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Effects of pH and Calcium Level on Extrusion-Textured Whey Protein Products

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EFFECTS OF PH AND CALCIUM LEVEL ON EXTRUSION-TEXTURED WHEY PROTEIN PRODUCTS

by

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ABSTRACT

Effects of pH and Calcium Level on Extrusion-

Textured Whey Protein Products

by

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The effects of altering pH and calcium level during whey protein extrusion were assessed by measuring the protein solubility and WHC of the textured whey protein (TWP). TWP samples were produced by extruding dry mixtures of 2/3 WPC 80 (80% protein) and 1/3 cornstarch using screw speed of 200 rpms, feed rate of 23 g/min, water flow rate of 11 g/min, and product temperature of 150°C. The levels of acid and base were adjusted by adding concentrated HCl or NaOH, respectively, to the water source. Calcium was added to the raw mix in the form of calcium chloride dihydrate before extrusion at levels of 0.4%, 0.88%, and 1.69% calcium per protein (w/w). It was shown that WHC of TWP was increased ($p < 0.05$) by extrusion with water. WHC was further promoted by extruding with increased levels of base. Solubility of whey protein in water was reduced by extrusion, especially when extruded with added acid or calcium.

The practicality of using hydrated TWP in beef patties was determined using sensory, physical, and instrumental analysis. An open consumer panel was conducted on six beef patty samples: 1) 100% beef, 2) 30% TWP extruded with 0.2 M NaOH ($TWP_{0.2MNaOH}$), 3) 30% TWP extruded with 1.69% calcium (w/w protein) added ($TWP_{1.69\%Ca2+}$), 4) 30% TWP extruded with water (TWP_{H2O}), 5) 30% TWP extruded with

0.1 M HCl ($TWP_{0.1MHCl}$), and 6) 30% textured soy protein (TSP). It was found that patties containing 30% $TWP_{0.2MNaOH}$ were equal ($p < 0.05$) to 100% beef patties in tenderness, juiciness, texture, flavor, and overall acceptability, and well above the scores for patties with 30% TSP.

$TWP_{0.2MNaOH}$ was then tested by sensory, physical, and instrumental analysis at three usage levels, 30, 40, and 50%, against 100% beef patties. Patties with up to 40% $TWP_{0.2MNaOH}$ were well accepted ($p < 0.05$) by consumers, and had higher cook yield, less diameter reduction, and less change in thickness than 100% beef patties. These results suggest great potential for the use of textured whey protein as a meat extender.

(74 pages)

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LIST OF SYMBOLS, NOTATION, DEFINITIONS

Abbreviation Key

α-La=alpha lactalbumin

ANOVA=analysis of variance

β-Lg=beta lactoglobulin

BME=beta-mercaptoethanol

BCA=bicinchoninic acid

BSA=bovine serum albumin

Ca^{2+}=calcium

EWP=expanded whey protein

HCl=hydrocholoric acid

Ig=immunoglobulin

LSD=least squares difference

mesh=number of openings per inch

NaCl=sodium choloride

NaOH=sodium hydroxide

$p < 0.05$=probability less than 5%

psi=pounds per square inch

rpm=revolutions per minute

SAS=statistical analysis system

SDS=sodium dodecylsulfate

TSP=textured soy protein

TWP=textured whey protein

WHC=water holding capacity

WPC=whey protein concentrate

CHAPTER I

INTRODUCTION AND OBJECTIVES

INTRODUCTION

Whey proteins are a common ingredient in emulsion-type meats (Mittal and Useborne, 1985; Parks and Carpenter, 1987) and have been used to formulate low-fat hamburger patties (El-Magoli et al., 1996). However, high levels of whey protein addition are detrimental to the texture of meat products. Thus, untextured whey protein cannot be used as a meat extender.

Over the years, proteins have been textured by different methods such as fiber spinning, microwave expansion, and thermoplastic extrusion (Burgess et al., 1978). Though dairy protein texturing technology has been researched, there are no commercially available dairy-based meat extenders. Other protein meat extenders, such as textured soy protein (TSP), are commercially available. TSP is used for meat replacement at levels up to 100%. However, such products may have limitations in texture and flavor.

Twin-screw extrusion permits extrusion of whey proteins (Burgess and Stanley, 1976), (Aguilera and Kosikowski, 1978; Cuddy and Zall, 1982; Martinez-Serna and Villota, 1992) and may facilitate the development of textured whey protein (TWP) for commercial use. TWP should meet specifications such as quick hydration before or during use, and stable texture during cooking and consumption.

The stability of TWP depends on the nature and extent of the protein cross-links formed during extrusion (Neumann et al., 1984; Noguchi, 1989; Camire, 1991; Tolstoguzov, 1993). Many types of protein interactions, both non-covalent and covalent, can occur in extrusion due to high shear (Ker and Toledo, 1992), temperature (DeWit and

Klarenbeek 1984), and moisture levels. In addition, research has shown that whey protein interactions are influenced by pH (Li-Chan, 1983; DeWit, 1989; Monahan et al., 1993) and added calcium (Schmidt et al., 1984; Barbut and Fogeding 1993; Tang et al., 1995; Parris et al., 1997; Kinekawa et al., 1998).

OBJECTIVES

There are two objectives in this research. The first objective is to determine the effects of altering pH and increasing calcium concentration during extrusion of whey proteins. These effects will be assessed by measuring the protein solubility and water holding capacity of TWP. The second objective is to determine practicality of using TWP produced in objective one using sensory, physical, and instrumental analysis. Acceptability and usage levels of TWP will be discerned using hedonic testing.

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CHAPTER II

LITERATURE REVIEW

Texturing whey proteins by thermoplastic extrusion combines technologies from several fields. It requires an understanding of extrusion technology, whey protein chemistry, whey protein texturization, and product analysis methods.

EXTRUSION TECHNOLOGY

Extrusion of food was first commercialized in 1935 to produce pasta (Kinsella, 1978). Initially, extrusion was only used for mixing and shaping and no cooking was involved. As extrusion cooking popularized, the variety of foods produced by extrusion exploded. Thermoplastic extrusion was first used to texture proteins in the 1960s (Areas, 1992).

Types of Extruders. Extruders are made for many of applications. Most extruders are one of two basic models, single or twin screw. These machines operate on the same principle, revolving rod(s) equipped with flights of screws and paddles that shear product, move it down the barrel, and push it out the die at the barrel's end.

Single-screw extruders were developed first and required that ingredients be mixed or preconditioned before entering the machine (Harper, 1981). Screw flights were filled with product, which was conveyed down the barrel by a single rotating screw. Harper (1981) completed a review of several single-screw extruders. Researchers have found single-screw extruders unsuitable for texturing milk proteins (Burgess et al., 1978). Whey protein dough is dilatant or sheer thickening and has low conveyance in these systems.

Twin-screw extruders were introduced in the 1970s. They have two screws that either corotate or counterrotate. Twin-screw extruders have higher conveyance and self-wiping screws, which allows extrusion of a greater variety of materials, including dilatant

whey protein dough. Because twin-screw extruders can extrude low moisture products, such as powders, they do not require preconditioning, or premixing, of the dough (Harper, 1981, 1989). A general schematic of a twin-screw extruder is shown in Figure 1.

In extrusion on twin-screw extruders, dry mix is added in the feed hopper, and fluids are added through the fluid line. Ingredients are mixed in the barrel by corotating screws. Screws and paddles can be set at different angles to promote conveyance or shearing of the product as it moves through the barrel (Harper, 1986).

Pressure in the extruder typically builds to around 250 - 900 psi (Harper, 1989). The barrel has thermocouples that can be set to desired temperatures, and a cooling jacket helps the machine maintain the set temperatures (Harper, 1989). The thermocouples are usually set to ambient temperature, in the first section, where the product is mixed. Thermocouples in the last sections are often set at much higher temperature to melt and cook product.

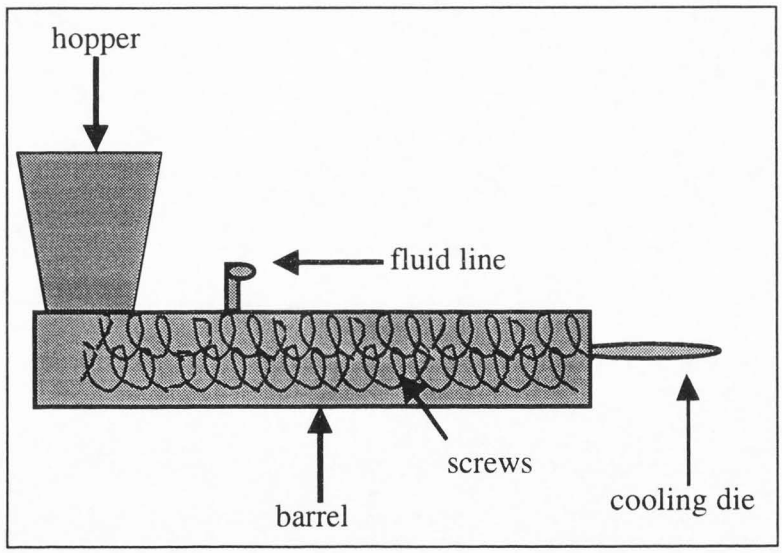

Figure 1. Model for a twin-screw extruder with a cooling die.

At the end of the barrel, product enters the cooling die. The cooling die is a long die designed to promote laminar flow, which leads to formation of fibrous texture (Harper, 1986). It allows product cooling, which reduces the expansion that is otherwise realized when pressurized steam flashes off as the product exits the die.

Textured Proteins. There are two types of textured proteins used in meat systems, meat extenders and meat analogs. Meat extenders are generally highly expanded, fibrous, and easily hydrate (Harper, 1981). They are added to foods such as hamburgers, tacos, and chili meat to cut costs. Meat extender texture is generally a poor match to meat texture, which may limit use.

Meat analogs, however, are designed to replace meat entirely. They are usually more dense than meat extenders, have structure that is more stable during food processing, and mimic meat texture (Harper, 1981). Examples of meat analogues include surimi and artificial bacon. In some cases, meat extenders and meat analogues are made using the same equipment, and from the same basic ingredients (Harper, 1981). Differences in processing techniques and flavorings make these two products unique.

Many researchers agree that the texture of extruded protein is formed as shown in Figure 2 (Harper, 1981; Camire, 1991; Tolstoguzov, 1993). First, proteins, carbohydrates, and liquid are mixed, which can occur in the first six flights of the screw (Noguchi, 1989). Then proteins and carbohydrates separate into different phases, with carbohydrates imbedded in a protein matrix. The high temperatures, pressures, and shear levels in extrusion denature proteins and fragment carbohydrates. Extrusion flow aligns denatured proteins, elongates carbohydrate pockets, and facilitates protein cross-linking. Layers of protein and carbohydrate give the meat extender its fibrous texture.

Proposed Protein Interactions. Research on extrusion-textured protein focuses mainly on soy and other plant proteins (Burgess and Stanley, 1976; Harper, 1981; Hagar, 1984; Camire, 1991; Tolstoguzov, 1993; Marsman et al., 1998). Textured soy protein

matrices are irreversible and much more stable than soy protein gels. This phenomenon has stimulated much controversy over the bonding mechanism that stabilizes these proteins.

The interactions stabilizing the structure of extrusion-textured proteins are complex and poorly understood. Various types of protein interactions have been proposed as the main stabilizing forces in textured soy protein, and different interactions result the various conditions used in each experiment, such as starting materials, temperature, shear rate, and extruder type.

Originally, scientists believed that disulfide bonding was primarily responsible for stability of textured plant proteins (Burgess and Stanley, 1976). The theory was supported by the importance of disulfide bonding in spun soy fibers, evidence of heat aggregation of soy proteins through disulfide bonding, and a patent for adding elemental sulfur or sulfur-containing adjuncts to improve the texturization of extruded soy protein (Jenkins, 1970).

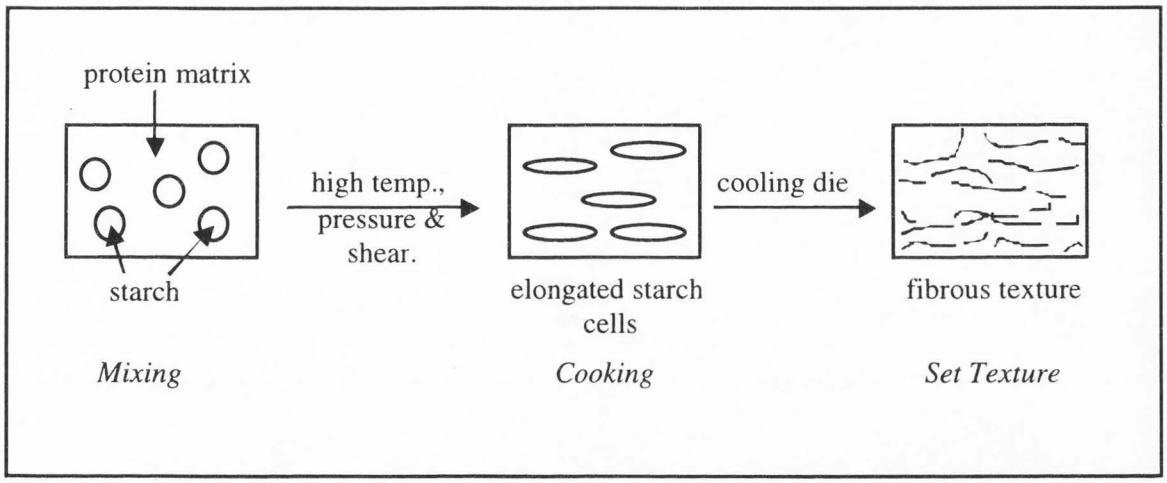

Figure 2. Proposed mechanism for extrusion-texturization of soy protein. The mechanism for extrusion-texturization of whey proteins may be similar to the proposed method for texturing soy protein.

Burgess and Stanley (1976) reported a decrease in disulfide bonds in soy protein extruded at 178°C, compared to unextruded soy protein. They proposed that intermolecular peptide bonds called isopeptides were more important than disulfide bonding in extrusion textured soy proteins.

Other studies have given further insight into the formation of protein interactions. In 1984, Hagar challenged the conclusions of Burgess and Stanley (1976). Hagar proposed that disulfide bonding was responsible for structure in low temperature extrusion (≤140°C) and that protein polymerization occurs only at higher extrusion temperatures such as those used by Burgess and Stanley (1976; Hagar, 1984). A more recent study by Marsman et al. (1998) demonstrated that in addition to temperature, shear forces influence the bonding type. The theory suggests that in low-shear extrusion, disulfide bonds are favored, and in high shear, covalent cross-links are favored.

WHEY PROTEIN

Composition. Whey proteins comprise about 20% of total milk protein. Whey is a by-product of cheese making. Processing 100 pounds of milk into cheese results in 10 pounds of cheese and 90 pounds of whey, this contains about 3/4 pound of protein (see Table 1).

There are two types of whey, sweet whey and acid whey. Sweet whey is a by-product of rennet cheeses, and acid whey comes from acid-set cheese. Each type of whey has a slightly different composition, as shown in Table 1.

Processing. In the past, whey was viewed as a waste product and dumped into fields or waterways. Environmental concerns over the high biological oxygen demand of discarded whey led to heavy restrictions on whey disposal (Smithers et al., 1996). Thus, processors explored alternatives such as concentration and spray drying.

Many different processes can be used to concentrate whey, and some affect functionality more than others. Any process involving heat is detrimental to whey protein functionality. The heat history of the milk proteins may include processes such as pasteurization, whey heat treatment, heating during isolation, fore warming, dehydration, evaporation, concentration, and possibly others (Schmidt et al., 1984). Different degrees of protein denaturation, and thus loss of functionality, result from the amount of heat and shear involved. Inconsistent functional properties are one of the major quality issues haunting whey protein application technologists.

Ultrafiltration is one of the most popular methods of concentration because no heating is involved. Thus, more proteins retain their native conformation and functionality. Advances in membrane separation technology have enabled separation of whey into specific fractions (Wade, 1994). Whey proteins are often separated by membrane filtration and marketed as nutritional and functional ingredients.

The major whey proteins include β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins (IG) as shown in Table 2. Together these proteins comprise about 80% of the whey proteins. The remaining 20% of whey proteins include proteose-peptones, lactoferrin, lactoperoxidase, and growth factors.

Table 1. Composition of Whey Varieties

Product	water %	protein %	fat %	lactose %	ash %
sweet whey [a]	93.0	0.8	0.2	4.9	0.5
dried sweet whey [b]	3.0	13.0	1.0	69.4	8.3
acid whey [a]	93.5	0.7	0.04	4.4	0.8
dried acid whey [b]	3.1	11.7	0.5	63.2	10.6
WPC 34 [c]	5.0	34.0	6.0	55.0	10.0
WPC 80 [d]	4.0	80.0	5.0	3.5	4.5
WPI [e]	4.5	92.0	1.0	.05	2.0

[a] Dybing and Smith, 1991; [b] Cuddy and Zall, 1982; [c] Davisco Foods International, Le Sueur, Minnesota; [d] AMPC, Ames, IA, this product contains 0.3 to 0.4% Ca^{2+}; [e] ADPI, Chicago, IL. WPC = whey protein concentrate, WPI = whey protein isolate

Table 2. Whey Protein Composition

protein	% of whey protein	conc. in milk (g/L)[a]	isoelectric point [b]	molecular wt (daltons) [c]	S-S bonds [d]	-SH content [d]
β-LG	45%	3.2	4.83	18,000	2	1
α-LA	20%	1.2	4.80	14,000	4	none
BSA	5%	0.4	5.6	66,000	17	1
IG	10%	0.8	6.8-7.8	≥145,000	varies	varies

T_d = thermal denaturation temperature, S-S bonds and -SH content based on MW. [a] DeWit, 1998; [b] Swaisgood, 1992; [c] Morr, 1992; [d] Monahan et al., 1993.

Whey proteins are a complete protein source. They have a protein efficiency ratio of 3.2 (Cuddy and Zall, 1982) and a protein digestibility corrected amino acid score of 1.0, the highest possible score. The nutritional properties of whey proteins have made it a popular protein supplement. Whey protein concentrates are used in dietary foods, infant formulas, and sports foods. The fat analogue Simpless 100 is made using whey proteins. In addition, specific whey proteins such as lactoferrin and lactoperoxidase are believed to provide non-immune protection against infection (Wade, 1994).

WHEY PROTEIN FUNCTIONALITY

Functionality of whey proteins has been studied extensively; they stabilize foams, whip to high volumes, emulsify, bind flavor, are soluble in their native state, and form gels. The functional properties of most interest in extrusion texturization of whey protein are solubility and gelation.

Solubility. Whey proteins are known as the water-soluble milk proteins because they are collected in the aqueous fraction when making cheese. However, only native whey proteins are readily water-soluble. Solubility of proteins in aqueous solutions is inversely related to the hydrophobicity of the protein, which can be manipulated. The major fractions of whey proteins, β-LG and α-LA, are albumins, and have low surface hydrophobicity in their native states. Thus, with low hydrophobicity, solubility is

promoted by the electrostatic repulsion amino acids of neighboring proteins. The lowest solubility of whey protein solutions is observed at pH 8 and 65°C (Lee et al., 1992).

Denatured whey proteins have low solubility, which is perpetuated by pH near the isoelectric points and at low salt concentration. The low solubility near the isoelectric points leads to aggregation known as isoelectric precipitation. Isoelectric precipitation occurs because the charge on proteins is neutral, which minimizes electrostatic repulsion; thus, hydrophobic patches attract and aggregate (Scopes, 1982). Low salt concentration perpetuates salting out of hydrophobic proteins, essentially an isoelectric precipitation (Scopes, 1982).

Gelation. Whey protein gelation depends on many factors, such as protein concentration, pH, ionic strength, time, and temperature. Depending on the parameters, whey protein gelation is stabilized by hydrogen bonds, hydrophobic interactions, electrostatic attraction, and disulfide bonds (Li-Chan, 1983; Monahan et al., 1993; Law et al., 1994; Zhu and Damodaran, 1994; Karleskind et al., 1995). General characteristics of molecular interactions are shown in Table 3 (Bryant and McClements, 1998).

Whey protein gelation requires two steps: protein unfolding and aggregation (DeWit, 1990). Whey protein unfolding occurs around 70°C, and exposes hydrogen bond sites and hydrophobic groups (Dybing and Smith, 1991; Monahan et al., 1993). Aggregation may follow one of at least two different mechanisms (DeWit, 1989). Globular or particulate aggregation occurs when proteins are highly charged, or salt is present (Bryant and McClements, 1998). Proteins aggregate into clumps, then associate to form gels that are opaque, wet, weak, and predominated by hydrophobic interactions (DeWit, 1989; Bryant and McClements, 1998).

Linear or fine-stranded aggregates form when whey proteins have little repulsion, generally at pH extremes or in the absence of salt (Bryant and McClements, 1998). Proteins aggregate into filamentous strands referred to as "string-of-beads" (Bryant and

McClements, 1998). These are associated into gels that are translucent and elastic or rubbery, and are more likely to be promoted by electrostatic interactions and stabilized by disulfide interactions (DeWit, 1989; Bryant and McClements, 1998).

WHEY PROTEIN STABILITY

The bonding mechanism of whey protein gels may give us some indication of how extruded whey proteins behave. Researchers have identified that physiochemical parameters such as temperature, pH, calcium level, and shear affect the formation and stability of whey protein gels (Li-Chan, 1983; Monahan et al., 1993; Law et al., 1994; Zhu and Damodaran, 1994; Karleskind et al., 1995). Adjusting physiochemical parameters in extrusion may increase protein interactions that stabilize the product.

Temperature. Each of the whey proteins has a different denaturation temperature which is pH dependent (Monahan et at., 1993). Immunoglobulins are the most heat labile followed by BSA, β-LG, α-LA, and proteose peptones (Li-Chan, 1983; Law et al., 1994). The precise temperature at which whey proteins denature and aggregate is influenced by the pH of the solution.

Thermal treatment of whey protein gels can be divided into three categories, low temperature (4 to 60°C), high temperature (60 to 100°C), and extreme temperature (100

Table 3. General Characteristics of Molecular Interactions Between Two Similar Protein Molecules in Aqueous Solution*

Type	affinity	strength	range	temp.	pH	I.S.
Hydrophobic	attractive	strong weak→	long short→	increases	no	no
Electrostatic	repulsive	strong †	long †	increases	yes	decreases
hydrogen bond	attractive	weak	short	decreases	no	no
Hydration	repulsive	strong	short	decreases	no ‡	no ‡
Van der Waals	attractive	weak	short	--	no	no
steric repulsion	repulsive	strong	short	--	no	no
disulfide bond	attractive	very strong	short	--	yes	no

* (Adapted from Bryant and McClements, 1998). † Depends on pH and ionic strength (I.S.). ‡ Indirectly depends on pH and I.S. because these factors influence the degree of protein hydration.

to 150°C) (DeWit and Klarenbeek, 1984). Heating whey protein solutions at low temperature results in reversible physiochemical changes such as hydrophobic association and partial unfolding (DeWit and Klarenbeek, 1984). Under these conditions, whey protein solutions become more viscous, but typically do not form gels (DeWit, 1990). Whey proteins heated to high temperature form irreversible gels.

Extrusion-texturization of whey proteins is conducted at extreme temperature (140 to 150°C). At such extreme temperatures, irreversible chemical changes occur such as Mailliard reactions, cys-breakdown, and possible breakdown of disulfide bonds (Li-Chan, 1983; DeWit and Klarenbeek, 1984). At high pH, cysteine breakdown increases, there is an increase in dehydro-alanine, and if lactose is present, lysine is destroyed (DeWit and Klarenbeek, 1984). These bond changes may result in formation of a more stable protein network.

pH. The method of aggregation, either linear or globular, is dependent upon the pH of whey protein solutions (Monahan et at., 1993). Whey proteins are not charged near their isoelectric point, pH 4.6; thus, they aggregate by the globular method. At pH extremes, less than pH 3 or greater than pH 7, whey proteins tend to repel one another and aggregate linearly (Monahan et at., 1993; Bryant and McClements, 1998).

The pH also influences the minimum temperature for gelation (Monahan et at., 1993). At pH 9 and 11, polymerization occurs at room temperature, and disulfide bonds predominate. At pH 3, 5, and 7, polymerization does not occur until solution has been heated to 85, 75, and 70°C, respectively, and evidence of disulfide bonding was present in pH 7 samples. Above 95°C, irreversible gelation occurs despite the pH effects (Li-Chan, 1983).

Ionic Strength. The effects of ionic strength on whey protein gel formation depend on the salt type, pH, and temperature used in processing. Whey protein gels are

strengthened by addition of sodium and calcium salts. Calcium salts are more effective than sodium salts (Tang et al., 1995). Maximum gel strength may be achieved with 11 mM $CaCl_2$ or 200 mM NaCl (Barbut and Foegeding, 1993). Salt addition is more effective at alkaline pH. Also, calcium promotes and mediates heat aggregation of whey proteins (Schmidt et al., 1984; Bryant and McClements, 1998).

Shear. Shear increases protein interactions by accelerating the unfolding stage in protein denaturation (Ker and Toledo, 1992). By shearing whey protein solutions before gelation, researchers have increased gel strength two-fold (Ker and Toledo, 1992). Sheared proteins were shown to hold more moisture and have lower cooking loss than unsheared proteins, in frankfurters (Ker and Toledo, 1992).

WHEY PROTEIN TEXTURE

History. Dairy proteins have been textured by different methods such as fiber spinning, microwave expansion, and thermoplastic extrusion (Burgess et al., 1978). Fiber spinning and microwave expansion preceded extrusion. The earlier methods lend to out knowledge of formation of stable protein networks.

In fiber spinning, proteins are mixed with sodium hydroxide into a viscous paste. The paste is then forced through small orifices into concentrated acid, which coagulates the strands of protein into thin fibers (Burgess et al., 1978). Due to hazards and difficulty in disposing of harsh chemicals, fiber spinning is no longer a popular method for texturing whey protein.

Microwave expansion involves cooking protein/water mixtures with microwave energy. The temperature of the solution rises to the boiling point of the water, and small steam pockets form that cause the mixture to expand (Burgess et al., 1978). Thermosetting whey proteins retain the expanded structure. The result is expanded whey protein (EWP).

EPW is described as a light brown substance that absorbs 2 to 3 times its weight in water and is very stable in boiling water (Burgess et al., 1978). EWP texture may not suitable for use as a meat analogue. However, EWP can be used as an extender at 10 to 20% levels in meat products such as sausage and beef patties (Burgess et al., 1978). EWP used in sausage reduced fat and water losses and did not affect sensory properties (Burgess et al., 1978). In further studies, water absorption in EWP was found to be negatively correlated with texture (Tuohy, 1980). Microwave expansion is also not suitable for continuous processing and is not used on a commercial scale.

Extrusion. Extrusion may advantages over other texturing methods. It is a simple process, parameters are readily adjusted, it lends itself to continuous processing and little or no waste is produced. However, there are still challenges to extruding whey proteins.

Whey proteins are accused of having detrimental effects on extrudate properties (Martinez-Serna and Villota, 1992). Among the offenses are difficulty in extrusion (Aguilera and Kosikowski, 1978; Burgess et al., 1978; Martinez-Serna and Villota, 1992), excessive product hardness (Cuddy and Zall, 1982; Martinez-Serna and Villota, 1992), and increased Malliard browning (Burgess et al., 1978; Cuddy and Zall, 1982; Martinez-Serna and Villota, 1992).

Difficulty in extrusion is explained by whey proteins tendency toward dilatancy with increasing sheer (Ker and Toledo, 1992; Martinez-Serna and Villota, 1992). As mentioned previously, whey protein extrusion is conducted on twin-screw extruders where self-wiping screws increase product conveyance. Screw configuration should be designed to minimize torque and maximize product conveyance. Even with increased conveyance, whey proteins are dilatant and difficult to extrude. Extrusion feasibility can be achieved by cutting the dry protein mix with starch, which separates the proteins and reduces dilatancy. Trial and error may be required to determine appropriate processing

parameters to keep the dough moving forward, rather than backing up and clogging the machine.

Interest in whey protein extrusion reaches into a variety of applications, such as ready-to-eat cereals and snack foods. Most attempts to extrude whey proteins, alone or in combination with other ingredients, are conducted using dry whey with about 10% protein (Cuddy and Zall, 1982; Wang et al., 1993), to as high as 36% protein (Aguliera and Kosikowski, 1978). At these relatively low protein levels, lactose is the major whey ingredient by weight (see Table 1), lending entirely different properties than extruding whey with higher protein content.

Martinez-Serna and Villota (1992) conducted significant work on extruding modified whey protein isolate at levels up to 50% with cornstarch. They investigated effects of pH modification, esterification, and acetylation on whey extruded at 110°C. Product with texture most similar to textured soy was obtained by increasing the pH. They found that in alkaline samples (pH adjusted to 8.5), disulfide bonding predominated followed by hydrophobic interactions. They claimed that when disulfide bonding predominated, extruded whey protein products became tough and inelastic. When hydrophobic interactions were stronger (see Table 3), extrudate became more brittle and less cohesive (Bryant and McClements, 1998). They concluded that a balance between disulfide and hydrophobic interactions must be reached when extruding at high levels of whey protein. Despite their success in extruding modified whey proteins, their modification methods were complex and are not used on an industrial scale.

ANALYSIS OF TWP

Water Holding Capacity. Water holding capacity (WHC) is the ability of a protein to take up water, trap, and hold it against gravitational forces (Damodaran, 1996). This trapped water is free only on a molecular level, and does not flow when food is

comminuted. Thus, WHC may indicate the optimal rehydration volume in meat extenders. In addition, foods with optimal rehydration volume have better freeze thaw stability (Fennema, 1996).

Some parameters may be adjusted to control WHC. Whey protein gels with higher protein concentrations have increased WHC (Bryant and McClements, 1998). Also, the linearly set, filamentous gels have higher WHC (Bryant and McClements, 1998).

There are many methods of determining WHC depending on the product (Tuohy, 1980; Berry et al, 1983 1983; Neumann et al., 1984). Methods for dry food proteins and carbohydrates involve saturating finely ground samples with water for a specified time. Then, the amount of water held by a sample is measured by weighing the hydrated sample directly, or measuring amount of supernatant not held by sample.

Protein Solubility. A TWP should hydrate quickly before or during use and should have stable texture during cooking and consumption. If proteins are too soluble, the extender dissolves into the juices when cooked. TWP must be held together by stable protein interactions. Many types of stabilizing non-covalent and covalent protein interactions can take place at extrusion temperatures and moisture levels (Burgess and Stanley, 1976; Hagar, 1984; Martinez-Serna and Villota, 1992).

Non-covalent interactions include hydrophobic interactions, hydrogen bonds, and ionic bonds, referred to as electrostatic interactions. Non-covalent interactions can be disrupted by water or buffers and are easily studied. A variety of covalent bonds are also promoted by extrusion conditions, including, non-enzymatic browning, isopeptide bond formation, and disulfide bond formation (DeWit and Klarenbeek, 1984). Covalent bonds resist disruption and retain product texture.

The type and extent of protein cross-links that contribute to the stability of aggregates are identified by comparing protein solubility in various solvents including

distilled water (dH_2O), sodium dodecyl sulfate (SDS), sodium chloride (NaCl), or beta-mercaptoethanol (BME) (Harper, 1986).

SDS is a detergent that disrupts tertiary and quatinary non-covalent interactions. It binds to proteins and prevents bonds from reforming. Protein solubility in SDS indicates the amount of protein held insoluble by the non-covalent interactions.

Low levels of salt destabilize ionic bonds among proteins (Scopes, 1982). Protein can be solubilized in low salt buffer, less than 1 M. This value can be compared with the protein solubility in water to reflect the amount of protein held insoluble solely by ionic bonds.

BME cleaves disulfide bonds. At high levels, greater than 0.01%, prevents reformation of disulfide bonds. Protein solubility in BME indicates the amount of protein held insoluble in aqueous solution by disulfide bonds.

Soluble protein is measured in a number of different methods, but all are based on the same principle (Burgess and Stanley, 1976; Neumann et al., 1984; Martinez-Serna and Villota, 1992). First, sample is finely ground and mixed with an excess of buffer. Then, the slurry is agitated for a specified time, and the amount of protein in the supernatant is determined.

Sensory Analysis. The first step in sensory analysis is to determine the objective of the tests. Once the objective is defined, sensory attributes that lead to the objective are identified, and a sensory method is chosen. When studying meat extenders, the objective is to determine the loss of sensory quality caused by adding a meat extender. This is measured by comparing acceptability of extended patties with all beef controls.

Meat extenders can be detrimental to sensory characteristics of ground meat products, especially texture. In ground beef patties, meat extenders replace fat and protein. Fat contributes beef flavor, juiciness, and tenderness; protein lends texture and binds water (Desmond et al., 1998).

Two major types of tests are used in sensory analysis, analytical and consumer tests. Consumer tests are efficient in assessing consumer acceptance of multiple products. Consumers are allowed to compare products, side by side, and preference can be indirectly determined from affective scores (Lawless and Heymann, 1998). Since the objective is to determine consumer acceptance rather than quantify a specific sensory attribute, it can be met by measuring the tenderness, juiciness, texture, flavor, and overall acceptability of samples in an open consumer panel.

Physical and Instrumental Analysis. The 1983 AMSA guidelines give methods for evaluation of ground beef (Berry et al., 1983). Physical measurement such as patty diameter reduction, change in thickness, and cooking yield are important in studying ground beef for commercial applications. Restaurants and other food service organizations are interested in convenience and cost of food products. Diameter reduction and change in thickness are related to the convenience of beef patties. Companies prefer patties that do not change shape during cooking because they are easier to fit to a bun or package. Patties with high cook yield are more cost effective, which is also attractive to buyers.

The ASMA guidelines recommend measuring texture by submitting the same ten patties used in physical analysis to shear tests (Berry et al., 1983). The literature shows that objective measurements, such as shear tests, can be collected from a variety of texture measurement machines including, Warner-Braztler Shear, Instron Universal Testing Machine, and other texturometers and penetrometers. These machines take precise measurements that should correlate with sensory data when intended as standards of comparison between studies.

SUMMARY

Technology in extrusion, whey protein chemistry, whey protein texturization and extrusion, and product analysis has advanced to a point where extrusion texturization of whey proteins may be feasible. Twin-screw extruders have sufficient conveyance to process whey protein dough. The high temperatures and pressures of extrusion will be sufficient to denature proteins. The pH and calcium levels influence the stability of whey protein networks, and may promote stable bond formation in extruded whey protein. Consumer acceptance and physical attributes can be determined by sensory, physical, and instrumental analysis. Truly, studying the effects of adjusting pH and calcium level on extrusion of whey proteins will be a complementary addition to existing technology.

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CHAPTER III

THE EFFECTS OF ALTERING PH AND CALCIUM LEVEL DURING THERMOPLASTIC EXTRUSION OF WHEY PROTEIN

ABSTRACT

This study examined the effects of adjusting the acid, base, or calcium levels during extrusion on the water holding capacity (WHC) and protein solubility of textured whey protein (TWP). To produce TWP, dry mixtures of 2/3 WPC 80 (80% protein) and 1/3 cornstarch were extruded with water. Acid and base were adjusted with 0.1, 0.2, or 1.0 M HCl or NaOH. Calcium was adjusted by adding calcium chloride dihydrate at levels of 0.4%, 0.88%, and 1.69% calcium per protein (w/w). The extruded products were ground and extracted into various solvents (distilled water, 0.5 N NaCl, 2% SDS, and 0.02% BME) from which protein solubility was determined. Extrusion increased ($p < 0.05$) the WHC of whey protein and cornstarch mixes. WHC was further promoted by adding base to the water source during extrusion, which had no relation to solubility in the various solvents. Extrusion reduced solubility of whey proteins in water. Protein solubility was decreased by extrusion with acid or calcium. These samples also had more protein solubility in 2% SDS, which indicates non-covalent interactions. This suggests that samples with lower protein solubility had more stabilizing non-covalent interactions. Results suggest that adjusting acid, base, or calcium level before extrusion can increase the stability of TWP.

INTRODUCTION

Over the years, dairy proteins have been textured by different methods such as fiber spinning, microwave expansion, and thermoplastic extrusion (Burgess et al., 1978). Thermoplastic extrusion was first used to texture proteins in the 1960s (Areas, 1992). Twin-screw extruders permit extrusion of whey proteins (Burgess and Stanley, 1976;

Aguilera and Kosikowski, 1978; Cuddy and Zall, 1982; Martinez-Serna and Villota, 1992), and may be used to develop extrusion TWP for use as a meat extender.

Originally, attempts at extruding whey proteins, alone or in combination with other ingredients, were conducted using dry whey with 10% to 36% protein (Aguilera and Kosikowski, 1978; Cuddy and Zall, 1982; Wang et al., 1993). Martinez-Serna and Villota (1992) conducted preliminary research on extruding 50% modified whey protein isolates with cornstarch. They investigated effects of pH modification, esterification, and acetylation on whey extruded at 110°C. They concluded that a balance between the disulfide and hydrophobic interactions of proteins must be reached when extruding at high levels of whey protein. Though a TWP would probably be best if developed using high protein levels, the protein modification methods used by Martinez-Serna and Villota were complex and impractical for industrial applications.

The stability of TWP depends on the nature and extent of the protein cross-links formed during extrusion (Neumann et al., 1984; Noguchi, 1989; Camire, 1991; Tolstoguzov, 1993). Many types of protein interactions, both non-covalent and covalent, can occur in extrusion due to high shear (Ker and Toledo, 1992), temperature (DeWit and Klarenbeek, 1984), and moisture levels. Perhaps varying physiochemical parameters during extrusion would promote formation of more stable interactions.

Research has shown that whey protein interactions are influenced by pH and added calcium. Lower pH (~ pH 4.5 - 6.0) promotes low solubility, high water absorption, high viscosity, and low gelation temperature (Schmidt et al., 1984). Higher pH (~ pH 7.0 - 9.0) results in lower solubility of whey proteins (Li-Chan, 1983), and promotes gels that are more elastic (Schmidt et al. 1984). Gels formed at an elevated calcium levels have increased aggregation speed and gel strength which may be due to calcium-mediated crosslinking between proteins (Schmidt et al., 1984, Dybing and Smith, 1991; Kinekawa et al., 1998).

The objective of this research was to study the effects of adjusting pH and calcium concentration on the texture and stability of extruded whey proteins. The WHC and protein solubility were both measured. Formation of protein interactions was measured by testing solubility of product in distilled water, sodium dodecyl sulfate (SDS), high salt (NaCl), and beta-mercaptoethanol (BME).

EXPERIMENTAL PROCEDURES

Materials. Dry mix of protein and carbohydrate was extruded with water to make textured whey proteins. The dry mix contained (w/w) 2/3 whey protein concentrate (AMPC 800, American Meat Packers Cooperation, Ames, IA) and 1/3 cornstarch (purchased locally). The composition of the whey protein concentrate was 80% protein, 4.6% fat, 4.5% ash (0.294% calcium), 4.4% carbohydrate, and 4.2% moisture. Both WPC 80 and the dry mix were used as controls in WHC and protein solubility experiments.

Experimental Design. The experiment was a completely randomized design, with a total of ten variables. Variables included three treatments (acid, base, and calcium) each applied at three levels, and one control (water). The treatment levels of acid and base were 0.1, 0.2, or 1.0 M, HCl or NaOH, respectively, and were administered by adding acid or base to the water before extrusion. Calcium was added at levels of 0.4%, 0.88%, and 1.69% calcium per protein (w/w). It was added to the dry mix before extrusion in the form of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, Malinckrodt, Paris, KY). The effects of extrusion were shown by comparing extruded samples to unextruded controls, namely dry mix and WPC 80.

Methods. Extrusion was conducted on a bench-top scale twin-screw extruder (MPF19 APV Baker, Grand Rapids, MI) with a cooling die. In the extruder, dry feed and fluid were added separately and mixed in the barrel. Extrusion parameters were screw

speed of 200 rpms, feed rate of 23 g/min, water-flow rate of 11 g/min, and product temperature of 150°C. Product temperature was measured during extrusion by a thermocouple placed at the end of the extrusion barrel. Observations were made on color, opacity, structure, and texture for each collected sample.

Collected samples were dried at room temperature overnight, then ground to a fine powder (< 60 mesh) using a Braun food grinder. The pH of 10% (w/v) slurries of powdered samples in distilled water was measured in duplicate, at room temperature using an Orion 520A pH meter.

Total protein in TWP was determined by analyzing one sample from each treatment group in triplicate using the Kjeldahl nitrogen method. A nitrogen to protein conversion factor of 6.38 was used (AOAC, 1996). WHC and protein solubility was measured for each sample.

Water Holding Capacity. The WHC of dry solids was determined in triplicate using a method adapted from Neumann et al. (1984). Briefly, 1.00-g (dry base, < 60 mesh) samples were measured into pre-weighed, 50-ml centrifuge tubes. Distilled water (10 ml) was added, and samples were vortexed on low speed for 30 sec. Samples were then incubated for 60 min at room temperature (20°C), and centrifuged at 5000 rpm for 30 min. Supernatants were decanted, and the remaining pellets were weighed in the 50-ml tubes. The residual water in the tube was adjusted for by calculating residual water in 10.0-ml distilled water blanks. Variables such as pellet weight and water retained by blank were determined by subtracting the final weight from the initial weight. WHC was calculated according to equation 1.

$$\text{WHC} = \frac{\text{wet pellet wt (g) - wt water retained by blank (g)}}{\text{total sample wt (g)}} \qquad (1)$$

Protein Solubility. Soluble protein in TWP products was extracted into four solvents, distilled water, 2% sodium dodecyl sulfate (SDS), 0.5 N sodium chloride (NaCl), and 0.02% beta-mercaptoethanol (BME). Solubility in water was the standard to which solubility in other solvents was compared.

Soluble protein was measured based on the method of Martinez-Serna and Villota (1992), with several adjustments. Slurries of protein and solvent, 3.85% (w/v), were shaken for 1.5 h at 150 rpms, centrifuged for 5 min at 5000 rpms, followed by 15 min at 9000 rpm to precipitate fine particles.

Protein content of the supernatants was determined spectrophotometrically (Shimadzu Biospec-1601) using the BCA assay (Pierce Chem. Co., Rockford, IL. Breifly, experimental samples were diluted with distilled water to 25 μg sample per ml BCA reagent. Experimental samples were diluted within the range of the BSA (bovine serum albumin, Pierce Chem. Co., Rockford, IL) standard curve, and buffer concentrations were sufficiently diluted to not interfere with the assay. Controls were diluted 1:10 (v/v) with distilled water. BSA standard curve samples were adjusted to the same buffer concentration as experimental samples.

Statistical Analysis. The differences between extruded samples and unextruded controls were measured. Within extruded samples, differences between acid, base, and calcium treatments were measured, with treatment level nested within treatment group. The significance of main effects was measured using the mixed procedure of analysis of variance (ANOVA) in SAS (Cary, NC). Differences were determined using least squares difference (LSD) with α=0.05.

RESULTS AND DISCUSSION

Methods. Extruded samples were initially analyzed by measuring pH and observing physical characteristics. The pH measurements showed that pH of the samples

ranged from 4.0 to 7.5 (Table 4). In general, pH decreased ($p < 0.05$) in samples extruded with acid, and pH increased in samples extruded with base. There was also a decrease in pH in samples extruded with calcium, which may be due the acidity of dissociated calcium chloride salt.

The acid, base, and added calcium level affected physical properties of TWP. Observations of physical characteristics imply that TWP differed in color, opacity, and structure. Color was darker at higher pH, most likely due to increased Malliard browning. TWP extruded at high pH contrasted other samples in opacity, structure, and texture, which may indicate a different protein structure in those samples.

In gelation, whey proteins exhibit either linear or globular aggregation mechanisms, depending on the physiochemical parameters. These aggregation mechanisms lead to different gel properties. During extrusion, the physiochemical parameters may influence the aggregation mechanism of whey proteins in the initial stages of extrusion. Perhaps the bonds formed in initial aggregation, though later replaced by more stable bonds, influence properties and functionality of the final product.

Table 4. The pH and Physical Properties of TWP Samples

variable	pH	color	opacity	structure
$TWP_{0.1MHCl}$	$6.25 \pm 0.02^{B, C, D}$	caramel brown	opaque	large cells
$TWP_{0.2MHCl}$	5.93 ± 0.09^{D}	light brown	opaque	small cells
TWP_{1MHCl}	4.02 ± 0.51^{E}	tan/orange	opaque	granular
$TWP_{0.1MNaOH}$	$6.58 \pm 0.02^{B, C}$	light yellow	opaque	large cells
$TWP_{0.2MNaOH}$	6.73 ± 0.11^{B}	dark brown	translucent	glassy
TWP_{1MNaOH}	7.48 ± 1.27^{A}	nearly black	translucent	glassy
$TWP_{0.4\%Ca2+}$	$6.37 \pm 0.09^{B, C, D}$	brown	opaque	small cells
$TWP_{0.88\%Ca2+}$	$6.18 \pm 0.10^{B, C, D}$	light brown	opaque	small cells
$TWP_{1.69\%Ca2+}$	$6.03 \pm 0.02^{C, D}$	grayish-brown	opaque	small cells
TWP_{H2O}	$6.45 \pm 0.04^{B, C, D}$	caramel brown	opaque	small cells

The pH values are the average of two replications with two samples in each replication. Statistics were calculated using analysis of variance in SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$. Other values are observations made on extruded and dried products.

There were no significant differences between the total protein of the samples as determined by the Kjeldahl nitrogen method. The average percent total protein content of TWP samples was 50.55% ± 0.56.

Water Holding Capacity. Extruded TWP had higher ($p < 0.05$) WHC than unextruded samples (Figure 3). This indicates that extrusion increased the sample affinity for water. This is likely due to protein unfolding and network formation, which increases the surface area and pockets available to trap and hold water.

Samples extruded with acid had lower WHC than other extruded samples (Figure 4). Level effects in Figure 5 show that samples treated with 1 M HCl and 0.4% Ca^{2+} had the least WHC of extruded samples. This may be indicative of the microstructure of the samples. Samples extruded with base may have initially aggregated linearly, which is favored in the absence of salt and at alkaline pH (Bryant and McClements, 1998). Whey protein gels formed by linear aggregation exhibit porous structure and high WHC (Bryant and McClements, 1998), which is supported by the consistently higher WHC observed in samples extruded with base. Thus, the acid/base condition may be important for initial aggregation that affects the functional properties of TWP.

Protein Solubility. Extruded samples had less soluble protein ($p < 0.05$) than unextruded samples (Figure 6). This indicates that protein interactions were increased by extrusion. Protein solubility in water was further decreased by extrusion with acid or calcium (Figure 7). High levels of acid or calcium result in the lower protein solubility (Figure 8). This may be due to a difference in the initial aggregation mechanism of the proteins. Globular aggregation is favored in the presence of salt or at pH near the isoeletric point, exhibited by samples extruded with acid (Bryant and McClements, 1998). In globular aggregation, proteins aggregate, unhindered, into large clumps, which results in low WHC and dense structure that may hinder solubilization of proteins.

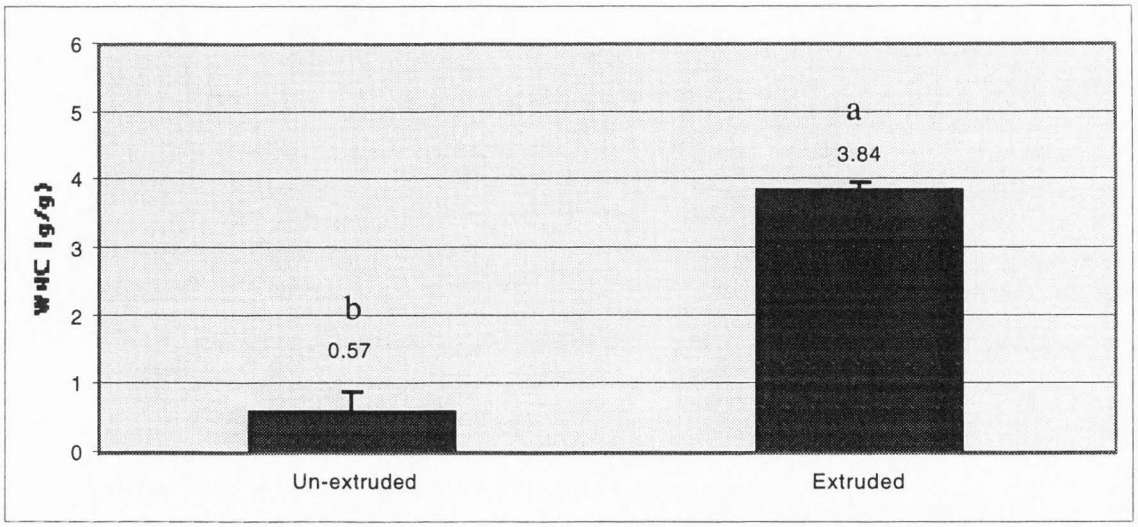

Figure 3. Water holding capacity of unextruded compared to extruded samples. The first column is the pooled mean of the two unextruded variables. The second column is the pooled mean of ten extruded samples. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

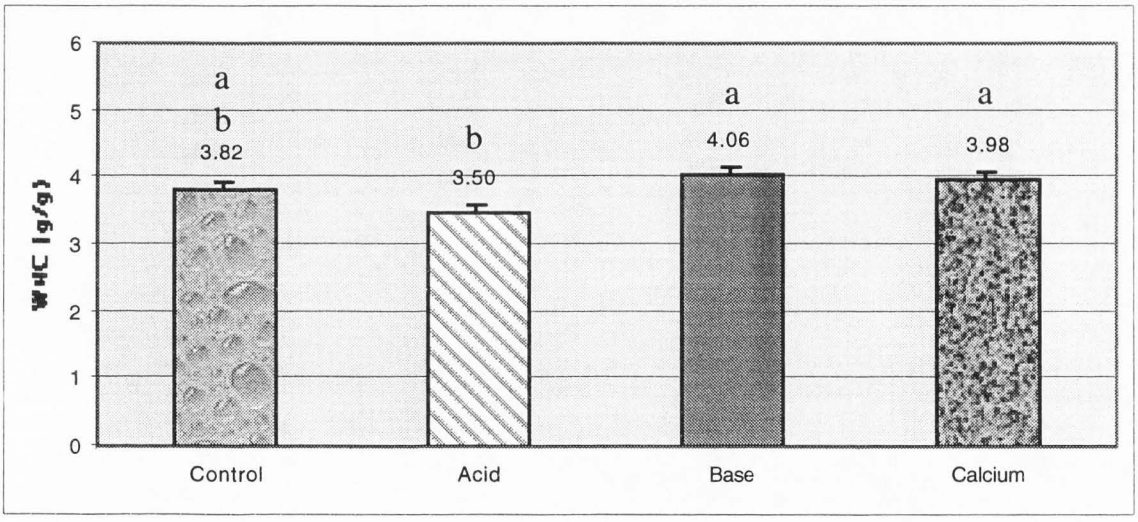

Figure 4. Water holding capacity of extrusion treatments. Columns are pooled means of all samples in each treatment category. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

Additional information on the types of bonds stabilizing insoluble aggregates was obtained by comparing protein solubility in 2% SDS (disrupts non-covalent interactions), 0.5 M NaCl (disrupts electrostatic interactions), and 0.02% BME (cleaves disulfide bonds). Water solubilizes protein held by only the weakest non-covalent interactions. The additional protein solubilized in each buffer (SDS, NaCl, and BME), as compared to the protein solubilized in water, reflects the relative extent of non-covalent, ionic, and disulfide bonds, respectively.

The additional protein solubilized by SDS in the base treatment group was not different from the water control (Figure 9), which indicates there was no significant difference in disruption of non-covalent interactions due to treatment with base. However, both acid and calcium samples had an increase in protein solubilized by SDS over the control. This indicates that more disruptable non-covalent interaction was formed in samples extruded with acid or additional calcium. Data collected at different treatment levels (Figure 10) show that the increase in disruptable non-covalent interaction in acid and calcium treated samples is more prevalent at higher levels.

Results of protein solubility in SDS complement the data collected on protein solubility in water. Samples that were less soluble in water have more disruptable non-covalent interactions. This indicates that non-covalent interactions lend stability to the protein structure of the product.

The amount of protein disrupted by 0.5 M NaCl compared to water (Figure 11) was higher than the control in samples extruded with acid and calcium. This is consistent with all protein solubility data discussed to this point. However, in the data on level effects (Figure 12), increased solubility was found in samples extruded with high levels of acid or calcium. Even then, the additional protein solubilized accounts for less than 2% of the total protein, which indicates electrostatic interactions were not predominant in stabilizing protein interactions.

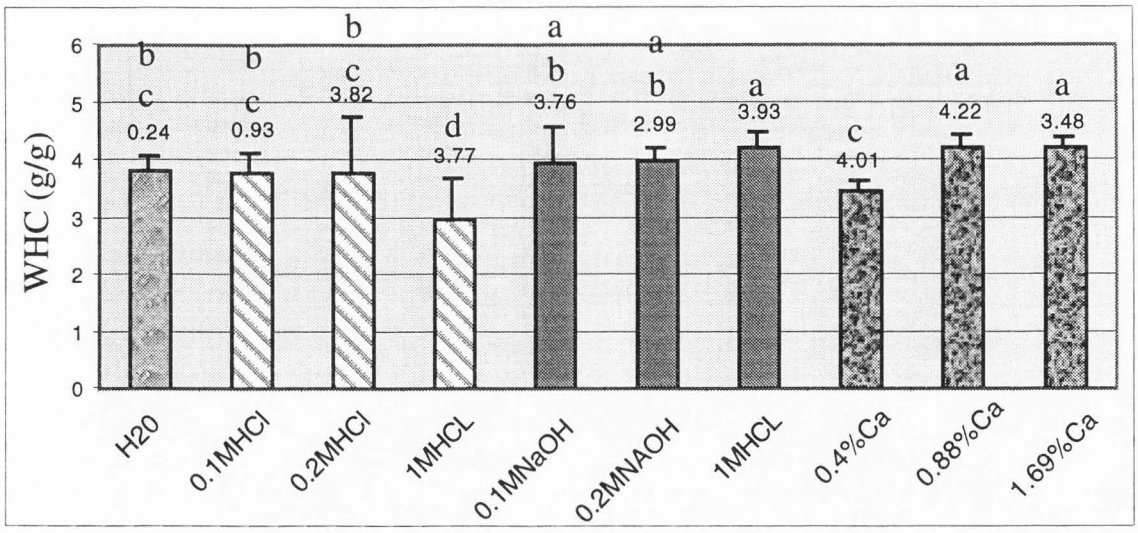

Figure 5. Water holding capacity of extrusion treatment levels. Columns are the pooled means labeled by extrusion treatment. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

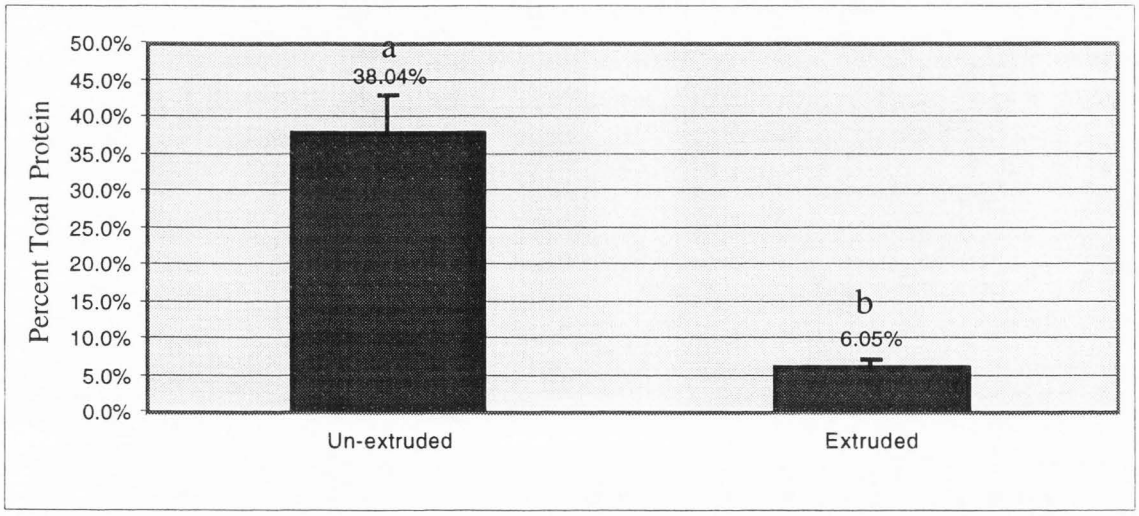

Figure 6. Percent soluble protein of unextruded compared to extruded samples in water. The first column is the pooled mean of two unextruded variables. The second column is the pooled mean of ten extruded samples. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

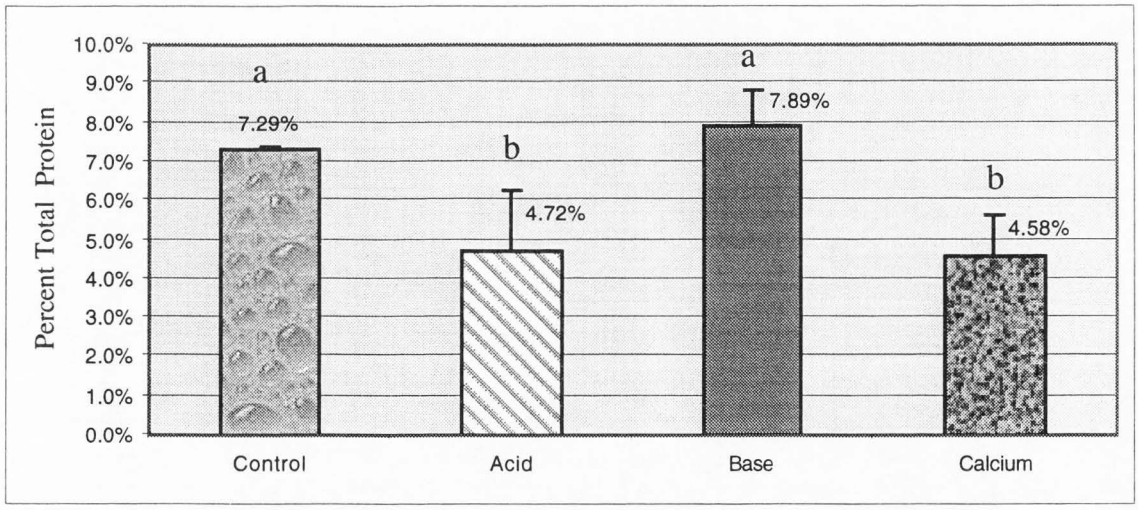

Figure 7. Percent soluble protein of extrusion treatments in water. Columns are pooled means of all samples in each treatment category. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

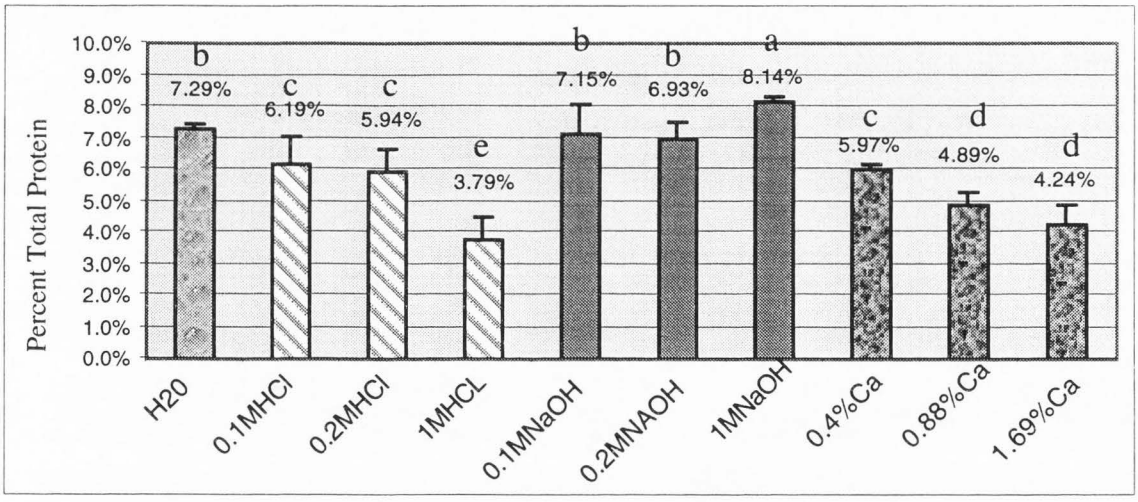

Figure 8. Percent soluble protein of extrusion treatment levels in water. Columns are the pooled means labeled by extrusion treatment. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

The amount of protein disrupted by 0.02% BME compared to water (Figure 13) was higher than the control in samples extruded with acid. Level effects shown in Figure 14 indicate an increase in disulfide bonds in samples extruded with low levels of base, all levels of acid, and high levels of calcium. Still, cleaving disulfide bonds solubilizes less than 1% of the total protein in these samples, and these bonds are not predominate in any sample. These results contrast those of Martinez-Serna and Villota, (1992), who suggested that a balance between disulfide and hydrophobic interactions must be reached when extruding at high levels of whey protein.

In this research, no more than 15% of the total protein was solubilzed by extraction in any one solvent. These results indicate that multiple types of bonds stabilized proteins. The remaining protein is most likely stabilized by nonspecific covalent bonds. Other researchers have suggested high temperatures (100 to 150°C) that are known to lead to covalent bond formation. These irreversible chemical changes include Malliard reactions, cys-breakdown, and possible breakdown of disulfide bonds (Li-Chan, 1983; DeWit and Klarenbeek, 1984). Also, at high pH, cysteine breakdown increases, dehydro-alanine forms, and if lactose is present, lysine is destroyed (DeWit and Klarenbeek, 1984). Perhaps initial bond formation mediates protein aggregation, and the bonds are later replaced by stronger covalent bonds that stabilize the networks.

CONCLUSIONS

Altering acid, base, and calcium in extrusion influenced the stability of textured whey proteins. Water holding capacity of whey proteins was increased by extrusion. Elevated WHC in extruded samples was promoted by extrusion with base or calcium, but there was no relationship between WHC and protein solubility. Extrusion also reduced protein solubility of whey proteins in water. The lower protein solubility was promoted by extrusion with acid or calcium. Samples extruded with acid and calcium had more

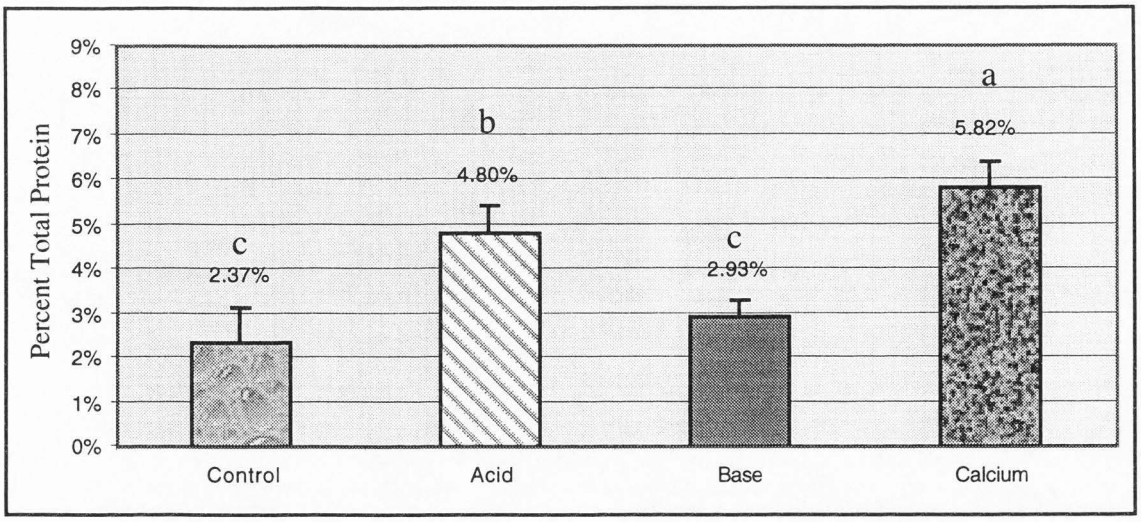

Figure 9. Percent soluble protein of extrusion treatments in SDS compared to water. Columns are pooled means of all samples in each treatment category. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

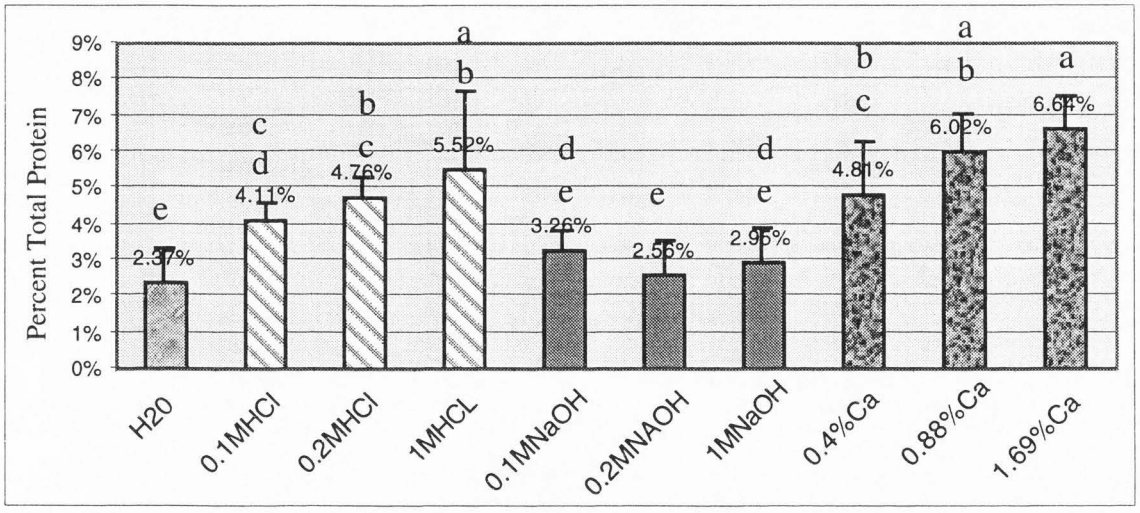

Figure 10. Increase in percent protein soluble in SDS compared to water. Columns are the pooled means labeled by extrusion treatment. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

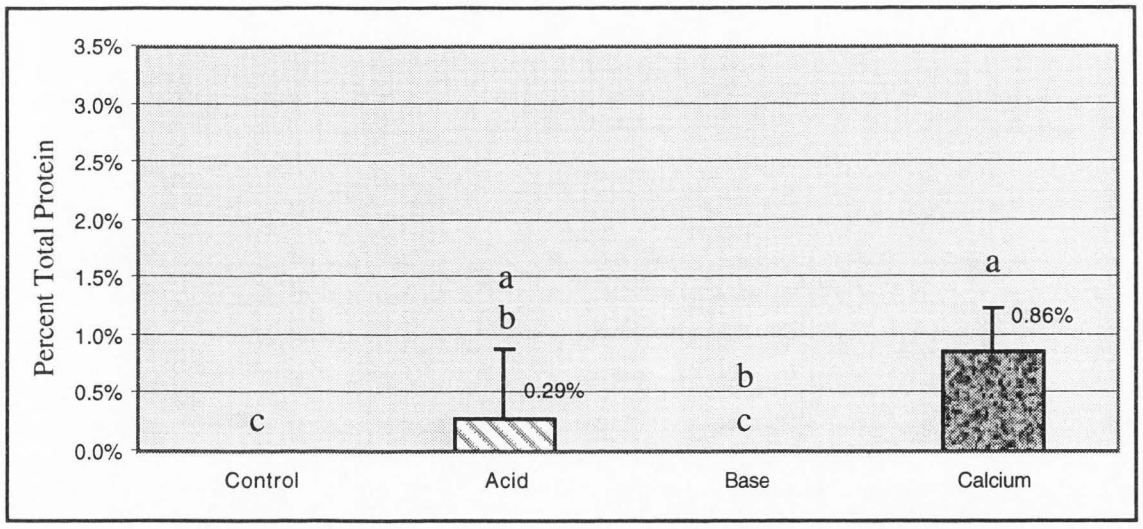

Figure 11. Percent soluble protein of extrusion treatments in NaCl compared to water. Columns are pooled means of all samples in each treatment category. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

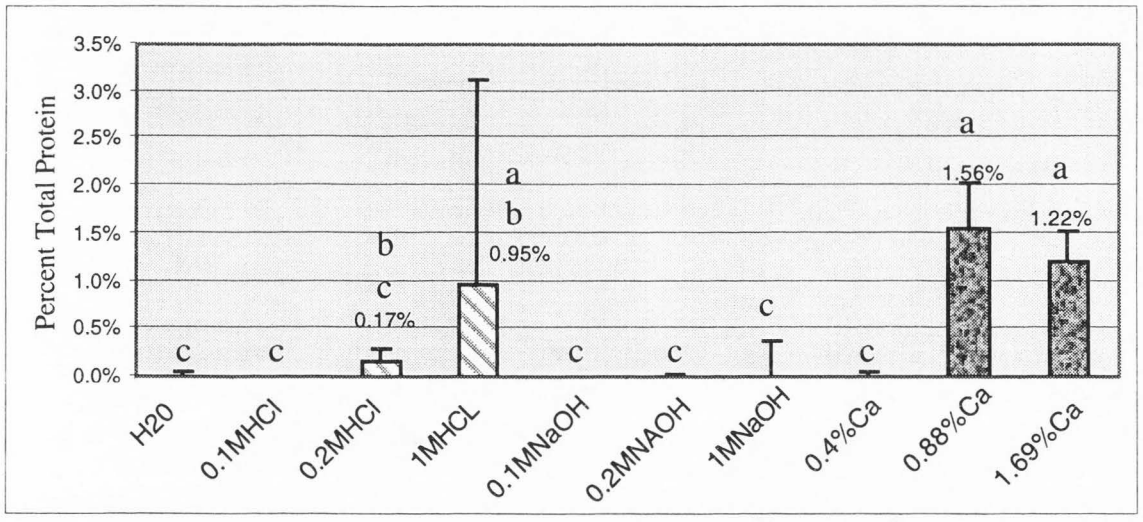

Figure 12. The increase in percent protein soluble in NaCl compared to water. Columns are the pooled means labeled by extrusion treatment. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

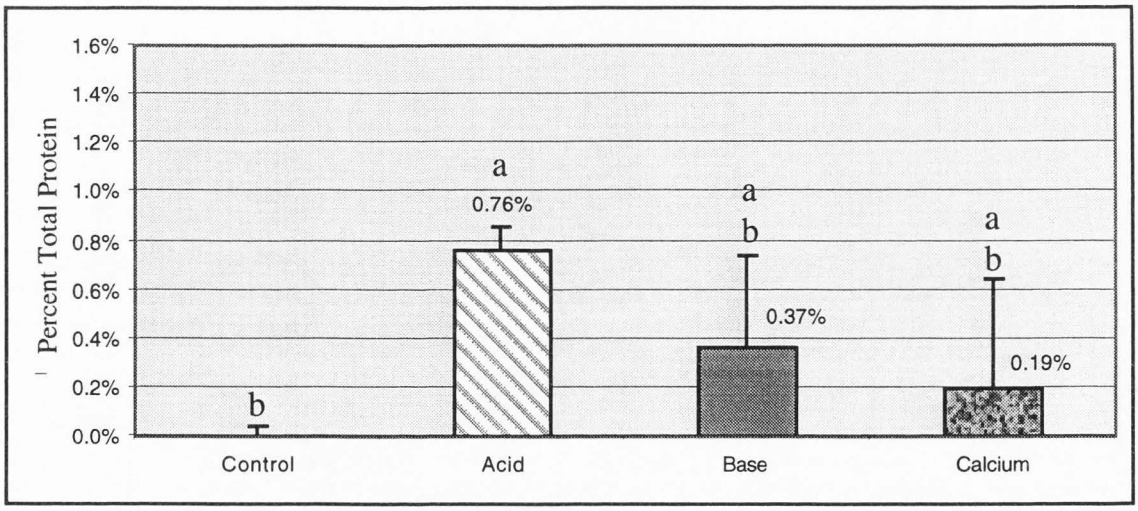

Figure 13. Percent soluble protein of extrusion treatments in BME compared to water. Columns are pooled means of all samples in each treatment category. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

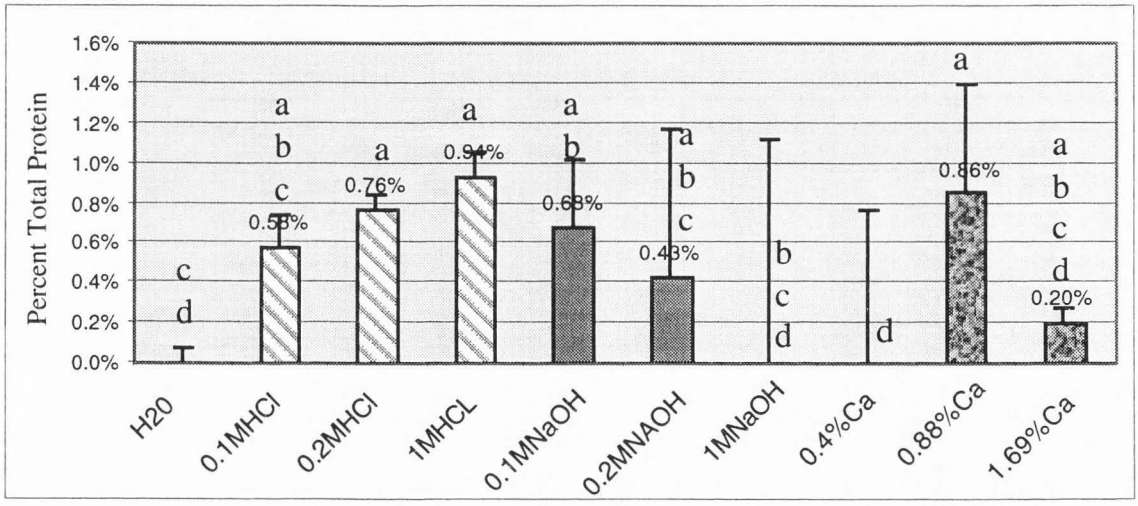

Figure 14. The increase in percent protein soluble in BME compared to water. Columns are the pooled means labeled by extrusion treatment. Statistics were calculated with analysis of variance using SAS (Cary, NC). Differences were calculated using least squared difference test (LSD, $p < 0.05$). Means sharing superscript letter are not different at $p > 0.05$.

stabilizing by non-covalent interactions. These results suggest that addition of acid, base, or calcium in extrusion of whey proteins promote formation of additional protein interactions. Increased stability may facilitate the use of TWP as a meat extender.

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CHAPTER IV
SENSORY AND INSTRUMENTAL EVALUATION OF HAMBURGER PATTIES CONTAINING EXTRUSION-TEXTURED WHEY PROTEIN

ABSTRACT

Textured whey protein (TWP) was made by extruding a dry mix of 2/3 whey protein concentrate (80% protein) and 1/3 cornstarch with water on a twin-screw extruder. The acid, base, and calcium level of the TWP formula was adjusted for comparison. The four, hydrated TWP samples were tested for acceptability in beef patties at the 30% level against 30% textured soy protein (TSP) and 100% beef patties. It was found that patties made with 30% TWP extruded with base (TWP_{base}) were equal to 100% beef in tenderness, juiciness, texture, flavor, and overall acceptability, and were preferred over TSP. The TWP_{base} was then tested against 100% beef at three usage levels, 30, 40, and 50%. Sensory analysis indicated high acceptance of TWP_{base} up to the 40% level in beef patties. Physical and instrumental analysis showed that TWP_{base} patties had higher cook yield and shape retention than 100% beef patties.

INTRODUCTION

Whey protein is a high quality protein that is readily available, has a clean, mild flavor, and has excellent functionality in foaming, emulsification, and gelation. It has been used as a binder in emulsion-type meats (Mittal and Useborne, 1985; Parks and Carpenter, 1987), and to formulate low-fat hamburger patties (El-Magoli et al., 1996). Whey protein can be detrimental to meat texture, which may limit its usage in meat products.

Textured whey proteins (TWP) were developed, as discussed in Chapter III, by adjusting acid, base, and calcium levels during extrusion of TWP. These products

exhibited different physical and functional properties, notably protein solubility and water holding capacity (WHC). The stability of TWP may allow use in beef patties at higher levels than other forms of whey protein.

The objective of this research was to determine the acceptability of and practicality of TWP in ground beef patties. Consumer acceptance was measured by sensory analysis. Mechanical properties were tested by physical and instrumental tests.

EXPERIMENTAL PROCEDURES

Two separate experiments were conducted to determine consumer acceptance and possible usage levels of TWP in beef patties. The objective of the first experiment was to determine which TWPs were acceptable to consumers. In the second experiment, the preferred sample from the first experiment was tested at three usage levels.

Materials. Textured whey proteins were made using a dry mix of (w/w) 2/3 whey protein concentrate with 80% protein (AMPC 800, American Meat Packers Cooperation, Ames IA) and 1/3 cornstarch (purchased locally). Dry mix was extruded with water on an APV Baker MPF19 twin-screw extruder (Grand Rapids, MI). In the MPF19, dry feed and fluid were added separately, and the components were mixed in the extruder barrel. The extrusion parameters were kept constant for each experiment at screw speed of 200 rpms, feed rate of 23 g/min, water-flow rate of 11 g/min, and product temperature of 150°C. Acid and base treatments were applied by substituting solutions of sodium hydroxide (NaOH) or hydrochloric acid (HCl) for the water source in extrusion. Calcium was added on a percent calcium per protein basis in the form of calcium chloride dihydrate (Malinckrodt, Paris, KY) to the dry mix before extrusion. Collected samples were dried at room temperature overnight. Samples were then fractured using pestle and mortar, sieved (< #4, > #8), and stored in air-tight containers for further use.

Experiment One. The objective of the first experiment was to determine consumer acceptability of TWP samples used as meat extenders in beef patties. A taste panel was used to screen four TWP samples developed by adding acid or base or calcium during extrusion. Only one extrusion replicate of each sample was paneled.

In the first panel, 83 panelists evaluated six samples, as identified in Table 5. Among the six samples, there were four variables, TWP extruded with base (TWP_{base}), TWP extruded with acid (TWP_{acid}), TWP extruded with added calcium (TWP_{Ca2+}), and TWP extruded with water (TWP_{H2O}). The remaining two samples, textured soy protein (TSP) and 100% beef patties served as controls.

The meat extenders were hydrated with water at 1.5:1 (w/w) ratio for 10 min, and mixed into the ground beef at the 30% level (w/w). Each of the variables was standardized to 20% fat using lean and fat meat mixes.

The panel was split between two days because only 52 panelists were recruited on the first day. Uncooked samples were wrapped in Saran and stored at -80°C overnight. The following day, the samples were thawed, and the panel was reopened to recruit additional judges for a total of 83 judges. The data from the panel were analyzed using a split plot design, and split between day one and day two. Scores between the two days were not different ($p > 0.05$) except in juiciness where the data collected on day two was slightly higher than day one. Increased perception of juiciness was expected because the samples were frozen and thawed. Still, the error was small ($p = 0.018$), so the data for the two days were pooled.

Experiment Two. Experiment two focused on TWP_{base}, the most successful sample from experiment one. The objective of panel two was to determine the maximum usage level of TWP. Thus, TWP_{base} was paneled a second time using a second extrusion run replicate to show a consistent product could be produced. TWP_{base} was tested in beef patties at three usage levels, 30, 40, and 50%, and compared to 100% beef patties. Each

patty mix was standardized to 10% fat because of the high levels of TWP added. Salt was added at 21 g per 2.5-kg batch of beef/meat extender. Samples were evaluated by 88 panelists within a 2-h panel period. Physical and instrumental analysis was completed for all samples used in the second panel.

Sample Preparation. Patties used in both sensory and instrumental analysis were prepared at the same time. Formulation was carefully calculated on a w/w basis for each panel (Tables 5 and 6). Final fat content (w/w) of patties in experiment one was 20% and 10% in experiment two. Formulations in experiment two had lower fat content due to the higher levels of TWP. Lean and fat ground beef mixes were purchased from the USU meat lab and fat content was determined using the Babcock for meat method. Textured Procon®, a textured soy protein concentrate (Central Soya, Fort Wayne, IN), was the control used in experiment one.

Dry TWP and TSP were hydrated with water at a 1.5:1 ration (w/v) for no less than 10 min. Extenders were then mixed with lean and fat ground beef and formed into patties. Patties (~ 113 g, raw wt) were shaped in a glass mold with a 10-cm diameter and 1.2-cm height. They were cooked on 168°C grills for 4 min on each side until juices ran clear. Patties were then salted (first experiment only), cut crosswise into four equal pieces, and served hot to panelists.

Sensory Methods. No less than 80 panelists were served samples in booths, under red lights, in an open consumer panel. Samples were assigned random three-digit

Table 5. Formulation for Experiment One (w/w)

treatment	30% fat beef	20% fat beef	meat extender*
100% beef	0%	100%	0%
TWP_{base}	60.2%	9.8%	30%
TWP_{acid}	60.2%	9.8%	30%
TWP_{Ca2+}	60.2%	9.8%	30%
TWP_{H2O}	60.2%	9.8%	30%
TSP	60.2%	9.8%	30%

* Meat extenders were hydrated with water at 1.5:1 (w/w) for 10 min.

Table 6. Formulation for Experiment Two (w/w)

treatment	27.2% fat beef	5% fat beef	meat extender*
100% beef	22.5%	77.5%	0%
30% TWP_{base}	29.3%	40.7%	30%
40% TWP_{base}	31.5%	28.5%	40%
50% TWP_{base}	33.8%	16.2%	50%

* TWP_{base} was hydrated with water at 1.5:1 ratio (w/w) for 10 min.

numbers and rotated in ballot position to prevent bias. Panelists were asked to score samples using a hedonic scale from 1 = dislike extremely, to 9 = like extremely, with a median of 5 = neither like, nor dislike. Panelists were encouraged to rinse mouth with water between samples, and given opportunity to comment on each sample

Samples were evaluated for tenderness, juiciness, texture, flavor, and overall acceptability. Though panels were strictly hedonic, the evaluated attributes were placed in a specific order on the ballot to help guide the panelist through the sample analysis. Tenderness was listed on the ballot before texture because, in this case, tenderness relates primarily to initial bite, and texture relates to mouthfeel of particles during chewing. Juiciness was sandwiched between tenderness and texture because moisture is released in initial chewing (Berry et al., 1983). Flavor and overall acceptability were listed last on the ballot because they are the last attributes to be perceived.

Physical and Instrumental Analysis. Physical parameters were determined as the difference between patty weight, diameter, and thickness for raw and cooked samples (Berry et al., 1983). Physical analysis was conducted by measuring diameter reduction, changes in thickness, and cook yield for ten patties in each treatment in experiment two. After initial measurements of weight, diameter, and thickness were made, patties were cooked according to the method used in sensory analysis, and cooled to room temperature for final measurements.

Instrumental analysis was conducted after physical analysis. Measurements of peak force were made using the USU Penetrometer, as described by Dobson et al. (1993).

Briefly, the patties were supported at their periphery and centered beneath a rod that resolved to a 1.9-cm steel ball. The rod was advanced at a maximum rate of 2 cm/min, and the load in grams was recorded manually every 5 sec until the rod penetrated the patty.

Statistical Analysis. In the first panel 83 judges evaluated six patties on five attributes. The design was a split plot, split between day one and day two. In the second panel, 88 judges evaluated four patties on five attributes. Two-way interactions between sample and presentation order were also tested for each panel.

Instrumental analysis was conducted on ten patties of each treatment group for the two panels. For the samples in panel two, physical analysis was conducted on the ten patties prior to the instrumental analysis.

The differences and interactions in sensory, instrumental, and physical analysis were calculated in SAS (Cary, NC) using the general linear model procedure for analysis of variance. Differences were determined using Fisher's least squares difference (LSD) test with $\alpha = 5\%$.

RESULTS AND DISCUSSION

Experiment One. There were significant differences ($p < 0.05$) in tenderness, juiciness, texture, flavor, and overall acceptability of patties containing TWP (Table 7). Scores for patties containing TWP_{base} were equal to 100% beef patties for all the measured sensory attributes, indicating that it was liked equal to 100% beef patties. Both TWP_{base} and 100% beef patties had higher scores than all other samples in all areas, except for juiciness of TWP_{H2O} patties. These results imply that TWP_{base} was preferred to TSP and other TWP samples. Also, TWP_{base} was the only sample with average scores above 6 (like slightly), in every category. TWP_{base} was the most accepted meat extender from panel one.

The 100% beef patties and 30% TSP patties had higher ($p < 0.05$) peak forces than 30% TWP patties (Table 7). This indicates they are more cohesive than patties with 30% TWP. Cohesive patties are less likely to break during cooking and serving.

Within patties with 30% TWP meat extenders, TWP_{base} had the highest peak force. The lower peak forces of 30% TWP patties indicate that patties are less cohesive, which may result in higher breakage, especially in samples other than TWP_{base}.

Experiment Two. In the sensory analysis of panel two (Table 8) no differences ($p < 0.05$) were found between the 100% beef patties and 30 and 40% TWP_{base} patties. However, 50% TWP_{base} patties received lower scores for texture, flavor, and overall acceptability. At 50% TWP_{base} addition, the texture becomes too soft, as indicated by panelists scores and comments.

Research has been conducted on use of dairy proteins as meat extenders (Tuohy et al., 1979), yet there are no commercially available products. Other products, such as textured soy protein (TSP), are used up to the 100% level in meat replacement, but these products may have limited acceptability due to poor texture and/or flavor.

TWP patties incurred less cook loss than the 100% beef patties (Table 9). The reduced percent cook loss can be attributed to the water holding and fat binding

Table 7. Means from Sensory and Instrumental Analysis of Experiment One

treatment	tenderness*	juiciness	texture*	flavor	accept.	peak force (g)
100% beef	6.17 ^{A}	5.87 $^{A, B}$	6.32 ^{A}	6.45 ^{A}	6.35 ^{A}	1418.3 ^{A}
30% TWP_{base}	6.67 ^{A}	6.19 ^{A}	6.16 ^{A}	6.28 ^{A}	6.32 ^{A}	1081.9 ^{B}
30% TWP_{acid}	4.86 ^{D}	4.90 ^{C}	3.43 ^{D}	4.73 ^{C}	4.69 ^{B}	419.2 ^{D}
30% TWP_{Ca2+}	5.77 $^{B, C}$	5.57 ^{B}	4.60 $^{B, C}$	5.51 ^{B}	5.14 ^{B}	693.6 ^{C}
30% TWP_{H2O}	5.64 $^{C, B}$	5.65 $^{A, B}$	4.53 ^{C}	5.27 $^{B, C}$	5.01 ^{B}	565.1 $^{C, D}$
30% TSP	5.23 $^{C, D}$	4.78 ^{C}	5.19 ^{B}	4.00 ^{D}	4.23 ^{B}	1530.9 ^{A}

* Tenderness relates to initial bite and texture relates to mouthfeel during chewing. Patties were evaluated in an open consumer panel by 83 panelists. All patties were adjusted to 20% fat. Statistics calculated with analysis of variance using SAS (Cary, NC). Within a column, mean values sharing a superscript letter are not different ($p > 0.05$).

Table 8. Means from Sensory Analysis of Experiment Two

treatment	tenderness	juiciness	texture	beef flavor	acceptability
100% beef	6.77 ^{NS}	6.48 ^{NS}	6.29 ^{A}	6.49 ^{A}	6.53 ^{A}
30% TWP_{base}	6.70 ^{NS}	6.49 ^{NS}	6.36 ^{A}	6.49 ^{A}	6.51 ^{A}
40% TWP_{base}	6.60 ^{NS}	6.45 ^{NS}	6.60 ^{A}	6.28 ^{A}	6.34 ^{A}
50% TWP_{base}	6.20 ^{NS}	5.98 ^{NS}	5.31 ^{B}	5.27 ^{B}	5.56 ^{B}

Patties were evaluated in a hedonic open consumer panel by 88 panelists. Patties were standardized to 10% fat due to high levels of TWP. Statistics calculated with analysis of variance using SAS (Cary, NC). Within a column, mean values sharing a superscript letter are not different ($p > 0.05$).

characteristics of whey proteins (El-Magoli et al., 1996). Decreased cook loss and shape retention result in less waste, higher nutritional value, and greater convenience for the final product. All of these are important given the meat extender may be sold for institutional, commercial, or retail use.

The original dimensions of the patties were a 10-cm diameter and a 1.2-cm height. Physical analysis indicated that patties with various levels of added TWP_{base} had better shape retention than for 100% beef patties. Patties with TWP_{base} had less diameter reduction, and patties extended with 40 and 50% TWP_{base} had less change in thickness than 100% beef patties.

Shape retention during cooking is desirable in the food industry because it allows process mechanization. El-Magoli et al. (1996) found that beef patties with added whey

Table 9. Means from Physical and Instrumental Analysis of Experiment Two

treatment	cook loss %	diameter*	thickness*	peak force (g)
100% beef	35.47 ± 1.10% ^{A}	7.8 ± 0.2 ^{C}	1.8 ± 0.2 ^{A}	1478.2 ± 241.02 ^{A}
30% TWP_{base}	29.53 ± 1.91% ^{B}	8.7 ± 0.8 ^{B}	1.7 ± 0.1 $^{A, B}$	602.33 ± 231.62 ^{B}
40% TWP_{base}	25.49 ± 0.93% ^{C}	8.8 ± 0.1 ^{B}	1.6 ± 0.1 $^{B, C}$	383.2 ± 76.61 ^{B}
50% TWP_{base}	24.25 ± 0.90% ^{C}	9.3 ± 0.1 ^{A}	1.4 ± 0.1 ^{C}	453.4 ± 37.95 ^{C}

* Measurement in centimeters for cooked patties. Peak force was measured using the USU Penetrometer. Patties were adjusted to 10% fat. Statistics calculated with analysis of variance using SAS (Cary, NC). Within a column, mean values sharing a superscript letter are not different ($p > 0.05$).

protein concentrate resist shrinkage due to their fat binding, emulsifying, and heat gelation properties. Resistance to shrinkage in TWP_{base} may also be related to the fat binding characteristics or WHC of whey proteins, though no measurements of fat binding or moisture content were made.

Instrumental analysis for experiment two (also in Table 9) showed that the peak force of patties with various levels of TWP_{base} was lower than the 100% beef control, indicating that they were less cohesive. Patty breakage during cooking is likely with higher levels of TWP_{base}, but could perhaps be remedied by adding additional meat binders or even increasing the fat content of the patties. The lower peak forces were not reflected in sensory scores of the TWP_{base} patties up to the 40% level.

There is a difference between the peak forces of TWP_{base} added at the 30% level in experiment one to experiment two. Peak forces of samples with 30% TWP_{base} added were different in each experiment. This can be attributed to differences in fat content of patties in each experiment. Decreased peak force was observed in samples with lower fat content experiment two. These results can be expected because TWP does not bind the meat pieces as readily as fat. Differences in peak force, which indicate cohesiveness, were not reflected in panel scores of texture or overall acceptability of the samples.

CONCLUSIONS

According to consumer panels, beef patties made with up to 40% TWP_{base} were equal to the 100% beef patties in tenderness, juiciness, texture, flavor, and overall acceptability. Beef patties formulated with up to 40% TWP_{base} had higher cook yield and less diameter reduction than 100% beef patties. Also, consumers prefer 30% TWP_{base} patties over patties made with 30% commercially available TSP. This research demonstrates great potential for use of textured whey proteins as a meat extender.

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CHAPTER V

SUMMARY

The first objective was to determine the effects of altering pH and increasing calcium concentration during extrusion of whey protein. It was found that altering the pH and calcium level in extrusion influenced the stability of the resulting textured whey protein (TWP). Extrusion increased ($p < 0.05$) the WHC of TWP. This was further promoted by adding base during extrusion. Extrusion also reduced protein solubility of whey proteins in water. Solubility was promoted by extrusion at increased levels of acid or calcium.

Approximately 90% of the total protein of pH and calcium-adjusted TWP was not solubilzed when extracted in 2% SDS, 0.5 M NaOH, or 0.02% BME. Nonspecific covalent bonds or multiple types of bonding most likely stabilized the remaining protein. Other research suggests that at high temperatures, 100 to 150°C, irreversible chemical changes occur such as Maillard reactions, cys-breakdown, and possible breakdown of disulfide bonds (Li-Chan, 1983; DeWit and Klarenbeek, 1984). Also, at high pH, cysteine breakdown increases; DHA (dehydro-alanine) forms, and if lactose is present, lysine is destroyed (DeWit and Klarenbeek, 1984). All of these reactions are likely to induce formation of covalent bonds, which are beyond the scope of the present study. Perhaps disulfide bonds form initially to mediate protein network aggregation, are broken down, and then replaced by other types of covalent bonds that stabilize the networks.

The second objective was to determine acceptability of TWP produced in the first objective by using sensory, physical, and instrumental analysis. An open consumer panel was conducted on six beef patty samples: 1) 100% beef, 2) 30% TWP extruded with 0.2 M NaOH ($TWP_{0.2MNaOH}$), 3) 30% TWP extruded with 1.69% calcium (w/w protein) added ($TWP_{1.69\%Ca2+}$), 4) 30% TWP extruded with water (TWP_{H2O}), 5) 30% TWP extruded with

0.1 M HCl ($TWP_{0.1MHCl}$), and 6) 30% textured soy protein (TSP). It was found that 30% $TWP_{0.2MNaOH}$ patties were equal to 100% beef patties in tenderness, juiciness, texture, flavor, and overall acceptability.

Hedonic testing indicated that beef patties extended with$TWP_{0.2MNaOH}$ were the most accepted ($p < 0.05$) of the TWP tested at the 30% level by consumers. Beef patties made with 30% $TWP_{0.2MNaOH}$ were scored equal to 100% beef patties in tenderness, juiciness, texture, flavor, and overall acceptability. Consumers preferred patties extended at the 30% level with $TWP_{0.2MNaOH}$ over those extended equally with TSP.

$TWP_{0.2MNaOH}$ was then tested against 100% beef patties at three usage levels, 30, 40, and 50%. $TWP_{0.2MNaOH}$ was well accepted by consumers up to the 40% level in beef patties. Extended samples had lower patty diameter reduction than 100% beef patties at all levels. Patties extended with 40 and 50% $TWP_{0.2MNaOH}$ had less increase in patty thickness than the 100% beef patties. There were no differences among the peak force of the patties with levels of added $TWP_{0.2MNaOH}$. Peak forces of samples with added $TWP_{0.2MNaOH}$ were lower than for 100% beef patties. Differences were not evident from the sample scores of texture or overall acceptability. These results indicated there is great potential for textured whey protein as a meat extender.

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APPENDICES

APPENDIX A.

ANALYSIS OF VARIANCE TABLES FOR CHAPER III

Table 10. Analysis of Variance of pH of TWP Samples

source	df	type I SS	mean square	F value	Pr > F
sample	9	27.96	3.12	15.53	0.0001

Table 11. Analysis of Variance of Water Holding Capacity

source	df	type I SS	mean square	F value	Pr > F
sample	11	66.70	6.06	72.42	0.0001

Table 12. Analysis of Variance of Percent Soluble Protein of Unextruded Compared to Extruded Samples in Water

source	df	type I SS	mean square	F value	Pr > F
sample	1	333114.82	333114.82	333.03	0.0001

Table 13. Analysis of Variance of Percent Soluble Protein of Extrusion Treatments in Water

source	df	type I SS	mean square	F value	Pr > F
sample	3	1770.78	590.26	24.84	0.0001

Table 14. Analysis of Variance of the Increase in Percent Protein Soluble in SDS Compared to Water

source	df	type I SS	mean square	F value	Pr > F
sample	9	123.97	13.77	10.94	0.0001

Table 15. Analysis of Variance of the Increase in Percent Protein Soluble in NaCl Compared to Water

source	df	type I SS	mean square	F value	Pr > F
sample	9	30.13	3.35	5.73	0.0001

Table 16. Analysis of Variance of the Increase in Percent Protein Soluble in BME Compared to Water

source	df	type I SS	mean square	F value	Pr > F
sample	9	11.62	1.29	3.23	0.0039

APPENDIX B.

ANALYSIS OF VARIANCE TABLES FOR CHAPTER IV

Table 17. Analysis of Variance of Tenderness in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	175.13	35.03	9.15	0.0001
order	5	6.67	1.33	0.35	0.8832
order*sample	25	97.79	3.91	1.02	0.4362
split	1	7.16	7.16	1.87	0.1721
split*sample	5	29.17	5.83	1.52	0.1810
split*order	5	8.58	1.72	0.45	0.8144
split*order*sam	25	159.92	6.40	1.67	0.0235

Table 18. Analysis of Variance of Juiciness in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	125.45	25.09	6.33	0.0001
order	5	8.51	1.70	0.43	0.8278
order*sample	25	69.36	2.77	0.70	0.8581
split	1	22.25	22.25	5.62	0.0182
split*sample	5	11.63	2.33	0.59	0.7096
split*order	5	5.16	1.03	0.26	0.9343
split*order*sam	25	110.18	4.41	1.11	0.3235

Table 19. Analysis of Variance of Texture in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	493.60	98.72	24.09	0.0001
order	5	11.34	2.27	0.55	0.7355
order*sample	25	97.27	3.89	0.95	0.5358
split	1	11.21	11.21	2.74	0.0989
split*sample	5	17.28	3.46	0.84	0.5193
split*order	5	16.89	3.38	0.82	0.5326
split*order*sam	25	77.00	3.08	0.75	0.8029

Table 20. Analysis of Variance of Flavor in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	355.96	71.19	17.78	0.0001
order	5	28.18	5.63	1.41	0.2202
order*sample	25	105.61	4.22	1.06	0.3928
split	1	15.08	15.08	3.77	0.0530
split*sample	5	29.00	5.80	1.45	0.2058
split*order	5	10.84	2.17	0.54	0.7447
split*order*sam	25	87.61	3.50	0.88	0.6407

Table 21. Analysis of Variance of Overall Acceptability in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	303.66	60.73	7.11	0.0001
order	5	38.31	7.66	0.90	0.4832
order*sample	25	203.79	8.15	0.95	0.5296
split	1	11.18	11.18	1.31	0.2534
split*sample	5	25.63	5.13	0.60	0.2058
split*order	5	10.84	2.17	0.54	0.7000
split*order*sam	25	123.65	4.94	0.58	0.9499

Table 22. Analysis of Variance of Peak Force in Experiment One

source	df	type I SS	mean square	F value	Pr > F
sample	5	10009503.59	2001900.72	41.03	0.0001

Table 23. Analysis of Variance of Tenderness in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	16.75	5.58	2.38	0.0700
order	3	16.23	5.41	2.30	0.0770
order*sample	9	28.81	3.20	1.36	0.2042

Table 24. Analysis of Variance of Juiciness in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	15.81	5.27	2.57	0.0546
order	3	30.72	10.24	4.98	0.0021
order*sample	9	13.51	1.50	0.73	0.6807

Table 25. Analysis of Variance of Texture in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	82.80	27.60	11.46	0.0001
order	3	10.56	3.52	1.46	0.2250
order*sample	9	34.36	3.82	1.59	0.1183

Table 26. Analysis of Variance of Flavor in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	88.05	29.35	9.67	0.0001
order	3	35.43	11.81	3.89	0.0094
order*sample	9	63.33	7.04	2.32	0.0153

Table 27. Analysis of Variance of Overall Acceptability in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	52.87	17.62	7.70	0.0001
order	3	34.64	11.55	5.04	0.0020
order*sample	9	28.24	3.14	1.37	0.2003

Table 28. Analysis of Variance of Peak Force in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	5	7592033.25	2530677.75	55.63	0.0001

Table 29. Analysis of Variance of Cook Loss in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	950.79	316.93	92.91	0.0001

Table 30. Analysis of Variance of Increased Thickness in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	1.08	0.36	9.81	0.0001

Table 31. Analysis of Variance of Diameter Reduction in Experiment Two

source	df	type I SS	mean square	F value	Pr > F
sample	3	15.49	5.16	77.37	0.0001

APPENDIX C.

SENSORY BALLOTS FOR CHAPTER IV

HAMBURGER PATTIES

September 29, 1999

Name_____

Evaluate each sample according to the scale presented below. Be sure to rinse mouth with water between samples.

9 - Like extremely
8 - Like very much
7 - Like moderately
6 - Like slightly
5 - Neither like or dislike
4 - Dislike slightly
3 - Dislike moderately
2 - Dislike very much
1 - Dislike extremely

Sample #	Tenderness	Juiciness	Texture	Flavor	Overall Acceptability	Comments
838						
359						
955						
798						
482						
410						

HAMBURGER PATTIES

November 4, 1999

Name_____

Evaluate each sample according to the scale presented below. For a proper evaluation, you must consume at least half of each given sample. Be sure to rinse mouth with water between samples.

9 - Like extremely
8 - Like very much
7 - Like moderately
6 - Like slightly
5 - Neither like or dislike
4 - Dislike slightly
3 - Dislike moderately
2 - Dislike very muchs
1 - Dislike extremely

Sample #	Tenderness	Juiciness	Texture	Beef Flavor	Overall Acceptability	Comments
357						
263						
871						
196						