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Development of low-fat ground beef patties with whey protein concentrates

Laroia, Serena, Ph.D.
The Ohio State University, 1994
DEVELOPMENT OF LOW-FAT GROUND BEEF PATTIES WITH WHEY PROTEIN CONCENTRATES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of the Ohio State University

By

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To My Parents
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INTRODUCTION

Dietary concerns have produced a demand for low-fat meat products. Approximately 3 billion pounds of ground beef are consumed every year in the United States. This accounts for approximately 50% of the total fresh beef consumption. Ground beef with 10% or less fat is not considered as palatable as beef with 20% fat or higher because of a decrease in juiciness, tenderness and flavor.

To overcome the texture problems, low-fat ground beef products have been formulated by addition of water in combination with specific water binders. Some of the binders which have been used include polysaccharides and various proteins. A formulation using iota-carrageenan and water as texture modifiers, and salt and hydrolyzed vegetable protein (HVP) as flavor enhancers was used by the McDonald's Corporation and promoted as McLean Deluxe. However, problems have been recognized with this formulation including an unwanted after-taste, a skin formation around the patty that diminishes the appearance and decreases the palatability.

In this study low-fat ground beef products were formulated using whey protein as a water-binder. The results have shown that whey proteins are effective for this purpose by providing for a low-fat product with satisfactory textural and flavor properties.
LITERATURE REVIEW

Nutritional Concerns

Nutritional and health concerns are influencing consumer choices with regard to food. A survey by the Economics and Statistics Service of the United States Department of Agriculture (USDA) (1979-1980) found that 28% of the households were making dietary changes due to concern about fat intake, 23% were concerned about cholesterol and salt, and 43% were interested in weight loss (National Research Council, 1988). Later reports by Haberstroh and Morris (1991) found that in 1989 the top two nutrition concerns were cholesterol and fat, with 61% and 58% of the respondents viewing these as serious health hazards.

Coronary heart disease (CHD) is reported to be the number one cause of death in the United States (Keeton, 1994). A decade ago, the Surgeon General's Report on Nutrition and Health (U.S. DHHS, 1988) urged Americans to change their lifestyles by reducing dietary intake of saturated fat, cholesterol, salt and sugar; to consume more complex carbohydrates; and to reduce obesity.

In 1990 the National Cholesterol Education Program (NCEP) published a report titled, "Population Strategies for Blood Cholesterol Reduction." This report was based on results from experimental laboratory work, extensive clinical and pathological research, and epidemiological studies. According to the report,
50% of the adults in the U.S. have cholesterol levels above the desirable range and are, therefore, at a greater risk for cardiovascular disease.

NCEP made recommendations for nutrient intakes for healthy Americans: (1) less than 10% of the total calories should be from saturated fatty acids; (2) an average of 30% of the total calories or less should be from all fat; (3) less than 300 mg of cholesterol should be consumed per day (NCEP, 1990).

The NCEP report also recommended to the food industry that efforts should be increased to design, modify, prepare, promote, label and distribute good-tasting food which would meet recommendations for lower levels of saturated fatty acids, total fat and cholesterol.

Harrington (1994) stated that "the Western diet is considered too high in fat (particularly saturated fat), sugar, and salt and too low in fruit, vegetables and complex carbohydrates." The popularity that some traditional and popular meat products enjoy has suffered due to these concerns. There are certain other factors that influence the purchase of these products such as income, availability, price, visual appeal, fitness, the choice of alternative foods, and eating satisfaction (Richardson et al., 1994). The willingness of consumers to make a repeat purchase depends on eating satisfaction and product consistency.

These trends have created a demand for development of leaner meat products. To satisfy consumer expectations these products should be convenient and healthy. Most importantly they should taste good (CAST, 1991). Thus methods and technologies need to be developed that will help decrease the fat and cholesterol content of meats and meat products.

The consumption of total beef has declined 19.9% from 1970 to 1991. Yet the consumption of hamburger or ground beef increased 21.9 to 28.5 lbs (10 to 13 kg)/capita, which is a 23.2% increase. Ground beef accounts for 60% of all beef used in food service applications. Since ground beef is a very popular item there are opportunities to lower dietary fat intakes by targeting this product.
Role of Fat in Meat

The eating quality of meat may be determined in terms of tenderness/texture, juiciness and flavor. The visual appearance such as color is also an important quality factor (Wood, 1990).

Wood (1990) has suggested that there are two types of toughness the first being background toughness due to amount and type of connective tissue. The second is myofibrillar toughness due the state of the muscle fibers. Fat may exert an independent modifying influence or may act through interactions with other components to affect toughness. Some products represented by a background matrix of mainly protein show reduced toughness due to the presence of fat. Thus, the amount of intermuscular fat is expected to play a role in the tenderness/toughness of meat (Cross et al., 1980).

The amount of liquid released during mastication both from the food and saliva is defined as juiciness (Gliese, 1992). Juiciness may be affected by formulation and by cooking. When fat is removed from meat there is a greater water loss during cooking and the meat may be perceived as dry.

Water and lipid soluble components of meat contribute to the flavor and aroma of meat. Many studies have related that the aqueous extracts contribute to the general meat flavor, while the fat-soluble fraction contributes to the species specific flavor such as beefy flavor (Pearson et al., 1973; MacLeod and Coppock, 1976; Chang and Peterson, 1977; Mottram et al., 1982; Mottram and Edwards, 1983).

The eating quality of meat is highly dependent on the fat content of the meat and fat reduction is likely to result in diminished flavor and texture properties (Wood, 1990). There are many ways to reduce fat and still maintain basic quality features, such as choosing the breed, sex, weights, ages, muscle type, slaughter method and carcass processing.
Decreasing the Fat Content

The simplest way to produce low-fat ground beef products would be to remove the visible fat (Huffman and Egbert, 1990). However, decreasing the fat content of ground beef patties to less than 10% does not give an acceptable product. The palatability, flavor intensity, juiciness, and tenderness of this product are greatly decreased (Egbert et al., 1991; CAST, 1991; Hoogenkamp, 1991; and Huffman and Egbert, 1990).

Fat Replacers

Fat replacers are ingredients that can be used to formulate low-fat foods without changing the flavor, mouthfeel, and other organoleptic properties found in full fat products (Keeton, 1994). These may be selected so that they contribute few or no calories to the food in which they are used. Morrison (1990) stated that a successful fat replacer should imitate the taste and texture provided by the fat being replaced. Most are used for partial fat replacement and can be categorized as follows: (a) water, (b) carbohydrates, including starches, carrageenan, maltodextrins, (c) protein based replacers including soy, oat, whey.

Added water

Beef patties with reduced fat content show diminished juiciness and tenderness (Berry and Leddy, 1984; Egbert et al., 1991). This problem can be overcome by addition of water to the meat. Water by itself is lost during cooking, so a water-binding agent is required. It should be emphasized that while addition of water will reduce the total fat per serving, this practice does not alter the percent calories originating from fat.

Some low-fat meat products have been formulated with fat replacements in conjunction with water. Starch is one such fat replacer that has been used in low-fat formulations.
Starches

Starch is one of the major food ingredients in the human diet, providing 75 - 80% of the total dietary calories. Starch is a plant polysaccharide found abundantly in seeds, roots and tubers (Whistler and Daniel, 1985). Polysaccharides, such as starch are macromolecules containing a large number of hydroxyl groups that interact with water. Hence, these compounds are categorized as hydrocolloids.

Due to their ability to absorb large amounts of water, starches have been used in the meat industry to improve the cooking yield (Figure 1), enhance juiciness, and decrease formulation costs. The primary function of the starch is to immobilize and retain water.

Figure 1: Low-Fat Sausage formulated with modified food starches, which are said to decrease cook loss (Giese, 1992).
Potato starch, sugarbeet fiber, wild rice and many other carbohydrate based fat replacers have been investigated for use in meat (Todd et al., 1989; Troutt et al. 1992). These fat replacers have had different rates of success. Troutt et al. (1992) tested dietary fibers, starches and polydextrose in 5 and 10% fat hamburgers. These samples were compared to controls with 5, 10, 20, and 30% fat. The experimental samples had reduced cooking losses. But the additions also reduced beef flavor intensity.

Minerch et al. (1991) formulated ground beef mixtures with added cooked wild rice. Grade-A Minnesota paddy wild rice was added at three different levels -- 0, 15, and 30% to low-, intermediate-, and high-fat ground beef patties. The experimental samples containing 15% and 30% cooked wild rice were preferred over 100% ground beef patties. The wild rice helped retain moisture in low-fat and fat in the high-fat patties. The patties with wild rice had significantly better cooking yields. It was concluded that the addition of wild rice to ground beef resulted in a more desirable product.

One of the advantages of using starches is the positive acceptance by consumers. Starch is a familiar term for consumers and thus they are more accepting of products containing starch. The low-cost is another advantage (CAST, 1991; Giese, 1992).

**Maltodextrins**

Maltodextrins are produced by the hydrolysis of amylose and amylopectin chains. The enzyme used for this cleavage is α-amylase. Maltodextrins with a low dextrose equivalent are most suitable as fat substitutes (Inglett and Grisamore, 1991).

The National Center for Agriculture Utilization Research developed an oat-based maltodextrin called Oatrim (Anonymous, 1990; Hardin, 1990). Oatrim produces a fat-like gel containing less than one calorie per gram. A fresh chub-packed beef product is available that uses Oatrim. This product contains 4 g of total fat, 1 g of saturated fat, 55 mg of cholesterol, and 240 mg sodium per 4
ounces. The total calories derived from fat are 28% and there are 130 calories per serving (Anonymous, 1992).

Carrageenan

Carrageenan is defined in the Food Chemicals Codex III as the product

"obtained by extraction with water or aqueous alkali from certain members of the class Rhodophyceae (red seaweeds). It is a hydrocolloid consisting mainly of the potassium, sodium, magnesium, calcium and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. The hexoses are alternately linked α-1,3 and β-1,4 in the polymer" (Gllcksman, 1983).

There are three main types of carrageenan: iota-, kappa-, and lambda-carrageenan (Whistler and Daniel, 1985). There are several structural differences in these carrageenan such as (1) degree and manner of sulfation; (2) proportion of 3,6-anhydrogalactose residues linked 1->4; (3) different pyranose ring conformations; and (4) the cations linked with the sulfate groups. Due to these structural differences the carrageenans also vary in their physical and chemical properties (Marrs, 1986).

The carrageenans are soluble in water at temperatures above 70°C. The most important function is the ability of kappa- and iota-carrageenan to form thermally-reversible gels. Certain cations are necessary for gel formation. Kappa-carrageenan produces strong gels in the presence of potassium. It also reacts synergistically with locust bean gum to give a strong, elastic gel with reduced syneresis. On the other hand, iota-carrageenan produces better gels in the presence of calcium cations. The gel formed is tender, elastic and syneresis free. These gels do not require refrigeration to form or even to remain gelled (Huffman et al., 1991; Giese, 1992; Therkelsen, 1993).

Carrageenan is used in many different food applications. The carrageenans have been used in ice-cream products, milk beverages, milk and non-dairy puddings, whipped cream products and a host of other products. They have also been used in meat applications.
The ability of iota-carrageenan to form strong, clear, elastic and syneresis free gels makes it ideal for low-fat ground beef applications. Huffman et al. (1991) formulated a lean beef patty using iota-carrageenan. Their lean patty was called AU (Auburn University) Lean. The formulated patty contained 0.5% iota-carrageenan, 10% water, 0.4% encapsulated salt, and 0.2% hydrolyzed vegetable protein (HVP). This formulation gave patties with 8% fat.

The composition of the AU Lean beef patties was compared to 20% fat beef patties. The raw AU Lean product contained significantly more moisture and less fat than the 20% fat beef patties. The cooked AU Lean and 20% fat patties were also compared. Once again AU Lean had significantly more moisture and less fat than the 20% fat beef patties. The AU Lean provided 172 calories per 100 grams as compared to 272 calories per 100 gram from the 20% fat beef patties (Egbert et al., 1991).

Another study used kappa-carrageenan in NaCl/phosphate structured beef rolls (Shand et al., 1994). This product contained 33% added water, 1% sodium chloride, 0.35% sodium tripolyphosphate and 0.5% and 1% kappa-carrageenan. The product had a fat content of 4-5%. The addition of kappa-carrageenan increased yield and improved textural properties.

Brewer et al. (1992) used carrageenan by itself and in combination with different soy products. The objective of this study was to investigate rehydrated soy and carrageenan effects on sensory, chemical and physical characteristics of low-fat ground beef patties. Two controls used were 20% and 8% fat beef patties. The experimental samples contained 8% fat, 20% soy protein isolate (SI), soy concentrate (SC), or soy flour (SF). Another sample (MIX) was also formulated with 0.2% sodium tripolyphosphate, 0.5% modified food starch, 0.5% carrageenan and 6% water. The MIX samples were less cohesive than the other samples. However, these samples were juicier and less rubbery than the 8% fat control. Beefy and off-flavor scores for MIX were similar to the 8% fat control. However, it should be noted that removal of fat results in reduced beefy flavor compared to 20% fat beef patties.
Foegeding and Ramsey (1986) replaced fat with water and water-gum suspensions in meat batters for frankfurters. They evaluated the cooking stability, textural and sensory properties of these formulations. The gums used in this study were iota-carrageenan, kappa-carrageenan, guar gum, locust bean gum, xanthan gum and methyl cellulose.

Samples with methyl cellulose had higher weight losses compared to the other formulations. The textural parameters were altered by xanthan and guar gum added at the 0.2% level. Sensory evaluation showed that the low-fat experimental frankfurters were as acceptable as the 27% fat control frankfurters.

**Soy Proteins**

Soy proteins have been used by the meat industry for a considerable period of time. Most often they are used as meat extenders. The ability of soy protein to absorb water and emulsify fat has also been utilized in meat applications (Jeng et al., 1988). Soy proteins can be classified on the basis of protein content: (1) soy flour with 50% protein, (2) soy concentrates with 70% protein, and (3) isolated soy protein (90%) with virtually no fat or carbohydrate (McMinides, 1991).

Huffman and Powell (1970) tested different fat and soy levels for tenderness of ground beef patties. This study showed that a formulation for ground beef patties with 25% fat and 2% soyabits gave a desirable product. However, this study did not compare any low-fat formulations. Other studies have also examined soy protein levels from a view to extending meat rather than to develop a low-fat product (Smith et al., 1976).

In another study two levels of tofu were substituted in place of fat in a comminuted meat-type product (Jeng et al., 1988). The water holding capacity was lowest for the samples prepared with tofu, this was expected since the tofu had higher initial levels of moisture. The sample containing 31.6% tofu contained 58.5% fewer calories than the all-meat sample. A trained sensory panel did not find any significant difference between the tofu and all meat.
samples. This study concluded that tofu can be used in emulsified meat products.

McMIndes (1991) reported on the application of isolated soy proteins (ISP) in low-fat meat products. The ISP did not have the characteristic beany flavor associated with soy because the carbohydrates and lipid fractions had been removed. The ISP was used to formulate a low-fat ground beef patty and the control used was a 20% fat beef patty. Sensory testing by a trained panel rated the soy protein samples better in flavor and overall quality. This product also had 50% less fat, 32% fewer calories, and 16-19% less cholesterol than the control.

**Milk Proteins**

The protein content of milk is approximately 3.2%. There are two major groups of milk proteins -- caseins and whey proteins (Swaisgood, 1985). The liquid remaining after casein is removed from skim milk is whey. Two kinds of whey are recognized, acid whey resulting from cottage cheese production and sweet whey from rennet cheese production. Whey contains lactose, proteins, minerals, vitamins and small amounts of fat. The combination of these components is dependent on the milk composition and method used to remove the casein. Whey proteins account for approximately 20% of the total milk proteins. The composition of proteins is shown in Table 1 (Swaisgood, 1987).
Table 1: Composition of Whey Proteins in Milk

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration in Whey</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Whey Protein</td>
<td></td>
<td>5.2</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Proteose Peptone</td>
<td></td>
<td>0.6</td>
</tr>
</tbody>
</table>


Morr (1992) described the manufacturing procedures used for production of whey protein concentrates (WPC) as follows:

"(1) fresh, sweet cheese whey (pH > 6.2) containing only about 0.5 - 0.7% protein is pasteurized; (2) the pasteurized whey is concentrated by ultrafiltration (UF) to achieve a volume:concentration ratio of 20 - 25%; (3) the UF retentate is diafiltered against >3 volumes of water; (4) the UF retentate is concentrated by vacuum evaporation (optional); and (5) the UF retentate concentrate is spray dried."

Mangino (1984) defined functionality as:

"any property of a food or food ingredient, except its nutritional ones, that affects its utilization."

Whey protein concentrates exhibit several functional properties, such as, emulsification, foaming and gelation that are useful in a range of food applications. The properties of milk proteins that are of most importance in meat products are immobilization of water; texture and consistency control; color improvement and enhancement of organoleptic properties (van den Hoven, 1987). One of the functional properties of interest in WPC is their ability
13
to form heat induced gels (Hiller et al., 1980; Schmidt and Morris, 1984; and deWit, 1984).

Gelation is an indication of protein denaturation. Mulvihill and Kinsella (1987) defined protein gels as "three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water by a small proportion of protein." Thermal gelation involves four steps -- (1) protein unfolding, (2) water binding, (3) protein-protein interactions, and (4) water immobilization (Mangino, 1984). The type of gel formed in turn depends on the type of protein, concentration of protein, pH, ionic environment, as well as, the method used for gelation (Morr, 1979).

In one study the effects of different dairy ingredients on the sensory properties of a sausage product were studied (Baardseth et al., 1992). Sausages were prepared with beef meat and pork fat. Five dairy ingredients -- ordinary or high-viscosity sodium caseinate (1.5% fat, 89% protein, 0.5% carbohydrate, 4.5% water), skim-milk powder (1% fat, 35.5% protein, 53% carbohydrate, 3.5% water), whey protein (7% fat, 78% protein, 4% carbohydrate, 5% water) and demineralized whey powders (2.1% fat, 11.6% protein, 82% carbohydrate, 3.5% water) were added at 3 different levels (1, 3, 5%) with potato starch at 2 different levels (2 and 4%). The control sample had 4% starch added, but no dairy ingredients. The results showed that sausages with sodium caseinate or skim-milk powder had higher elasticity, firmness and whiteness scores. Samples prepared with whey protein and demineralized whey powder had high scores for smokiness, fatness and juiciness. The whey protein and demineralized whey powder samples were most similar to the control. These samples were also regarded as more desirable compared to the other samples.

Casella (1983) reported on several investigations that used whey in meat products. Most of these investigations used whey as an extender, but they still showed the cementing ability of whey. In one study the sensory and physical properties of meat loaf processed with WPC and non-fat dry milk (NFDM) were compared. Dry whey (13% protein) and two WPC (19% and 33% protein) replaced NFDM. The moisture and fat content of these samples did not differ.
Instron measurements also showed that whey products and NFDM had the same binding ability. The 33% WPC had the highest juiciness and flavor ratings, followed by dry whey, control and the 19% WPC sample. The higher ratings for the 33% WPC were probably due to superior water binding. This study showed that 33% WPC could give a more juicy and flavorful product compared to NFDM.

Another study used delactosed whey, different whey products, NFDM and soy as texture binders in frankfurters. The delactosed whey gave a product similar to the control. Therefore, it was successful in achieving fat stability and replacing the beef. The only product with a defined off-flavor was the one formulated with soy isolate.

Ensor et al. (1987) compared WPC, soy protein isolate (SPI), and calcium-reduced nonfat dry milk (RNFDM) in an emulsion type sausage. A knockwurst type product was formulated with 25% water, 2% sodium chloride, 0.5% dextrose, 0.4% liquid flavoring, 0.06% sodium erythrobate and 0.015% sodium nitrite. The levels of WPC used were 0, 1.75, 2.0, and 3.5%. The level of RNFDM used was, 3.5%. SPI was at the 2% level. All samples were formulated to contain 24% fat. The 2% WPC samples had less fat, gel water and total losses compared to SPI. Within the different levels of WPC the 0% sample had the greatest amount of released fat. This establishes the ability of WPC to bind fat. The WPC samples were just as cohesive as SPI and RNFDM samples. A sensory panel rated samples with 2% WPC most desirable. Compared to SPI and RNFDM they had similar stability, texture and sensory attributes. It can be concluded that WPC can be effective binders in emulsion-type meat products.

There are new protein-based fat substitutes such as Simplesse. Simplesse is the first fat substitute approved as Generally Recognized as Safe (GRAS) by the FDA. It is a milk and egg protein based product which undergoes a unique process called microparticulation (McCormick, 1988; Morrison, 1990; Schlicker and Regan, 1990; Anonymous, 1991; Berglund, 1991; Fredericq, 1991 and Segal, 1991). In this process the proteins are heated till they begin to gel, but undergo high-shear blending before the gel forms. This shearing subjects the
protein to form small bead-like spheres which are 0.1 to 0.2 microns in diameter. The tongue is not able to detect these as individual spheres and thus perceives them as a creamy fluid. Several types of Simplesse are available such as Simplesse 100 and Simplesse 550. Simplesse 100 is microparticulated WPC while Simplesse 550 is a combination of WPC and hydrolyzed oat flour. The nutritional composition of Simplesse 100 and 550 are shown in Table 2.

The suggested uses for Simplesse are in products such as frozen desserts, sugar-free desserts, cream cheese, baked goods, natural cheese, cultured dairy products and salad dressings. There is potential for using Simplesse in low-fat meat applications. Its performance as a fat substitute in these products is yet to be determined.
Table 2: Nutritional Composition for 100 Grams of Simplesse Dry 100 and 550

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Simplesse 100</th>
<th>Simplesse 550</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>3.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>50.5</td>
<td>32.2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>4.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>5.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>380</td>
<td>369</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>36.6</td>
<td>53.7</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>137</td>
<td>85</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>675</td>
<td>809</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>0.94</td>
<td>0.52</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.18</td>
<td>0.64</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1207</td>
<td>1530</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.18</td>
<td>1.28</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>589</td>
<td>729</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.27</td>
<td>0.34</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>&lt;1.50</td>
<td>--</td>
</tr>
</tbody>
</table>

Source: The NutraSweet Company, Box 730, 1751 Lake Cook Road, Deerfield, Illinois 60015

Other Additives

Sodium chloride has several beneficial functions when added to meat. Some of the benefits are flavor enhancement, food preservation, protein solubilization and tenderization (Keeton, 1983).

Salt functions as a preservative because it inhibits the growth of bacteria due to the osmotic differences between the interior of the cell and the external environment. Davidson and Juneja (1990) suggested that the tolerance of microorganisms to sodium chloride is dependent on the type of
food product, and microbial growth is actually stimulated at low concentrations, but high concentrations are inhibitory.

Sodium chloride enhances water binding properties of meat by extracting the salt soluble proteins (Knipe et al., 1985). This increases tenderness because of an increase in ionized groups in the muscle tissue allowing the protein to swell with water (Huffman et al., 1981; Matlock et al., 1984; Wheeler et al., 1990).

The one big disadvantage of using sodium chloride is that it is a prooxidant. Impurities in salt such as metals and minerals are the cause of the oxidative rancidity. This problem can be overcome to some extent by using purified salt and encapsulated salt. Encapsulated salt is sodium chloride which is coated with a partially hydrogenated oil. This coating only dissolves at elevated temperatures such as used for cooking. Thus the sodium chloride is not in direct contact with the meat till it is heated. USDA (1992) has stipulated the requirements that should be met if encapsulated salt is to be used in low-fat beef patties. The requirements are follows:

Table 3: Requirements for Encapsulated Salt for Use in Low-Fat Beef Patties

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate Composition</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Substrate Content</td>
<td>70% ± 2%</td>
</tr>
<tr>
<td>Coating Composition</td>
<td>Partially Hydrogenated Cottonseed or Soybean Oils.</td>
</tr>
<tr>
<td>Coating Content</td>
<td>30% ± 2%</td>
</tr>
<tr>
<td>Coating Melting Point</td>
<td>140 - 150° F</td>
</tr>
<tr>
<td>Appearance</td>
<td>White Free Flowing Granules</td>
</tr>
<tr>
<td>Particle Size</td>
<td>2% Maximum on #10 Mesh Screen</td>
</tr>
</tbody>
</table>

Source: USDA, 1992
Processed meat products are all-meat-based products which have undergone different treatments (van den Hoven, 1987). These can be categorized as non-comminuted, coarsely comminuted, and finely comminuted. Table 4 shows the classification of different comminuted meat products (van den Hoven, 1987).

Table 4: Classification of meat products by their degree of comminution.

<table>
<thead>
<tr>
<th>Degree of Comminution</th>
<th>Meat Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Hams (cooked, smoked, dry); bacon, cooked loins; shoulders</td>
</tr>
<tr>
<td>Some</td>
<td>Reformed hams</td>
</tr>
<tr>
<td>Coarse</td>
<td>(Semi)dry sausages (salami, cervelat); fresh sausages (bratwurst); hamburgers; meat patties; meat balls; chicken nuggets</td>
</tr>
<tr>
<td>Fine</td>
<td>Frankfurters; hot dogs; polony; pariser; bologna; mortadella; liver sausages/spreads; meat loaves</td>
</tr>
</tbody>
</table>

There are three types of ground beef available in the stores, which differ in their total fat content. Regular ground beef has 27% fat, lean ground beef has a nominal content of 21%, extra-lean ground beef has a nominal fat content of 17% (Berry, 1984; Giese, 1992).

The USDA (1991) policy for labeling low-fat ground beef products was published in 1991. This policy states that "low-fat products which combine hamburger or ground beef with other non-fat ingredients may be descriptively labeled. The finished product may contain no more than 30% of a combination of fat and added substances and no more than 10% fat." An example of the above policy would be "Low Fat Ground Beef with an X% Solution of ..." If a product is not low-fat it must be labeled as "Imitation Ground Beef or Beef Patty or Beef Patty Mix."
SCOPE OF INVESTIGATION

The objective of this research has been to develop a low-fat ground beef patty with whey protein concentrate as a water binder. The specific aims have been:

1. To formulate low-fat beef patties with whey protein (WP).

2. To select a protocol for preparation and cooking of the WP patties.

3. To establish procedures to assess water, fat, and cooking losses during the various stages of preparation, frozen storage and cooking.

4. To evaluate the quality of the cooked beef patties using objective as well as sensory testing procedures.

5. To develop quality specifications for meat to be used in low-fat beef patties based on WP.
EXPERIMENTAL PROCEDURE

Materials

1% osmium tetroxide in buffer -- Jenella Chemical Co., Cincinnati, OH
1:1 Propylene oxide/Spurr
Calcium Chloride (CaCl₂.2H₂O) -- Spectrum Chemical Mfg. Corp.,
Gardena, CA
Cheesecloth
Coarsely ground beef -- Karn Meats Inc., and Kroger, Columbus, OH
Crackers (Saltines)
Distilled Water (pilot plant)
Drinking water (tap water)
EDTA (Ethylenediaminetetraacetic acid)
Encapsulated salt with Durkote coating, product number 1134-15-85 --
Morton Salt, Morton International, Inc., Chicago, IL
Fresh curd sodium caseinate, Alanate 180 -- New Zealand Milk Products
(North America), Inc., Santa Rosa, CA
Glutaraldehyde (3%) in 0.1M phosphate buffer, pH 6.8
Glymol
Graded ethanol (50%, 70%, 80%, 95%, 100%)
Hydroxypropyl Methylcellulose, Methocel, K100LV -- Dow Chemical Co.,
Midland, MI
Iota-carrageenan, Viscarin ME389 -- FMC Corporation, Marine Colloids
Division, Philadelphia, PA
Lactose, Monohydrate Powder (C₁₂H₂₂O₁₁.H₂O) -- J.T. Baker, Inc.,
Phillipsburg, NJ
Paper plates
Potassium phosphate
Propylene oxide
Raw and cooked ground beef samples
Raw, cooked and extracted muscle protein samples
Saran wrap -- DowBrands L.P., Indianapolis, IN
Sensory evaluation sheets
Simplesse 100, 500 and 550 -- NutraSweet Co., Chicago, IL
Solo P49, poly-lined souffle cups -- Solo Cup Co., Urbana, IL
Solo Ultra Clear Cups -- Solo Cup Co., Urbana, IL
Spurr resin
Sulfuric Acid for Babcock test -- Fisher Scientific, Fair Lawn, NJ
Triflex Sterile Latex Surgeon's Gloves, 2D7254 -- Baxter Healthcare Corp., Valencia, CA
Tripolyphosphate -- Stauffer Chemical Co., Food Ingredient Division, Westport, CT
Ultra pure helium
Untrained panelists (selected among faculty, staff and students from the Food Science and Technology Department)
Whey Protein Concentrates, Alacen 837, 878, 890 -- New Zealand Milk Products (North America) Inc., Santa Rosa, CA
Ziploc Freezer bags -- DowBrands L.P., Indianapolis, IN

Equipment

3/8" and 3/16" grinding plate -- Hobart, Troy, OH
21X micrologger -- Campbell Scientific, Inc., USA
Babcock reading light
Beakers
Burger Press (HEUCK CIN'TI, US Pat D191367)
Calipers
Cream bottles, 0% to 50%:9g, 15.24 cm with 0.5% divisions
Disposable aluminum weighing dish -- VWR Scientific, Philadelphia, PA
Drying Oven, Gasoline Special 1N -- Gotham Scientific CO., New York, NY
Electric stir plates, Model 4810, Cole Parmer Instrument Co., Chicago, IL
Fused silica capillary column (DB-WAX; 30m x 0.25mm i.d. x 0.25 um film thickness) -- J&W Scientific Inc., Folsom, CA
Garver Shaker, Model 240 -- Garver Manufacturing Co., Union City, IN
Garver "Super" Babcock Centrifuge, Model 556 -- Garver Manufacturing Co., Union City, IN
Hewlett Packard Chemstation NIST/EPA/MSDC 49K Mass Spectral Database -- Palo Alto, CA
Hobart Grinder, Model A2001 -- Hobart, Troy, OH
Hypodermic probes, thermocouple probe model HVP-2-21-1-V2-TG-48-OST-M -- Omega, Stamford, CT
Magnetic stir bars
Model LSC 2000 purge and trap concentrator -- Tekmar, Cincinnati, OH
N-1428 Laser 486 DX system
Coarsely ground beef (10%, 20% and 27% fat) was purchased from a local meat supplier. Low-fat formulations were mixed by adding the water binder as a solution or a powder. Formulations with powdered water binders had the water and salt added separately to the meat. The meat was mixed well manually and ground first with a 3/8" plate and a second time with a 3/16" plate. The high fat controls (20% and 27% fat) had salt added and were mixed before grinding. Once the meat was ground it was divided into 114 g portions and manually formed into patties using a mold.

The patties were frozen quickly using liquid nitrogen. For this purpose, a wide neck thermos was used to hold the liquid nitrogen. The patty was placed in a wire basket with a handle and dipped in the thermos where it was allowed to freeze for 40-50 seconds. The frozen patties were individually wrapped in plastic wrap and stored in batches of 3 or 4 in freezer bags at -18°C.
Several formulations were tested during this study, all based on meat with 10% fat, with added 10% water and 0.5% encapsulated salt and incorporating varying amounts of water binders. Controls without water binders included meat with 10%, 20% and 27% fat with or without added water and salt. Table 6 shows the formulations and water binders used for this experiment.

The WPC solutions were reconstituted by adding the required amount of WPC powder to the required water with agitation. This mixture was mechanically stirred for 20 minutes to allow the WPC to dissolve. The solution was covered and stored at 4°C. This process was used for the duration of the study. The sodium caseinate (NaCas) and hydroxypropyl methylcellulose (HMC) samples were prepared by adding the powders, water and encapsulated salt directly to the meat. The meat was mixed well and then ground as described in the section on processing.

In the initial experiments carrageenan was added as a solution to the meat. This solution was prepared by adding carrageenan powder to the water with stirring and heating to 70°C. This did not give satisfactory results since the carrageenan formed a gel before dissolving. Subsequent to the experiments involving the formulations shown in Table 5, carrageenan, water and salt were added separately to the meat.

Different levels of water were tested to determine the correct level of water for the 4% WPC samples. These samples were formulated with 10% fat (90% lean) ground beef.
Table 5: Formulations used for regular and low-fat ground beef patties with different whey protein concentrates. Values shown are for a 114g ground beef patty.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Meat</th>
<th>Water</th>
<th>Salt</th>
<th>WPC</th>
<th>Carr</th>
<th>Simplesse</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% fat</td>
<td>113.43</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20% fat</td>
<td>113.43</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27% fat</td>
<td>113.43</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1%WPC (Alacen878)</td>
<td>100.89</td>
<td>11.4</td>
<td>0.57</td>
<td>1.14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2%WPC (Alacen878)</td>
<td>99.75</td>
<td>11.4</td>
<td>0.57</td>
<td>2.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3%WPC (Alacen878)</td>
<td>98.61</td>
<td>11.4</td>
<td>0.57</td>
<td>3.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4%WPC (Alacen878)</td>
<td>97.47</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.25% Carr</td>
<td>102.88</td>
<td>10.26</td>
<td>0.57</td>
<td>-</td>
<td>0.29</td>
<td>-</td>
</tr>
<tr>
<td>0.20% Carr</td>
<td>102.60</td>
<td>10.26</td>
<td>0.57</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
</tr>
<tr>
<td>0.75% Carr</td>
<td>102.31</td>
<td>10.26</td>
<td>0.57</td>
<td>-</td>
<td>0.86</td>
<td>-</td>
</tr>
<tr>
<td>Simplesse 100</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>2.28</td>
</tr>
<tr>
<td>Simplesse 550</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>2.28</td>
</tr>
<tr>
<td>34% WPC</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>2.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>56% WPC (Alacen837)</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>2.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>79.5% WPC (Alacen878)</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>2.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>92% WPC</td>
<td>100.89</td>
<td>10.26</td>
<td>0.57</td>
<td>2.28</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Simplesse is a specially formulated and microparticulated WPC
Table 6: Formulations for water binders in combination and without whey protein concentrate. The values shown are for a 114g ground beef patty

<table>
<thead>
<tr>
<th>Sample</th>
<th>Meat</th>
<th>Water</th>
<th>Salt</th>
<th>WPC</th>
<th>TPP</th>
<th>NaCas</th>
<th>HMC</th>
<th>Lactose</th>
<th>CaCl2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%WPC+TPP</td>
<td>97.13</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>0.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4%WPC+CaCl2</td>
<td>97.13</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>-</td>
</tr>
<tr>
<td>4%WPC+HPM</td>
<td>97.13</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10%fat+1.2% lactose</td>
<td>112.06</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4%WPC+1.2% lactose</td>
<td>96.38</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20%fat+4%WPC</td>
<td>97.47</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20%fat+4%WPC+1.2%</td>
<td>96.38</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27%fat+4%WPC</td>
<td>97.47</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27%fat+4%WPC+1.2%</td>
<td>96.38</td>
<td>11.4</td>
<td>0.57</td>
<td>4.56</td>
<td>-</td>
<td>-</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 7: Low-fat ground beef patties with 0%, 3.3%, 6.7% and 10% water, and 0%, 1%, and 2% WPC formulated with and without Encapsulated salt.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water</th>
<th>WPC</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>3.3</td>
<td>0</td>
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Cooking Protocol

Patties were thawed at 4°C for 8 - 10 hours before cooking. The cooking time was determined by monitoring the temperature with hypodermic probes. The probes were placed horizontally through the side of the patty with the sensing tip at the center of the patty (as shown in Figure 2). The probes were connected to a micrologger. The raw patties were placed on a preheated (177°C) electric grill. The patties were cooked 3 minutes and 2 minutes on each side. A hole was poked in the middle of the patty after 5 minutes, and three holes near the periphery after 8 minutes of cooking. The cooking was completed by cooking an additional 1 minute on one side, and turned and cooked half a minute on the last side. This cooking protocol achieved an internal temperature of 71°C.

Moisture

The moisture content of the raw and cooked samples was determined in duplicate by the gravimetric oven-drying method of AOAC (1990). Approximately 5 g of meat was spread on a disposable aluminum weighing dish and dried for 18 hours in a 100°C oven. Samples were cooled in a dessicator for 1 hour, then weighed again. Moisture was calculated according to the following formula:

\[
\text{Equation 1} \quad \text{Moisture (\%)} = \frac{\text{wet meat weight} - \text{dried meat weight}}{\text{wet meat weight}} \times 100
\]

Cooking Yield and Shrinkage

Cooking yield was determined in duplicate for each treatment. It was determined by weighing the patty before and after cooking. Shrinkage was determined by averaging three measurements of the diameter and height of the raw and cooked patties. This was done for two patties per treatment. The
percent retention of a component (fat and moisture) was also determined. The following formulas were used to determine the cooking yield, shrinkage and component retention:

Equation 2

\[
\text{Cooking yield (\%) } = 100\% \times \frac{\text{Raw weight} - \text{Cooked weight}}{\text{Raw weight}} \times 100
\]

Equation 3

\[
\text{Shrinkage (\%) } = \frac{(\text{Raw diameter} - \text{Cooked diameter}) + (\text{Raw height} - \text{Cooked height})}{\text{Raw diameter} + \text{Raw height}} \times 100
\]

Equation 4

\[
\text{Retention (\%) } = \frac{\text{\% component in cooked meat} \times \text{cooked weight}}{\text{\% component in raw meat} \times \text{raw weight}} \times 100
\]

Fat Content

The AOAC (1950) soxhlet method was used for preliminary experiments. After the specific whey protein was determined for use in this study the fat content of the raw and cooked samples was determined in duplicate by the Babcock cream method (Bradley et al., 1992) with modifications in the following manner. Approximately 4.5 g of meat was weighed into a cream bottle, 9 ml of distilled water was added to the bottle and the sample was mixed thoroughly by swirling the bottle. To this mixture, 17.5 ml sulfuric acid for Babcock test was added within 20 seconds. The bottles were immediately swirled. A mechanical shaker was used to shake the bottles for another 1 minute.

The bottles were then placed in a heated (60°C) centrifuge for 5 minutes. After 5 minutes, hot (60°C) distilled water was added till the base neck markings of the bottles. The bottles were centrifuged another two minutes. Hot (60°C) distilled water was added till the 45% graduation markings. Samples were centrifuged another 1 minute.
The bottles were transferred to a hot water bath for 5 minutes. The level of water was adjusted to be above the fat column. One or two drops of glymol were added and allowed to run down the neck of the bottle. The fat column was immediately measured using Babcock reading light and calipers.

**Protein Extraction**

Meat proteins were extracted by the method described by Perry and Corsi (1958). The procedure for protein extraction was done at refrigerated temperatures. Muscle tissue was homogenized in a blender, for 1 minute in 5 volumes of borate-KCl solution (0.025M KCl, 0.039M borate (or 0.05M potassium phosphate), 5mM EDTA, pH 7.1). The connective tissue was removed by filtering the homogenized tissue though cheesecloth. The filtrate was centrifuged at 600g for 15 minutes. The pellet was then resuspended in buffer. It was centrifuged again till the supernatant was clear (generally 2-3 times).

The extracted myofibril protein was mixed with WPC solution and heated at 70°C to form a firm gel. The different ratios of myofibril protein to WPC used were 10:30, 20:20, 30:10, and 40% WPC, 100% myofibril protein. These gels were then processed for TEM.

**Texture Profile Analysis (TPA)**

The samples were cooked and allowed to cool for one hour at 25°C. Two whole patties per treatment were used for this analysis. Each patty was compressed in 3 separate locations (as shown in Figure 2).

The test consisted of two consecutive compressions. Samples were compressed to 75% of the original sample height using a 1/2 inch plunger. The speed and distance were 2 mm/min and 14 mm, respectively. The TPA parameters hardness, chewiness, and springiness were computed using the installed computer software.
Ground beef patties were cooked and immediately 4.5 - 5 g transferred into 100 ml serum bottles. Teflon-coated rubber septa and aluminum caps were used to seal the bottles air-tight. The samples were stored at -18°C until ready for analysis. Samples were equilibrated at room temperature for one hour before dynamic headspace analysis (DHA).

The procedure described by Laye et al. (1993) was used for analysis of volatile compounds. The sample bottle was connected to a purge and trap concentrator. Ultra pure helium was used to purge the headspace volatiles. Volatiles were purged for 10 minutes with the flow rate at 40ml/min. A Tenax TA trap was used for adsorption of the volatiles. These were then desorbed by flash heating, cryogenically focused at the liquid nitrogen cooled capillary interface and automatically injected into the gas chromatograph/mass spectrophotometer (GC/MS) by heating 1 minute at 180°C.

A gas chromatograph and a mass selective detector were used for fractionating the volatile compounds. These volatiles were then separated with a fused silica capillary column. The carrier gas, ultrahigh purity helium, was used at a flow rate of 1ml/min. The oven temperature was increased from 32°C to 80°C at 2°C/min, to 160°C at 5°C/min, to 220°C at 10°C/min, and maintained at 220°C for 2 minutes. The MS ion source was maintained at 180°C throughout the analysis.

Mass spectra were acquired with an ionization energy of 70eV and within the mass range of m/z 19 - 300.

The Mass Spectral Database was used to tentatively identify full or partial mass spectral data.
Figure 2: Placement of Thermocouple Probes for Temperature Monitoring and Location of Vent Holes and TPA Probes
Transmission Electron Microscopy

All sample preparation and microscopy was done by The Ohio State University Image Analysis Laboratory. Raw, cooked and extracted muscle protein samples were cut into approximately 1 cm cubes and placed in glutaraldehyde in 0.1M phosphate buffer, pH 6.8. Sections were taken from each sample after one hour of fixation and put in fresh fixative. These were cut into 1 mm thickness and fixed overnight at 4°C.

Phosphate buffer (0.1 M, pH 6.8) was used to rinse the samples 3 times over 45 minutes. These sections were then post-fixed at room temperature in 1% osmium tetroxide in buffer for 90 minutes. They were then rinsed 3 times over a 90 minute period in buffer. Graded ethanol (50%, 70%, 80%, 95%, 100%, 100%) was used for dehydration. The samples were changed twice over 15 minutes into fresh graded ethanol for each step. Samples were then placed in propylene oxide for 15 minutes.

The samples were stored overnight in 1:1 propylene oxide/Spurr at room temperature. The Spurr resin was changed twice the next day. The samples were then embedded in Spurr and polymerized at 60°C overnight. Sample sectioning at 70 nm was conducted by the Image Analysis laboratory and observed with a transmission electron microscope at 60 kv.

Sensory Evaluation

The method was the forced ranking method. The parameters evaluated by this method were flavor, texture, juiciness, and overall acceptability (see Appendix A for sample evaluation sheet). An untrained panel was used for these evaluations. Judges were given 4-5 samples and asked to rank them with 1 being the least preferred sample and 5 being the preferred sample. The data was analyzed by the method by Kramer (1956) described in the Merck Technical Bulletin (Merck, 1963).
Statistical Analysis

The data was analyzed by StatWorks. Individual degree of freedom analysis was computed by the method described by Ostle (1956). Most variations are contributed by some treatments and not others. At times significance is not indicated by the statistical analysis utilized, but there could be significant differences if treatments were compared to each other. This method allows the comparison of various treatments to each other by the subdivision of sum of squares into individual degree of freedom. This method is illustrated in the example shown in Appendix B.
RESULTS

Initial levels of whey protein concentrate (2%), added water (9%) and salt (0.5%) used in this research were established by the OSU Technical Problem Solving class in 1991. Using these levels the first goal was to determine which WPC would give the best product organoleptically. The cooking protocol established by the class was also used at this time.

I. Preliminary Experiments

In an initial experiment, low-fat (10% fat) ground beef was formulated with two different WPCs (56% and 92% protein) and two different Simplesse (100 and 550). Twenty percent fat ground beef (nothing added) was used as the control.

These specific samples were chosen because they allowed the comparison of a medium and high protein WPC or Simplesse. Simplesse is a microparticulated, heat denatured WPC and the number designates the different formulations. Using two different Simplesse samples allowed for a comparison of the Simplesse to each other as well as to the WPC. Four parameters were evaluated by eight untrained panelists, aroma/flavor, texture, saltiness and color (see Appendix A for evaluation sheet).

The panelists first evaluated the control by itself. They then evaluated coded experimental and control samples. A rank of one was assigned to the least preferred sample and a rank of five for the most preferred. Kramer's (1956) rank sum method (Table 8, Appendix C) was then used to analyze the significance of the data.
Figure 3: Rank Sums for 20% Fat Ground Beef Patties and Low-Fat Patties Containing WPC and Simplesse
Figure 3 shows the rank sums for the five samples with the upper and lower limit of significance. The line at 32 rank sum is the upper limit and samples with rank sums equal to or greater than this are preferred for that parameter, while the line through rank sum 17 is the lower limit and samples with rank sums equal to or below this are preferred less for that parameter. It is seen that the saltiness of the control sample is not preferred, while the 92% WPC and Simplesse 100 samples are preferred for their saltiness. Lastly, the color of the Simplesse 100 sample is below the lower limit.

In the initial experiment the following observations were made. Grinding the samples through the 1/8" grinding plate resulted in improper grinding because the grinding plate holes became clogged. Furthermore, when the manually formed patties were frozen and stored at -18°C upon cooking these patties were observed to deform because of the development of a large pita pocket. The control sample was processed without added salt which could have made it the least preferred sample for saltiness. Therefore, the following changes were made in subsequent trials. The same meat was processed again, but was ground twice. The meat was first ground coarsely through a 3/8" and then more finely through a 3/16" plate. The control sample was processed with 0.5% added salt.

These patties were also evaluated for the same parameters as the first experiment. Figure 4 (Table 9, Appendix C) show the rank sums for the samples prepared by grinding the meat twice. The texture for the 92% WPC sample was preferred over the Simplesse and control samples. The Simplesse 550 sample had a rank sum for texture significantly lower. Though Simplesse is a WPC based product, it is a precooked product. Its functionality lies in the microparticles which give a sensation of creaminess. This function is lost when the Simplesse is heated. On the other hand, the WPC entraps water by gelling in the meat system during cooking. The difference in texture may be attributed to the fact that these two products function differently when heated. The graph also shows that the control was preferred for its saltiness. Color did not differ greatly among the samples.
The addition of salt to the control sample prevented an obvious bias against this sample. Grinding the meat twice produced a more uniformly ground product, but the patties still deformed during cooking. Further experiments were then designed to overcome the deformation of the patties.

The fat for these initial trials was measured by the Soxhlet method. However, this method is time consuming and allows analysis of a limited number of samples at one time. The Babcock fat method was then tested since it is fast, easy to use and allows measurements for a large number of samples at one time. The data collected by these two methods for raw 10% fat samples was not significantly different (p>0.345). The fat readings by the Soxhlet method were 15.21 and 16.75 while the Babcock readings were 15.5 and 12.5. The precision for the Babcock fat measurements as determined by the standard error of the means of 11 duplicates was 0.56.

Compared to the other experimental samples the 92% WPC sample was preferred the most for the parameters evaluated. It had the highest rank sum for aroma/flavor for both the first and second trial. Its texture, one of the most important parameters, was also preferred the most for the second trial. Lastly, the saltiness of this sample was the most preferred in the first trial. After comparing the data obtained, the 92% WPC sample was chosen for further experimentation.

Even though the 92% WPC was chosen for further use, another WPC (79.5% protein, Alacen 878) was used for all other experiments. A small group of panelists (consisting of OSU FST faculty) evaluated both the 92% WPC and 79.5% WPC samples. This group determined that the 79.5% WPC samples were equal to the 92% WPC samples in aroma/flavor, texture, color and saltiness. This change was constituted due to economical reasons. The 92% WPC costs approximately 6.50/pound compared to only 2.40/pound for the 79.5% WPC. This drastic reduction in cost without sacrificing quality was considered strategically sensible.
Figure 4: Rank Sums for 20% Fat Ground Beef Patties and Twice Ground Low-Fat Patties Containing Whey Protein Concentrate and Simplesse
II. Cooking Protocol

In the preliminary stages of this research the cooking protocol established by the OSU Technical Problem Solving Class of 1991 was used -- 3 minutes, 2 minutes and 1 minute on each side. Samples cooked by this method and using the available equipment were often overcooked and therefore dry. The electric grill being used did not maintain a uniform temperature. This problem was overcome by using new electric grills which maintained a uniform temperature. The cooking time was established by monitoring the internal temperature of the patties with hypodermic probes. Patties were considered cooked when an internal temperature of 71°C was achieved. To achieve this temperature patties were cooked 3 and 2 minutes on each side. A hole was poked in the middle of the patty after 5 minutes, and three holes near the periphery after eight minutes of cooking. The cooking was completed by cooking an additional one minute. The holes probably shortened the cooking time from 12 minutes to 11 minutes by allowing the heat to enter the interior of the patties during cooking.

III. Effect of Water, WPC and Carrageenan on the Shape of Cooked Beef Patties

a. Effect of WPC, Water and Salt

The effect of WPC, water and salt on the shape of the cooked beef patties was evaluated. Earlier trials showed that low-fat samples (90% lean) formulated with WPC, water and salt deform during the cooking process.

The low-fat patties formed a bread-like pita pocket in the center, with all the water migrating away from this area. The next set of experiments were attempted to identify if any of the variables (WPC, water, or salt) were responsible for this deformation. Four levels of water (0, 3.3, 6.7, and 10%) were each formulated with three levels of WPC (0, 1, and 2%). Each of these samples was formulated with and without added salt (Table 10).

It is seen in Figure 9 that the samples with 1% and 2% WPC have higher cooking yields. Two-way analysis of variance showed that there was an effect
on cooking yield due to the WPC (p<0.01) (Table 10, Appendix C). The interaction of water and WPC, WPC and salt and water and salt was not significant. Salt by itself did effect (p<0.01) cooking yield.

Moisture retention was also effected (p<0.01) by the WPC (Table 10, Appendix C). Once again Figure 8 shows that the moisture retention was higher for samples containing WPC. The interaction of water and WPC, WPC and salt, and water and salt did not effect the moisture retention.

Fat retention was not effected by any of the added compounds or their interactions (Table 10, Appendix C) this is not readily observed looking at Figure 10. Percent shrinkage seen in Figure 11 was also not significantly effected by water, WPC and salt (Table 10, Appendix C).

It was observed that the low-fat patties puffed up after the first three minutes of cooking. When these patties were cut in half they had a noticeable pita pocket in the middle. Figures 5, 6 and 7 show the pita pocket formed in the experimental samples.

Other observations made during this experiment were that the patties expelled a considerable amount of liquid during cooking. However, this liquid did not form any layer on the grill. Lastly, the unsalted samples seemed dry and tough compared to the salted samples.

The cause of deformation was not determined by this experiment. The deformation may be caused by the processing conditions rather than the added ingredients.
Figure 5: Deformation of a Low-Fat Ground Beef Patty Formulated with 3.3% Water, 2% WPC and 0.5% Salt
Figure 6: Deformation of a Low-Fat Ground Beef Patty Formulated with 6.7% Water, 2% WPC and 0.5% Salt
Figure 7: Deformation of a Low-Fat Ground Beef Patty Formulated with 10% Water, 2% WPC and 0.5% Salt
Figure 8: Percent Moisture Retention for Low-Fat Ground Beef Patties Formulated with Different Levels of WPC, Water and Salt
Figure 9: Percent Cooking Yield for Low-Fat Ground Beef Patties Formulated with Different Levels of WPC, Water and Salt
Figure 10: Percent Fat Retention for Low-Fat Ground Beef Patties Formulated with Different Levels of WPC, Water and Salt
Figure 11: Percent Shrinkage for Low-Fat Ground Beef Patties Formulated with Different Levels of WPC, Water and Salt
b. Effect of Iota-Carrageenan

It has been observed that while cooking lean samples (90% lean) formulated with water, WPC, and salt the patties deform (form a *pita* pocket in the middle) during the cooking process. This set of experiments were performed to determine if carrageenan has a similar effect on cooked patties.

Two-way ANOVA showed that carrageenan had a significant (*p*<0.001) effect on cooking yield (Table 11, Appendix C). The effect of salt and the interaction of carrageenan and salt was also significant (*p*<0.05). Single degree of freedom analysis showed that the 0.25% carrageenan sample had a lower yield (*p*<0.01) than the 0.5% and 0.75% carrageenan samples. The 0.5% and 0.75% carrageenan samples did not differ significantly. Figure 12 shows that the 0.75% carrageenan sample had the highest cooking yield and the 0.25% carrageenan sample the lowest. Both these samples had salt added to them. It was observed that the samples with added salt had increasing cooking yield from 0.25% to 0.75%. The samples with no salt added had similar cooking yields.

Moisture retention was effected (*p*<0.05) by carrageenan and salt. The effect due to the interaction of carrageenan and salt was not significant. Single degree of freedom analysis showed that the 0.25% carrageenan sample had lower (*p*<0.05) moisture retention compared to the 0.5% and 0.75% carrageenan samples (Table 12, Appendix C). There was no difference between the 0.5% and 0.75% carrageenan samples. Figure 15 shows that the 0.75% carrageenan sample with salt added had the highest moisture retention with the 0.25% carrageenan sample with salt added the least moisture retention. The samples with no salt added had very similar moisture retention.

The interaction of carrageenan and salt had a significant (*p*<0.05) effect on the fat retention. Individually these variables did not effect fat retention. Single degree of freedom analysis also showed that there was no difference in the fat retention of the three samples. Figure 14 shows that the 0.25% carrageenan sample with added salt had the lowest fat retention and the 0.75% sample with added salt had the highest fat retention. It was observed that the samples with
no added salt had decreasing fat retention with increasing carrageenan while the opposite was observed for the samples with added salt.

As the level of carrageenan increased shrinkage decreased (p<0.01) (Table 11, Appendix C). Salt and the interaction of carrageenan and salt was not significant. The 0.25% carrageenan sample had significantly (p<0.01) greater shrinkage. Figure 13 shows that the 0.5% carrageenan sample with added salt had the least shrinkage with the 0.75% carrageenan with salt added sample with the second lowest shrinkage. The samples with salt added to them had the highest shrinkage for the 0.25% and lowest for the 0.75% carrageenan sample.

There were several observations made during the duration of this experiment. The observations of importance were carrageenan at the 0.5% and 0.75% concentrations did not hydrate as desired. These formulations formed a gel before going into solution, and this further hindered the incorporation of them with meat.

It was observed that the uncooked samples with 0.5% and 0.75% carrageenan and no salt had pieces of gel that resembled _candied fruit_. These pieces of gel were still visible in these samples after cooking. The samples containing salt with the same carrageenan concentration did not have these pieces of gel.
Figure 12: Percent Cooking Yield for Low-Fat Ground Beef Patties Formulated with 9% Water, 0% and 0.5% Salt and 0.25%, 0.5% and 0.75% Carrageenan
Figure 13: Percent Shrinkage for Low-Fat Ground Beef Patties Formulated with 9% Water, 0% and 0.5% Salt and 0.25%, 0.5% and 0.75% Carrageenan
Figure 14: Percent Fat Retention for Low-Fat Ground Beef Patties Formulated with 9% Water, 0% and 0.5% Salt and 0.25%, 0.5% and 0.75% Carrageenan
Figure 15: Percent Moisture Retention for Low-Fat Ground Beef Patties Formulated with 9% Water, 0% and 0.5% Salt and 0.25%, 0.5% and 0.75% Carrageenan
All the samples had partial pita pockets after cooking, cooling, and cutting the patty in half, except for the sample with 0.75% carrageenan and no salt. These pockets were not as large as the pockets in the WPC samples.

The data indicates that the sample containing 0.75% carrageenan and 0.5% salt had the most desirable results where the least amount of shrinkage and highest cooking yield, fat retention and moisture retention occurred.

IV. Determining the Ideal Levels of WPC and Water

a. Level of Whey Protein Concentrate

In this experiment low-fat ground beef patties were prepared with 3-10% WPC (Alacen 878) with 10% water and 0.5% salt. Appropriate levels for WPC and water had to be established.

Figure 16 shows the cooking yield for the samples with 3-10% WPC. The 3% WPC sample had the lowest cooking yield compared to all the other samples. The cooking yield was significantly (p<0.001) effected by the level of WPC added to the sample (Table 13, Appendix C). Single degree of freedom analysis revealed that the 3% WPC sample had a lower yield (p<0.01) compared to all the other samples. The 5% WPC sample had a significantly (p<0.01) lower yield compared to the 6-10% WPC samples. Similarly the 7% WPC sample had a lower cooking yield (p<0.05) compared to the 8-10% WPC samples (Table 14, Appendix C).

Figure 17 shows the shrinkage for samples containing 3-10% WPC. Shrinkage was not effected by the level of WPC added. Single degree of freedom analysis also did not show any differences between samples (Table 15, Appendix C).

Figure 18 shows that the 3% WPC sample had the lowest moisture retention. Moisture retention was effected (p<0.05) by the level of WPC added. (Table 13, Appendix C). As the level of WPC increased so did moisture retention. Thus the 3% WPC sample had the lowest moisture retention (p<0.05), the next
Figure 16: Percent Cooking Yield for Low-Fat Ground Beef Patties Formulated with 3-10% WPC, 10% water and 0.5% Salt
Figure 17: Percent Shrinkage for Low-Fat Ground Beef Patties
Formulated with 3-10% WPC, 10% Water and 0.5% Salt
Figure 18: Percent Moisture Retention for Low-Fat Ground Beef Patties with 3-10% WPC, 10% Water and 0.5% Salt
Figure 19: Percent Fat Retention for Low-Fat Ground Beef Patties with 3-10% WPC, 10% Water and 0.5% Salt
lowest retention was observed for the 4% WPC sample (p<0.05). The 6-10% WPC samples had a higher moisture retention (p<0.05) compared to the 5% WPC sample. Lastly the 7% WPC had a significantly (p<0.05) lower moisture retention compared to the 8-10% WPC samples (Table 16, Appendix C).

The fat retention was significantly (p<0.05) effected by the level of WPC. Once again the 3% WPC sample had the lowest fat retention (p<0.01) among all the samples, and the 5% WPC (p<0.05) the lowest fat retention compared to the 6-10% WPC samples. Figure 19 clearly shows that the 3% WPC sample had the lowest fat retention (Table 17, Appendix C).

The data indicated that the higher levels of WPC had better cooking yields, moisture and fat retention and lower shrinkage then the lower levels of WPC. These higher WPC level samples were not desirable in all aspects. They were much harder because as the concentration of protein increases a stronger gel forms. The 4% WPC sample was chosen for further investigation. This sample did not form a pita pocket observed in some of the other samples. It also did not give as hard a product as the higher WPC samples.

It was observed that as the concentration of WPC increased the solution became more viscous. This prevented the magnetic stir bars from functioning properly. The meat also became very sticky when the solution was added to it and thus harder to grind. The 3% WPC sample had a pita pocket in the middle after cooking. This pocket was smaller compared to the 0%, 1% and 2% WPC samples.

b. Level of Water

Six additional (10%, 12%, 14%, 16%, 18% and 20%) levels of water were also tested. The level of WPC was maintained at 4% and 0.5% encapsulated salt was also added.

One-way analysis of variance showed that there was no significant effect of water on the cooking yield (Table 18, Appendix C). The sample with 16% water had a significantly lower cooking yield compared to the 18% and 20% water
samples (Table 19, Appendix C). Figure 20 shows that the 16% water sample did have a lower cooking yield compared to the 18% and 20% water samples.

Shrinkage was not significantly affected by the level of water added to the meat (Table 18, Appendix C). Figure 21 shows that the 14% water sample had the least shrinkage. The shrinkage for this sample was significantly lower compared to the 16%, 18% and 20% water samples (Table 20, Appendix C).

Figures 22 and 23 show the moisture and fat retention for samples prepared with different levels of water. Moisture and fat retention did not differ significantly among the samples (Table 18, Appendix C). Comparing the samples to each other also did not give significant results (Tables 21 and 22, Appendix C).

The observation was made that as the level of water increased the meat became softer. This meat was harder to work with during the grinding, freezing and cooking processes. The 10% water sample was the easiest to work with compared to the other water added samples. It also did not differ significantly in its cooking yield, shrinkage, fat and moisture retention from the other samples. This was the level of water used for other trials.
Figure 20: Percent Cooking Yield For Low-Fat Ground Beef Patties Formulated with 4% WPC and Different Levels of Water
Figure 21: Percent Shrinkage For Low-Fat Ground Beef Patties Formulated with 4% WPC and Different Levels of Water
Figure 22: Percent Moisture Retention For Low-Fat Ground Beef Patties Formulated with 4% WPC and Different Levels of Water
Figure 23: Percent Fat Retention For Low-Fat Ground Beef Patties Formulated with 4% WPC and Different Levels of Water
V. Effect of Processing Conditions on Shape

a. Adding iota-Carrageenan as a Powder vs Solution

Several problems were encountered with the carrageenan samples. The first problem was dissolving the carrageenan without forming a gel. The other problem was to uniformly distribute the gel that did form in the meat. To overcome this problem the next experiment compared the addition of carrageenan as a powder verses as a solution to low-fat ground beef. The samples were prepared with 0.75% carrageenan, 9% water and 0.5% encapsulated salt. The solution was prepared by adding the carrageenan to water with stirring and heating to 70°C. The second sample was prepared by sprinkling carrageenan powder over the meat and to this meat the water and salt were added separately.

The two samples did not differ significantly in their cooking yield, shrinkage, fat and moisture retention (Table 23, Appendix C). The samples prepared by adding powder are much easier to work with. This method also required less time for sample preparation. Future formulations were processed by adding carrageenan as powder.

It was observed as before that the solution gelled and was hard to distribute evenly throughout the meat. It was much easier to evenly distribute the carrageenan powder throughout the meat. These patties also did not have the gelled spots observed in the samples with carrageenan solution added. However, the samples with powder added started to crumble after the first three minutes of cooking which was not observed for samples prepared earlier. The level of carrageenan to add to these samples was determined earlier, but using gelled solutions. The level of carrageenan to add as a powder to meat could be different due to the method of addition. These samples may require less carrageenan since it will be gelling in the meat and not being added as a gel.

The cause of deformation (pita pocket formation) was still not determined. Now the samples had also started to crumble and this was an added deformation in the powder added carrageenan samples. The next experiment dealt with quick
freezing the samples in liquid nitrogen. The rationale was that slow freezing allowed liquid loss during the thawing process.

**b. Quick Freezing Samples**

Three samples -- 4% WPC (10% water, 0.5% salt), 0.75% carrageenan (9% water, 0.5% salt) and a 20% fat (0.5% salt) were processed for this experiment. After grinding the meat was formed into 114 g (4 oz) patties and then quick frozen in liquid nitrogen.

A pattern was observed among the sample for cooking yield and fat retention (Figure 24 and 27, respectively). The experimental samples had higher cooking yields and fat retentions (p<0.01) than the control sample (Table 24, Appendix C). Between the experimental samples the WPC had the highest cooking yield and fat retention (p<0.01) (Table 25, Appendix C). A similar pattern was observed for shrinkage (Figure 25) except the experimental samples had less (p<0.01) shrinkage than the control, and the WPC less (p<0.01) than the carrageenan sample. Moisture retention (Figure 26) followed the same pattern except for the 17th day when the carrageenan sample had the lowest moisture retention.

The number of days the samples were stored also followed a pattern (Table 26, Appendix C). The fat retention decreased with increase in age with the lowest retention for the 17th day (p<0.05) (Table 27, Appendix C). Cooking yield also followed the same pattern with the lowest cooking yield observed for the 17th day (Figure 24). Moisture did decrease with increase in age, but much more gradually compared to the fat retention (Figure 26). In all cases the WPC samples had the highest fat/moisture retention and cooking yield, and the lowest shrinkage.

The quick freezing did prevent the formation of a *pita* pocket in the middle of the patties, which was caused by the method of freezing. When the samples were slowly frozen the outer layer cooled faster than the core of the patty. Due to the temperature difference the moisture from the core was migrating to the surface of the patty. When the patty was thawed the ice crystals melted and
Figure 24: Percent Cooking Yield for Quick Frozen Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan
Figure 25: Percent Shrinkage for Quick Frozen Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan
Figure 26: Percent Moisture Retention for Quick Frozen Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan
Figure 27: Percent Fat Retention for Quick Frozen Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan
were observed as the excess liquid lost during thawing. When the quick frozen samples were thawed there was less loss of liquid compared to the slowly frozen samples. The *pita* pocket formed since the core had lost most of its moisture.

**VI. Addition of Lactose for Flavor**

**a. Comparing Alacen 837 to Alacen 878**

A low protein and high lactose WPC (Alacen 837; 56.4% protein, 30.7% lactose, 4.6% ash, 4% moisture and 4.3% fat) was compared to Alacen 878 (79.5% protein, 6% lactose, 4.5% ash, 4.2% moisture and 4.6% fat). The rationale for testing a high lactose WPC was that lactose is a reducing sugar and it might enhance the browning reaction and produce additional flavor components. Samples were formulated with 0-4% WPC, but no additional lactose was added to the samples.

Figure 29 shows the fat retention for samples formulated with Alacen 878 and 837. It is observed that at all levels the Alacen 878 samples had higher fat retentions. The ability to retain fat could be attributed to the protein content (*p*<0.05) and not to the presence of lactose (Table 28, Appendix C). The lactose may interfere with gelation and thus hinder fat retention.

The moisture retention was also higher for the Alacen 878 samples (Figure 30). As mentioned earlier the lactose level could interfere with gelation and thus binding of water. The level of lactose did have an effect (*p*<0.05) on moisture retention, but the level of protein did not (Table 28, Appendix C).

Cooking yield for samples with both Alacen 878 and 837 increased as the level of added WPC increased (Figure 31). The Alacen 878 samples had higher cooking yield (*p*<0.05) than the Alacen 837 samples. This may be attributed once again to the higher protein content and therefore better gelation ability of Alacen 878.
Figure 28: Sensory Evaluation of Low-Fat Ground Beef Patties with Alacen 837 Using the Forced Ranking Method
Figure 29: Percent Fat Retention for Low-Fat Ground Beef Patties
Formulated with Alacen 837 and Alacen 878
Figure 30: Percent Moisture Retention for Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 31: Percent Cooking Yield for Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 32: Percent Shrinkage for Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 33: Texture Profile Analysis of Hardness for Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 34: Texture Profile Analysis of Chewiness of Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 35: Texture Profile Analysis of Springiness of Low-Fat Ground Beef Patties Formulated with Alacen 837 and Alacen 878
Figure 32 shows the percent shrinkage for the two samples. There was no pattern observed for shrinkage. The level of lactose or protein did not effect the shrinkage (Table 28, Appendix C).

Figures 33, 34 and 35 show the hardness, chewiness and springiness, respectively, of the samples formulated with two different WPCs. The hardness was effected by the level of lactose (p<0.05), but not the level of WPC added. Similarly the chewiness was effected by the level of lactose (p<0.001) and not the level of WPC added.

The Alacen 837 samples were evaluated for their sensory properties. The parameters evaluated were flavor, texture, juiciness, and overall acceptability. Figure 28 shows that the 4% WPC samples were preferred the most compared to all the other samples. The juiciness and overall acceptability of this samples was above the limit of significance (p<0.05). The juiciness of the 10% fat sample was below the limit (p<0.05), and the texture and overall acceptability of the 1% WPC was preferred the least (p<0.05) (Table 27, Appendix D).

The data indicated that Alacen 878 had higher cooking yields, fat and moisture retention than the Alacen 837 samples. The lower protein content seemed to effect the ability of the WPC to bind moisture and fat. Using a high lactose WPC (Alacen 837) decreased the cooking yield, fat and moisture retention and did not enhance the flavor as desired. The addition of lactose to Alacen 878 may be beneficial since the protein level is not being lowered.

b. Addition of Lactose to Alacen 878 Patties

The next set of samples were formulated using Alacen 878. Three fat levels -- 10%, 20% and 27% fat -- were formulated with 4% WPC (0.6% lactose) and 4% WPC with 1.2% lactose added. The rationale for this experiment was that the higher protein WPC functioned more efficiently than the low protein (high lactose, Alacen 837) WPC. The addition of lactose to the high protein WPC may help enhance browning and flavor in these samples and yet maintain good cooking characteristics.
The level of fat as seen in Tables 29, 30 and 31 (Appendix C) did effect the fat retention (p<0.001), moisture retention (p<0.05), and cooking yield (p<0.001). The 10% fat samples (that includes samples with 4% WPC and 4% WPC with 1.2% lactose added) had higher fat retentions (p<0.01) and cooking yields (p<0.01). The low-fat samples have less fat loss during cooking and the higher fat retentions and cooking yields could be attributed to this factor. The 10% fat samples on the other hand had lower moisture retentions (p<0.01). These samples have less moisture to begin with and there is more moisture loss during cooking which accounts for the lower moisture retentions.

Table 30 (Appendix C) shows that only one cooking characteristic, shrinkage, was effected by the level of lactose (p<0.05). The 0% lactose samples had greatest shrinkage (p<0.05). This supports the fact that the addition of WPC (by itself and with lactose) does bind water and, therefore, these samples have less shrinkage. The cooking yield was highest for the 4% WPC (0.24% lactose) samples (p<0.05). Once again the WPC helps in binding water in the low-fat patties and this would have an effect on the overall yield of the samples. Even though the 4% WPC samples with 1.2% lactose added have the same protein level the lactose may interfere with the gelation of the protein and thus the yield may be lower for these samples.

The interaction of fat and lactose did effect the moisture retention (p<0.001), cooking yield (p<0.001), and shrinkage (p<0.05) of the samples (Table 29, Appendix C). It is known that the gelation of WPC is dependent upon other components present in the system. Some of these components enhance gelation while others would interfere with gelation. The addition of lactose is detrimental for gelation. The higher the fat level the greater the fat loss during cooking and this would give lower yield and greater shrinkage in the high fat samples. Together, fat and lactose, interfere with the gelation process.

It was observed that the Alacen 878 samples with added lactose did have enhanced browning. This browning was not necessarily desirable because it made the samples look overdone. Samples with added lactose were also reported to have a pleasant odor. However, this improvement was not reported to be preferred over the 4% WPC samples. The data indicated that the 4%
WPC (Alacen 878) samples were comparable to the 4% WPC with added lactose samples. The addition of lactose did enhance browning, but was detrimental for the appearance of the sample.

VII. Comparing Other Water Binders to Whey Protein Concentrate

a. Water binders in Combination with WPC

The performance of tripolyphosphate (TPP), calcium chloride (CaCl2) and hydroxypropyl methylcellulose (HPM) was investigated by adding these water binders to the WPC samples. The water binders were added at the 0.3% level to low-fat patties with 4% WPC, 10% water and 0.5% salt. The purpose of this experiment was to determine if the addition of other water binders with WPC would help improve the cooking characteristics as well as the sensory attributes.

Figure 37 shows the percent cooking yield differs for the four samples (p<0.01) (Table 33, Appendix C). It is observed that the WPC sample and TPP had the highest yields (p<0.05 and p<0.01, respectively) and the CaCl2 sample the lowest yield (p<0.05). A similar pattern was also observed (Figure 40) for moisture retention with WPC and TPP having similar moisture retentions (p<0.05 and p<0.01, respectively). As seen in Figure 38 the shrinkage was lowest for the TPP sample (p<0.05). Fat retention was not effected by the water binders added (Table 34 (Appendix C), Figure 39). The hardness, chewiness and springiness were not significantly different for the four samples and this is observed in Figures 41, 42 and 43.

Figure 36 shows the sensory evaluation ranks of the samples and it is seen that the sample preferred the most for flavor and overall acceptability was the HPM sample and the TPP sample was least preferred for the same parameters (Table 32, Appendix C). The WPC sample was preferred the most for its juiciness.

The results indicate that the addition of TPP improved the cooking yield, moisture retention and decreased the shrinkage of the samples. However,
these samples were preferred less than the WPC samples for their sensory attributes. The HPM samples were preferred the most for their sensory attributes, but did not improve the cooking characteristics significantly. Another observation made was that the HPM samples were hard to process. When the WPC-HPM solution was added to the meat it formed an extremely sticky mass. This meat was hard to grind and shape into patties. The HPM patties also left a residue on the grill after cooking. Samples prepared with CaCl2 had inferior cooking characteristics compared to the other samples. When cooking these samples an undesirable odor was also observed.

This data suggests that the performance of the WPC samples is comparable to that of the samples with other water binders. The ease of processing, the equivalent cooking characteristics and the preference for its juiciness would suggest using this formulation for other trials.
Figure 36: Rank Sums for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders
Figure 37: Percent Cooking Yield for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=trisodium phosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
Figure 38: Percent Shrinkage of Low-Fat Ground Beef Patties Formulated In Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=triphosphosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
Figure 39: Percent Fat Retention for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC= whey protein concentrate, TPP= tripolyphosphate, HPM= hydroxypropyl methylcellulose, CaCl₂= calcium chloride)
Figure 40: Percent Moisture Retention in Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=trisodium phosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
Figure 41: Texture Profile Analysis of Hardness for Low-Fat Ground beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=tripolyphosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
Figure 42: Texture Profile Analysis of Chewiness for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=tripolyphosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
Figure 43: Texture Profile Analysis of Springiness for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders (WPC=whey protein concentrate, TPP=trisodium phosphate, HPM=hydroxypropyl methylcellulose, CaCl2=calcium chloride)
b. Other Protein and Carbohydrate Water Binders

This next experiment allowed the comparison of other protein and carbohydrate water binders to WPC. This data helped establish the performance of WPC as a water binder. The WPC and sodium caseinate (NaCas) were added at the 4% level and hydroxypropyl methylcellulose (HPM) at the 0.5% level. These samples had 10% water and 0.5% encapsulated salt added to them. Two controls, a 10% fat and 10% fat with 10% water (both with 0.5% salt) were also processed.

Figure 44 shows the sum of ranks for the samples. It is seen that the 4% WPC samples were preferred (p<0.05) for their flavor while the NaCas samples were preferred the least (p<0.05) for the same attribute (Table 36, Appendix C). The texture of the 4% WPC and HPM sample is very close to the upper limit of significance and thus preferred more.

Figure 45 shows the cooking yield for low-fat ground beef patties formulated with different water binders. A pattern was observed among the yields for the samples. The samples do differ in their yields (p<0.05, Table 37, Appendix D). The 10% fat sample has a higher yield (p<0.05) than the 10% fat with 10% water added sample (Table 38, Appendix C). The added water is not bound by any added ingredient in this sample and thus is lost during cooking. The experimental samples had better yields (p<0.05) than the two controls. Once again this is because the added water binders help retain the water better during cooking. Among the experimental samples the 4% WPC sample had the highest yields (p<0.05). This pattern where the 10% fat had a higher yield than the 10% fat with 10% water added, and the WPC had a higher yield compared to NaCas and HPM is clearly seen in Figure 45.

The shrinkage and moisture retention were not significantly different for the samples (Table 37 and 38, Appendix C). The fat retention was better (p<0.05) for the 10% fat with 10% water added sample compared to the 10% fat sample (Figure 47).
The hardness and the chewiness of these samples was significantly different $p<0.001$ and $p<0.01$, respectively. As seen in Table 39 for both parameters the WPC sample was harder and more chewy ($p<0.01$ and $p<0.01$, respectively) than the NaCas and HPM samples. The NaCas was harder than the HPM sample ($p<0.05$). Figure 49 also shows the hardness and Figure 50 the chewiness of the 4% WPC samples.

Several observations were made during the processing and cooking of these samples. Once again the meat for the HPM samples was very sticky and thus hard to grind, shape and freeze. The NaCas samples were considerably harder to cook. These samples formed a paper thin, but sticky coat around themselves when cooked. This sticky coat was also left on the grill. The ease of working with the WPC sample and its comparable cooking characteristics and sensory attributes suggest continued use of this formulation.
Figure 44: Sensory Evaluation of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (NaCas = sodium caseinate, HPM = hydroxypropyl methylcellulose)
Figure 45: Percent Cooking Yield For Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (WPC=whey protein concentrate, NaCas=sodium caseinate, HPM=hydroxypropyl methylcellulose)
Figure 46: Percent Shrinkage For Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (WPC= whey protein concentrate, NaCas = sodium caseinate, HPM = hydroxypropyl methylcellulose)
Figure 47: Percent Fat Retention For Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders. (WPC = whey protein concentrate, NaCas = sodium caseinate, HPM = hydroxypropyl methylcellulose)
Figure 48: Percent Moisture Retention For Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders. (WPC=whey protein concentrate, NaCas=sodium caseinate, HPM=hydroxypropyl methylcellulose)
Figure 49: Texture Profile Analysis for Hardness of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (WPC = whey protein concentrate, NaCas = sodium caseinate, HPM = hydroxypropyl methylcellulose)
Figure 50: Texture Profile Analysis for Chewiness of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (WPC = whey protein concentrate, NaCas = sodium caseinate, HPM = hydroxypropyl methylcellulose)
Figure 51: Texture Profile Analysis for Chewiness of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders (WPC=whey protein concentrate, NaCas=sodium caseinate, HPM =hydroxypropyl methylcellulose)
VIII. Volatile Profile Analysis

Fat is a vital component of flavor in most foods. When fat is replaced there is a change in the flavor profile of the low-fat product. The acceptance of a food depends on sensory properties such as flavor. Thus it is important to determine if the flavor profile of the low-fat product matches that of the full fat product. In this trial the volatile flavors of 10%, 20% and 27% fat were determined using the dynamic headspace technique. These samples were also processed with 4% WPC (10% water and 0.5% salt) and 4% WPC with 1.2% lactose (10% water and 0.5% salt). The values for the identified compounds were computed as a ratio of 2-propanone, which was standardized to one hundred percent.

Table 40 shows the ratio of the compounds to 2-propanone. Table 45 gives the percent area and method used for identification of the volatile compounds. Table 41 shows the characterization of some of the volatile compounds identified in our samples. It seems that most of the compounds were not specific to beef, but were compounds identified in other meat sources such as chicken, mutton, and pork. Also the odor characterization of these compounds using data from Shahidi et al. (1991) revealed that most of them were not desirable. However, the odor characterization is based on sensory evaluation and this explains the variations in characterization. The compound 2-methyl furan is described as pleasant and also sickly which are contradictory to each other.

A large number of compounds were identified in the 10% fat samples (by itself and with 4% WPC and 4% WPC with 1.2% lactose) which were not identified in the other samples. The samples for this experiment were not analyzed at the same time. Even though the same source of meat and type of meat (ground round) was used for all the experiments there could be differences in the composition of the meat. This could be one of the causes of variation. Also, the 20% and 27% fat samples were prepared by adding tallow to the 10% fat meat. This is another variable that could cause differences in the volatile components identified.
Dynamic headspace analysis showed that the low-fat samples and the high fat samples did not have the same volatile profile. The WPC and lactose by interacting with other components may act as precursors of some volatile flavors. They could also mask other volatile compounds. It can be concluded that the volatile compounds differ in the low-fat and high fat ground beef patties.

IX. Ultrastructure of the WPC Low-Fat Ground Beef Patties

The ultrastructure of low-fat beef patties is of particular importance since it gives an insight into the mechanism of water binding. In this particular study the ability of WPC to function as a water binder was explored using transmission electron microscopy (TEM). Samples of WPC gels (solutions of 15, 30 and 40% WPC which represent 11.3%, and 22.5% and 30% whey protein, respectively), WPC combined with bovine myofibril protein (BMP) (combined solutions at a ratio of 30:10, 20:20, 10:30 of WPC:BMP [w/w]), and low-fat beef patties with 2% and 4% WPC, 10% water and 0.5% encapsulated salt were also prepared and cooked at three different temperatures (60°, 70° and 80°C).

Figures 52 and 53 show transmission electron micrographs (TEM) of 15% WPC gels. Void spaces are seen separating areas with dark clusters of protein. As the concentration of WPC was increased (30% and 40%) the void spaces decreased and the clusters were more compact and dense (Figure 54 (30% WPC) and 55 (40% WPC)). The scans of the higher WPC concentration gels also have dense white spots dispersed among the protein clusters. These spots could be WPC which did not dissolve. Higher magnification of the 15% and 40% gels also showed that the protein clusters have a network of fine thread like projections (Figures 53 and 57). The ability of WPC to function as a water binder may be attributed to the thread like projections which may make it possible to entrap water.

Bovine myofibril protein (BMP) was extracted and heated at 71° C for 15 minutes. The TEM scans of BMP before heating show a defined myofibril structure (Figure 58). In this scan the sarcomeres are seen with the I-bands
Table 40: Volatile Flavor Compounds as a Ratio of 2-Propanone in Ground Beef Patties Containing Whey Protein Concentrate and Lactose with Different Levels of Fat

<table>
<thead>
<tr>
<th>Compound</th>
<th>SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%fat</td>
</tr>
<tr>
<td>Pentane</td>
<td>42.95</td>
</tr>
<tr>
<td>Hexane</td>
<td>7.85</td>
</tr>
<tr>
<td>Heptane</td>
<td>8.82</td>
</tr>
<tr>
<td>Menthae Thiobis</td>
<td>2.68</td>
</tr>
<tr>
<td>Octane</td>
<td>9.91</td>
</tr>
<tr>
<td>2-butanolone</td>
<td>3.22</td>
</tr>
<tr>
<td>2-methyl butanal</td>
<td>4.26</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>15.41</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>-</td>
</tr>
<tr>
<td>Hexanal</td>
<td>-</td>
</tr>
<tr>
<td>2-butanolone-3-hydroxy</td>
<td>4.82</td>
</tr>
<tr>
<td>4,4-dimethyl-2-oxetanal</td>
<td>8.11</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
</tr>
<tr>
<td>2-propanol</td>
<td>10.97</td>
</tr>
<tr>
<td>Methyl benzene</td>
<td>4.73</td>
</tr>
<tr>
<td>2-methyl-1-propane</td>
<td>20.66</td>
</tr>
<tr>
<td>2,5-dihydroxy furan</td>
<td>1.37</td>
</tr>
<tr>
<td>Thiourea</td>
<td>-</td>
</tr>
<tr>
<td>3-methyl-2-butanone</td>
<td>-</td>
</tr>
<tr>
<td>2-ethyl furan</td>
<td>-</td>
</tr>
</tbody>
</table>

*+Sample had 4% WPC, 10% water and 0.5% encapsulated salt added
++Sample had 4% WPC, 10% water, 0.5% encapsulated salt and 1.2% lactose added*
Table 41: Characterization of Identified Volatile Compounds of Ground Beef Patties Containing Whey Protein Concentrate and Lactose with Different Levels of Fat

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Meat Source</th>
<th>Odor Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>Hydrocarbon, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>-</td>
</tr>
<tr>
<td>Heptane</td>
<td>Hydrocarbon, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>Cooked Meat</td>
</tr>
<tr>
<td>Octane</td>
<td>Hydrocarbon, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>Meaty</td>
</tr>
<tr>
<td>Pentane</td>
<td>Hydrocarbon, aliphatic, acyclic</td>
<td>Chicken, Beef</td>
<td></td>
</tr>
<tr>
<td>Methyl Benzene</td>
<td>Hydrocarbon, aromatic</td>
<td>Mutton, Chicken, Beef</td>
<td>Strong, Fruity, Becoming Dank, Bitter</td>
</tr>
<tr>
<td>Hexanal</td>
<td>Aldehyde, aliphatic</td>
<td>Mutton, Chicken, Beef</td>
<td>Strong, Rancid, Unpleasant, green, Pungent, Sickly</td>
</tr>
<tr>
<td>2-methyl butanal</td>
<td>Aldehyde, aliphatic</td>
<td>Beef</td>
<td>Burnt, Sickly</td>
</tr>
<tr>
<td>2-propanone</td>
<td>Ketone, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>-</td>
</tr>
<tr>
<td>2-butanone, 3-hydroxy</td>
<td>Ketone, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>Buttery</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>Ketone, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef, Pork</td>
<td>Buttery, Sweet, Sickly, Meaty, Burnt, Green</td>
</tr>
<tr>
<td>2-butaneone</td>
<td>Ketone, aliphatic, acyclic</td>
<td>Mutton, Chicken, Beef</td>
<td>Sickly</td>
</tr>
<tr>
<td>2-butaneone, 3-methyl</td>
<td>Ketone, aliphatic, acyclic</td>
<td>Chicken, Beef</td>
<td>-</td>
</tr>
<tr>
<td>2-methyl furan</td>
<td>Furan</td>
<td>Chicken, Beef, Pork</td>
<td>Pleasant, Slightly Sulfurous, Meaty, Sickly</td>
</tr>
<tr>
<td>Dimethyl Disulfide</td>
<td>Sulfur Compound</td>
<td>Mutton, Chicken, Beef</td>
<td>Very strong, Sulfurous, Garlic, Onion, Sickly, Cooked Cabbage</td>
</tr>
</tbody>
</table>

and A-bands and Z-lines. Upon heating the BMP the sarcomere is not seen clearly anymore. They seem to have separated and the Z-lines are no longer visible (Figure 59).

Scans of combined gels of WPC and BMP (30:10, 20:20 and 10:30 WPC:BMP (w/w)) were also obtained. In the 30:10 samples the WP formed the continuous phase with the BMP distributed through it (Figure 60). In this case the WP provided the matrix in which the BMP was held. When the two proteins were present in an equal ratio (20:20) the BMP was present in larger clusters and the WP surrounded these clusters (Figure 61). Whey protein still seemed to provide the matrix to hold the BMP. At a ratio of 10:30 (WPC:BMP) the WP is present between the BMP sarcomeres (Figure 62). It is observed that at this ratio the BMP is more clearly defined into sarcomeres. Also the WP no longer provides the matrix for holding the BMP. Higher magnification of the same scan (Figure 63) shows that the WP and BMP may be interacting together to form a gel between the interstitial spaces of the myofibrils.

TEM scans were obtained of the 10% fat and low-fat ground beef patties containing 2% and 4% WPC before and after cooking at 60°, 70° and 80° C. The raw samples of 10% fat, 2% and 4% WPC (Figures 64, 65 and 66, respectively) showed the defined structure of the myofibrilar protein. As the cooking temperature increased the distinct structure of the myofibrils became less defined (60°C, Figure 67 (10% fat), 68 (2% WPC) and 69 (4% WPC); 70°C, Figure 70 (10% fat), 71 (2% WPC) and 72 (4% WPC)) with barely any discernible myofibrilar structure at 80°C for the 10% fat sample (Figure 73). In the 4% WPC sample heated to 80°C some myofibrilar structure was still observed (Figure 74).

Pools of fat were observed in samples containing lower levels of WPC. The fat globules rupture during processing and form larger pools of globules. However, the 4% WPC samples contained large fat droplets surrounded by WPC (Figure 75). This may explain the mechanism by which WPC retains fat within the sample.
The TEM micrographs helped elucidate a possible mechanism by which the WPC functions as a water binder in a low-fat ground beef system. The level of WPC seems to determine what type of gel formed. It was observed that the WPC forms aggregated clusters with thread like projections which form the gel network. This network is essential since it helps entrap water and fat and also holds the BMP together.
Figure 52: TEM of 15% Whey Protein Concentrate Gel, scale for the image
1.6 cm = 2 um

Figure 53: TEM of 15% Whey Protein Concentrate Gel, scale for the image
3.1 cm = 1 um
Figure 54: TEM of 30% Whey Protein Concentrate Gel, scale shown
1.6 cm = 2 um

Figure 55: TEM of 30% Whey Protein Concentrate Gel, scale shown
3.14 cm = 1 um
Figure 56: TEM of 40% Whey Protein Concentrate Gel, scale for the image 1.6 cm = 2 um

Figure 57: TEM of 40% Whey Protein Concentrate Gel, scale for the image 3.9 cm = 1 um
Figure 58: TEM of Bovine Myofibril Protein Gel Before Heating, scale for the image 2.3 cm = 5 um

Figure 59: TEM of Bovine Myofibril Protein Gel After Heating, scale for the image 2.3 cm = 2 um
Figure 60: TEM of a Combined 30:10 WPC to BMP Gel, scale for the image 2.3 cm = 2 um

Figure 61: TEM of a Combined 20:20 WPC to BMP Gel, scale for the image 2.3 cm = 2 um
Figure 62: TEM of a Combined 10:30 WPC to BMP Gel, scale for the image 2.0 cm = 2 um

Figure 63: TEM of a Combined 10:30 WPC to BMP Gel, scale for the image 3.1 cm = 1 um
Figure 64: TEM of a Raw 10% Fat Ground Beef Patty, scale for the image $2.3 \text{ cm} = 2 \text{ um}$

Figure 65: TEM of a Raw Low-Fat Ground Beef Patty with 2% WPC Added, scale for the image $2.3 \text{ cm} = 2 \text{ um}$
Figure 66: TEM of a Raw Low-Fat Ground Beef Patty with 4% WPC Added, scale for the image 2.3 cm = 2 um

Figure 67: TEM of a 10% Fat Ground Beef Patty Cooked to an Internal Temperature of 60°C, scale for the image 2.3 cm = 2 um
Figure 68: TEM of a Low-Fat Ground Beef Patty with 2% WPC Added Cooked to an Internal Temperature of 60°C, scale shown 2.3 cm = 2 um

Figure 69: TEM of a Low-Fat Ground Beef Patty with 4% WPC Added and Cooked to an Internal Temperature of 60°C, scale shown 2.3 cm = 2 um
Figure 70: TEM of a 10% Fat Ground Beef Patty Cooked to an Internal Temperature of 70°C, scale for the image 2.3 cm = 2 um

Figure 71: TEM of a Low-Fat Ground Beef Patty with 2% WPC Added Cooked to an Internal Temperature of 70°C, scale shown 2.3 cm = 2 um
Figure 72: TEM of a Low-Fat Ground Beef Patty with 4% WPC Added and Cooked to an Internal Temperature of 70°C, scale shown 2.3 cm = 2 um

Figure 73: TEM of a 10% Fat Ground Beef Patty Cooked to an Internal Temperature of 80°C, scale for the image 2.3 cm = 2 um
Figure 74: TEM of a Low-Fat Ground Beef Patty with 4% WPC Added and Cooked to an Internal Temperature of 80°C, scale shown 2.3 cm = 2 um

Figure 75: TEM of a Fat Droplet in a Low-Fat Ground Beef Patty with 4% WPC Added, scale shown 3.9 cm = 1 um
X. Nutritional Composition and Cost Analysis

The nutritional composition of the raw WPC, 27% fat and 0.5% carrageenan patties was done using the ESHA Food Processor II computer software. Table 43 shows the nutritional composition of the three samples. The 4% WPC sample had 45.71% less calories than the 27% fat sample and 4.75% more calories than the carrageenan sample. The WPC sample had 65.9% less calories from fat than the 27% fat sample and 1% less than the carrageenan sample. The WPC sample has considerably higher protein content with 65.49% and 18.83% more than the 27% fat and carrageenan sample, respectively.

The prices used for these analysis are based on 1994 estimates obtained from the ingredient supplier. The 10% fat meat is an estimate based on 1993 pricing used by Karn Meats. Cost analysis (Table 44) of the samples shows that the prices do not differ greatly. However, the WPC sample is cheaper to produce than the carrageenan samples. These prices are subject to change depending on the quantity purchased.
Table 43: Nutritional Composition* of Raw WPC, 27% fat and Carrageenan Ground beef Patties

<table>
<thead>
<tr>
<th></th>
<th>27% fat</th>
<th>Carrageenan</th>
<th>WPC</th>
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<tr>
<td>Calories</td>
<td>350.00</td>
<td>181.00</td>
<td>190.00</td>
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<td>Total fat (g)</td>
<td>30.00</td>
<td>9.96</td>
<td>10.20</td>
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<td>Calories from fat</td>
<td>273.00</td>
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<td>93.10</td>
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<tr>
<td>Protein (g)</td>
<td>18.80</td>
<td>20.70</td>
<td>23.50</td>
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<td>Calories from protein (g)</td>
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<tr>
<td>Carbohydrates (g)</td>
<td>0</td>
<td>0.485</td>
<td>0.277</td>
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<tr>
<td>Calories from carbohydrates</td>
<td>0</td>
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<td>0.003</td>
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<tr>
<td>Sodium (mg)</td>
<td>298</td>
<td>295</td>
<td>291</td>
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</tbody>
</table>

*Analysis based on 114 g portion

Table 44: Cost Analysis for WPC, 27% fat and Carrageenan Ground Beef Patties

<table>
<thead>
<tr>
<th>WPC Sample</th>
<th>% Ingredient</th>
<th>Price ($ per pound)</th>
<th>Cost Contribution ($)</th>
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<tbody>
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<td>Ingredient</td>
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<td></td>
<td></td>
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<tr>
<td>WPC</td>
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<td>0.0240</td>
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<tr>
<td>Encapsulated Salt</td>
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<td>0.95</td>
<td>0.0012</td>
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<tr>
<td>Meat (10% fat)</td>
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<td>1.35</td>
<td>0.2895</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.3147</td>
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</table>

<table>
<thead>
<tr>
<th>27% Fat Sample</th>
<th>% Ingredient</th>
<th>Price ($ per pound)</th>
<th>Cost Contribution ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encapsulated Salt</td>
<td>0.5</td>
<td>0.95</td>
<td>0.0012</td>
</tr>
<tr>
<td>Meat (27% fat)</td>
<td>99.5</td>
<td>1.29</td>
<td>0.3219</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.3231</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carrageenan Sample</th>
<th>% Ingredient</th>
<th>Price ($ per pound)</th>
<th>Cost Contribution ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.5</td>
<td>8.04</td>
<td>0.0101</td>
</tr>
<tr>
<td>Encapsulated Salt</td>
<td>0.5</td>
<td>0.95</td>
<td>0.0012</td>
</tr>
<tr>
<td>Meat (10% fat)</td>
<td>90.0</td>
<td>1.35</td>
<td>0.3047</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.3160</td>
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</table>
DISCUSSION

This study was conducted to determine the feasibility of using whey protein concentrates (WPC) as water binders in low-fat ground beef patties. The lowering of fat in meat systems has undesirable consequences such as a reduction in juiciness. To overcome these problems water and water binders are added to the low-fat products. The ability of WPCs to form heat induced gels was the functional property that indicated its use as a water binder in low-fat meat systems.

The initial experiments concentrated on determining the specific WPC or whey protein product (Simplesse 100 and 550) to use in this study. These experiments helped establish Alacen 878 as the WPC of choice. Even though Simplesse is a whey protein based product, it is a pre-cooked (microparticulated) product (McCormick, 1988; Morrison, 1990). This unique process gives it the much-sought smooth and creamy texture (Schlicker and Regan, 1990). However, upon heating Simplesse loses this desirable feature. As observed samples prepared with Simplesse were not preferred organoleptically.

The low-fat patties were frozen at -18°C in 72 hours. However, these patties deformed during cooking. At first the cause of deformation was not clear. Different WPC and water levels were tested, but the patties still deformed. Eventually, the cause of deformation was found to be the method of freezing. When the meat is slowly frozen (-18°C in 72 hours) ice crystals form first in the interstitial spaces and then in the fibers. The lower vapor pressure of ice crystals causes migration of water from the fibers to the interstitial spaces (Hultin, 1985). In our samples the core of the patties was freezing at a slower
rate than the outside of the patty. The water from the core was migrating to the outer layers and thus the *pita* pocket formed in the middle. By quick freezing the samples in liquid nitrogen this problem was overcome. Berry (1993) conducted experiments to determine the effect of the fat level and freezing temperature on the sensory, shear, cooking and composition properties of ground beef patties. Slowly frozen samples were found to be tougher than quick frozen samples. This toughness could be attributed to the increased exudate due to slow freezing.

Most applications using WPC in meat have been to replace meat, that is to use WPC as extenders, and not as such for their water binding ability. Ensor et al. (1987) found knockwurst formulated with 2% WPC had lower fat, gel water and total losses compared to a soy protein isolate formulation. In the same study the 0% WPC sample had the greatest losses thus showing the ability of WPC to bind water and fat. Our data also showed that samples prepared with 4% WPC, 10% water and 0.5% encapsulated salt had higher cooking yield, fat and moisture retention and less shrinkage compared to a 20% fat (0.5% salt) control and a 0.75% carrageenan (9% water, 0.5% salt) sample.

McLean Deluxe, a low-fat patty with carrageenan as the water binder, was introduced in the market by McDonald's Corporation. This product was developed by Egbert et al. (1991) at Auburn University. The McLean Deluxe had approximately 17% less fat loss and 36% lower moisture loss compared to the 4% WPC patties. The carrageenan burger did have lower cooking losses, but it has been reported to have other drawbacks. The McLean Deluxe when held in the holding cabinet for 25 minutes loses its juiciness and crumbles easily. However, the WP samples were able to retain their juiciness and shape after 25 minutes. The McLean Deluxe has also required the addition of flavoring agents which the protein sample has not. Lastly, carrageenan is an expensive ingredient and can cost as much as $8.04/lb while the WPC costs only $2.39/lb.

Samples were also prepared with a WPC high in lactose (Alacen 837) and its performance was compared to a high protein low lactose WPC (Alacen 878). The high lactose WPC had a lower cooking yield, fat and moisture retention
compared to the high protein WPC (Alacen 878). It is known that gelation of WPC is dependent on several factors such as the temperature, protein concentration, salt concentration and calcium concentration. The protein concentration determines if a gel will form and if it does the characteristics of the gel (Mangino, 1984). The gel formation is also dependent on the presence of other compounds. Mulvihill and Kinsella (1987) stated that when WPC prepared by gel filtration and having a protein-to-lactose ratio of 2:1 and 10% w/v protein gelled in 1.25 min at 100°C. When the protein to lactose ratio was changed to 100:1 the gel strength increased. The high protein content of Alacen 878 allowed a better gel to form and thus bind water and entrap fat compared to the Alacen 837 samples. Partially delactosed whey was used in frankfurters and the data showed that this sample had better fat stability compared to a control, soy and regular whey samples (Casella, 1983). In our samples the high lactose level probably also interfered with gelation.

Then samples with different fat levels (10%, 20% and 27%) were formulated with 4% WPC and 4% WPC and 1.2% lactose. The purpose for adding lactose in these samples was to enhance flavor and browning. There was enhanced browning in these samples, but it had an adverse affect. The samples looked overdone and were tougher. Lactose is often added to cured meat products to increase the total solids content of brine to aid in protein extraction. It helps mask the flavor of other additives while providing flavor itself. However, the use of lactose is limited due to browning reactions (van den Hoven, 1987). Also the addition of lactose interferes with gelation. The samples with 0.6% lactose (4% WPC; Alacen 878) had the best overall cooking characteristics. The samples with 1.2% lactose looked overdone, but did have a better flavor.

Texture is one of the important parameters of a meat product. Hardness (the "first bite"), springiness (elasticity), and chewiness (hardness x cohesiveness x springiness) are some of the parameters of interest for evaluating in new meat formulations. Many factors effect the tenderness or textural properties of a product such as the fat level, storage conditions and temperature of storage. The texture profile analysis (TPA) of our 4% WPC samples showed us that the 4% WPC samples were harder and more chewy compared to the control (20% and 27% fat). Sample prepared with a high lactose (lower protein
concentration, Alacen 837) and a high protein (low lactose, Alacen 878) WPC had similar TPA values, but the lactose did have a significant effect on the hardness and chewiness. When samples were formulated in combination with other water binders the 4% WPC sample was not as hard as compared to the hydroxypropyl methylcellulose and CaCl2 sample. The addition of CaCl2 to WPC helps increase the gel strength up to a certain level after which it decreases gel strength. This decrease is due to excessive cross-linking of the protein. In our sample the gel strength probably increased, but this gave a hard product. The 4% WPC sample by itself had the highest hardness values so the addition of CaCl2 just increased this hardness.

Correia et al. (1991) reported that samples formulated with micro-crystalline cellulose and soy protein concentrate were harder compared to other fillers and the WPC sample was the least hard. A sausage product was used for this study which is an emulsion based product. The method of processing for these products is very different from ground beef patties. This may be one of the reasons that the WPC gave a softer product compared to the other fillers. Sofos and Allen (1977) also found that as texturized soy protein (TSP) replaced fat the hardness of the samples increased. However, in another study Sofos et al. (1977b) reported that TSP-substitution for fat resulted in a gradual decrease in hardness. The method of analysis is important. These researchers used sections and not the whole product for analysis. In our analysis whole patties were evaluated.

Transmission electron microscopy (TEM) of WPC gels revealed that as the concentration of protein increased the type of gel formed also changes. At higher concentrations the gel was more compact. Overall there was a network of aggregates connected to each other by fine threads, this was also reported by Langton and Hermansson (1992). Yost and Kinsella (1992) stated that the void spaces and crevices observed are formed due to the removal of water during sample preparation.

Fat droplets surrounded by WPC were observed for the scans of the 4% WPC sample. Many mechanisms have been postulated relating the microstructure of gels with fat. One study concluded that there is an increase in gel strength and
decrease in gel syneresis because the fat globules hydrostatically restrain the protein matrix from moving. Another hypothesis is that fat droplets simply fill the pores of the gel, but do not actually interact with the protein. It is also possible that at first the fat globules adsorb protein molecules which as a emulsifying membrane. Later they interact with the protein from the surrounding area and form continuous, heat-induced protein gels (Yost and Kinsella, 1992). This last hypothesis has been confirmed to be the mechanism by which fat is retained in protein gels (Yost and Kinsella, 1992). Also the 4% WPC sample had more protein to cover the fat and so fat globules were observed instead of the pools of fat observed in the 2% WPC sample.

Ground beef flavor intensity is related to the fat level. Berry (1992) stated that samples with high levels of fat (16% and 20% fat) had higher beef flavor intensity compared to low-fat samples. Kregel et al. (1986) and Berry and Leddy (1984) concluded that the method of cooking affected flavor intensity and not the level of fat. This conclusion was also supported by Cross et al. (1980).

The identification of volatile components of our samples (10%, 20% and 27% fat with 4% WPC and 4% WPC and 1.2% lactose) showed that the volatile profiles of the samples are very different. Mottram and Edwards (1983) noted that the water soluble fraction of muscle contributes the basic meaty flavor (for example beef, pork and lamb), but it is the fatty tissue that provides the species specific aroma and flavor. Many of the compounds identified in our samples are from the water soluble fraction since they have been identified in different meat sources. To obtain fat levels of 20% and 27% fat for our volatile component analysis tallow was added to 10% fat meat. This also would effect the volatile flavors. When beef and pork samples were analyzed their volatile profiles differed due to the differences in the lipid-derived volatiles. The addition of fat to low-fat meat did not give the same volatile profile as the regular meat (that is meat with no fat removal). This is because the intramuscular and intracellular structural lipids are the important source of the meat flavor volatiles (Mottram and Edwards, 1983).

The samples with WPC and WPC and lactose had higher levels of some volatile components and not others. Jasinski and Kilara (1985) studied the flavor
binding ability of whey proteins. The chemistry, structure, and hydrophobicity are some of the factors that influence their ability to bind flavor. The different whey proteins such as β-lactoglobulin differ in their ability to bind flavors. Overall WPC have excellent binding ability. However, it could be possible that some compounds are bound but not released when bound to WPC. This may explain the differences in the volatile profiles of the controls compared to samples with WPC. Also the ability of WPC to bind flavor compounds was investigated in a model system. In an actual food this ability may be hindered due to the presence of other components.

As mentioned the purpose of incorporating lactose with the WPC was to enhance browning and flavor production. Ferretti and Flanagan (1971) studied off-flavor development in dairy products as a consequence of lactose-casein browning. Forty compounds were identified of which some were lactones (3), pyrazines (5), and furans. None of the compounds were identified in our meat samples. This difference could be attributed to the different systems studied. These researchers used a dairy based system whereas ours was a meat based product. Also the protocol used, that is the method of investigation can also give different results.

The low-fat product developed in this study has been cooked on a commercial fast-food grill. The low-fat patties achieved an internal temperature of 71°C when cooked at the same time and temperature as the regular hamburger patty sold at this fast food chain.
SUMMARY

There appears to be much potential for the use of whey protein concentrates (WPC) in low-fat meat products. The ability of WPCs to emulsify, foam and form gels are all important functional properties that can be utilized advantageously in different meat applications. The ability of WPCs to form heat induced gels was successfully used to produce a low-fat ground beef patty.

After testing several whey protein concentrates and whey protein products, Alacen 878 (79.5% protein, 6% lactose) was chosen for further trials. When added at the 4% level with 10% water and 0.5% encapsulated salt it gave a low-fat ground beef patty with good cooking characteristics. These patties were also organoleptically preferred to the control and patties formulated with other water binders.

This study utilized WPC from one source, and other WP products were not tested. Research should be done to compare the performance of WPC from different sources. Factors such as the method of processing and milk source should be investigated to determine their effect on the product.

The type of meat used determines the quality of the product produced. In this study ground round was used for producing the low-fat ground beef patties. There are other cheaper sources of meat which may be used in a formulation such as the one developed in this study. Future research needs to be done to determine the impact of the type of meat on the quality of the product.

The low-fat product developed in this study has already been tested for its cooking performance in a fast-food chain. This product holds its shape and
retains juiciness even after holding in the oven for 30 minutes. There would not be need for many modifications. However, this sample has only been processed at the pilot plant scale. The next step should be to process the low-fat WPC patties on a commercial scale.
REFERENCES


Appendix A

Sample Sensory Evaluation Sheet
Sensory Evaluation of Hamburgers

Instructions: You will be given five samples. You are asked to rank these samples according to the attributes given. This is forced ranking and you must assign a sample to each rank provided. A recommended procedure is to taste the samples and place them in the appropriate rank order. The samples may then be tasted once more to assign the final rank. It is recommended that you clean your palate after each sample with the crackers and water provided.
Ranking Analysis: (Forced Ranking)

For each attribute rank the samples according to the best sample to worst sample. Every sample has to be assigned a rank for each attribute. You may not use the same rank twice or use fractions.

Ranking of 1 = Best Sample  
Ranking of 5 = Worst Sample

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Flavor</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
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COMMENTS:
Appendix B

Single Degree Of Freedom Analysis
Table 45: Percent Shrinkage of Quick Frozen Ground Beef Patties Formulated with Whey Protein Concentrate and Carrageenan

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Control</th>
<th>WPC</th>
<th>Carrageenan</th>
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</thead>
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<td></td>
<td>14.58</td>
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</tr>
<tr>
<td></td>
<td>13.54</td>
<td>9.24</td>
<td>12.25</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>233.94</strong></td>
<td><strong>102.05</strong></td>
<td><strong>156.97</strong></td>
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</table>
Table 46: Two-Way Analysis of Variance of Shrinkage for Quick Frozen Ground Beef Patties Formulated with Whey Protein Concentrate and Carrageenan

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degree of Freedom</th>
<th>Mean Square</th>
<th>F-Ratio</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
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<td>Between Age</td>
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<td>4</td>
<td>7.666</td>
<td>2.030</td>
<td>0.115</td>
</tr>
<tr>
<td>Between Sample</td>
<td>585.235</td>
<td>2</td>
<td>292.617</td>
<td>77.473</td>
<td>0.000</td>
</tr>
<tr>
<td>Interaction</td>
<td>146.043</td>
<td>6</td>
<td>18.255</td>
<td>4.833</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>113.311</td>
<td>30</td>
<td>3.777</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>875.252</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 47: Symbolic Representation of Selected Treatment Comparisons for Data of Table 43

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>WPC</th>
<th>Carrageenan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-2</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-1</td>
<td>+1</td>
</tr>
</tbody>
</table>
Equation 5

\[ \text{Sum of Square}(1) = -2(233.94) + 1(102.05) + 1(156.97) = 484.6944 \]

Equation 6

\[ \text{Sum of Square}(2) = -1(102.05) + 1(156.97) = 100.5402 \]

In this example the two-way ANOVA tells us that there is a significant difference between samples, but it does not tell us which sample is different from the others. To determine which samples differ two comparisons (since there are two degrees of freedom these are broken into individual degrees of freedom, each corresponding to one comparison) are chosen (1) the shrinkage of the control sample to that of the experimental samples and (2) the shrinkage of the WPC sample to that of the carrageenan sample. The sum of squares (the sum of squares for the comparisons should add up to the sum of squares between samples in Table 43 and in this case 484.6944+100.542=585.235) are calculated and then the F-value can be determined as well. These calculated F-values are compared to the F-distribution. The individual degree of freedom analysis shows us that the control has significantly greater shrinkage than the experimental samples, and the carrageenan significantly more than the WPC sample. This ability to compare samples within certain treatments gives detailed information which is not obtained by computing two-way ANOVA.
APPENDIX C

Statistical Tables Relevant to the Results
Table 8: Sensory Evaluation of Ground Beef Patties Containing WPC and Simplesse using the Forced Ranking Test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aroma/Flavor</th>
<th>Texture</th>
<th>Saltiness</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>18</td>
<td>16**</td>
<td>26</td>
</tr>
<tr>
<td>56% WPC</td>
<td>28</td>
<td>26</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>92% WPC</td>
<td>28</td>
<td>30</td>
<td>31*</td>
<td>22</td>
</tr>
<tr>
<td>Simplesse100</td>
<td>22</td>
<td>24</td>
<td>31*</td>
<td>14**</td>
</tr>
<tr>
<td>Simplesse550</td>
<td>22</td>
<td>25</td>
<td>22</td>
<td>24</td>
</tr>
</tbody>
</table>

The minimum and maximum rank sums attainable for eight panelists are 8-40, respectively.

* at the 5% level is significantly worse
** at the 5% level is significantly better

Table 9: Sensory Evaluation of Ground Beef Patties which Have Been Ground Twice and Contain WPC and Simplesse

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aroma/Flavor</th>
<th>Texture</th>
<th>Saltiness</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>19</td>
<td>32*</td>
<td>25</td>
</tr>
<tr>
<td>56% WPC</td>
<td>21</td>
<td>30</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>92% WPC</td>
<td>28</td>
<td>32*</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Simplesse100</td>
<td>26</td>
<td>22</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>Simplesse550</td>
<td>20</td>
<td>17**</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

The minimum and maximum rank sums attainable for eight panelists are 8-40, respectively.

** at the 5% level is significantly worse
* at the 5% level is significantly better
Table 10: Two-way Analysis of Variance for Hamburger Patties with Added WPC, Water and Salt

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>Water</td>
<td>0.520</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.751</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.003</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.075</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.575</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.010</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.129</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Water</td>
<td>0.147</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.010</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.529</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.012</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.691</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.272</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.191</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.704</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.177</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Water</td>
<td>0.711</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.249</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.253</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.253</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.533</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.120</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.752</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.558</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.488</td>
<td>NS</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Water</td>
<td>0.051</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.111</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.970</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC</td>
<td>0.106</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.987</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.085</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.052</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.987</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.763</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
Table 11: Two-Way Analysis of Variance for Low-Fat Ground Beef Patties with Added Carrageenan, Water and Salt

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>Carrageenan</td>
<td>0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.040</td>
<td>p&lt;0.050</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.017</td>
<td>p&lt;0.050</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Carrageenan</td>
<td>0.030</td>
<td>p&lt;0.050</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.025</td>
<td>p&lt;0.050</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.116</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Carrageenan</td>
<td>0.679</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.208</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.015</td>
<td>p&lt;0.050</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Carrageenan</td>
<td>0.010</td>
<td>p&lt;0.010</td>
</tr>
<tr>
<td></td>
<td>Salt</td>
<td>0.632</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.110</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
Table 12: Individual Degree of Freedom Analysis for Low-Fat Ground Beef Patties with Added Carrageenan, Water and Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Parameter</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25% Carrageenan vs 0.5% and 0.75%</td>
<td>Cooking Yield</td>
<td>17.3908*</td>
<td>0.25% lower</td>
</tr>
<tr>
<td>0.5% Carrageenan vs 0.75% Carrageenan</td>
<td>Moisture Retention</td>
<td>11.6828**</td>
<td>0.25% lower</td>
</tr>
<tr>
<td>0.25% Carrageenan vs 0.5% and 0.75%</td>
<td>Fat Retention</td>
<td>0.3231</td>
<td>NS</td>
</tr>
<tr>
<td>0.5% Carrageenan vs 0.75% Carrageenan</td>
<td>Shrinkage</td>
<td>11.8246*</td>
<td>0.25% lower</td>
</tr>
<tr>
<td>0.5% Carrageenan vs 0.75% Carrageenan</td>
<td></td>
<td>0.1159</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant

*Significant at the 1% level
**Significant at the 5% level
Table 13: One-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated with 3-10% WPC, 10% Water and 0.5% Salt

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>WPC</td>
<td>0.001</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>WPC</td>
<td>0.423</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>WPC</td>
<td>0.016</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>WPC</td>
<td>0.029</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

NS = Not significant

Table 14: Individual Degree of Freedom Analysis of Cooking Yield for Ground Beef Patties Formulated with 3-10% WPC, 10% Water and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% WPC vs others</td>
<td>17.6421*</td>
<td>3% WPC lower yield</td>
</tr>
<tr>
<td>4% WPC vs 5,6,7,8,9 &amp; 10% WPC</td>
<td>2.2591</td>
<td>NS</td>
</tr>
<tr>
<td>5% WPC vs 6,7,8,9 &amp; 10% WPC</td>
<td>10.2957*</td>
<td>5% WPC lower yield</td>
</tr>
<tr>
<td>6% WPC vs 7,8,9 &amp; 10% WPC</td>
<td>0.4207</td>
<td>NS</td>
</tr>
<tr>
<td>7% WPC vs 8,9 &amp; 10% WPC</td>
<td>6.2853**</td>
<td>7% WPC lower yield</td>
</tr>
<tr>
<td>8% WPC vs 9% &amp; 10% WPC</td>
<td>0.3004</td>
<td>NS</td>
</tr>
<tr>
<td>9% WPC vs 10% WPC</td>
<td>0.4072</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant

* Significant at the 1% level
** Significant at the 5% level
Table 15: Individual Degree of Freedom Analysis of Shrinkage for Ground Beef Patties Formulated with 3-10% WPC, 10% Water and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% WPC vs others</td>
<td>0.0515</td>
<td>NS</td>
</tr>
<tr>
<td>4% WPC vs 5,6,7,8,9 &amp; 10% WPC</td>
<td>0.3492</td>
<td>NS</td>
</tr>
<tr>
<td>5% WPC vs 6,7,8,9 &amp; 10% WPC</td>
<td>2.5562</td>
<td>NS</td>
</tr>
<tr>
<td>6% WPC vs 7,8,9 &amp; 10% WPC</td>
<td>0.1001</td>
<td>NS</td>
</tr>
<tr>
<td>7% WPC vs 8,9 &amp; 10% WPC</td>
<td>0.1964</td>
<td>NS</td>
</tr>
<tr>
<td>8% WPC vs 9% &amp; 10% WPC</td>
<td>3.6794</td>
<td>NS</td>
</tr>
<tr>
<td>9% WPC vs 10% WPC</td>
<td>0.4172</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
Table 16: Individual Degree of Freedom Analysis of Moisture Retention for Ground Beef Patties Formulated with 3-10% WPC, 10% Water and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% WPC vs others</td>
<td>9.308**</td>
<td>3% WPC lower retention</td>
</tr>
<tr>
<td>4% WPC vs 5,6,7,8,9 &amp; 10% WPC</td>
<td>6.223**</td>
<td>4% WPC lower retention</td>
</tr>
<tr>
<td>5% WPC vs 6,7,8,9 &amp; 10% WPC</td>
<td>10.396**</td>
<td>5% WPC lower retention</td>
</tr>
<tr>
<td>6% WPC vs 7,8,9 &amp; 10% WPC</td>
<td>3.096</td>
<td>NS</td>
</tr>
<tr>
<td>7% WPC vs 8,9 &amp; 10% WPC</td>
<td>9.934**</td>
<td>7% WPC lower retention</td>
</tr>
<tr>
<td>8% WPC vs 9% &amp; 10% WPC</td>
<td>0.3344</td>
<td>NS</td>
</tr>
<tr>
<td>9% WPC vs 10% WPC</td>
<td>3.3280</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant

** Significant at the 5% level
Table 17: Individual Degree of Freedom Analysis of Fat Retention for Ground Beef Patties Formulated with 3-10% WPC, 10% Water and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% WPC vs others</td>
<td>14.6441*</td>
<td>3% WPC lower retention</td>
</tr>
<tr>
<td>4% WPC vs 5,6,7,8,9 &amp; 10% WPC</td>
<td>1.7704 NS</td>
<td></td>
</tr>
<tr>
<td>5% WPC vs 6,7,8,9 &amp; 10% WPC</td>
<td>9.5260**</td>
<td>5% WPC lower retention</td>
</tr>
<tr>
<td>6% WPC vs 7,8,9 &amp; 10% WPC</td>
<td>0.2065 NS</td>
<td></td>
</tr>
<tr>
<td>7% WPC vs 8,9 &amp; 10% WPC</td>
<td>0.3357 NS</td>
<td></td>
</tr>
<tr>
<td>8% WPC vs 9% &amp; 10% WPC</td>
<td>2.5387 NS</td>
<td></td>
</tr>
<tr>
<td>9% WPC vs 10% WPC</td>
<td>1.0562 NS</td>
<td></td>
</tr>
</tbody>
</table>

NS=Not significant

* Significant at the 1% level
** Significant at the 5% level
Table 18: One-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated with 10,12,14,16,18 and 20% Water, 4% WPC and 0.5% Salt

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>Water</td>
<td>0.150</td>
<td>NS</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Water</td>
<td>0.098</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Water</td>
<td>0.296</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Water</td>
<td>0.547</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant

Table 19: Individual Degree of Freedom Analysis of Cooking Yield for Low-Fat Ground Beef Patties Formulated with 10,12,14,16,18 and 20% Water, 4% WPC and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% water vs others</td>
<td>1.1355</td>
<td>NS</td>
</tr>
<tr>
<td>10% water vs 12,14,16,18 and 20% water</td>
<td>4.3970</td>
<td>NS</td>
</tr>
<tr>
<td>12% water vs 14,16,18 and 20% water</td>
<td>0.0314</td>
<td>NS</td>
</tr>
<tr>
<td>14% water vs 16,18 and 20% water</td>
<td>0.0774</td>
<td>NS</td>
</tr>
<tr>
<td>16% water vs 18 and 20% water</td>
<td>7.7594**</td>
<td>16% water lower yield</td>
</tr>
<tr>
<td>18% water vs 20% water</td>
<td>0.3831</td>
<td>NS</td>
</tr>
</tbody>
</table>

**significant at the 5% level

NS=Not Significant
Table 20: Individual Degree of Freedom Analysis of Shrinkage for Low-Fat Ground Beef Patties Formulated with 10,12,14,16,18 and 20% Water, 4% WPC and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% water vs others</td>
<td>2.3207</td>
<td>NS</td>
</tr>
<tr>
<td>10% water vs 12,14,16,18 and 20% water</td>
<td>0.0421</td>
<td>NS</td>
</tr>
<tr>
<td>12% water vs 14,16, 18 and 20% water</td>
<td>1.2885</td>
<td>NS</td>
</tr>
<tr>
<td>14% water vs 16, 18 and 20% water</td>
<td>7.5961**</td>
<td>14% water less shrinkage</td>
</tr>
<tr>
<td>16% water vs 18 and 20% water</td>
<td>5.1278</td>
<td>NS</td>
</tr>
<tr>
<td>18% water vs 20% water</td>
<td>0.7570</td>
<td>NS</td>
</tr>
</tbody>
</table>

** significant at the 5% level
NS=Not Significant

Table 21: Individual Degree of Freedom Analysis of Moisture Retention for Low-Fat Ground Beef Patties Formulated with 10,12,14,16,18 and 20% Water, 4% WPC and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% water vs others</td>
<td>0.2714</td>
<td>NS</td>
</tr>
<tr>
<td>10% water vs 12,14,16,18 and 20% water</td>
<td>2.9892</td>
<td>NS</td>
</tr>
<tr>
<td>12% water vs 14,16, 18 and 20% water</td>
<td>0.7778</td>
<td>NS</td>
</tr>
<tr>
<td>14% water vs 16, 18 and 20% water</td>
<td>0.0003</td>
<td>NS</td>
</tr>
<tr>
<td>16% water vs 18 and 20% water</td>
<td>4.4244</td>
<td>NS</td>
</tr>
<tr>
<td>18% water vs 20% water</td>
<td>0.6706</td>
<td>NS</td>
</tr>
</tbody>
</table>

** significant at the 5% level
NS=Not Significant
Table 22: Individual Degree of Freedom Analysis of Fat Retention for Low-Fat Ground Beef Patties Formulated with 10,12,14,16,18 and 20% Water, 4% WPC and 0.5% Salt

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% water vs others</td>
<td>0.0037</td>
<td>NS</td>
</tr>
<tr>
<td>10% water vs 12,14,16,18 and 20% water</td>
<td>4.2204</td>
<td>NS</td>
</tr>
<tr>
<td>12% water vs 14,16, 18 and 20% water</td>
<td>0.0640</td>
<td>NS</td>
</tr>
<tr>
<td>14% water vs 16, 18 and 20% water</td>
<td>0.5508</td>
<td>NS</td>
</tr>
<tr>
<td>16% water vs 18 and 20% water</td>
<td>0.1418</td>
<td>NS</td>
</tr>
<tr>
<td>18% water vs 20% water</td>
<td>0.3763</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant

Table 23: One-Way Analysis of Variance for Ground Beef Patties Prepared with Carrageenan Added as a Powder and as a Solution

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>Preparation</td>
<td>0.210</td>
<td>NS</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Preparation</td>
<td>0.367</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Preparation</td>
<td>0.134</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Preparation</td>
<td>0.690</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
Table 24: Two-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Retention</td>
<td>Age</td>
<td>0.063</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Age</td>
<td>0.111</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.643</td>
<td>NS</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Age</td>
<td>0.376</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Age</td>
<td>0.115</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

NS=Not significant
Table 25: Individual Degree of Freedom Analysis Among Samples for Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Retention</td>
<td>Control vs WPC and Carrageenan</td>
<td>71.3601*</td>
<td>Control lower</td>
</tr>
<tr>
<td></td>
<td>Carrageenan vs WPC</td>
<td>57.2240*</td>
<td>Carrageenan lower</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Control vs WPC and Carrageenan</td>
<td>92.3415*</td>
<td>Control lower</td>
</tr>
<tr>
<td></td>
<td>Carrageenan vs WPC</td>
<td>30.5085*</td>
<td>Carrageenan lower</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Control vs WPC and Carrageenan</td>
<td>512.1885*</td>
<td>Control lower</td>
</tr>
<tr>
<td></td>
<td>Carrageenan vs WPC</td>
<td>245.6345*</td>
<td>Carrageenan lower</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Control vs WPC and Carrageenan</td>
<td>128.3279*</td>
<td>Control lower</td>
</tr>
<tr>
<td></td>
<td>Carrageenan vs WPC</td>
<td>26.6191*</td>
<td>Carrageenan lower</td>
</tr>
</tbody>
</table>

* significant at the 1% level
Table 26: Individual Degree of Freedom Analysis Among Age for Low-Fat Ground Beef Patties Formulated with WPC and Carrageenan

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Retention</td>
<td>3 days vs 7, 10, and 17 days</td>
<td>0.958</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>7 days vs 10 and 17 days</td>
<td>6.6431**</td>
<td>7 days less retention</td>
</tr>
<tr>
<td></td>
<td>10 days vs 17 days</td>
<td>0.7569</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>3 days vs 7 and 17 days</td>
<td>4.1948**</td>
<td>3 days more retention</td>
</tr>
<tr>
<td></td>
<td>7 days vs 17 days</td>
<td>0.0564</td>
<td>NS</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>3 days vs 7, 10 and 17 days</td>
<td>6.4119**</td>
<td>3 days more yield</td>
</tr>
<tr>
<td></td>
<td>7 days vs 10 and 17 days</td>
<td>2.9399</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10 days vs 17 days</td>
<td>8.3335*</td>
<td>10 days more yield</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>3 days vs 7, 10, 14 and 17 days</td>
<td>1.3963</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>7 days vs 10, 14 and 17 days</td>
<td>0.1981</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10 days vs 14 and 17 days</td>
<td>5.7381**</td>
<td>10 days less shrinkage</td>
</tr>
<tr>
<td></td>
<td>14 days vs 17 days</td>
<td>0.7860</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not significant
* significant at the 1% level
** significant at the 5% level
Table 27: Sensory Evaluation of Low-Fat Ground Beef Patties Formulated with Alacen 837 using the Forced Ranking Test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flavor</th>
<th>Texture</th>
<th>Juiciness</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Fat</td>
<td>32</td>
<td>29</td>
<td>25**</td>
<td>31</td>
</tr>
<tr>
<td>1% WPC</td>
<td>30</td>
<td>25**</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>2% WPC</td>
<td>33</td>
<td>36</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>3% WPC</td>
<td>31</td>
<td>34</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>4% WPC</td>
<td>36</td>
<td>39</td>
<td>46*</td>
<td>42*</td>
</tr>
</tbody>
</table>

The minimum and maximum rank sums attainable for 11 panelists are 11-55, respectively.

** at the 5% level is significantly worse

* at the 5% level is significantly better
Table 28: One-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated with Alacen 837 and 878

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Retention</td>
<td>Lactose</td>
<td>0.809</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.013</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Lactose</td>
<td>0.003</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.137</td>
<td>NS</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Lactose</td>
<td>0.073</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.050</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Lactose</td>
<td>0.440</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.543</td>
<td>NS</td>
</tr>
<tr>
<td>Hardness</td>
<td>Lactose</td>
<td>0.020</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.634</td>
<td>NS</td>
</tr>
<tr>
<td>Chewiness</td>
<td>Lactose</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.155</td>
<td>NS</td>
</tr>
<tr>
<td>Springiness</td>
<td>Lactose</td>
<td>0.064</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>0.104</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
Table 29: Two-Way Analysis of Variance for Low-Fat Ground Beef Patties with Different Levels of Fat and Lactose

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Retention</td>
<td>Fat</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.964</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.189</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>Fat</td>
<td>0.011</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.122</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Fat</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.069</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Fat</td>
<td>0.209</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.027</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.020</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

NS=Not Significant
Table 30: Individual Degree of Freedom Analysis for Ground Beef Patties with Different Levels of Fat and Lactose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking</td>
<td>All 10% vs all 20% and 27% fat Samples</td>
<td>40.510**</td>
<td>10% fat samples higher</td>
</tr>
<tr>
<td></td>
<td>All 20% fat samples vs all 27% fat samples</td>
<td>3.3549</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0% lactose vs 0.24% and 1.2% lactose samples</td>
<td>0.5954</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0.24% lactose vs all 1.2% lactose samples</td>
<td>6.7066*</td>
<td>0.6% lactose samples higher</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>All 10% vs all 20% and 27% fat Samples</td>
<td>3.0873</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 20% fat samples vs all 27% fat samples</td>
<td>0.6529</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0% lactose vs 0.24% and 1.2% lactose samples</td>
<td>9.6368*</td>
<td>0% lactose samples higher</td>
</tr>
<tr>
<td></td>
<td>All 0.24% lactose vs all 1.2% lactose samples</td>
<td>1.4057</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
*significant at the 5% level
**significant at the 1% level
Table 31: Individual Degree of Freedom Analysis of Cooking Parameters for Ground Beef Patties with Different Levels of Fat and Lactose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Retention</td>
<td>All 10% vs all 20% and 27% fat Samples</td>
<td>15.2639**</td>
<td>10% fat samples higher</td>
</tr>
<tr>
<td></td>
<td>All 20% fat samples vs all 27% fat samples</td>
<td>0.2366</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0% lactose vs 0.24% and 1.2% lactose</td>
<td>0.8584</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0.24% lactose vs all 1.2% lactose samples</td>
<td>4.4972</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>All 10% vs all 20% and 27% fat Samples</td>
<td>287.3113**</td>
<td>10% fat samples lower</td>
</tr>
<tr>
<td></td>
<td>All 20% fat samples vs all 27% fat samples</td>
<td>4.9451</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0% lactose vs 0.24% and 1.2% lactose</td>
<td>0.0188</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All 0.24% lactose vs all 1.2% lactose samples</td>
<td>0.0553</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
*significant at the 5% level
**significant at the 1% level
Table 32: Sensory Evaluation of Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders Using the Forced Ranking Test

<table>
<thead>
<tr>
<th>Sample Flavor</th>
<th>SUM OF RANKS</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Flavor</td>
<td>Texture</td>
</tr>
<tr>
<td>4% WPC</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>4% WPC+TPP</td>
<td>23**</td>
<td>29</td>
</tr>
<tr>
<td>4% WPC+HPC</td>
<td>41*</td>
<td>35</td>
</tr>
<tr>
<td>4% WPC+CaCl2</td>
<td>35</td>
<td>33</td>
</tr>
</tbody>
</table>

The minimum and maximum rank sums attainable for 13 panelists are 13-52, respectively.

** at the 5% level is significantly worse
* at the 5% level is significantly better

Table 33: One-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Retention</td>
<td>Sample</td>
<td>0.737</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Sample</td>
<td>0.004</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Sample</td>
<td>0.005</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Sample</td>
<td>0.075</td>
<td>NS</td>
</tr>
<tr>
<td>Hardness</td>
<td>Sample</td>
<td>0.297</td>
<td>NS</td>
</tr>
<tr>
<td>Chewiness</td>
<td>Sample</td>
<td>0.570</td>
<td>NS</td>
</tr>
<tr>
<td>Springiness</td>
<td>Sample</td>
<td>0.009</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

NS=Not Significant
Table 34: Individual Degree of Freedom Analysis for the Cooking Characteristics of Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>WPC vs Other Water Binders</td>
<td>13.4865*</td>
<td>WPC better yield</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>48.5444**</td>
<td>TPP better yield</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>9.1136*</td>
<td>HPM better yield</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>WPC vs Other Water Binders</td>
<td>0.0743</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>9.6209*</td>
<td>TPP less shrinkage</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>5.5076</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>WPC vs Other Water Binders</td>
<td>0.1569</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>2.9006</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>0.4804</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>WPC vs Other Water Binders</td>
<td>16.1118*</td>
<td>WPC better retention</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>65.3647**</td>
<td>TPP better retention</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>3.9661</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
*significant at the 5% level
**significant at the 1% level
Table 35: Individual Degree of Freedom Analysis of the Texture Profile Parameters of Low-Fat Ground Beef Patties Formulated in Combination with Whey Protein Concentrate and Other Water Binders

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>WPC vs Other Water Binders</td>
<td>0.5742</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>4.4988</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>0.1412</td>
<td>NS</td>
</tr>
<tr>
<td>Chewiness</td>
<td>WPC vs Other Water Binders</td>
<td>0.2996</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>1.9959</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>0.0045</td>
<td>NS</td>
</tr>
<tr>
<td>Springiness</td>
<td>WPC vs Other Water Binders</td>
<td>0.0000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPP vs HPM and CaCl2</td>
<td>0.0000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>HPM vs CaCl2</td>
<td>0.0000</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
Table 36: Sensory Evaluation of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders Using the Forced Ranking Test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flavor</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Fat</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>10% Fat + 10% Water</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>4% WPC</td>
<td>38*</td>
<td>36</td>
</tr>
<tr>
<td>NaCas</td>
<td>21**</td>
<td>24</td>
</tr>
<tr>
<td>HPM</td>
<td>33</td>
<td>36</td>
</tr>
</tbody>
</table>

The minimum and maximum rank sums attainable for 10 panelists are 10-50, respectively.

* at the 5% level is significantly better

** at the 5% level is significantly worse

Table 37: One-Way Analysis of Variance for Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>F-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Retention</td>
<td>Sample</td>
<td>0.104</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>Sample</td>
<td>0.172</td>
<td>NS</td>
</tr>
<tr>
<td>Cooking Yield</td>
<td>Sample</td>
<td>0.030</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>Sample</td>
<td>0.466</td>
<td>NS</td>
</tr>
<tr>
<td>Hardness</td>
<td>Sample</td>
<td>0.000</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Chewiness</td>
<td>Sample</td>
<td>0.002</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Springiness</td>
<td>Sample</td>
<td>0.598</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not Significant
Table 38: Individual Degree of Freedom Analysis for the Cooking Characteristics of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Yield</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>15.7166*</td>
<td>10% fat better yield</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>4.6231</td>
<td>WPC better yield</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>5.3709</td>
<td>Experimental samples better yield</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>1.2598</td>
<td>NS</td>
</tr>
<tr>
<td>Shrinkage</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>2.5304</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>0.0692</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>0.8488</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>0.7591</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Retention</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>10.4103*</td>
<td>10% fat with 10% water better retention</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>1.3793</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>1.1996</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>0.7294</td>
<td>NS</td>
</tr>
<tr>
<td>Moisture Retention</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>4.5588</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>0.2289</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>3.3990</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>1.7768</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
*significant at the 5% level
Table 39: Individual Degree of Freedom Analysis for the Texture Profile Analysis of Low-Fat Ground Beef Patties Formulated with Whey Protein Concentrate and Other Protein and Carbohydrate Water Binders

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>F-Value</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>0.0766</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>292.1875**</td>
<td>WPC harder</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>0.8626</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>15.3141*</td>
<td>NaCas harder</td>
</tr>
<tr>
<td>Chewiness</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>0.0643</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>91.9339**</td>
<td>WPC greater chewiness</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>0.1543</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>5.4018</td>
<td>NS</td>
</tr>
<tr>
<td>Springiness</td>
<td>10% fat vs 10% fat with 10% water</td>
<td>0.0000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>WPC vs NaCas and HPM</td>
<td>0.0000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10% fat and 10% fat with 10% water vs WPC, NaCas and HPM</td>
<td>0.0000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NaCas vs HPM</td>
<td>0.0000</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=Not Significant
*significant at the 5% level
** significant at the 1% level
Appendix D

Percent Area and Identification of Volatile Compounds
Table 43: Percent Area and Method of Identification of Volatile Compounds in Ground Beef Patties Containing Whey Protein Concentrate and Lactose with Different Levels of Fat

<table>
<thead>
<tr>
<th>Compound</th>
<th>10% fat</th>
<th>10% fat+</th>
<th>10% fat++</th>
<th>20% fat</th>
<th>20% fat+</th>
<th>20% fat++</th>
<th>27% fat</th>
<th>27% fat+</th>
<th>27% fat++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane</td>
<td>16.16*</td>
<td>9.216***38</td>
<td>5.69***38</td>
<td>6.80*</td>
<td>3.37*</td>
<td>2.17*</td>
<td>5.80*</td>
<td>4.63*</td>
<td>3.54***12</td>
</tr>
<tr>
<td>Hexane</td>
<td>9.25*</td>
<td>0.24***12</td>
<td>0.20***20</td>
<td>0.03*</td>
<td>0.13*</td>
<td>0.09*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Heptane</td>
<td>3.32***38</td>
<td>1.25*</td>
<td>0.96***28</td>
<td>1.84***25</td>
<td>1.72*</td>
<td>1.76***25</td>
<td>3.42*</td>
<td>3.21***17</td>
<td>4.12***21</td>
</tr>
<tr>
<td>Methane Thiobis</td>
<td>1.01*</td>
<td>2.39***40</td>
<td>1.83***39</td>
<td>0.85*</td>
<td>0.86*</td>
<td>0.77*</td>
<td>0.73*</td>
<td>1.10*</td>
<td>0.73*</td>
</tr>
<tr>
<td>Octane</td>
<td>3.73*</td>
<td>1.32***78</td>
<td>1.08***39</td>
<td>2.61***38</td>
<td>4.06***38</td>
<td>2.59***45</td>
<td>0.54*</td>
<td>3.69***47</td>
<td>3.82*</td>
</tr>
<tr>
<td>2-butanone</td>
<td>1.21*</td>
<td>1.55*</td>
<td>1.44*</td>
<td>1.32*</td>
<td>1.63*</td>
<td>3.82*</td>
<td>1.09*</td>
<td>1.26*</td>
<td>6.97*</td>
</tr>
<tr>
<td>2-methyl butanal</td>
<td>1.60***32</td>
<td>3.37***43</td>
<td>6.01***43</td>
<td>2.73*</td>
<td>4.08*</td>
<td>7.44*</td>
<td>2.05*</td>
<td>3.02*</td>
<td>10.04*</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>5.80***45</td>
<td>-</td>
<td>6.21*</td>
<td>15.45*</td>
<td>23.07*</td>
<td>20.76*</td>
<td>14.94*</td>
<td>17.91*</td>
<td>13.18*</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>-</td>
<td>-</td>
<td>0.79***91</td>
<td>1.98***83</td>
<td>1.25*</td>
<td>1.83*</td>
<td>1.38*</td>
<td>1.63*</td>
<td>2.74*</td>
</tr>
<tr>
<td>Hexanal</td>
<td>-</td>
<td>-</td>
<td>17.8*</td>
<td>5.36*</td>
<td>3.17*</td>
<td>19.97*</td>
<td>2.91*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-butanone</td>
<td>1.81*</td>
<td>2.23*</td>
<td>2.59***90</td>
<td>7.00***64</td>
<td>7.29*</td>
<td>7.62*</td>
<td>4.05*</td>
<td>1.24*</td>
<td>3.46*</td>
</tr>
<tr>
<td>3-hydroxy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4,4-dimethyl-2-oxetanal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.31*</td>
<td>2.49*</td>
<td>3.77*</td>
<td>5.21*</td>
<td>3.85*</td>
<td>6.36***60</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.88*</td>
<td>3.29*</td>
<td>3.71*</td>
<td>1.90*</td>
<td>5.18*</td>
<td>2.71*</td>
</tr>
<tr>
<td>2-propanol</td>
<td>4.13*</td>
<td>4.58***64</td>
<td>3.93*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl benzene</td>
<td>1.78***86</td>
<td>1.57*</td>
<td>1.31*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-methyl-1-propene</td>
<td>7.77***30</td>
<td>1.71*</td>
<td>1.49***35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>propene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2,5-dihydroxy furan</td>
<td>0.52*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thiourea</td>
<td>-</td>
<td>3.27***74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-methyl-2-butanal</td>
<td>-</td>
<td>6.04*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-ethyl furan</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-propanone</td>
<td>21.36*</td>
<td>40.68*</td>
<td>29.91*</td>
<td>19.33*</td>
<td>18.21*</td>
<td>23.52*</td>
<td>24.85*</td>
<td>29.48*</td>
<td>32.80*</td>
</tr>
</tbody>
</table>

- Sample had 4% WPC, 10% water and 0.5% encapsulated salt added. ++Sample had 4% WPC, 10% water, 0.5% encapsulated salt and 1.2% lactose added. *Identification by retention time, **identification by computer and # is the % chance of matching.