also been reported by Baer et al. (1976).

de Wit (1981) and de Wit and Klarenbeek (1984) noted denaturation temperatures (Td) of 68°C and 78°C for α-lactalbumin and β-lactoglobulin respectively. This is different from what is normally said about the heat resistance of α-lactalbumin. To explain this discrepancy these authors suggested that the high reversibility to renaturation is responsible for the molecule to be thermostable against protein aggregation. Their results come from differential scanning calorimetry (DSC) studies, while the general view is based on denaturation determined by protein solubility measurements at pH 4.6. Although the observed low Td for α-lactalbumin was explained, no reasons were given to explain why the Td for β-lactoglobulin was higher than the one based on solubility measurements.

Whey Protein Concentrates

Whey from bovine milk is a dilute fluid containing about 5% solids of which the major constituents are lactose (70-80%) and protein (9%). Traditionally, the proteins from

whey have been isolated by heat denaturation and acid precipitation to form a product commercially known as lactalbumin which has a high nutritive value but, because it is denatured and insoluble in water, lacks the functional properties of native whey proteins (Marshall, 1982). Whey protein concentrate (WPC) is a relatively recent term which applies to soluble forms of whey protein products containing from 25% to 90% protein on a dry basis, having a high protein efficiency ratio (PER) of 3.1 compared to 2.5 for casein, and displaying excellent functional properties.

Several processes have been designed for production of WPCs for commercial or research purposes. These include electrodialysis (Stribley, 1963), metaphosphate complex (Hartman and Swanson, 1966), Sephadex gel filtration (Morr et al., 1967), reverse osmosis (Marshall et al., 1968), ethanol precipitation (Morr and Lin, 1970), ultrafiltration (Fenton-May et al., 1971), methyl cellulose complex (Hansen et al., 1971), iron complex (Jones et al., 1972) and ion exchange (Nichols and Morr, 1985). Of these processes, ultrafiltration has been the most widely used method for production of WPCs. In 1981, about 8% of the total world's whey was processed by ultrafiltration (Marshall, 1982). This method has the advantage that it can be controlled to obtain a product with the desired protein level. However, the disadvantage is that not only protein but also fat and bacteria are concentrated (de Boer et al., 1977). Even more, fouling of the membranes used in the process as a

result of protein-protein, protein-lipid, or protein-calcium complexes and calcium-phosphate precipitation becomes a major problem (Schmidt et al., 1984)

Interaction Between Myosin and Non-Meat Proteins

Since myosin is the most abundant myofibrillar protein in meat, it has been considered to play a very important role in determining the functional properties of processed meat products (Samejima et al., 1969). Numerous types of non-meat proteins have been used in meat systems with the purpose of extending to lower the cost or to improve some of the functional properties of processed meat products. Soy and milk proteins have been commonly used, but proteins from other sources such as plasma and gluten have been used as well. Consequently, the manner in which these proteins interact with meat proteins in meat systems has created considerable interest among researchers.

King (1977) studied the interaction of β-conglycinin (a soybean 7S globulin fraction) with myosin using viscometry, gel chromatography, and density gradient centrifugation. No evidence of complex formation was obtained at temperatures of 2°C and 25°C. However, they observed that protein interaction appeared to be induced at temperatures in the range 75-100°C as indicated by a) an increase in specific viscosity of the protein mixture, b) a change in composition of the soy protein component of the aggregate formed, and c)

a decrease in sedimentation coefficient. Further studies on the interaction between myosin and soy protein (the 11S protein fraction) were conducted by Peng et al. (1982a,b). Solubility and turbidity tests, gel electrophoresis and gel filtration chromatography were carried out after heating the single or combined proteins at temperatures between 4°C and 100°C. It was found that interaction of myosin heavy chains with partially dissociated 11S protein or with its basic subunit ocurred only between 85-100°C.

In attempting to promote complexing of myosin with either casein, soy protein, or gluten, Kurth (1983) and Kurth and Rogers (1984) used the enzyme transglutaminase to induce covalent crosslinks between the proteins. Enzymatic linkage was tried because of its higher possibility for approval by regulatory agencies than chemical crosslinking. Myosin was immobilized on Sepharose 4B beads and made to react with free radiolabeled non-meat proteins. Gamma counting after washing appropriately to remove unreacted protein showed that in fact transglutaminase induced crosslink formation between myosin and the non-meat proteins. Electrophoretic analysis of reaction mixtures also evidenced association between the proteins after transglutaminase action.

CHAPTER III

MATERIALS AND METHODS

Good grade beef chucks were purchased from a local meat packing plant. Upon arrival at the meat laboratory, Oklahoma State University, chucks were deboned and trimmed of visible fat and connective tissue to an end product as lean as possible. The meat was then cut into chunks of approximately one square inch and mixed thoroughly in a paddle type mixer (Leland, 100 lb capacity, Square D Company, Milwaukee, Wisconsin). Approximately one hundred grams of meat was randomly chosen from different positions in the mixer, ground twice (12.7 and 3 mm plates), packed in a plastic Whirl-Pak bag, and used for proximate analysis. The rest of the meat was wrapped with freezer wrap paper into eight packages (3632 g each) randomly assigned to each of eight treatments to be applied in making structured steaks, and five packages (1500 g each) assigned at random to each of five temperatures to be tested in the meat model system study. The packaged meat was then put in a freezer at -28^{o}C until used.

Whey protein concentrates (WPC) used in this research were supplied by New Zealand Milk Products, Petaluma, California. These products are commercially manufactured

and sold under the trade name of Alacen 882 and Alacen 878, and were chosen due to the manufacturer's claim of a lower gelation temperature than ordinary whey protein concentrates. Throughout this thesis they will be referred to as WPC A and WPC B, respectively. Other than being manufactured by means of ultrafiltration, no additional information about the production process of these WPC's was detailed by the supplier.

Chemical Analyses

Protein, moisture, and fat contents were determined in 1-2 g samples of raw meat and WPC. Protein determinations were done by the Kjeldahl procedure using a Kjeltec Auto 1030 Analyzer (Tecator, Herndon, Virginia). Nitrogen conversion factors of 6.25 for the meat samples and 6.38 for the WPC samples were used to calculate percent protein. Determination of moisture content of both meat and WPC samples was carried out by the oven drying method. Samples were dispersed on aluminum pans and allowed to dry in an oven at 105^{o}C for 24 hours. The percent weight loss after drying was expressed as percent moisture content. Fat content of the samples was determined by the Soxhlet extraction method, using petroleum ether as the extractant and refluxing for 24 hours. Samples, wrapped in Whatman # 1 filter paper, were oven dried at 105^{o}C for 24 hours before extracted in the Soxhlet apparatus. After extraction, the samples were dried again as before, and the weight loss of

the extracted samples used to calculate percent fat as follows:

$$\% \text{ fat} = \frac{\text{wt. of dry samples before extraction} - \text{wt. of dry samples after extraction}}{\text{wt. of sample before drying}}$$

Ash content was determined by placing samples in porcelain crucibles, then drying for 24 hours in an oven at 105°C and for additional 24 hours in a furnace at 540°C. The weight of the residual material was expressed as percent ash.

The lactose content of the WPC's was determined following the method of Teles et al. (1978) with slight modifications for the whey protein powders. One half gram of WPC was diluted to 100 ml with distilled water and mixed well. A 2.5 ml aliquot of the diluted sample was placed in a test tube and 0.2 ml of 5% zinc sulfate plus 0.2 ml of 4.5% barium hydroxide were added. The tubes were centrifuged for 5 minutes in a Babcock centrifuge. From each tube, 1 ml of the clear supernatant was transferred to a screw-cap test tube and 2.5 ml of Teles' reagent (1% phenol, 5% NaOH, 1% picric acid and 1% sodium bisulfite mixed in a ratio 1:2:2:1) were added. The tubes were immersed in a boiling water bath for 6 minutes, cooled in tap water, the volume brought to 12.5 ml with distilled water, and mixed well. Absorbance readings at 520 nm against a similarly treated reagent blank in which 2.5 ml of distilled water substituted for the sample were done using a

Gilford Model 240 spectrophotometer. The concentration of lactose (mg/2.5 ml) in the diluted samples was calculated using the equation Y=0.147+0.093X, derived from average absorbance readings of standard lactose solutions. The percent lactose in the original sample powders was calculated dividing the amount of lactose (g) in 100 ml of the sample dilution by 0.5 g (weight of sample) and multiplying by 100.

Preparation of Structured Steaks

A flow diagram indicating the steps followed in the manufacture of and the parameters evaluated in structured steaks is shown in Figure 1. Structured steaks were made to represent each of the formulations indicated in Table I. Meat batches (3632 grams each) were allowed to thaw in a cooler at 5°C. The WPC's were mixed with the water in a one-liter beaker and let hydrate for approximately one hour before being mixed with the meat in a Hobart N-100 kitchen-type mixer (The Hobart Mfg. Co., Troy, Ohio). Mixing time (one minute) was found to be adequate to permit uniform distribution of the WPC on the meat surfaces. After mixing, the meat product was stuffed into fibrous casings (10 cm diameter) to form two meat logs (about 1830 g each) per treatment, for a total of 16 logs per meat block. The meat logs were appropriately identified and placed in a blast freezer at -30°C for 24 hours, tempered at -5°C in a Freas 815 Low Temperature Incubator (GCA/Precision

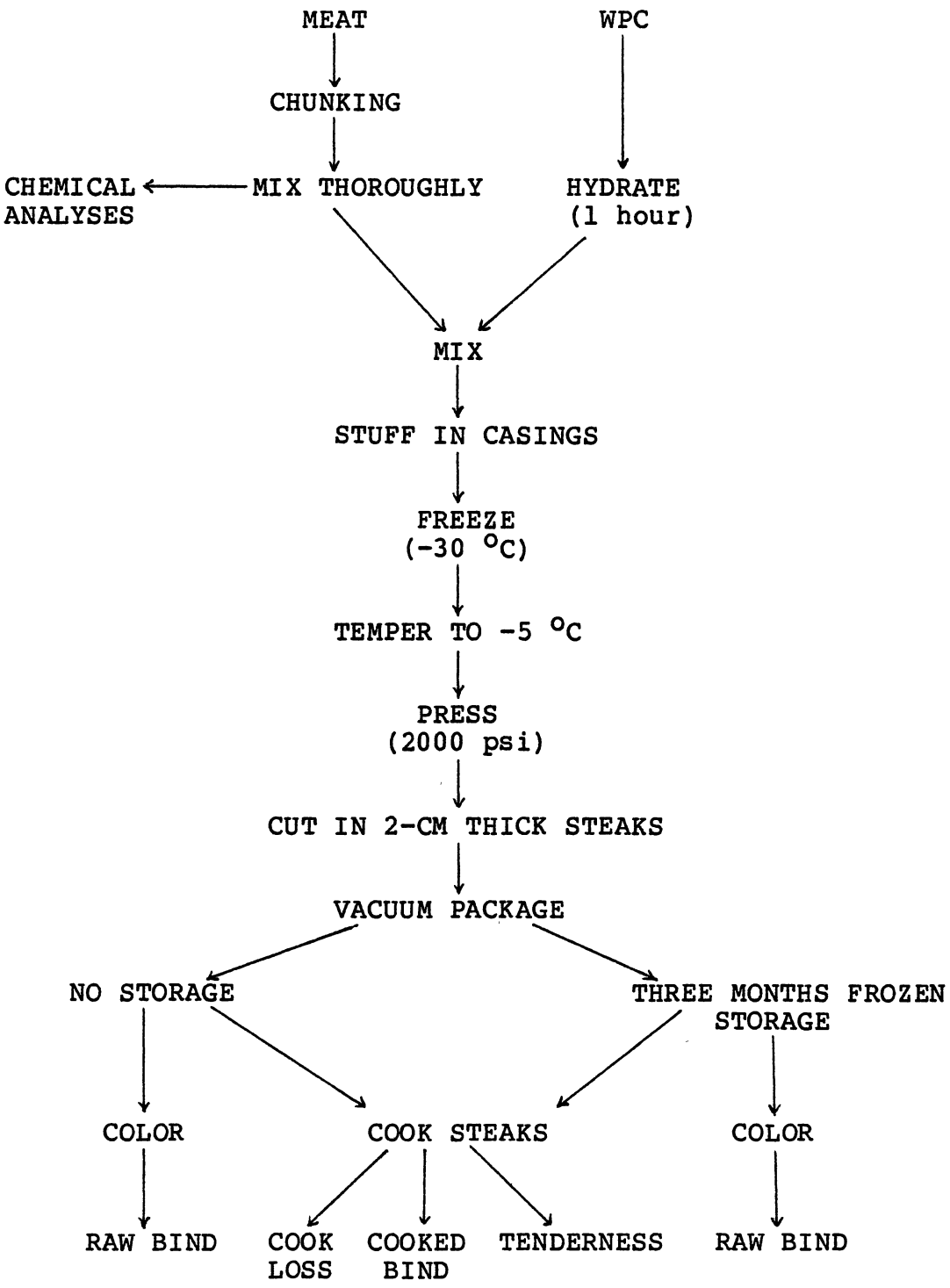

Figure 1. Flow Diagram of Experimental Procedure for Preparation and Evaluation of Structured Beef Steaks.

TABLE I

FORMULATIONS USED IN PREPARATION OF STRUCTURED STEAKS

TREATMENT[a]	MEAT (g)	SALT (g)	WPC A (g)	WPC B (g)	WATER (g)
MEAT ONLY	3632	-	-	-	108.96
0.5% SALT	3632	18.16	-	-	108.96
1% WPC A	3632	-	36.32	-	108.96
2% "	3632	-	72.64	-	108.96
3% "	3632	-	108.96	-	108.96
1% WPC B	3632	-	-	36.32	108.96
2% "	3632	-	-	72.64	108.96
3% "	3632	-	-	108.96	108.96

a. Percent based on weight of meat.

Scientific, Chicago, Illinois) for 24 hours, placed in a Soiltest apparatus (a cylindrical stainless steel mold commonly used in engineering work for testing of soil samples), and pressed in a Carver press (Fred S. Carver, Inc., Summit, New Jersey) at 2000 psi for 2 minutes. After pressing, the meat logs were placed in a freezer at -13°C for few hours to allow hardening of the meat before being sliced. Slicing was done using a Biro band saw cutter (The Biro Mfg. Co., Marblehead, Ohio) set up to produce slices (steaks) 2 cm in diameter. The steaks obtained from two logs of each treatment were randomly assigned to the evaluation procedures, vacuum packaged (0.98 bar), properly labelled, and stored in wax-lined carton boxes in a freezer at -13°C until evaluated.

Evaluation of Structured Steaks

Binding among the meat pieces in raw and cooked state, Kramer shear values, cook loss, and Hunter "L", "a", and "b" color measurements were assesed in the structured steaks within four days after preparation (0 month storage) and after three months of storage in a freezer at -13°C. At each storage period, three steaks per treatment were used for evaluation of bind after cooking (cooked bind), three steaks for Kramer shear value, and three steaks for color measurement and bind evaluation in the raw state (raw bind). Cook loss was determined from the six steaks used for evaluation of cooked bind and Kramer shear value.

Cooking Procedure

Frozen steaks were taken out of the vacuum bags, weighed, and placed on the rack of a Farberware "Open Hearth" electric broiler (Farberware, Bronx, New York). Copper-constantan thermocouples, attached to an Omega OM-202 temperature logger (Omega, Stamford, Connecticut), were inserted at the geometrical center of each steak to monitor the internal temperature of the steaks during cooking. Steaks were cooked from the frozen state (-3 to -5°C) to an internal temperature of about 50°C, then turned over and cooked to a final internal temperature of 70°C. Steaks were placed in a tray, covered with freezer wrap paper, allowed to cool for two hours at room temperature, weighed to determine cook loss, and subjected to the corresponding evaluation procedure.

Bind Evaluation

Binding among the meat pieces forming the structured steaks was evaluated in both the raw and cooked state according to the method described by Brewer et al. (1984) using an Instron Model 1122 Universal Testing Machine (Instron Corporation, Canton, Massachussetts). Raw bind was measured in steaks previously used for color measurements after being allowed to equilibrate to room temperature (24°C). The raw steaks were placed on top of a device consisting of a circle of needles (6.8 cm diameter) mounted 2 mm apart around the cross sectional edge of a plexiglass

cylinder. A compression anvil, modified as a plunger with a round end (2.5 cm diameter), was mounted under the Instron's crosshead and moved down to press and penetrate through the center of the steak at a speed of 100 mm/min until the round end of the plunger had completely emerged through the opposite side of the steak, separating the meat pieces. The peak force (Kg), considered an indication of the degree of binding, was monitored with a 50-Kg cell calibrated to one kg full scale load, and recorded at a chart speed of 100 mm/min. Cooked bind was evaluated in the same manner as raw bind except that steaks were pressed in rather than placed on the circle of needles, and the full scale load was set to 10 kg.

Tenderness Evaluation

Kramer shear values, considered a measure of tenderness, were determined in a 4 cm x 4 cm sample obtained from the central portion of each steak. The sample was weighed, placed in the Kramer shear cell attached to the Instron, and sheared at a crosshead speed of 100 mm/min. The peak force (Kg) required to shear the sample was measured with a 500-Kg cell set at full scale load of 500 Kg, and recorded at a chart speed of 100 mm/min. Results were expressed as Kg/g of sample.

Cook Loss

The difference in weight of the steaks before cooking (at frozen state) and after 2 hours at room temperature following cooking, expressed as percent of the uncooked weight, was reported as percent cook loss.

Color Measurements

Frozen steaks were removed from the vacuum plastic bags, placed on plastic party plates, and exposed to the room environment (approximately 50 minutes) to allow partial thawing until the frost layer formed on the surface of the steaks, which could interfere with the color readings, was no longer visible. Hunter "L", "a", and "b" color values were measured at three positions on the exposed surface of each steak using a Hunterlab Tristimulus Colorimeter Model D25/L-9 (Hunter Associates Laboratory, Reston, Virginia). During measurement of color values, a glass plate was placed on the exposed surface of the steaks to obtain more uniform color readings.

Meat Model System

Five packages (1500 g each) of thoroughly mixed raw chunked chuck meat, obtained from each meat block, were assigned at random to five temperatures (70, 75, 80, 85, and 90^{o}C). The meat from each package was fine ground (3 mm plate), mixed thoroughly, and distributed into eight 180 g

TABLE II

FORMULATIONS FOR TREATMENTS IN MEAT MODEL SYSTEM STUDY

TREATMENTS[a]	MEAT (g)	SALT (g)	WPC A (g)	WPC B (g)	WATER (g)
MEAT ONLY	180	-	-	-	5.4
0.5% SALT	180	0.9	-	-	5.4
1% WPC A	180	-	1.8	-	5.4
2% "	180	-	3.6	-	5.4
3% "	180	-	5.4	-	5.4
1% WPC B	180	-	-	1.8	5.4
2% "	180	-	-	3.6	5.4
3% "	180	-	-	5.4	5.4

a. Percent based on weight of meat.

portions that were randomly assigned to be included in the eight treatment formulations indicated in Table II.

The components of each treatment formulation were mixed for one minute in a Hobart NP-50 kitchen-type mixer (Hobart Corporation, Troy, Ohio). Sixty-gram portions of the mixture were packed into each of three glass tubes (3.3 cm I.D., 3.7 cm O.D., 11 cm long) stoppered at one end with a No. 7 rubber stopper. The mixture was carefully packed in order to avoid air pockets. After packing the mixture, the other end of the tube was stoppered with a No. 7 rubber stopper bearing a glass cylinder (1 cm I.D., 30 cm long) to act as a condenser. Once all the glass tubes from the eight treatments (24 tubes) were packed, they were heated for 30 minutes in a water bath at the corresponding temperature. The tubes were withdrawn from the heating water bath, placed in ice water for 10 minutes, and allowed to stand at room temperature for 2 hours before evaluation. The condenser was then removed and the meat logs withdrawn from the tubes. Removing the lower end stopper allowed the log to slip by gravity out of the tubes. The fluid and particulate matter draining off was collected, weighed and expressed as percent fluid loss. The end portions of each meat log were then cut to obtain a smaller log 4 cm long which was placed standing on a stainless steel plate under the crosshead of the Instron. The meat logs were compressed using a compression anvil (35 mm base diameter) attached to the Instron's crosshead and moving down at a speed of 100 mm/min. The

compression peak force (Kg) was measured with a 50-Kg load cell calibrated to 10 kg full scale load and recorded at a chart speed of 100 mm/min. Since the peak force recorded coincided with the breaking point of the meat log, it was considered a measure of binding among the meat particles.

Assessment of the Interaction Between Myosin and Whey Proteins

The interaction between myosin and the major whey proteins β-lactoglobulin and α-lactalbumin was studied in a model system by high pressure liquid chromatography (HPLC). Commercially purified myosin, β-lactoglobulin and α-lactalbumin (Sigma Chemical Co., St. Louis, Missouri) were used in this experiment. Individual protein solutions (1 mg/ml) or their mixtures (1 mg of each protein/ml) were prepared in 0.05 M potassium phosphate, 0.6 M potassium chloride buffer, pH 6.5, freshly before use. One ml portions were delivered into thoroughly cleaned vials, left unheated, or heated to 70, 80, or 90°C for 15 minutes in a circulating water bath and immediately cooled in ice water for two minutes. All samples were centrifuged (10500 x g, 10 minutes, 0°C) and filtered through a membrane filter 0.45 μm pore diameter before chromatography.

Samples (20 microliters) were injected into a Waters high pressure liquid chromatography system (Waters Associates, Milford, Massachusetts) composed of a Model 6000A solvent delivery system, a U6K injector, a Model 680

Automated Gradient Controller, a Model 440 absorbance detector, a Model 730 data module, and a I-125 protein analysis column. The mobile phase consisted of 0.05 M potassium phosphate, 0.6 M potassium chloride buffer, pH 6.5, flowing at a rate of 2 ml/min. Eluants were detected by their absorption at 280 nm. The resultant peaks were recorded with a sensitivity of 0.02 AUFS at a chart speed of 0.5 cm/min. Triplicate injections were made from each treatment.

Statistical Designs and Analyses

The experiment involving evaluation of structured steaks was designed as a randomized block experiment consisting of three blocks with a split plot arrangement of treatments in which eight formulations represent main unit treatment factors, two storage periods the sub-unit treatment factors, steaks are sub-samples, and color measurements within each steak are sub-sub-samples.

The meat model system experiment was also designed as a randomized block experiment composed of three blocks with a split plot arrangement of treatments. Five temperatures are main unit factors, eight formulations are sub-unit treatments, and meat logs are sub-samples.

Analysis of variance for the data was carried out in a Model 3081 IBM computer at the Computer Center, Oklahoma State University, using the Statistical Analysis System (Barr and Goodnight, 1972). When appropriate, the Least

Significant Difference (LSD) test was used to compare differences among means.

CHAPTER IV

RESULTS

Table III shows the chemical composition of three meat blocks and whey protein concentrates (WPC) used in this research. The meat blocks were in practical terms fairly similar in protein, moisture and ash. In fact, they closely maintained the 4:1 moisture to protein ratio generally reported for meat (deHoll, 1978; Ockerman, 1980). Fat content was similar in meat blocks 1 and 2, and relatively high in block 3. Nevertheless, no attempt was made to uniform the proximate composition of the meat blocks, under the assumption that doing so could interfere with the effect of the whey protein concentrates on the bind between the meat pieces. Furthermore, the meat was lean enough to suggest that interaction, if any, of the whey protein preparations would occur mainly with the protein at the interface of the meat pieces. Chemical analyses of the whey protein concentrates (Table III) indicated that, on the average, WPC B had a slightly higher protein and fat content but lower moisture, ash, and lactose content than WPC A.

TABLE III

CHEMICAL COMPOSITION OF MEAT AND WHEY PROTEIN CONCENTRATES (WPC)

Chemical Component (%)	Meat Block[a]			WPC[b]	
	1	2	3	A	B
Protein	18.69	19.59	18.55	71.47	75.45
Moisture	75.54	74.52	72.83	4.79	3.84
Fat	3.96	3.82	6.11	7.47	7.70
Ash	1.03	1.05	1.02	7.89	4.92
Lactose	-	-	-	8.54	7.56

a. Protein values are means from five determinations. Moisture, Fat, and Ash values are means from 10 determinations.

b. All values are means from duplicate determinations.

Structured Steaks

Effect of WPC on Bind, Tenderness, and Cook Loss

Binding among raw meat pieces in structured steaks was significantly different ($P<0.05$) among tratments depending upon the period of storage as indicated by the presence of a significant Treatment x Storage interaction (Table VIII, Appendix A). However, the mean raw bind values at each period of storage were too low (Table XVII, Appendix B) to be considered of any practical importance and were not further analyzed.

Cooked bind, tenderness, and cook loss of structured steaks were not significantly ($P<0.05$) affected by the interaction of Treatment x Storage (Tables IX, X, and XI, Appendix A, respectively). In addition, the analysis of variance showed no significant ($P>0.05$) differences in cooked bind or in tenderness among structured steaks due to Treatment (Tables IX and X, Appendix A, respectively). However, results presented in Table IV indicated that steaks with WPC A added at the 2% and 3% levels had a tendency to greater cooked bind than steaks with other tratments. The opposite seemed to be true for steaks with added WPC B, which had the lowest mean cooked bind values. In addition, steaks containing added salt or WPC A were the most tender since they had the lowest mean Kramer shear value (9.55 Kg/g

TABLE IV

BIND, TENDERNESS AND COOK LOSS OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE

Parameter	Treatment[a,b]							
	Meat Only	0.5% Salt	WPC A 1%	WPC A 2%	WPC A 3%	WPC B 1%	WPC B 2%	WPC B 3%
Cooked Bind[c] (Kg)	2.14	2.12	2.05	2.22	2.24	1.96	1.82	1.82
Tenderness[d] (Kg/g)	10.87	9.55	9.87	9.71	9.55	10.47	10.54	10.27
Cook Loss[e] (%)	40.91a	36.99cd	38.90b	36.10d	33.94e	38.48bc	35.93d	35.72d

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882; WPC B = Alacen 878.

b. Means in same row followed by different letters are statistically different ($P<0.05$), otherwise they are similar ($P>0.05$)

c. Values are means from 18 determinations

d. Values are means from 18 determinations

e. Values are means from 36 determinations

in the case of both 0.5% salt and 3% WPC A) while steaks containing WPC B or only meat were the least tender of all.

Significant differences ($P<0.05$) in cook loss were found among structured steaks due to a treatment effect (Table XI, Appendix A). Comparison among the mean percent cook loss values (Table IV) revealed that cook losses were lowest ($P<0.05$) in steaks with 3% WPC A (33.94%), followed by steaks with 2% WPC A and 2% and 3% WPC B which had similar ($P>0.05$) cook losses. Steaks with salt or with 1% WPC B had rather intermediate cook loss values, while steaks with 1% WPC A or with only meat showed the highest ($P<0.05$) cook loss (40.91% in steaks with only meat)(Table IV).

Frozen storage for three months had a significant ($P<0.05$) effect on cooked bind, tenderness, and cook loss of structured beef steaks (Tables IX, X, and XI, Appendix A). Mean values for these parameters before (0 months) and after three months of frozen storage are shown in Table V. In general, there was an increase ($P<0.05$) in cooked bind after frozen storage for all steaks regardless of treatment, with the exception of steaks with 1% WPC A, which showed a decrease in cooked bind after storage (Table XVIII, Appendix B). An increase ($P<0.05$) in Kramer shear values was observed after frozen storage, which indicated that keeping the steaks frozen for three months had an adverse effect on tenderness. It should be noted, however, that in steaks containing 3% WPC A the effect was in the opposite direction. They were more tender after frozen storage

TABLE V

EFFECT OF FROZEN STORAGE ON BIND, TENDERNESS, AND COOK LOSS OF STRUCTURED STEAKS WITH OR WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC)

Parameter	Storage (Months)[a] 0	3
Cooked Bind (Kg)[b]	1.93a	2.17b
Tenderness (Kg/g)[b]	9.83a	10.37b
Cook Loss (%)[c]	37.88a	36.36b

a. Means in a row followed by different letters are statistically different ($P<0.05$).

b. Each value is the mean from 72 measurements

c. Each value is the mean from 144 measurements

(Table XIX, Appendix B). A similar pattern occurred with the effect of frozen storage on cook loss. The statistical analysis indicated a significant ($P<0.05$) decrease in the percent cook loss, with mean values over all treatments decreasing from 37.88% before storage to 36.36% after three months of frozen storage (Table V). However, a closer look at the data in Table XX, Appendix B, shows that a decrease in cook loss happened only in steaks containing added WPC, while in steaks prepared with only meat or containing 0.5% salt there was actually an increase in cook loss.

Effect of WPC on Color

As shown in Tables XII, XIII, and XIV, Appendix A, the interaction between the effects of Treatment and Storage, as well as the main effect of Treatment on Hunter "L", "a" and "b" color values of structured steaks were not significant ($P>0.05$). "L" values indicate the degree of lightness-darkness in a scale ranging from 0 for black to 100 for white (Francis and Claydesdale, 1975). No significant differences ($P>0.05$) were found among "L" values of steaks from all treatments, before or after frozen storage (Table XII, Appendix A). The overall mean "L" value was 26.56 (Table VI). Mean Hunter "a" and "b" values, an indication of redness-greeness (+100 = red, 0 = gray, -80 = green) and yellowness-blueness (+70 = yellow, 0 = gray, -70 = blue) respectively, were not statistically different ($P>0.05$) among treatments (Tables XIII and XIV, Appendix A,

TABLE VI

HUNTER COLOR VALUES OF STRUCTURED STEAKS BEFORE AND AFTER THREE MONTHS OF FROZEN STORAGE

Hunter Value	Storage (Months)[a] 0	3	Overall Means[c]
"L"	26.30[b]	26.81	26.56
"a"	9.18a	7.05b	8.12
"b"	7.20a	6.38b	6.79

a. For each color characteristic, means followed by different letters are statistically different ($P<0.05$); otherwise they are statistically similar ($P>0.05$)

b. Each value is the mean from 216 determinations

c. Each value is the mean from 432 determinations

respectively). However, three months of frozen storage significantly ($P<0.05$) reduced redness of the steaks from a mean value over treatments of 9.18 before storage to 7.05 after frozen storage, while yellowness was reduced from a mean of 7.20 to 6.38 during the same period (Table VI).

It is important to point out that although the mean "L" values were fairly similar among the steaks from the various treatments in consideration, before and after frozen storage (Table XXI, Appendix B), the same was not completely true for "a" values despite the similarity indicated by the analysis of variance. A look at the mean "a" values of steaks from each treatment at each period of storage (Table XXII, Appendix B) revealed a tendency of the steaks containing added WPC to be redder than steaks with only meat or containing 0.5% added salt. Such a tendency was more notorious after three months of storage, in particular when the means for steaks with WPC A are compared with that of steaks containing 0.5% salt, which appeared much more discolored.

Meat Model System

Effect of WPC on Bind

Table XV, Appendix A, indicates that there was no significant ($P>0.05$) effect of the interaction Temperature x Treatment on meat bind when using a model system. It means that the effect of one factor (i.e. treatment) followed the same pattern at all "levels" of the other factor (i.e.

temperature). The main effects of treatment and temperature on bind were, however, significant ($P<0.05$). The mean bind value for each treatment, obtained across all temperatures is shown in Figure 2. Incorporation of WPC A at 2% and 3% levels in the meat model system produced meat logs that required the greatest ($P<0.05$) force to be broken, therefore having the highest bind of all treatments. Mean bind values were 3.85 and 4.46 Kg for 2% and 3% WPC A respectively. Meat logs from systems with 0.5% added salt had lower ($P<0.05$) binding than those with 2% or 3% WPC A but were not significantly ($P>0.05$) different from meat logs containing 1% added WPC A. On the other hand, meat formulated with WPC B had lower ($P<0.05$) bind than meat having added salt; meat logs containing 1% and 2% WPC B being no different ($P>0.05$) from logs with only meat (Figure 2).

Figure 3 shows the increasing ($P<0.05$) effect of temperature on binding among meat particles in a model system. The test for differences among means revealed that mean bind values across all treatments, obtained by heating the meat at 75, 80 and 85^{o}C increased significantly ($P<0.05$) with rspect to those at 70^{o}C, but were not different ($P>0.05$) among themselves. Mean bind values at 90^{o}C were, in turn, greater ($P<0.05$) than those at 75, 80 or 85^{o}C (Figure 3).

The particular effects of 2% and 3% WPC A on meat binding in model system at the various temperatures selected for this research are compared in Figure 4 with the effect

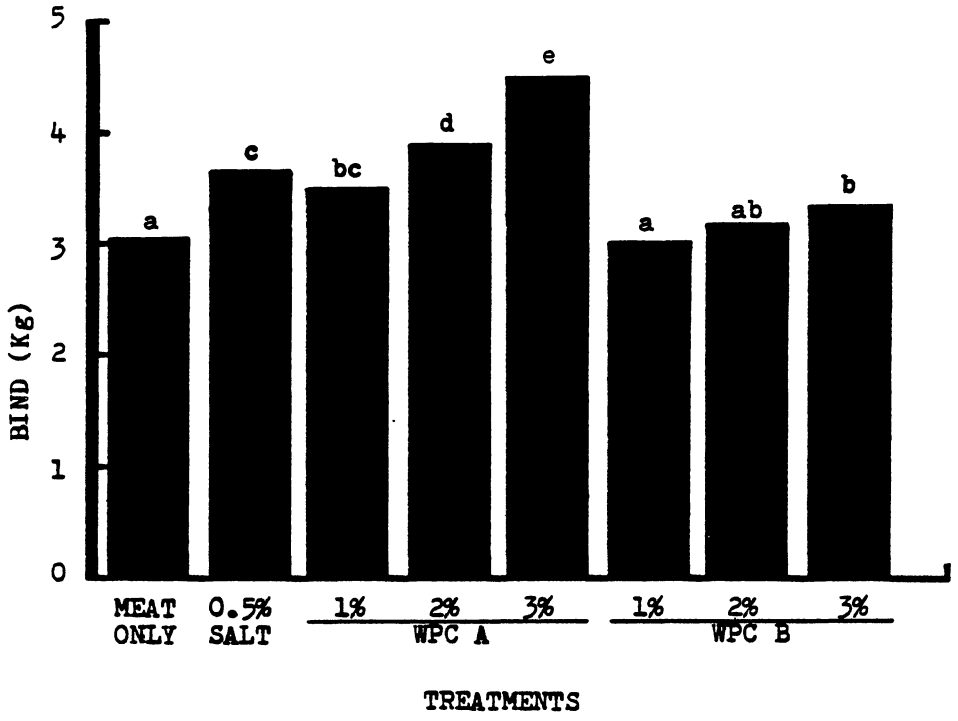

Figure 2. Meat Binding Effect of Whey Protein Concentrate in Model System. (Each bar represents a mean of 45 determinations. Bars with different letters are statistically different at $P<0.05$. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882; WPC B = Alacen 878).

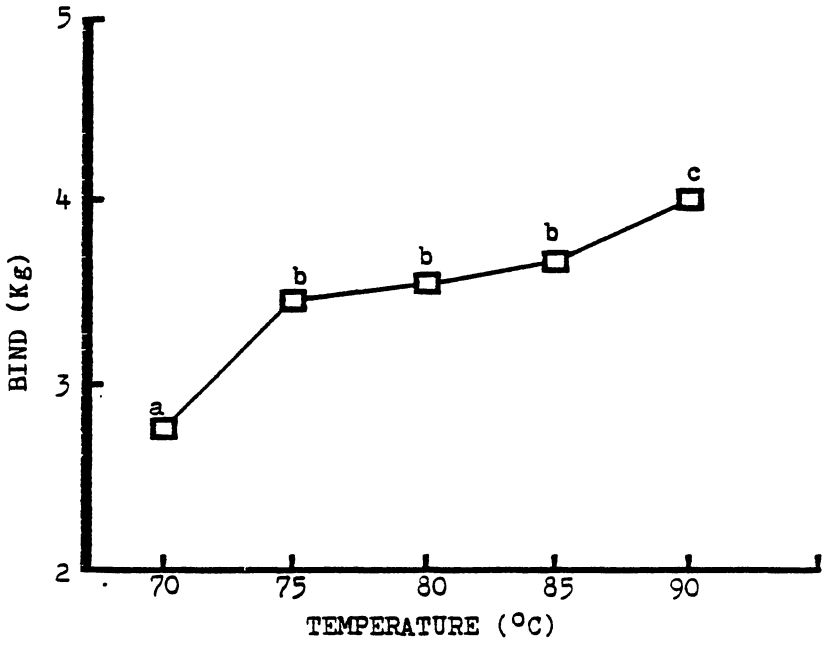

Figure 3. Effect of Heating on Meat Binding in Model System. (Each point represents a mean bind value from all treatments. Points with different letters are statistically different at $P<0.05$).

of adding 0.5% salt or with the effect of using only meat. A clear superior binding effect of 3% WPC A over 0.5% salt or only meat is shown. Although binding was similar when meat with added 2% WPC A or 0.5% salt was heated to 70 or 75°C, the effect of 2% WPC A on binding was greater than the effect of 0.5% salt when the heating temperature was raised to 80, 85, or 90°C. Systems with only meat had lower binding than those containing added salt or WPC A (Figure 4).

Effect of WPC on Fluid Loss

The analysis of variance for data on fluid loss (Table XVI, Appendix A) indicated the presence of a significant ($P<0.05$) interaction of Temperature x Treatment on the loss of fluid from the meat in model system. This means that the differences in fluid loss among treatments were not similar from one temperature to another. Therefore, the mean values for each treatment, obtained over all temperatures (main effects) are not representative of the effect of treatment and cannot be used for comparison. Instead, the mean values for each treatment at each temperature (simple effects) are the ones to be used for establishing appropriate comparisons.

Mean fluid loss values among treatments at each temperature are presented in Table VII and graphically represented in Figure 5. As clearly seen, fluid loss increased ($P<0.05$) in all treatments as the temperature of

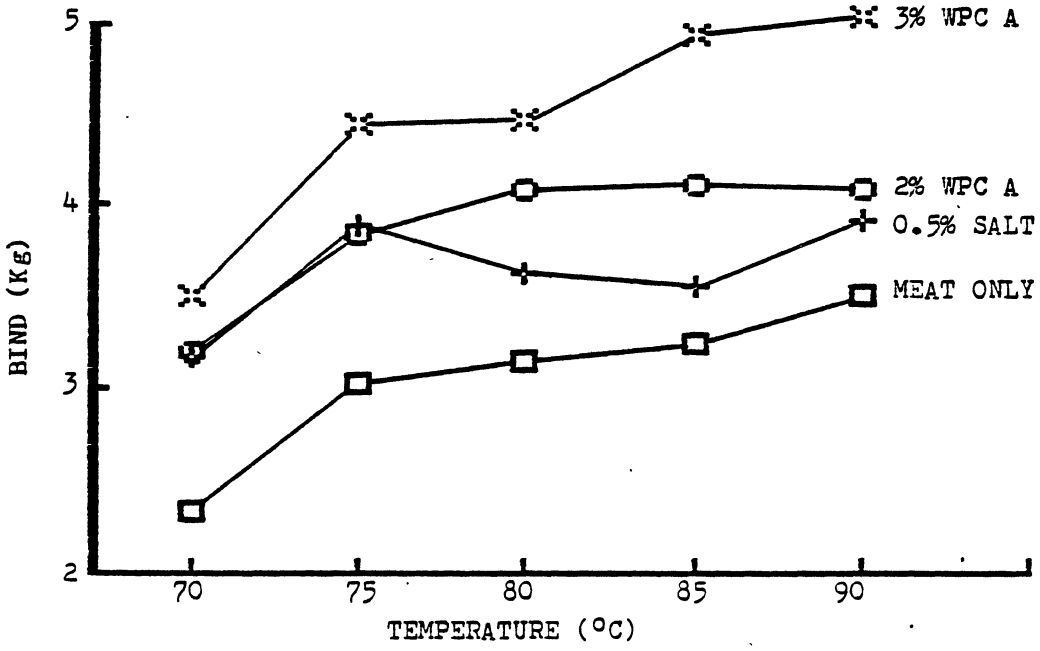

Figure 4. Effect of WPC A on Heat Induced Meat Binding in Model System. (Each point represents a mean bind value from nine determinations).

TABLE VII

FLUID LOSS UPON HEATING OF MEAT MODEL SYSTEM WITH OR WITHOUT ADDED WHEY PROTEIN CONCENTRATE

Temperature (°C)	Treatment[a,b]							
	Meat Only	0.5% Salt	WPC A 1%	WPC A 2%	WPC A 3%	WPC B 1%	WPC B 2%	WPC B 3%
70	15.98e	15.06e	12.39d	9.57bc	7.66ab	10.55c	8.62b	6.90a
75	20.27d	17.38c	17.38c	13.91b	12.12a	16.93c	14.25b	12.51a
80	22.25d	19.36c	19.10c	16.48b	14.21a	18.98c	16.22b	13.71a
85	22.93e	21.06d	19.77c	17.09b	15.56a	19.57c	17.00b	14.44a
90	24.21e	22.39d	21.38d	17.87b	15.95a	22.34d	19.54c	17.26b

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882, WPC B = Alacen 878.

b. Expressed as percent values. Each value is a mean from nine measurements. Means in same row followed by different letters are different ($P<0.05$).

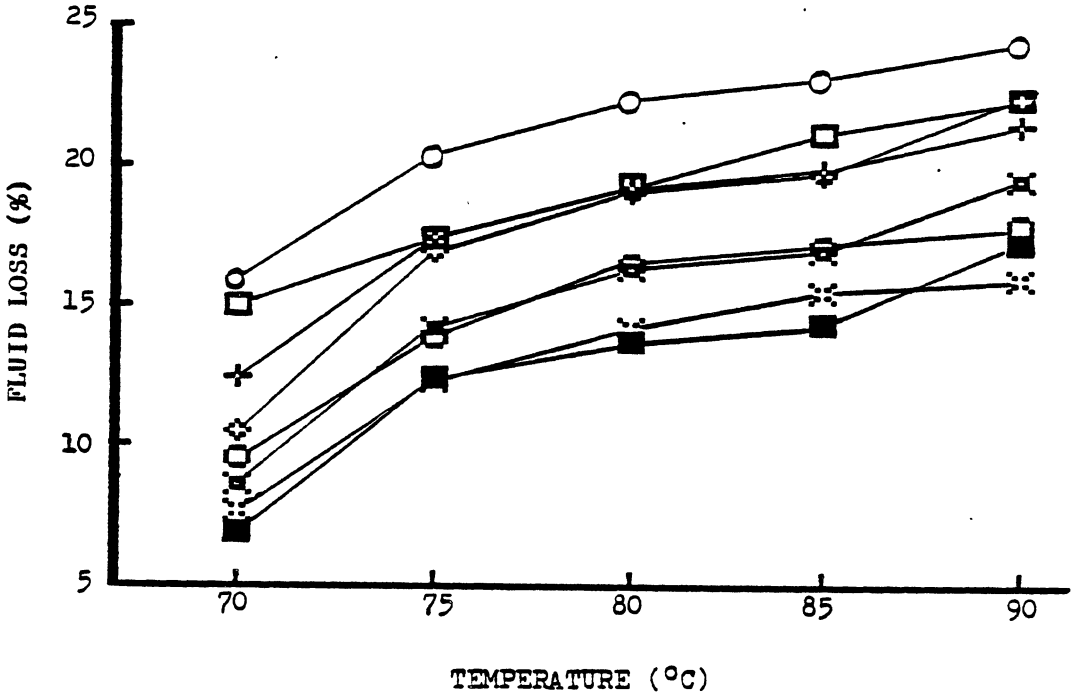

Figure 5. Effect of Whey Protein Concentrate on Fluid Loss in Meat Model System Upon Heating. (Symbols represent treatments formulated to have meat + 3% added water + indicated ingredient as follows: ○ = Meat only, □ = 0.5% salt, ✛ = 1% WPC A, ⬡ = 2% WPC A, ⁜ = 3% WPC A, ✧ = 1% WPC B, ⧓ = 2% WPC B, ■ = 3% WPC B. Each point is a mean from nine determinations).

heating was increased. However, more important to note is the effect upon fluid loss caused by the addition of WPC to the meat system. Fluid loss in meat systems containing 2% and 3% added WPC was significantly ($P<0.05$) reduced in comparison with the other treatments; the 3% level of WPC causing a still greater ($P<0.05$) reduction in fluid loss than the 2% level. At each of these levels, both WPC A and WPC B had a similar effect on fluid loss at all temperatures, except at 90°C, where the effect of WPC A was more beneficial ($P<0.05$) than the effect of WPC B (Figure 5). At 70°C the meat systems with 1% added WPC also showed a significant ($P<0.05$) reduction in fluid loss when compared to systems containing 0.5% salt or meat only. However, as the temperature was increased both WPC's at 1% level had similar ($P>0.05$) effect on fluid loss from the meat as 0.5% added salt. Treatments containing only meat (no added salt or WPC) showed the greatest ($P<0.05$) loss of fluid upon heating (Figure 5).

High Pressure Liquid Chromatography of Myosin, Whey Proteins and Their Mixtures

Individual Protein Solutions

Figures 6, 7 and 8 show the chromatograms for individual solutions of myosin, β-lactoglobulin and α-lactalbumin, respectively. In the unheated state, myosin eluted at about 2.9 minutes after injection as a single peak

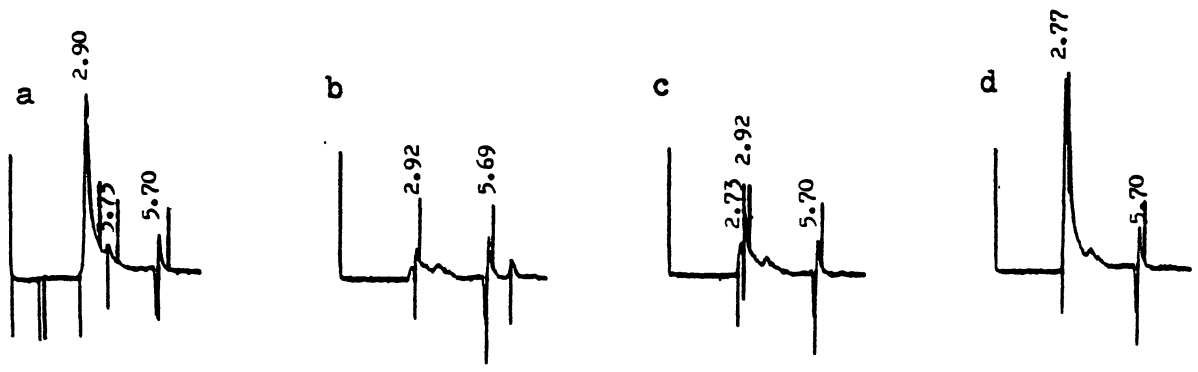

Figure 6. Gel Filtration HPLC Elution Profile of Myosin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

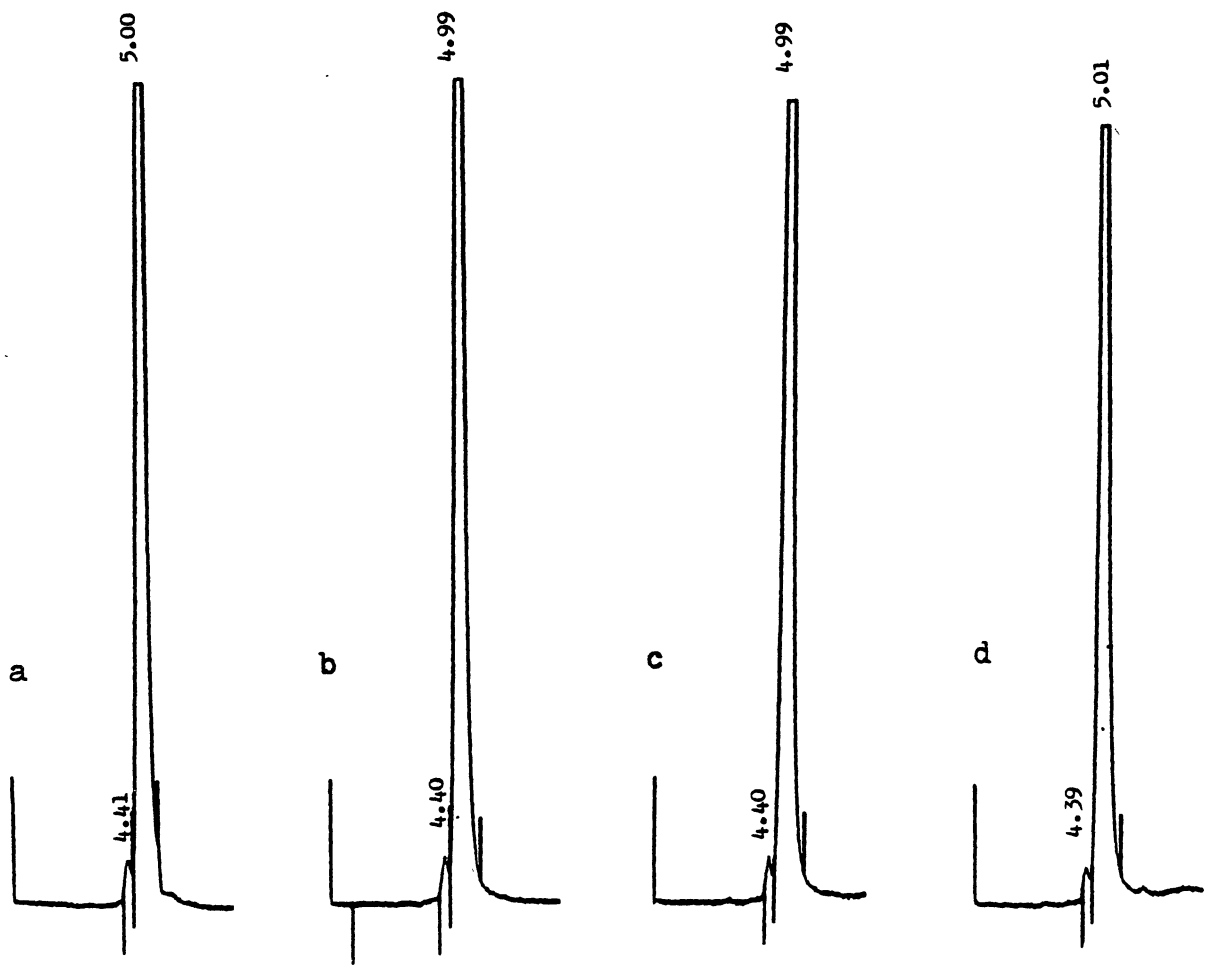

Figure 7. Gel Filtration HPLC Elution Profile of α-Lactalbumin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

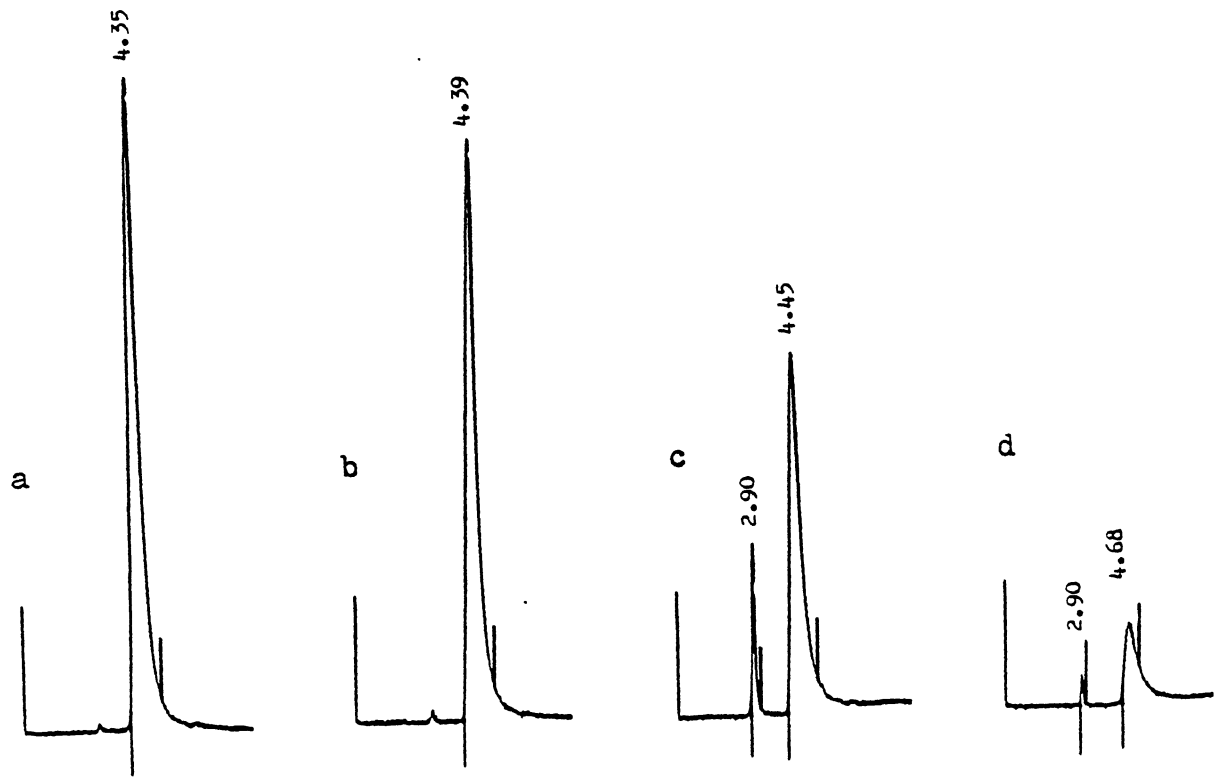

Figure 8. Gel Filtration HPLC Elution Profile of β-Lactoglobulin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

(Figure 6a) which was significantly reduced when the solution was heated at 70°C (Figure 6b). When heated at 80°C, myosin eluted as two peaks, one at about 2.73 minutes and one at about 2.92 minutes (Figure 6c). It should be noted that at the 2.73 minutes region was already visible in the chromatogram of the solution heated at 70°C (Figure 6b). At 90°C (Figure 6d), only one peak eluted at about 2.77 minutes. This peak was greater in size than the peaks observed in the chromatograms of the myosin solution heated at 70 or 80°C (Figures 6b and 6c respectively).

Chromatograms presented in Figure 7 show that α-lactalbumin eluted as a single peak at 5.00 minutes and remained practically unchanged in size in either the heated or the unheated samples. Only at 90°C appeared to occur a very slight reduction in peak size (Figure 7d). On the other hand, β-lactoglobulin eluted as a single peak at about 4.40 minutes when unheated samples or samples heated at 70°C were chromatographed (Figures 8a,b). However, upon heating samples at 80 or 90°C (Figures 8c,d) β-lactoglobulin eluted as two separate peaks (one new peak appearing at about 2.90 minutes) which were greatly reduced in size when samples were heated at 90°C (Figure 8d). It should be noted that there was a small peak at about 2.90 minutes in the chromatograms corresponding to the β-lactoglobulin solution unheated or heated at 70°C (Figures 8a,b respectively).

Mixture of Myosin and α-Lactalbumin

Figure 9 contains the chromatograms of the solution mixture of myosin and α-lactalbumin. Upon injection of unheated samples of this mixture two peaks corresponding to myosin and α-lactalbumin eluted at about 2.89 and 4.83 minutes respectively (Figure 9a). However the myosin peak was greater than the corresponding peak obtained when unheated myosin solution was injected (Figure 6a). Upon heating the myosin-α-lactalbumin mixture at 70°C the corresponding chromatogram showed a greatly increased new peak appearing at 2.78 minutes while the peak corresponding to α-lactalbumin was reduced in size (Figure 9b). A greater reduction in the α-lactalbumin peak ocurred in samples heated at 80 and 90°C but the new peak was only slightly reduced at 90°C (Figures 9c,d).

Mixture of Myosin and β-Lactoglobulin

The chromatographic patterns of the mixture myosin β-lactoglobulin are presented in Figure 10. In the unheated state two main peaks appeared, one at about 2.91 minutes, corresponding to myosin, and the other at about 4.33 minutes which corresponded to β-lactoglobulin. Like in the case of the unheated mixture of myosin and α-lactalbumin (Figure 9a), the myosin peak shown in the chromatogram of the mixture of myosin and β-lactoglobulin (Figure 10a) was greater than the corresponding peak in the chromatogram of the unheated individual myosin solution (Figure 6a).

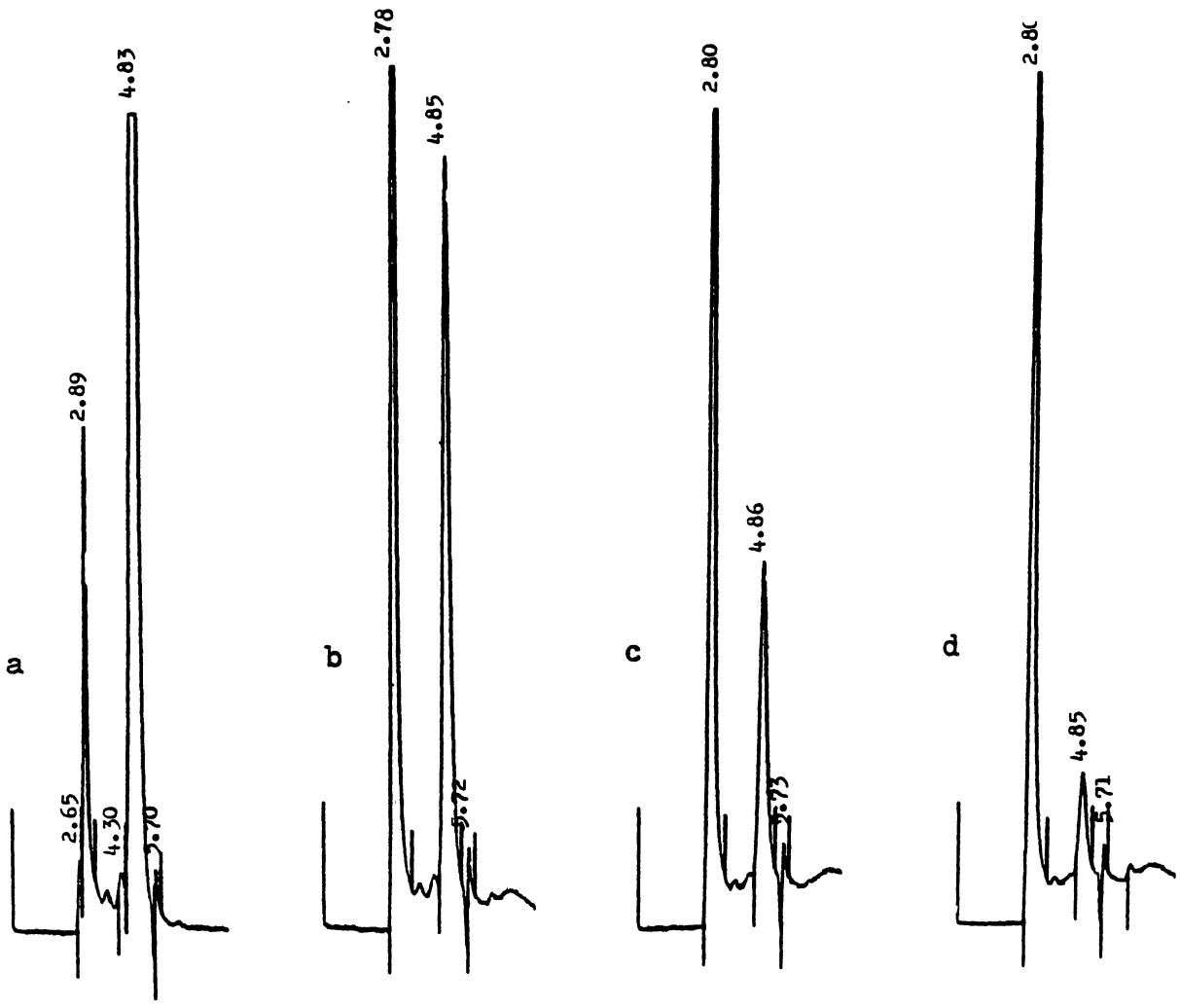

Figure 9. Gel Filtration HPLC Elution Profile of a mixture of myosin and α-Lactalbumin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

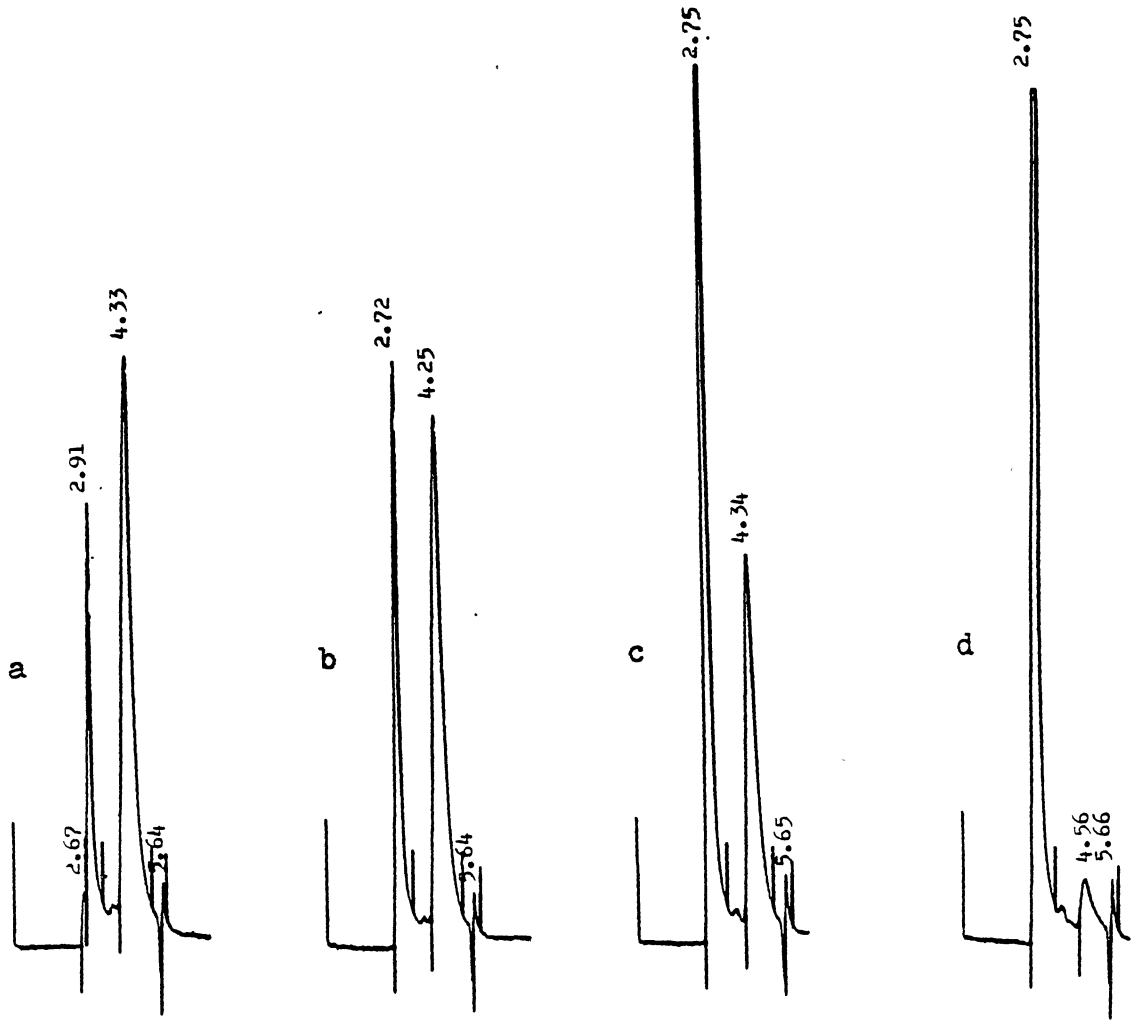

Figure 10. Gel Filtration HPLC Elution Profile of a mixture of myosin and β-Lactoglobulin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

Heating the myosin-β-lactoglobulin mixture at 70°C produced a change in the elution pattern (Figure 10b). The myosin peak increased in size and eluted earlier (at about 2.72 minutes) than its unheated counterpart (Figure 10a); while the β-lactoglobulin peak decreased in size. This effect became increasingly more evident in samples heated at 80 and 90°C (Figures 10c,d). In fact, when heating the mixture at 90°C the peak corresponding to β-lactoglobulin became so small that it was not integrated.

Mixture of α-Lactalbumin
and β-Lactoglobulin

Figure 11 contains the chromatographic patterns of the α-lactalbumin-β-lactoglobulin mixture before and upon heating. The unheated mixture (Figure 11a) and the mixture heated at 70°C (Figure 11b) revealed elution patterns for α-lactalbumin and β-lactoglobulin very much like the elution patterns of the unheated individual protein solutions (Figures 7a,b and 8a,b, respectively). A very small peak (not integrated in the chromatogram from the unheated mixture) showed up at about 2.93 minutes when the mixture was heated at 70°C (Figure 11b). This peak, which corresponds to a similar peak observed when heating the solution of β-lactoglobulin at 70°C (Figure 8b), became greater when the mixture was heated at 80°C (Figure 11c). In addition, the β-lactoglobulin peak (shown eluting at 4.45 minutes in Figure 11b) was not resolved from the mixture

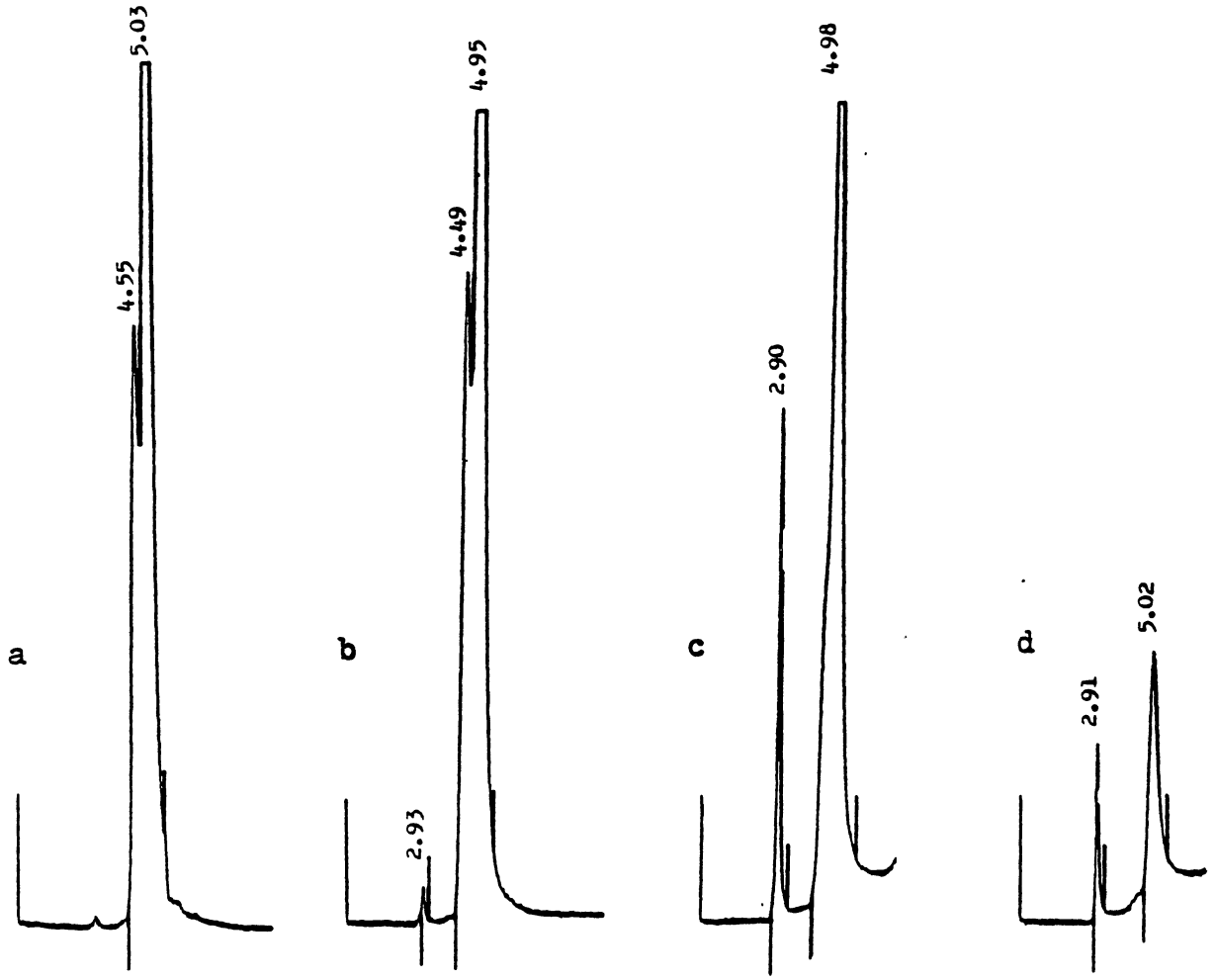

Figure 11. Gel Filtration HPLC Elution Profile of a mixture of α-Lactalbumin and β-Lactoglobulin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

heated at 80°C. It appeared as a shoulder in the front side of the peak corresponding to α-lactalbumin (Figure 11c). In the chromatogram of the mixture of α-lactalbumin and β-lactoglobulin heated at 90°C (Figure 11d) both the peak corresponding to the fraction of β-lactoglobulin eluting at about 2.91 minutes and the α-lactalbumin peak appeared of considerable reduced size, which indicates that at this temperature a great deal of the whey protein in the mixture had precipitated.

Mixture of Myosin, α-Lactalbumin, and β-Lactoglobulin

The elution pattern of the myosin mixture with α-lactalbumin and β-lactoglobulin is presented in Figure 12. The chromatogram for the unheated protein mixture showed three peaks which eluted at 2.96, 4.55 and 5.00 minutes, corresponding to myosin, β-lactoglobulin, and α-lactalbumin, respectively (Figure 12a). The myosin peak appeared reduced in size when compared with the myosin peak from the unheated individual myosin solution (Figure 6a). β-lactoglobulin and α-lactalbumin eluted as two peaks which were not completely resolved (fused peaks). Upon heating the mixture at 70°C, the size of the myosin peak slightly increased, while that of the β-lactoglobulin peak slightly decreased; however, the α-lactalbumin peak was significantly reduced in size (Figure 12b). The elution pattern of the mixture heated at 80°C or 90°C showed only two peaks (Figures 12c,d). At 80°C the

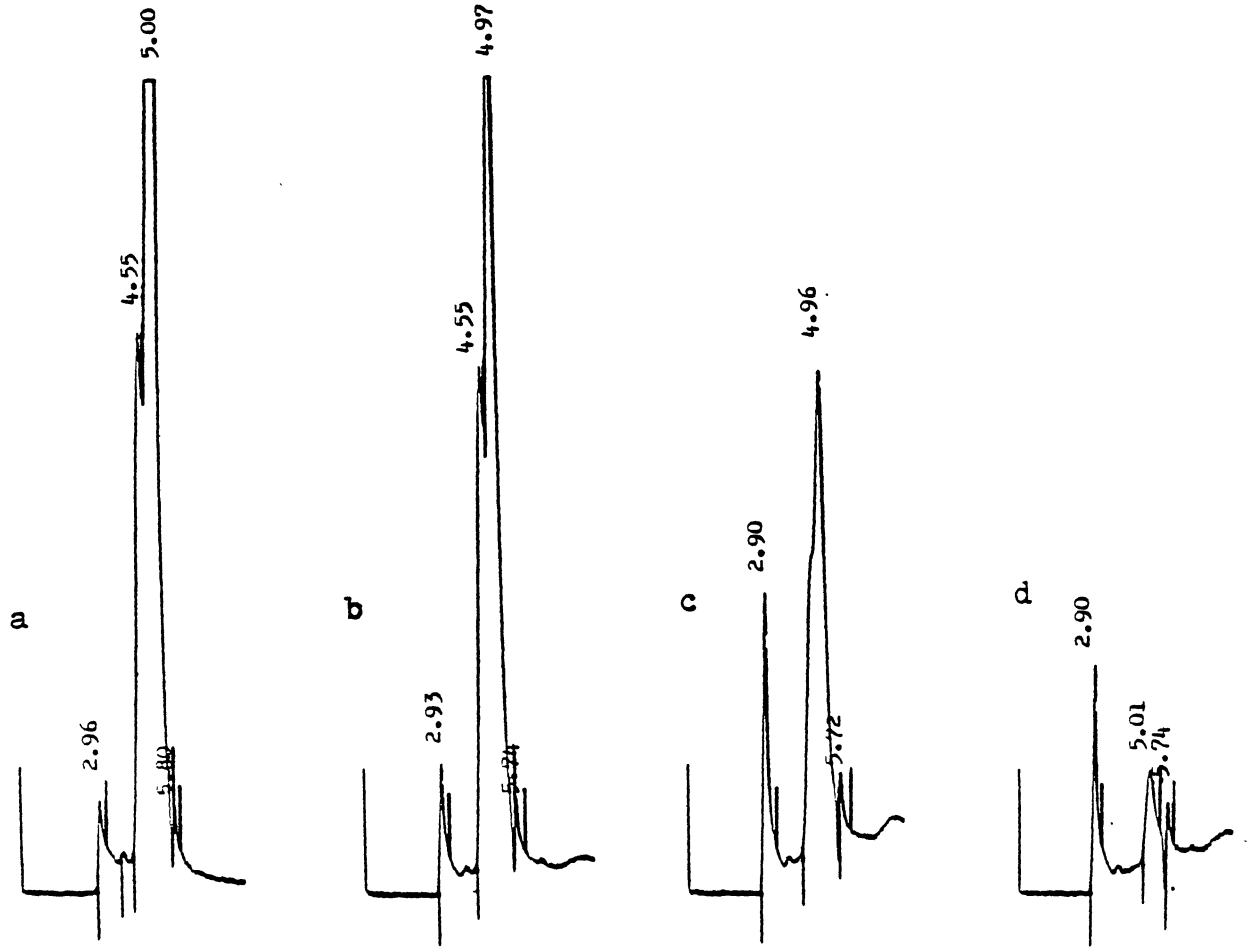

Figure 12. Gel Filtration HPLC Elution Profile of a mixture of myosin, α-Lactalbumin and β-Lactoglobulin. (a, unheated; b, heated at 70°C; c, heated at 80°C; d, heated at 90°C)

peak corresponding to myosin further increased in size when compared to the same peak in the mixture heated at 70°C. On the other hand, the peak corresponding to β-lactoglobulin was not resolved, rather seeming to appear as a shoulder in front of the peak corresponding to α-lactalbumin, which was notably smaller in size than the α-lactalbumin peak at 70°C (Figure 12c). Heating at 90°C caused a much stronger effect on the proteins in the mixture, as the myosin peak was reduced to about 50% its size at 80°C, while the α-lactalbumin peak was very small and no evidence of the presence of β-lactoglobulin was observed (Figure 12d).

CHAPTER V

DISCUSSION

Two reasons seem to account for the development and/or the increasing success of structured beef steak. One is the high price of whole muscle steak, evolved as a consequence of the growing consumer desire for more steak and less roast (Mandigo, 1982a). The other reason is represented by the interest of meat producers and processors for better utilization and value enhancement of those portions of the beef carcass which are currently deemed to be of relatively low value (Breidenstein, 1982).

As of today, structured beef steak is produced mainly for distribution and use in a frozen state by the hotel, restaurant, and institutional (HRI) trade and by the fast food industry. Lack of stable binding among meat pieces in the unfrozen state has not allowed, so far, merchandising of raw refrigerated structured steak thus hampering their access to the retail market in the fresh meat area.

The effect of whey protein concentrate (WPC) as raw binder in structured beef steak was evaluated as part of this research. The results showed that meat binding was practically negligible in either the products with or without added WPC since the meat pieces were not held

together strong enough to maintain the integrity of the steaks during handling. Thus, in regard to the conditions of this experiment, application of heat during cooking continues to be the only way to induce stable acceptable binding among meat pieces in structured steaks. However, it is important to note that Means and Schmidt (1986) reported on the production of structured beef steaks having effective bind in the raw refrigerated state using a combination of sodium alginate (0.8-1.2%) and calcium carbonate (0.144-0.216%) as a meat binder. There is no doubt that retail marketing of refrigerated structured raw steaks will increase the sales volume of these products. However, for that to be successfully accomplished, the products must keep a natural meat identity to the eye of the buyers. In addition, incorporation of ingredients not traditionally used for a function in meat systems may have to face rigorous testings imposed by the regulatory agencies before they are approved for use in structured meat products.

Despite the absence of statistically significant differences among steaks due to the effect of treatment, cooked bind in structured steaks containing WPC A added at 2% or 3% levels was perceived to be greater and had greater mean values than in steaks from any of the other treatments in study. Statistical differences were not found probably because of high variability observed among individual values within treatments, which would overlap making the failure of the statistical test to detect the perceived differences.

Two main factors could be held reponsible for such variability, both of which are directly or indirectly related to a lack of uniformity among steaks within a particular treatment: a) Chunks of meat forming a log do not arrange themselves in an organized manner within the casing during stuffing. Therefore, when slicing the meat logs to obtain steaks, the meat pieces within a steak are in different arrangement and have a different surface area of contact than the meat pieces in other steaks from the same meat log. b) As a consequence, the plunger used for evaluation of bind does not "penetrate" the steaks at a point with similar characteristics every time. In some instances the plunger hits the center of the whole meat chunks while in other cases it hits the area of contact between two or more chunks of meat. Thus, the system used in this research for evaluation of meat binding does not seem to be the most appropriate one for chunked and formed steaks. The unit would be good for bind evaluation in flaked and formed steaks or in steaks formed from ground meat, which would be structurally more uniform than chunked and formed steaks. Nevertheless, this method of bind evaluation appears to be one of the most acceptable at the present time. Other methods that have been utilized for the evaluation of meat bind involve using an Instron machine to pull apart strips or sections of cooked structured meat held by means of pneumatic grips (Booren et al., 1981b; Huffman et al., 1984) or by a device in which the meat samples are

secured between two plates by pins (Gillett et al., 1978). These methods, however, are more time consuming and have problems, associated with handling of the meat samples and/or with calibration of the Instron, not found in the method used in this research.

Cooked bind increased after the steaks were kept frozen for three months. This result is in agreement with the findings of Ockerman and Organisciak (1979), who observed a slight non-significant increase in panel scores for cohesiveness of structured steaks that were also frozen for three months. Schnell et al., (1970) suggested cell disruption as one of the factors influencing the binding ability of meat. Mixing is one processing step which favors cell disruption, promoting release of intracellular contractile proteins and making them available for binding. In this research, mixing was done for one minute, not enough time to allow a significant extraction of myofibrillar proteins (Booren et al., 1981b,c). It is possible, however, that cell disruption ocurred as a consequence of freezing and/or thawing during cooking thus accounting for the increase in bind after frozen storage. Ockerman and Organisciak (1979) did not detect a significant difference in binding between frozen and unfrozen steaks probably because most cell disruption already occurred before freezing, during the sequential tumbling of the meat done by these authors.

The statistical analysis indicated that no differences existed in tenderness among structured steaks due to a treatment effect. However, average Kramer shear values were lower (meaning more tenderness) for steaks containing added salt or WPC A than for steaks from the other treatments. As with cooked bind, variability among tenderness values within treatments was probably high enough to avoid its detection by the statistical method of analysis. This variability was probably produced by pieces of connective tissue being sheared by the blades of the Kramer shearing device. Connective tissue (pieces of tendon, fascia, or septa) will remain in some chunks of meat even after every effort is made to remove it. That steaks containing salt appeared more tender than steaks with only meat is in agreement with previous reports (Furumoto and Stadelman, 1980; Coon et al., 1983) of a beneficial effect of salt on meat tenderness. However, since no salt was included in steaks with 2% or 3% WPC A, and water retention is ruled out because steaks with WPC B had good water retention but were less tender than steaks with salt, a clear explanation for the increase in tenderness observed in steaks with 2% and 3% WPC A is not available. The fact that steaks containing WPC A were more tender and had better bind than steaks with added WPC B indicates that the properties of the protein in these products are different. Although no information was supplied by the manufacturer, it seems obvious from the differences in appearance and functional properties observed

that some sort of modification was induced during manufacture of these whey protein concentrates.

Determining cook loss in meat products is important because it reflects the ability of the system's proteins to hold water. Percent cook loss was significantly lower in structured steaks containing 2% and 3% added WPC than in steaks from the other treatments. These results are not surprising because whey proteins are known to have good water binding ability (de Wit, 1984; Mangino, 1984). They show, in addition, that a concentration of 2% or 3% could be used in meat systems.

After frozen storage there was a reduction in tenderness of the structured steaks. One could expect that an increase in cook loss, produced in part by promoted cell disruption due to freezing (Franks et al., 1983), would lead to such reduction in tenderness (Lawrie, 1979); however, this possibility has to be ruled out because a decrease in cook loss was rather observed in the structured steaks after frozen storage. Nevertheless, it should be noted that such explanation holds for structured steaks with meat only or with added 0.5% salt, which suffered a reduction in tenderness (Table XXIII, Appendix B) with an increase in cook loss after frozen storage.

It is not clear why a reduction in tenderness was detected in steaks with added WPC. Possibly frozen storage induced some interaction between whey proteins and meat components in such a way that the product became tougher.

Nevertheless, tenderness of steaks with added WPC A was acceptable and similar to that of steaks with added salt.

Cook loss was further reduced in the structured steaks after frozen storage possibly because there was more opportunity for the whey proteins to interact with muscle intracellular water as a result of more cell damage due to freezing.

Color is a prime factor influencing customers' decision to buy or not to buy raw meat or meat products (Secrist, 1982). Regardless of treatment, color values of the structured steaks manufactured in this research were not different, which indicated that addition of WPC did not make the steaks look lighter, as one might expect, than steaks without added WPC.

Usually redness is the meat color attribute of concern to producers and consumers. Despite Hunter "a" values, which indicate the degree of redness, being statistically not different among treatments, structured steaks containing WPC, in particular WPC A, appeared redder to the naked eye than steaks without added WPC. Probably the WPC components, mainly whey proteins, directly or indirectly prevent reduction of the pigments in meat. However this is something to be proven.

Frozen storage had an adverse effect on "a" and "b" color values of the structured steaks. Such effect was more pronounced in "a" values of steaks containing salt, which were highly discolored. These results are in agreement with

previous reports (Ockerman and Organisciak,1979; Booren et al., 1981a; Chastain et al., 1982; Huffman et al., 1984; Means and Schmidt, 1986) indicating the negative effect of salt on meat color.

In evaluating protein functionality, model food systems have been suggested to be more realistic than systems involving individual proteins (Porteous and Quinn, 1979). Potential effects of non protein components in the actual food on the functionality of added protein products are included in food model systems but not in mixtures of individual proteins. The meat model system utilized in this research corroborated the perceived effectiveness of WPC A as a meat binder when added at 2% or 3% levels to structured steaks. Similar findings have been reported by Burgarella et al., (1985a) who observed that WPC A (Alacen 882) added to minced fish (surimi) in proportions of 20% or 40% was responsible for producing meat gels that were at least twice as rigid as mixtures of minced fish with egg white at the same combination levels.

It is worth mentioning that WPC A promoted good bind in structured meat despite mixing time being only one minute. This is important because a reduction in mixing time during processing of structured meat products would lead not only to time and energy savings but also to reduced incorporation of oxygen into the meat system. Limiting the amount of oxygen will limit development of oxidative reactions in lipids and pigments in the meat. In addition to the good

binding ability demonstrated for WPC A, the observed reduction in fluid loss from treatments containing added WPC in the food model system reaffirmed the excellent water binding capacity of the whey protein products.

Increasing the heating temperature in the range of 70°C to 90°C produced an increase in bind among meat particles in the model system. This result was expected because meat binding is a phenomenom associated with a heat-induced gelation mechanism involving the proteins in the meat system (Schnell et al., 1970), which becomes more pronounced as the heating temperature is increased (Acton, 1972; Siegel and Schmidt, 1979a). The fact that 3% WPC A was better than 0.5% salt in promoting meat binding at all temperatures tested and that 2% WPC A promoted greater bind than 0.5% salt after 75°C demonstrated that at these concentrations WPC A has good gelation characteristics which are maintained at temperatures generally used in heat processing of meat products. This suggested that the utilization of WPC A in processed meat is warranted.

Water binding, as assessed with a meat model system, was much greater in meat containing either WPC A or WPC B, in particular at either the 2% or 3% levels, than in meat from any other treatment at all temperatures investigated. It has been reported that meat products showing decreased cook loss or increased juiciness, and therefore increased water retention, showed also good binding or increased cohesiveness (Schnell, et al., 1970; Ockerman and

Organisciak, 1979; Huffman, 1979). In addition, it has been suggested a relation of meat binding with hydration of the myofibrillar proteins at the meat surfaces (Terrell et al., 1982). The observed beneficial effect of added WPC A on meat binding was not a result of the increased water holding ability of this whey protein product because WPC B, which also had good water retention, had a poor effect on bind. Possibly the good gelation capacity along with an improved ability of the whey proteins in WPC A to interact with the meat proteins, as a result of protein modification, may have resulted in improved binding. Siegel et al. (1979) have also reported that the ability of added nonmeat proteins to bind fat and water was not related to their ability to bind meat pieces. In this respect, Burgarella et al. (1985b) suggested that a "filler effect" produced upon gelation of an added protein in the interstitial spaces of a meat (fish) gel network is reponsible for an increase in structural strength.

The chromatographic information presented in Figures 6-12 was collected in order to determine qualitatively the effect of heat on myosin and whey proteins and whether or not there was any protein interaction upon heating of their mixtures. It should be pointed out, however, that in trying to establish appropriate conditions for chromatography of these proteins, in particular myosin, several problems arose which are worth mention. Myosin is a troublesome molecule for HPLC studies because of its shape, high molecular weight

Stoke's radius, and low solubility. Thus, at the beginning of this research a Bio-Sil TSK 400 column (Bio-Rad Laboratories, Richmond, California) was considered to have suitable specifications to carry out gel filtration HPLC of myosin and whey proteins. However, apparently due to problems related to myosin and/or to the high salt concentration in the buffer and the inability of the gel in the column to allow use of pressures higher than 1,000 psi, the column lost its efficiency and could not be further used.

A Waters I-125 protein analysis column, showed good stability to the high salt-containing buffer. In addition, it was capable of resisting pressures of up to 3,000 psi and therefore the flow rate could be increased to allow greater flow of solvent. The manufacturer specifications for the Waters column indicated that it would have a resolution in the molecular weight range of 500 to 80,000. Therefore, it should have resolved the whey proteins without problems, but myosin and high molecular weight aggregates would elute in its void volume, a characteristic that must be kept in mind for interpretation of the respective chromatograms.

Heating myosin at 70^{o}C caused a great deal of protein denaturation and precipitation. This result is in agreement with those observed from studies using DSC (Samejima et al., 1983), gel electrophoresis (Cheng and Parrish, 1979) or gel filtration chromatography (Peng et al., 1982b). However, upon heating at 80^{o}C the peak height increased slightly and

a small peak was detected at about 2.73 minutes, which indicated that upon heating at this temperature molecular aggregation occured. This effect was more noticeable in the chromatogram corresponding to 90°C (Figure 6d), in which the original "myosin peak" dissappeared almost completely while the peak shown at 2.77 minutes was as high as the unheated myosin peak. The reason for this behavior is not clearly interpreted. It seems to indicate that upon heating to 90°C solubility of myosin or its products is regained; however, this explanation is not in agreement with the traditional biochemical concept of insolubility and precipitation after heat induced aggregation. A similar phenomenom could be observed in the chromatograms presented by Peng et al., (1982b), although these authors did not discuss the phenomenon.

The fact that the chromatograms for α-lactalbumin (Figure 7) showed little change in the temperature range of 70-90°C confirms previous reports (Larson and Rollieri, 1955; de Wit, 1981) indicating that α-lactalbumin is the most heat stable whey protein. On the other hand, the chromatographic behavior of β-lactoglobulin (Figure 8) is also in agreement with reports from DSC studies (Hegg, 1980; de Wit, 1981; de Wit and Klarenbeek, 1984) which indicated a denaturation temperature of about 70°C for this protein. The effect of heat on β-lactoglobulin, which was already noticed at 70°C (Figure 8a), was more pronounced with further increase in the temperature to 80°C or 90°C. The

detection of a peak at about 2.90 minutes suggested that protein aggregation was favored at 80°C.

The increase in the height of the myosin peak observed in the chromatogram from the unheated myosin-α-lactalbumin mixture (Figure 9a) when compared to the peak from the unheated individual myosin solution (Figure 6a) appears to indicate that solubility of myosin is increased in the presence of α-lactalbumin or that actually some interaction already occurs in their native state, or both. Protein interaction is, however, suggested by the results presented in Figures 9b, 9c, and 9d. As a consequence of heating, a new peak with a significant increase in height appeared at about 2.80 minutes while the height of the α-lactalbumin peak decreased, which indicated that a stable association between myosin and α-lactalbumin ocurred in the temperature range of 70-90°C. A similar phenomenom occurred when myosin was in the presence of β-lactoglobulin. In the unheated mixture (Figure 10a) the myosin peak appeared increased in size in comparison with the myosin peak from the unheated individual myosin solution (Figure 6a), which indicated that in the unheated state β-lactoglobulin increased myosin solubility. The new peak eluting at about 2.72 minutes upon heating of the mixture at 70°C (Figure 10b) suggested that a myosin-β-lactoglobulin interaction developed as a result of heating because such peak does not form in the unheated individual β-lactoglobulin solution heated at 70°C (Figure 8b). Chromatograms from the mixtures of myosin and

β-lactoglobulin heated at 80 and 90°C (Figures 10c,d) revealed that further interaction along with progressive precipitation of β-lactoglobulin occurred at these temperatures.

It is interesting to notice that in the unheated mixture of myosin and α-lactalbumin (Figure 9a) as in the unheated mixture of myosin and β-lactoglobulin (Figure 10a) a small peak was detected at about 2.65 minutes. Since such peak did not appear in the chromatograms of any of the involved proteins in the unheated state, it may be an indication of some protein interaction occurring between myosin and whey proteins in the unheated state.

Interaction between α-lactalbumin and β-lactoglobulin was reported by Hunziker and Tarassuk (1965) to occur upon heating at 75°C for 30 minutes. These authors based this conclusion on the fact that the amount of α-lactalbumin decreased 14% when heated alone but 84% when heated in the presence of β-lactoglobulin. Although such decrease could have really happened due to interaction with β-lactoglobulin, it could also have been the result of α-lactalbumin precipitation. Results in Figure 11 indicate that some interaction between α-lactalbumin and β-lactoglobulin started to occur at 70°C because the peak shown at 2.93 minutes (Figure 11b) was not detected in the chromatogram of β-lactoglobulin heated at 70°C (Figure 8b). Such interaction is more noticeable in the chromatogram of Figure 11c which shows a peak at 2.90 minutes of greater

size than its counterpart peak originated from heating β-lactoglobulin at 80°C (Figure 8c). A chromatogram comparable to that of Figures 11b,c was presented by Baer et al., (1976), who used radioactive α-lactalbumin to demonstrate its presence in the new peak appearing upon heating.

The elution profiles of the mixtures of myosin with α-lactalbumin and β-lactoglobulin (Figure 12) showed no interaction of the whey proteins with myosin because no peak was detected eluting at about 2.70 minutes. However, the chromatographic behavior of the whey proteins in this mixture followed the same pattern observed for the mixture α-lactalbumin-β-lactoglobulin (Figure 11). Thus, it appears that when both whey proteins are in the presence of myosin the interaction with themselves is preferred to the interaction with myosin. One reason for this result could be related to the number of each type of protein molecules per unit volume of the mixture. Although this number is not known for this particular mixture, it is obvious that myosin molecules being of much larger molecular weight and size will be in smaller number than whey proteins. Therefore, the probability of whey proteins to interact with themselves is greater than the probability of interacting with myosin, because of the increased surface area of the proteins (de Wit, 1981).

From the results shown in Figure 12 it would be thought that the incorporation of whey proteins in a meat system,

such as in structured steaks or other processed meats, would result in no interaction between whey proteins and myosin. However, it should be taken into consideration that in a meat system the number of myosin molecules available for reaction with the whey proteins is much greater than under the experimental conditions of this chromatographic research. Therefore, for the same reasons explained before, a great possibility for interaction of myosin with the whey proteins exist.

Although interaction of the individual whey proteins with myosin and with themselves was shown to occur, no attempt was done to investigate the mechanisms by which such interactions are maintained. It has been reported that formation of disulfide bonds occurs during interaction of α-lactalbumin with β-lactoglobulin (Hunziker and Tarassuk, 1965) or with casein (Doi et al., 1983b), while both disulfide and hydrophobic bonds are formed during interaction of β-lactoglobulin with casein (Doi et al., 1983a). On the other hand, disulfide bonds are involved in the interaction of myosin with actin (Gergely, 1966). It is possible that interaction of myosin with whey proteins could occur through disulfide bridge formation or hydrophobic association, since this molecule has more than 40 thiol residues (Lowey et al., 1969) and good surface hydrophobicity (Borejdo, 1983; Li-Chan et al., 1984).

It deserves consideration that results of this study, which are in agreement with those of Hunziker and Tarassuk

(1965) and de Wit (1981), revealed that α-lactalbumin increases its heat sensitivity in the presence of β-lactoglobulin (Figure 11). In addition, the same phenomenom was observed in the presence of myosin (Figure 9). Differential scanning calorimetry (DSC) studies have suggested that the thermal behavior of whey protein concentrate is governed or controlled by that of β-lactoglobulin (de Wit, 1981; Bernal and Jelen, 1985). However, since the heat sensitivity of α-lactalbumin is increased in the presence of other proteins, it is possible that the observed deflection in the DSC thermograms obtained by these authors be actually the result of overlapped denaturation temperatures of α-lactalbumin and β-lactoglobulin. If this is true, then the thermal behavior of WPC would be actually determined by the two main whey proteins.

The area of structuring meat, as newly perceived in the sense of formed meat rather than the traditional sausage concept, has developed increasing interest among all sectors of the meat industry because of the feasibility not only of producing new meat products of varied composition, but also because of the potential for increasing profitability from those parts of the animal carcasses traditionally considered of low value. By 1982 there were no figures for the volume of structured meat produced in the U.S.A. but Western European countries were producing 11,000 to 13,500 tons per year (Field, 1982). However, Mandigo (1982a) reported

military purchases of 6.5 million lb of flaked veal steaks in 1981 and expected purchases of 10.5 million lb of flaked pork and 400,000 lb of flaked lamb products. New technologies in the structured meats area may represent also an opportunity for better utilization by developing countries of other more-available meats such as rabbit, goat, mutton, or fish.

Production of whey protein concentrate, on the other hand, has significantly increased in the last years as a result of pollution regulations which ban disposal of liquid whey into municipal drain systems, and as a result of development of ultrafiltration membranes of good quality and shelf life. By 1983, an estimated 15.4 million lb of WPC were produced in the U.S.A. (Morr, 1984).

This research has demonstrated the efficiency of a whey protein concentrate, WPC A, with particular functional properties in improving binding and other quality characteristics of chunked and formed beef steaks. This is important because it expands the potential for marketing of both structured meat products and whey protein concentrate. In the near future, more scientific work will be conducted to further insure the success of structured meat products. In particular, binding technology will be an area of continuing exploration, with emphasis on the search for new binding materials or modification of the binders already known, in order to obtain an acceptable degree of cohesiveness which permits refrigerated retail marketing of

structured steaks. In this respect, the observed interaction of whey proteins with myosin in the unheated state may hold promise for WPC manufacturers and for meat processors. While research aimed to solve problems associated with connective tissue, color and shelf life of structured meat will continue, new products will be developed through new processing technologies and combinations of different types of meat, including poultry and fish.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This research evaluated the effect of two whey protein concentrates, WPC A and WPC B, on meat bind (raw and cooked), tenderness, cook loss, and color of structured steaks, before and after three months of frozen storage; and on meat bind and fluid loss in a meat model system at temperatures of 70-90°C. Good grade beef chuck meat was used for manufacturing the structured steaks and for the meat model system. In addition, high pressure liquid chromatography (HPLC) experiments were carried out to assess protein interaction in unheated or heated combinations of commercially purified myosin, α-lactalbumin, and β-lactoglobulin.

Structured steaks were manufactured from chunked beef and formulated to contain WPC added at 1%, 2%, or 3% levels. Steaks with only meat or with 0.5% added salt (NaCl) were also prepared, thus making a total of eight treatment formulations. Mixing time was one minute for all formulations. After preparation, the steaks were vacuum packaged and half their number from each treatment were evaluated within the next four days for bind and color in the raw state, and for bind, tenderness and cook loss after

being cooked to an internal temperature of 70°C. Steaks constituting the remaining half of each treatment were evaluated in the same manner after being held in storage for three months at -13°C.

Binding in raw state (raw bind) was not strong enough to hold the meat pieces together; therefore it was considered inexistent.

Although no statistically significant differences were detected among treatments, binding in the cooked state (cooked bind) was perceived to be higher in steaks containing 2% or 3% added WPC A than in steaks from other treatments. Lack of statistical difference was attributed to high variability among bind values within treatments, caused by absence of uniformity among steaks. Cooked bind increased after frozen storage of the steaks possibly due to release of intracellular proteins after cell disruption caused by freezing and thawing.

No significant differences were detected in tenderness among structured steaks as an effect of treatment. However, steaks containing added WPC A or 0.5% salt appeared more tender than steaks with added WPC B or with only meat. In this case, lack of significant differences was attributed to high variability in tenderness values within treatments, caused by the presence of pieces of connective tissue in the meat chunks. After frozen storage there was a significant reduction in tenderness, which in steaks with only meat or with added 0.5% salt was attributed to an increase in cook

loss after frozen storage. It is not clear why a reduction in tenderness occurred in steaks with added WPC.

Cook loss was significantly lower in steaks with added 2% or 3% WPC than in steaks from other treatments as a result of the good ability of the whey proteins to hold water. A further reduction in cook loss observed after frozen storage may have resulted from increased interaction of the whey proteins with water released from the cells due to cell damage by freezing.

Hunter "L", "a", and "b" color values were not significantly different among structured steaks due to the effect of treatment. However, steaks containing added WPC appeared redder than steaks without WPC added. Frozen storage had no effect on "L" values, but produced a decrease in "a" and "b" values of the steaks. In particular, steaks containing salt looked more discolored than steaks from other treatments.

The effect of added whey protein concentrate on bind and fluid loss in a ground meat model system was evaluated upon heating the system in the temperature range of 70-90^{o}C. Treatments consisted of ground meat only, ground meat plus 0.5% salt, or ground meat plus WPC added at 1%, 2%, or 3% level. The meat mixtures (60 grams) were placed in glass tubes and heated in a water bath at 70, 75, 80, 85, and 90^{o}C for 30 minutes. Upon cooling, fluid loss was determined and Instron evaluation of bind was carried out. Treatments with 2% or 3% WPC A had greater bind than other treatments.

Adding WPC B to the meat system resulted in poor bind among the meat particles. The effect on bind increased as the temperature was raised. Treatments containing WPC added, in particular at 2% or 3% levels, had significantly less fluid loss than treatments with 0.5% salt or with only meat.

Good bind was promoted in meat by added WPC A despite mixing time being only one minute. Therefore, savings in time and energy along with reduced incorporation of oxygen into the system are potential benefits during manufacture of structured meat products containing WPC A.

Studies on the interaction between myosin, α-lactalbumin, and β-lactoglobulin were carried out in a model system. Solutions of commercially purified myosin, α-lactalbumin, and β-lactoglobulin and their mixtures were prepared and left unheated or heated at 70, 80, and 90°C for 15 minutes. Upon cooling of the solutions, appropriate samples were subjected to analysis by high pressure liquid chromatography. Heating the individual protein solutions indicated that α-lactalbumin was the most heat stable protein, while a great deal of myosin precipitation already ocurred at 70°C. Interaction of myosin with α-lactalbumin and myosin with β-lactoglobulin ocurred in either unheated and heated solution mixtures. However, when myosin was in the presence of both whey proteins in the same mixture, no interaction with myosin ocurred.

Although α-lactalbumin was demonstrated to be very stable to heat when heated individually, such stability

decreased when α-lactalbumin was in the presence of either myosin or β-lactoglobulin. This result leads to think that both whey proteins, and not β-lactoglobulin alone, determine the thermal behavior of whey protein concentrates.

In the near future more research will be done in the area of binder technology with the purpose of finding a means to maintain acceptable binding in the refrigerated state.

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APPENDIXES

APPENDIX A

ANALYSES OF VARIANCE

TABLE VIII

ANALYSIS OF VARIANCE FOR BIND IN RAW STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	143	0.5146		
Whole Unit				
Block (B)	2	0.0063	0.82	0.4597
Treatment (T)	7	0.1646	6.10	0.0021
Error (a)	14	0.0540		
Sub - Unit				
Storage (S)	1	0.0097	6.29	0.0233
T x S	7	0.0230	2.78	0.0426
Error (b)	16	0.0246	0.65	0.8306
Subsampling	96	0.2255		

TABLE IX

ANALYSIS OF VARIANCE FOR BIND IN COOKED STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	143	40.3645		
Whole Unit				
Block (B)	2	5.6559	10.27	0.0018
Treatment (T)	7	3.4470	1.79	0.1680
Error (a)	14	3.8560		
Sub - Unit				
Storage (S)	1	2.0856	11.19	0.0041
T x S	7	2.6085	2.00	0.1189
Error (b)	16	2.9812	0.91	0.5639
Subsampling	96	19.7304		

TABLE X

ANALYSIS OF VARIANCE FOR KRAMER SHEAR VALUES OF STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	143	277.5091		
Whole Unit				
Block (B)	2	30.6407	8.48	0.0039
Treatment (T)	7	31.8048	2.51	0.0673
Error (a)	14	25.2955		
Sub - Unit				
Storage (S)	1	10.2400	7.61	0.0140
T x S	7	4.4139	0.47	0.8431
Error (b)	16	21.5244	0.84	0.6370
Subsampling	96	153.5899		

TABLE XI

ANALYSIS OF VARIANCE FOR COOK LOSS OF STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	287	4738.1028		
Whole Unit				
Block (B)	2	126.4090	5.22	0.0202
Treatment (T)	7	1221.1410	14.41	0.0001
Error (a)	14	169.4320		
Sub - Unit				
Storage (S)	1	167.9486	13.12	0.0023
T x S	7	154.7641	1.73	0.1728
Error (b)	16	204.8309	1.14	0.3183
Subsampling	240	2693.5771		

TABLE XII

ANALYSIS OF VARIANCE FOR HUNTER "L" COLOR VALUES OF STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	431	3504.3567		
Whole Unit				
Block (B)	2	21.6421	0.47	0.6359
Treatment (T)	7	185.1252	1.14	0.3919
Error (a)	14	323.8772		
Sub - Unit				
Storage (S)	1	28.2594	0.45	0.5134
T x S	7	162.9856	0.37	0.9077
Error (b)	16	1011.8195	16.97	0.0001
Subsampling	96	697.1464	1.95	0.0001
Subsubsampling	288	1073.5014		

TABLE XIII

ANALYSIS OF VARIANCE FOR HUNTER "a" COLOR VALUES OF STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	431	1839.1023		
Whole Unit				
Block (B)	2	282.5867	31.32	0.0001
Treatment (T)	7	63.8170	2.02	0.1244
Error (a)	14	63.1642		
Sub - Unit				
Storage (S)	1	489.4741	23.34	0.0002
T x S	7	78.3052	0.53	0.7969
Error (b)	16	335.5720	17.75	0.0001
Subsampling	96	185.9522	1.64	0.0010
Subsubsampling	288	340.2307		

TABLE XIV

ANALYSIS OF VARIANCE FOR HUNTER "b" COLOR VALUES OF STRUCTURED BEEF STEAKS

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	431	557.6586		
Whole Unit				
Block (B)	2	127.5668	32.52	0.0001
Treatment (T)	7	9.0213	0.66	0.7041
Error (a)	14	27.4609		
Sub - Unit				
Storage (S)	1	73.4250	12.52	0.0027
T x S	7	76.0019	1.85	0.1456
Error (b)	16	93.8353	20.17	0.0001
Subsampling	96	66.6146	2.39	0.0001
Subsubsampling	288	83.7327		

TABLE XV

ANALYSIS OF VARIANCE FOR BIND IN MEAT MODEL SYSTEM

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	359	189.1484		
Whole Unit				
Block (B)	2	16.9109	10.64	0.0056
Temperature (C)	4	60.3810	18.99	0.0004
Error (a)	8	6.3583		
Sub - Unit				
Treatment (T)	7	75.4171	65.20	0.0001
C x T	28	7.2526	1.57	0.0668
Error (b)	70	11.5664	3.52	0.0001
Subsampling	240	11.2622		

TABLE XVI

ANALYSIS OF VARIANCE FOR FLUID LOSS IN MEAT MODEL SYSTEM

Source of Variation	Degrees of Freedom	Sum of Squares	F value	PR>F
Total Corrected	359	7480.9564		
Whole Unit				
Block (B)	2	780.7237	63.47	0.0001
Temperature (C)	4	3651.6551	148.44	0.0001
Error (a)	8	49.2014		
Sub - Unit				
Treatment (T)	7	2685.4217	213.30	0.0001
C x T	28	110.9971	2.20	0.0041
Error (b)	70	125.8988	5.60	
Subsampling	240	77.0585		

APPENDIX B

MEANS

TABLE XVII

RAW BIND IN STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a,b]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[d]
MEAT ONLY	0.20[c]	0.20	0.20
0.5% SALT	0.17	0.25	0.21
1% WPC A	0.12	0.14	0.13
2% WPC A	0.13	0.16	0.15
3% WPC A	0.13	0.15	0.14
1% WPC B	0.18	0.19	0.19
2% WPC B	0.14	0.13	0.14
3% WPC B	0.11	0.11	0.11
Means Across Treatments[e]	0.15	0.16	0.16[f]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Values are expressed in Kg

c. Means from nine measurements.

d. Means from 18 measurements.

e. Means from 72 measurements.

f. Overall mean from one 144 measurements.

TABLE XVIII

COOKED BIND IN STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a,b]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[d]
MEAT ONLY	2.06[c]	2.22	2.14
0.5% SALT	1.89	2.36	2.12
1% WPC A	2.27	1.84	2.05
2% WPC A	2.03	2.40	2.22
3% WPC A	2.06	2.42	2.24
1% WPC B	1.80	2.12	1.96
2% WPC B	1.60	2.04	1.82
3% WPC B	1.70	1.93	1.82
Means Across Treatment[e]	1.93	2.17	2.05[f]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Values are expressed in Kg

c. Means from nine measurements.

d. Means from 18 measurements.

e. Means from 72 measurements.

f. Overall mean from 144 measurements.

TABLE XIX

TENDERNESS OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a,b]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[d]
MEAT ONLY	10.43[c]	11.31	10.87
0.5% SALT	9.33	9.76	9.55
1% WPC A	9.37	10.37	9.87
2% WPC A	9.45	9.97	9.71
3% WPC A	9.61	9.48	9.55
1% WPC B	10.28	10.65	10.47
2% WPC B	10.38	10.69	10.54
3% WPC B	9.38	10.71	10.27
Means Across Treatments[e]	9.83	10.37	10.10[f]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Tenderness was measured as Kramer shear values expressed in Kg/g

c. Means from nine measurements.

d. Means from 18 measurements.

e. Means from 72 measurements.

f. Overall mean from 144 measurements.

TABLE XX

COOK LOSS OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a,b]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[d]
MEAT ONLY	40.82[c]	41.00	40.91
0.5% SALT	36.92	37.06	36.99
1% WPC A	40.81	36.98	38.90
2% WPC A	36.95	35.26	36.10
3% WPC A	34.93	32.94	33.94
1% WPC B	39.04	37.92	38.48
2% WPC B	36.08	35.77	35.93
3% WPC B	37.52	33.92	35.72
Means Across Treatments[e]	37.88	36.36	37.12[f]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Cook loss is expressed as percent values.

c. Means from 18 measurements.

d. Means from 36 measurements.

e. Means from 144 measurements.

f. Overall mean 288 measurements.

TABLE XXI

HUNTER "L" COLOR VALUES OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[c]
MEAT ONLY	27.46[b]	27.03	27.25
0.5% SALT	25.26	25.85	25.58
1% WPC A	27.71	26.57	27.14
2% WPC A	25.68	27.06	26.37
3% WPC A	26.64	25.33	25.98
1% WPC B	25.39	27.27	26.33
2% WPC B	26.61	28.56	27.59
3% WPC B	25.61	26.78	26.20
Means Across Treatments[d]	26.30	26.81	26.55[e]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Means from 27 measurements.

c. Means from 54 measurements.

d. Means from 216 measurements.

e. Overall mean from 432 measurements.

TABLE XXII

HUNTER "a" COLOR VALUES OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[c]
MEAT ONLY	8.25[b]	7.73	7.99
0.5% SALT	8.53	5.89	7.21
1% WPC A	8.88	7.62	8.25
2% WPC A	9.52	7.51	8.51
3% WPC A	9.67	7.33	8.50
1% WPC B	9.25	7.06	8.15
2% WPC B	9.81	6.29	8.05
3% WPC B	9.52	6.97	8.25
Means Across Treatments[d]	9.18	7.05	8.11[e]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Means from 27 measurements.

c. Means from 54 measurements.

d. Means from 216 measurements.

e. Overall mean from 432 measurements.

TABLE XXIII

HUNTER "b" COLOR VALUES OF STRUCTURED STEAKS WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC), BEFORE AND AFTER FROZEN STORAGE

Treatment[a]	Storage (Months) 0	Storage (Months) 3	Means Across Storage[c]
MEAT ONLY	6.84[b]	7.32	7.08
0.5% SALT	6.69	6.58	6.63
1% WPC A	7.10	6.32	6.71
2% WPC A	7.01	6.69	6.85
3% WPC A	7.18	6.62	6.90
1% WPC B	7.36	5.95	6.66
2% WPC B	7.81	5.50	6.66
3% WPC B	7.60	6.03	6.81
Means Across Treatments[d]	7.20	6.38	6.79[e]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.

b. Means from 27 measurements.

c. Means from 54 measurements.

d. Means from 216 measurements.

e. Overall mean from 432 measurements.

TABLE XXIV

HEAT INDUCED BIND AMONG MEAT PARTICLES IN A MEAT MODEL SYSTEM WITH AND WITHOUT ADDED WHEY PROTEIN CONCENTRATE (WPC)

Temperature (°C)	Treatment[a,b]								Means Across Treatments[d]
	MEAT ONLY	0.5% SALT	WPC A 1%	WPC A 2%	WPC A 3%	WPC B 1%	WPC B 2%	WPC B 3%	
70	2.33[c]	3.16	2.73	3.19	3.51	2.31	2.36	2.46	2.76
75	3.01	3.88	3.45	3.83	4.43	2.93	3.06	3.07	3.46
80	3.13	3.62	3.43	4.07	4.45	3.03	3.17	3.54	3.55
85	3.24	3.56	3.74	4.09	4.91	3.02	3.36	3.33	3.66
90	3.50	3.91	3.95	4.08	5.00	3.72	3.86	4.05	4.01
Means Across Temperatures[e]	3.04	3.63	3.46	3.85	4.46	3.00	3.16	3.29	3.49[f]

a. Treatments were formulated to contain meat + 3% added water + indicated level of ingredient. WPC A = Alacen 882 WPC B = Alacen 878.
b. Values are expressed in Kg
c. Means from nine determinations.
d. Means from 72 determinations
e. Means from 45 determinations
f. Overall mean from 360 determinations

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