ABSTRACT

FIREBAUGH, JONATHAN DAVID. Characterization and application of a derivatized whey ingredient. (Under the direction of Christopher R. Daubert.)

Whey protein is a nutritious ingredient that is manipulated to impart texture in many foods. Thermal treatment is commonly used as a means of inducing whey protein aggregation, which under suitable conditions may form gels. Unfortunately, many foods cannot or should not withstand the temperatures required to induce protein gelation, thereby limiting native whey as a thickening agent. A process for manufacturing cold-gelling whey proteins has been developed, and the resulting derivatized whey ingredient provides the food industry with more alternatives in product formulation, including superior nutritional value when compared with carbohydrate-based stabilizing systems.

The current cold-gelling whey protein derivatization procedure involves protein hydration, pH adjustment, thermal treatment, and spray drying. This process yields an easily dispersed protein powder that does not require heat or addition of salts to function as a thickening agent. Dispersions containing 9% protein (w/w) of the derivatized ingredient in deionized water display apparent viscosities of approximately 1.0 Pa s at shear rates of 50 s^{-1}. For comparison, the viscosity of a 9% protein (w/w) solution of unmodified whey is approximately 0.002 Pa s. Therefore, the derivatization process yields an ingredient with an apparent viscosity approximately 3 orders of magnitude greater than unmodified whey protein.

The derivatized whey ingredient could potentially serve a number of different functions in food systems: thickening agent, stabilizer, fat-mimicker, emulsifier, and texturizer. To test this potential, a basic understanding of ingredient interactions was needed; hence the modified whey ingredient was characterized under conditions generally encountered in food systems. The effects of protein concentration, pH, and salt on the modified whey protein ingredient were determined using model systems prepared as dispersions or solutions at controlled pH, temperature, and salt concentration. Water holding and viscosity imparted by the modified whey ingredient were dependent on protein concentration, pH, and salt concentration. The effective concentration, or the concentration at which dilute dispersions enter a concentrated regime, was approximately 7% (w/w) protein. Effective concentration increased, and water holding and viscosity decreased as the pH of modified protein dispersions approached the isoelectric point (pI) of whey protein (~5.2). Furthermore, water holding and viscosity decreased as salt concentration of modified whey protein dispersions increased.

Additional studies characterized the emulsifying and foaming properties of the modified whey protein ingredient. Emulsifying capacity, or the amount of oil a protein can adsorb, of the modified whey protein ingredient was similar to that of unmodified whey at pH 3.4 and 6.8. However, the modified whey ingredient created a more stable emulsion than unmodified whey at an equivalent pH. The modified whey protein ingredient created more stable foams that had lower overrun than unmodified whey protein foams. Unlike unmodified whey protein foams, stiffness of modified whey protein foams was improved through pH adjustment.

Dairy food applications traditionally stabilized with starch were targeted for application of the modified whey protein ingredient because the modified ingredient had proven to hold water and add viscosity similar to modified starch. Comparisons of yogurt containing 2% (w/w) unmodified whey protein and yogurt containing 2% (w/w) of the derivatized whey ingredient showed the modified ingredient improved yogurt water holding capacity. Yogurt in which starch was completely replaced with modified protein displayed higher yield stress than yogurt formulated with starch. The derivatized whey ingredient's ability to stabilize sour cream was also investigated. Increasing the level of modified whey protein from 2% (w/w) protein to 4% (w/w) protein in sour cream resulted in increased yield stress, and viscosity. However, neither modified whey protein formulation achieved yield stress or syneresis results similar to sour cream formulated with 2% (w/w) starch. Although preliminary results were promising, further optimization of yogurt and sour cream formulations with the modified whey ingredient is needed.

Based on this research, derivatized whey ingredients are likely to prove an extremely useful ingredient in dairy food applications. Replacing non-protein stabilizers with a derivatized whey protein ingredient may provide food processors the opportunity to tailor ingredient labels for diet conscious consumers. For example, replacing carbohydrate-based thickeners with protein thickeners would provide a more appealing label for low-carbohydrate dieters. Furthermore, substituting stabilizers with the derivatized whey protein ingredient will give dairy food manufacturers the option of claiming an "all-dairy" product.

CHARACTERIZATION AND APPLICATION OF A DERIVATIZED WHEY INGREDIENT

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

DEPARTMENT OF FOOD SCIENCE

Raleigh

2004

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BIOGRAPHY

Jonathan David Firebaugh was born on April 1, 1979 in Greensboro, North Carolina. He is the son of Robert and Nancy Firebaugh, natives of Michigan. Jon was raised in Climax, North Carolina and graduated from Southeast Guilford High School in June of 1997. Two months later, Jon moved to Raleigh, NC to pursue a bachelor's degree at NC State University. The ensuing four years provided the author plenty of fun and opportunities. Summers during Jon's undergraduate tenure were spent interning with Milkco (Asheville, NC), General Mills (Buffalo, NY and Reed City, MI) and The Department of Agriculture and Rural Development (Belfast, Northern Ireland). Jon obtained his B.S. in Food Science in May of 2001.

After graduation the author spent a tumultuous six months growing up in the mid-west, realizing his values, setting his priorities and ultimately returning to the land of sweet tea and pig pick'ns. Jon pursued an M.S. in Food Science at NC State University under the guidance and direction of Dr. Christopher Daubert.

ACKNOWLEDGEMENTS

- I am lucky to have worked under the *best* advisor at NC State. Thank you, Dr. Daubert, for providing a "meal ticket" for me to return to North Carolina. I am grateful for your guidance and willingness to allow me to foster a research project in which I can take ownership. Although our weekly meetings often ended in more questions than answers I always appreciated your commitment and willingness to offer suggestions. Two of my favorite memories include helping paint your deck one Friday afternoon and your insistence that someone always eat the last piece of pizza at Amedeo's during our lab lunches.

- Mom and Dad, I love you. The older I get, the more I realize your unfailing commitment to your three children and the sacrifices you've made along the way. It's probably safe to say that without your love and encouragement none of us would have achieved our current levels of success.

- To Joe and Julie - my older brother and younger sister: Knowing that I have a strong, supportive family gives me courage to face the challenges of life. You guys are the greatest!

- To Amanda Dees, one of the best friends I made while at NC State. I'll always remember Dairy Bar and your dislike of upside-down roller coasters. I look forward to continuing our friendship beyond our Shaub Hall years.

- To college bowl guru and former roommate, Jeff Resch, for his friendship and assistance learning the ins-&-outs of derivatized whey proteins. I won't soon forget The Big Bad Show, Wednesday nights at The Flying Saucer, or Skeeter, the black cat that terrorized our apartment for a short while. Good luck at the Big G, and I look forward to visiting you and all of our other cronies in Minneapolis!

- To Katie Cleary, I'll miss our occasional lunches and helping you move odds and ends (washers, dryers, tomato plants) in and out of your apartment. Good luck at Kraft.

- To Scott, for lightening things up when I came home frustrated about classes, research, and job hunting. I always looked forward to dropping and forgetting about everything on the weekends and just having fun. I am grateful for your encouragement and support over the last two years.

- To my friends outside of Schaub Hall: Del, Jay, Roger, Bennie, James, Todd, Brock, Wendy, and Joy.

- To the Food Rheology Lab (my fellow "*deformers of matter*") as I knew it: Michelle, Tom, Noel, Den, Qixin, Jeff, Junhua, Lisa, Melissa, Deepti, Sharon, and Tristan. Thanks for making graduate school fun!

- I am grateful for the encouragement of the Foegeding lab, Paige, Jack, Dany, Matt, and others. They were instrumental in helping me with the technicalities of this project as well as lending an ear when things weren't going just right.

- I'd like to acknowledge my fellow dungeon dwellers as well as the dairy plant guys; I'll miss our lunch time conversations.

- To the Southeast Dairy Foods Research Center (SDFRC) for their financial support of this project.

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LITERATURE REVIEW

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1.1 – INTRODUCTION

Milk and dairy-based ingredients are components of many food products. Understanding the physical and chemical properties of dairy-based ingredients provides food technologists options during food formulation and product development. The following chapter is a review of literature relevant to the characterization and application of whey ingredients. In particular, topics reviewed include milk proteins, milk composition, physicochemical properties of whey proteins, food thickeners, whey protein modification technologies, foams and emulsions, and dairy foods formulated with thickeners. The aim of this review is to provide a clearer understanding of how milk proteins are utilized for specific function within formulated foods.

1.2 – MILK OVERVIEW

Throughout this manuscript, discussion of milk is limited to bovine milk. Nevertheless, there are other mammals, including goats and sheep, whose milk is consumed by humans (Chandan, 1997). According to United States law, “Milk is the lacteal secretion, practically free of colostrum, obtained by the complete milking of one or more healthy cows” (Code of Federal Regulations, 2003(a)). Physically, milk is an opaque, whitish fluid of numerous dispersed phases: fat exists in the form of an oil-in-water emulsion; lactose, vitamins, and minerals exist in true solution; while casein and other proteins exist in the colloidal phase (Chandan, 1997). Chemically speaking, milk is a complex fluid composed of water, fat, protein, lactose, and ash. The amounts of each component vary depending on source and lactation period, yet

an approximate analysis is water (86.6%), fat (4.1%), protein (3.6%), lactose (5.0%), and ash (0.7%) at pH 6.7 (Swaisgood, 1996).

1.3 – MILK PROTEINS AS FOOD INGREDIENTS

Milk supplies a variety of proteins categorized into the whey and casein fractions. Current separation technologies allow for the isolation and purification of dairy proteins into natural food ingredients, many of which exhibit excellent functional properties, including foaming, emulsifying, thickening, texturization, and gelation (Imafidon, 1997). Common milk protein products derived from milk are illustrated in Figure 1. These value-added food ingredients help the food industry meet specific demands that cannot be fulfilled with milk itself (Huffman, 1999).

1.3.1 – Molecular and Physical Properties of Milk Proteins

Milk proteins have been extensively researched and reviewed. Initially, milk was purported to contain only one type of protein. But in the early 1900's a German scientist, Hammarsten, found that milk proteins could be fractionated into two well-defined groups (Fox and McSweeney, 1998). Hammarsten established that when milk was acidified to pH 4.6 at ~30°C, approximately 80% of the total protein content precipitated from solution. This precipitate is known as casein, and the remaining 20% of soluble protein is referred to as whey (Fox and McSweeney, 1998). Further differences between caseins and whey proteins are significant from both an industrial and scientific viewpoint and are outlined in the following sections.

1.3.2 – Caseins

In addition to serving a biological and nutritional role, caseins are important because of their structure, charge, and physical properties (Chandan, 1997). The casein fraction is comprised of four principal proteins: α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN (Swaisgood, 1996) typically found at a ratio of 4:1:4:1, respectively (Dalgleish, 1997). Caseins are able to undergo posttranslational phosphorylation due to unique primary and tertiary structures (Swaisgood, 1996). This chemical modification results in the formation of anionic clusters in the calcium-sensitive caseins (α_s and β-casein). Binding of Ca^{2+} in the clusters alters the balance of hydrophobic and electrostatic interactions (Brunner, 1981). These ionic alterations, along with the amphiphilic structure of caseins, allow the molecules to interact with one another to form large spherical micelles ranging in diameter from approximately 30 to 300 nm (Swaisgood, 1996). The spherical casein micelles are stabilized by calcium insensitive κ-caseins which locate on the micellar surface due to an interaction with the molecules' hydrophobic tail (Fox and McSweeney, 1998). The κ-casein layer is also known as the "hairy" layer due to it's appearance under magnification.

Various models for casein micelle structure have been proposed over the past 40 years (Fox and McSweeney, 1998) and at least three basic models for the internal structure of casein micelles have been reported (Lucey, 2002). One model proposed that the micelle core is divided into discrete submicelles with distinctly different properties from the "hairy" layer (κ-casein) on the outside (Schmidt, 1982; Walstra, 1990). In 1992, Holt proposed a second model in which the internal substructure was a mineralized, entangled chain of casein molecules. The third, and

most recent, model submits a dual-binding mechanism for gel assembly (Horne, 1998).

Casein micelles are the primary constituent of gel formation in many dairy foods, including cheese and yogurt (Lucey, 2002). When fresh milk sours, casein aggregates. This natural aggregation results from bacterial production of lactic acid, which acidifies the milk. The overall net charge on the casein molecules becomes more negative as acid levels increase. Aggregation increases as the overall net charge on the molecules approaches zero. The pH at which a molecule has a net charge of zero is known as the isoelectric point (pI) (Figure 2). If the casein aggregates are allowed to settle without stirring or agitation, a gel is formed. As the gel cures, some whey separation generally occurs, forming a simple cheese – cheese making in its most primitive form. However, in the present day, milk is usually clotted by the addition of agents such as chymosin (rennet), an extract of calf stomach (Walstra et al., 1999). In cheese production, added chymosin cleaves κ-casein from the surface of the casein micelle, ultimately destabilizing the system (Huffman, 1999) and allowing the micelles to aggregate and gel. Similar to cheese, the physical structure of yogurt is a network of aggregated casein particles in conjunction with heat denatured serum proteins. Therefore, the casein fraction of milk plays an important role in the production of a number of dairy foods.

1.3.3 – Whey

Whey is a general term that describes the watery, natural byproduct expressed during cheese manufacture. Historically this side stream of cheese production has been considered a waste product and was discarded. Collected

immediately after cheese processing, whey is normally 93% water and only ~0.6% protein. This protein fraction is comprised of a number of individual proteins including β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins. Of these individual proteins, β-lactoglobulin and α-lactalbumin are found in the highest concentrations and therefore have the greatest impact on functionality of whey protein ingredients. These proteins account for approximately 20% of the total protein found in milk and, unlike casein, remain soluble at pH 4.6 and 30°C (Huffman, 1996).

During the past 10 years, considerable research in protein functionality and nutrition have shown whey proteins to possess unsurpassed functional and nutritional properties (Morr and Foegeding, 1990), making them excellent candidates for use in manufactured food products. Newer processing technologies have made it economically possible to concentrate and exploit whey protein as a food ingredient (McIntosh et al., 1998; Morr and Foegeding, 1990; Huffman, 1996). Currently available whey protein ingredients are lactalbumin, whey protein concentrate (WPC), and whey protein isolate (WPI) (Huffman 1996). Lactalbumin is a powder containing all of the major whey proteins, isolated by heat precipitation at greater than 90°C. Since lactalbumin is already denatured, this protein functions quite differently from other whey ingredients such as WPC and WPI (Huffman 1996).

Whey protein concentrates are commonly manufactured using filtration techniques to concentrate protein based on molecular weight differences. During ultrafiltration, lactose, salts, and other low molecular weight materials pass through a membrane in the permeate, while higher molecular weight components such as

protein are concentrated in the retentate. The concentrated protein solution is then spray dried to form a WPC powder, which generally ranges in protein concentration from 34 to 89%. High protein whey protein concentrates (>80% protein) are used in a wide range of foods, including meats, bakery, surimi, and confections, because of their ability to impart viscosity, hold water, gel, foam, and emulsify (Huffman, 1996 and Morr, 1982).

Whey protein isolates contain >90% protein and can be prepared by either ion exchange or microfiltration membrane processes. The process by which WPI itself is manufactured will affect the overall composition of individual proteins, which influences functional properties such as gelation, foaming, and emulsification. Differences in protein composition also give rise to somewhat different functionality between WPC and WPI. For example, it is not clear what effect the glycomacropeptide from κ-casein plays on the functionality of WPCs. This glycomacropeptide is released into the whey during the renneting, or enzyme treatment, of milk. The WPI manufactured using ultrafiltration contains virtually no glycomacropeptide fraction, whereas WPI manufactured using ultrafiltration of rennet whey will contain 15-20% glycomacropeptide (Jost, 1993). As previously mentioned, β-lactoglobulin and α-lactalbumin have the greatest impact on whey functionality. Therefore, if the total protein content remains equal, yet the ratio of β-lactoglobulin and α-lactalbumin is lower due to the presence of glycomacropeptide, a difference in functionality is likely to surface.

1.4 – PHYSICSOCHEMICAL PROPERTIES OF WHEY PROTEINS

Whey protein conformation and physicochemical properties depend on a number of factors, including pH, solute concentration, ionic strength, temperature, and others (Cayot and Lorient, 1997, Kinsella and Whitehead, 1989). The pH of the dispersing medium affects the net charge of proteins (Figure 2). Thus due to the polar nature of water, a net negative or positive charge on protein molecules makes them more water soluble than if the net charge is minimal, i.e. at the isoelectric point (Kinsella and Whitehead, 1989). Salt concentration also affects the physicochemical properties of whey proteins. As the concentration of dissolved ions in solution increases, charges on the protein molecules are shielded, thus affecting protein hydration and electrostatic interactions between proteins. Salt concentrations up to 0.1M enhance whey protein solubility, but concentrations beyond 0.15M can result in salting out of the protein (Kinsella and Whitehead, 1989; Zayas 1997(b)). Additionally, ionic strength influences the gelling ability of milk proteins (Boye, 1997). Thus, when comparing properties affected by the physicochemical nature of whey proteins, experimental results must be obtained under identical pH and salt conditions.

1.5 – WHEY THICKENERS

Food thickeners, typically starch, hydrocolloids, and gelatin, are commonly added to products such as soups, dressings, and sauces to increase viscosity and improve mouth feel. The suitability of modified whey proteins as a food thickener has been investigated.

An ingredient must be able to form weak gel structures to function as an efficient thickener. The thickening of solutions or formation of weak gel structures depends on the ability of proteins to form aggregates (Bryant and McClements, 1998). Proteins in solution are typically characterized as being in either a native or denatured conformation. In a native form, whey proteins exist as individual entities with compact, organized globular conformation (Kinsella, 1989). Denatured whey proteins are more randomly oriented and less globular. Environmental conditions such as concentration, pH, ionic strength and temperature can alter the conformation of whey proteins such that they favor a native or denatured state. Aqueous solutions of whey proteins heated beyond a denaturing temperature will promote protein unfolding, exposing previously buried non-polar amino acids, thus increasing hydrophobic attraction between proteins. If favorable salt, protein concentration, and pH conditions exist, hydrophobic attraction may lead to aggregation of individual proteins (Figure 3). Aggregation leads to an increase in viscosity because the aggregates have an effective volume greater than the effective volume of the individual whey protein molecules. A gel can be formed if the protein concentration exceeds some critical level allowing the formation of a three-dimensional network of aggregated or entangled molecules (Bryant and McClements 1998).

Cold gelling whey ingredients have received considerable attention in recent years. These ingredients have previously been formed by a two-stage process: (1) preparation of a heat-denatured whey protein solution; and (2) induction of gelation at low temperatures (Bryant and McClements, 1998; Barbut and Foegeding, 1993, McClements and Keogh, 1995; Nakamura et al., 1995; Sato et al., 1995). In these

studies, whey protein solutions of low ionic strength were heated to achieve partial protein unfolding, but gels were not formed due to the repulsion of the highly charged molecules. Subsequent to heating, the solutions were cooled to room temperature and $CaCl_2$ or NaCl was added to increase the ionic strength, thereby inducing gelation (Barbut and Foegeding 1993, McClements and Keogh 1995). Acidification with glucono-delta-lactone (Kawamura *et al.,* 1993) and incubation with enzyme (Ju and Kilara, 1998) was also utilized to achieve cold-set gelation following a thermal pre-treatment. The common theme to these approaches is the application of heat as an initial denaturing treatment, followed by a further processing step to induce gel formation.

Other studies were conducted on cold-gelling whey proteins in a dried form. Thomsen (1994) described a commercially available modified whey protein concentrate powder produced by heat treatment during homogenization of a whey protein concentrate at slightly alkaline pH, followed by immediate drying. The dried powder reportedly thickened upon dispersion in a salt solution (Elofsson *et al.,* 1997).

1.6 – STARCH AND OTHER INSTANT THICKENERS

Several ingredients are used in foods to impart unique functional characteristics such as general thickening properties and stabilization for emulsions, suspensions, and foams. Instant thickeners, or cold-gelling ingredients, can be used in applications where heat-induced gelation is inappropriate or undesired. Many of these thickeners are derived from natural sources and are chemically or

enzymatically modified to achieve desired functional characteristics, among these are starch and various classes of gums (Lindsay, 1996).

Food starches and starch derivatives are used by the dairy industry for a variety of applications. Traditionally, starches have been used as bulking agents, to control consistency, and as texture enhancers (Friedman, 1995). Food applications often demand that starch withstand high shear rates, as well as temperature and pH extremes. Additional considerations include shelf life, ingredient interactions, and storage conditions such as freezing and thawing. The properties of native starches usually do not meet these demands, therefore modification is required to expand the utility of starch.

Chemical and physical modifications are used to provide starches with improved functional properties (Eliasson, 1996). Pregelatinized or instant starches are a common category of physically modified starches that develop viscosity in cold or warm water without additional heating. Unmodified, or cook-up, starch is not suitable in many processed food applications, in which case a pregelatinized starch is required to provide functionality. For example, preparation of an instant pudding using cold water or milk would require an instant thickener instead of an unmodified starch. Pregelatinized starch is composed of small gel particles, that when dissolved at ambient or refrigerated temperatures will yield solutions of high viscosity (BeMiller & Whistler, 1996). The process of pregelatinization involves hydrating and heating native starch, allowing the granules to swell and gelatinize, followed by drying the starch to a powder (BeMiller & Whistler 1996). Additionally, some heated preparations require a pregelatinized starch because either the temperature or time

period is insufficient to properly gelatinize a conventional cook-up starch (Light, 1990).

1.7 – DERIVATIZATION OF WHEY PROTEINS

Whey proteins were previously excluded as an instant-viscosifying agent due to the necessary thermal treatment or salt addition needed to create structure. Earlier research developed a process to "pregelatinize" whey protein isolates (Hudson *et al*., 2000) and concentrates (Resch and Daubert, 2002) in an attempt to garner similar functional properties and stability to modified food starches. Hudson *et al.* (2000) described a derivatization process for creating a whey protein powder capable of forming cold-set weak gel structures without the addition of salts or heat (Figure 4). Whey protein isolate was modified through a procedure consisting of solubilization of whey protein isolate (WPI) in deionized water, subsequent pH adjustment, heating to a gel, freezing, freeze-drying, and milling to a powder. Powders from this process at 10% protein (w/w) displayed uncharacteristic stability for proteins to both temperature (5-90°C) and pH adjustment (4-8) while under shear. Furthermore, the hydrated powders demonstrated weak gel properties during small strain rheological analysis. Although this process produced an ingredient with the desirable cold-gelling and thickening functionality, the commercial viability was limited by the expense of WPI and freeze drying operation. Resch and Daubert (2002) addressed the expense of WPI by applying the same derivatization process to several lower cost whey protein concentrates (WPC). This study concluded that derivatized whey protein concentrates were also capable of imparting viscosity, holding water, and forming weak gels at room temperature when compared with

unmodified WPC starting materials. However, the economical limitations of the freeze-drying step remained. Addressing this process limitation, Resch and Daubert (2003) were successful in producing derivatized whey powders manufactured with a more economical process that replaced the freezing and milling operations with spray drying (Figure 4). Results of this study showed that spray-dried derivatized whey proteins attained cold-set thickening and gelling behavior comparable to the powder modified by the original freeze-drying technique. Replacement of freeze-drying with spray-drying improved the efficiency and cost effectiveness of the derivatization process while preserving, and in many cases improving, the functionality of the original derivatized ingredient (Resch and Daubert, 2003).

The effect of particle size on the rheological characteristics of derivatized whey protein dispersions was also investigated. Increasing particle size led to increased gel strength, intrinsic viscosity, zero shear viscosity, and decreased zeta potential and swelling capacities. Therefore, manipulating dWPI powders of defined particle size could potentially permit enhanced control of powder functionality (Hudson, 2001).

1.8 – FOOD FOAMS AND EMULSIONS

Proteins in general, and milk proteins in particular, are widely used as functional ingredients for the formation and stabilization of food foams and emulsions (Dickinson, 1999). As new food ingredient sources and processing methods are developed, the need to understand and predict the behavior of protein components in foods remains important. A key property of proteins is their ability to act as

surfactants, stabilizing the air/water interface of foams and the oil/water interface of emulsions.

1.8.1 – Foams

The ability of proteins to aid in the formation of stable foams is important in the production of a variety of foods. In short, a liquid foam is a two-phased system in which distinct gas bubbles are surrounded by a continuous liquid lamellar phase (Phillips *et al.*, 1990). Food foams are usually complex systems, including a mixture of gases, liquids, solids, and surfactants that can be formed by a number of actions, the most common being through whipping, injecting, or shaking of aqueous solutions (Richert, 1979). The basic function of proteins in foams is to decrease interfacial tension, to increase viscous and elastic properties of the liquid phase, and to form strong films (Zayas, 1997(a)). During the formation of a foam, surfactants within the continuous phase adsorb to the liquid/air interface, thereby reducing surface tension (Figure 5). This reduction in surface tension allows for easier bubble, and thus foam, formation (Wilde, 2000). After formation, foam stability depends on the ability of the proteins to unfold at the interface and interact with one another, forming a viscoelastic network around the gas bubbles (Dickinson, 1999). Depending on mobility, protein molecules unfold at the interface with polar groups directed toward water and non-polar groups toward air. Instability of foams is indicated by liquid separation and drainage, and by an increase in bubble size (Richert, 1979). Liquid may separate from a foam either by drainage from the lamellae or by rupture of bubbles. In this case, surface viscosity and capillary forces become very important. Thinning of films may occur as a result of drainage, thus leading to rupture of

bubbles and foam collapse. Therefore, protein or surfactant selection is key in forming and stabilizing foams.

Foaming properties of proteins are influenced by protein source, methods and thermal parameters of processing, temperature, pH, protein concentration, mixing time, and method of foam formation (Zayas, 1997(a)). The pH of the initial protein solution markedly affects foaming, particularly foam stability, by its effects on net charge and conformation of the protein (Kinsella, 1981). Previous research by Mita *et al.* (1977, 1978) has shown that surface tension of wheat proteins was lowest at pH levels close to their isoelectric points (pH 6.5 - 7.5). Electrostatic attractions are maximum at the isoelectric point (pI), thereby allowing more protein to adsorb at the water-air interface, resulting in decreased surface tension (Kinsella, 1981). Films are thickest at the pI, and interfacial elasticity tends to be highest because of electrostatic bonding between molecules, resulting in more stable foams (Mita *et al*., 1977, 1978). However, while maximum stability of foams usually occurs at the pI, minimum solubility of the protein is also observed. Many proteins easily coagulate in the pI region, and the presence of coagulated protein reduces foam stability, resulting from reduced film strength. Cherry and McWatters (1981) suggested that proteins in the liquid lamellar phase should 1) be in the soluble state in aqueous media; 2) be concentrated at the liquid/air interface; and 3) be in the denatured state to possess high viscosity and strength. However, protein particles in a dispersion, as opposed to a solution, can also stabilize foams as a result of location near the liquid/air interface and serving as a physical barrier to bubble coalescence (Zayas, 1997(a)). Knowledge of these foam properties enables scientists to formulate foams meeting specific consumer and processing demands.

1.8.2 – Experimental Approach to Foams

Foams are inherently difficult structures to study because of delicate instabilities. However, the importance of foams in many food products has motivated development of somewhat standardized, reliable methods for foam characterization. Some common properties measured in protein foams include overrun, stability (drainage), and yield stress. However, the complex nature of foam formation suggests that overrun and stability measurements may have little value beyond tools for foam comparison. (Halling, 1981). Phillips *et al*. (1990) developed a standard method for preparing and characterizing food foams formed through whipping, limited to overrun and stability measurements. Overrun was calculated by measurement of density, and stability was related to drainage rates. In this study the following equation was used to define overrun:

$$\%\ Overrun = \frac{(wt\ 100\ mL\ protein) - (wt\ 100\ mL\ foam)}{(wt\ 100\ mL\ foam)} \times 100 \qquad (1.0)$$

Foam stability was measured by monitoring drainage over time (Figure 6). The time required to attain 50% drainage was used as an index of foam stability according to Halling (1981).

Finally, foam yield stress can also be measured. Yield stress (σ_o) is defined as the minimum shear stress required to initiate flow in a material (Steffe, 1996). The material responds as a solid below the yield stress and as a liquid above this parameter. The concept of structural deformation and breakage of network bonds may explain one characteristic of materials with yield stress (Dzuy and Boger, 1983,

1985). From this, two yield stress measurements have been reported: static and dynamic (Cheng, 1986). Dynamic yield stress is a result of the chemical structure within the material that is insensitive to shearing. When the sample is at rest, a secondary, weaker structure forms due to weak time-dependant secondary forces: hydrogen bonding, electrostatic interactions, and Van der Waal's forces. The additive effect of the dynamic yield stress to this secondary structure determines the static yield stress, resulting in a higher yield stress than the dynamic yield stress (Steffe, 1996).

The vane method is a relatively easy, technique used as an alternative to uniaxial compression tests (Kovalenko and Briggs, 2002) to measure large strain properties of viscoelastic materials. A vane consists of 4 to 8 blades attached to a cylindrical shaft, connected to a rheometer that rotates the vane at a constant speed (Figure 7). Initially, samples with a yield stress will resist deformation (acting as a solid), increasing the torque required to maintain the constant rotational speed of the vane. Eventually, as the rotational distance increases, the material will begin to flow. The torque response at the time material flow initiates is converted to yield stress. For the single point method, this conversion assumes that the stress contribution from the top and bottom of the vane is negligible. This assumption is usually satisfactory, especially when making quality control comparisons or when timely results are necessary (Steffe, 1996). Yield stress using the single point method is calculated using the following equation:

$$\sigma_o = \frac{2M_o}{\pi d^3}\left(\frac{h}{d}+\frac{1}{3}\right)^{-1} \qquad (2.0)$$

where:

σ_o = yield stress, Pa
M_o = maximum torque, Nm
d = vane diameter, m
h = vane height, m

Typical foods displaying a yield stress include ketchup, mayonnaise, peanut butter, and foams. Product formulators are often required to design products with or without a yield stress to meet consumer expectations and processing needs.

Pernell *et al*. (2000) successfully applied the vane method to measure the yield stress of protein foams. In this study, a four-bladed vane attachment was submerged into a whipped foam and rotated at 0.3 rpm. A Brookfield model DV-I 25xLVTDV digital viscometer was used to measure the yield point. Based on the reproducibility and agreement between point and slope method evaluation, it was concluded that the vane method was a reliable method for characterizing yield properties of protein foams.

1.8.3 – Emulsions

In a sense, oil-in-water emulsions are much like foams; both are dispersions of hydrophobic fluids in a hydrophilic liquid (Walstra, 1996). Emulsions are classified by type, that is, oil-in-water (o/w) or water-in-oil (w/o), and this nomenclature differentiates between continuous and disperse phases. An oil-in-water emulsion, for example, would consist of oil dispersed within a continuos water phase (Becher, 1965). Similar to foams, emulsions can be stabilized by surface-active agents known as emulsifiers. The surface activity of emulsifiers serves to decrease interfacial tension, thus increasing emulsion stability. Additionally, emulsion stability can be

increased through addition of other agents, such as stabilizers, which serve to hold water and increase the viscosity of the continuous phase of the emulsion (Becher, 1965).

A number of food ingredients act as emulsifiers and or stabilizers. For example, commercial dairy proteins are used in meats as extenders to reduce formulation costs and improve functionality, mainly for emulsion formation and control (Mittal and Usborne, 1985; Hung and Zayas, 1992). According to Swift (1965) both emulsifying capacity (EC) and emulsion stability (ES) are essential measurements for determining the emulsification value of food ingredients. Emulsifying capacity is determined as the maximum quantity of oil that can be dispersed in an aqueous solution, containing a specified concentration of protein, to produce and oil-in-water emulsion without the emulsion breaking down or inverting to a water-in-oil emulsion (Swift, 1961). Emulsifying capacity is usually expressed as oil volume emulsified by an aqueous solution containing a given weight of protein (Acton, 1972). Webb *et al.* (1970) described a method of determining EC through measurement of electrical resistance. This method is based on the principal that fats and oils are nonconductors, whereas protein and water are good conductors. Resistance is relatively low in an o/w emulsion in which the protein-water phase is continuous. However, when fat droplets begin to aggregate, electrical resistance abruptly increases. When the continuous phase fails to maintain the matrix structure to support the disperse phase, the system collapses, resulting in an easily recorded change in electrical resistance (Webb, 1970). Figure 8 represents an experimental setup to measure EC.

Emulsion stability is the ability of emulsion droplets to remain dispersed without coalescing, flocculating, or creaming, and is a measure of the emulsion's ability to remain stable and unchanged. One method of measuring ES is dispersion analysis to register the size distribution of the fat particles as a function of factors, such as time and temperature. Dispersion analysis is often measured through microscopic observation (Zayas, 1997(a)), but other methods to measure ES have been reported. Imm and Regenstein (1997) evaluated emulsion stability by observing drainage, over time, of cream layers sandwiched between a polyester mesh (PECAP #7-105/35). Huidobro *et al.* (1998) measured the emulsion stability of ultrafiltered protein from minced fish wash water by centrifuging emulsions containing 80% of the oil needed to collapse the emulsion. The supernatant was carefully decanted from the centrifuged sample and emulsion stability was reported as:

$$\% \text{ Emulsion Stability} = \frac{\text{Weight of precipitate}}{\text{Initial weight of emulsion}} \times 100 \qquad (3.0)$$

1.9 – DAIRY FOODS WITH THICKENERS

A variety of food ingredients such as modified food starch, gums, and hydrocolloids are used as thickeners or stabilizers in dairy-based foods. These thickeners play a number of functional roles such as limiting syneresis, improving texture and appearance, and aiding in manufacturing processes, such as pumping and filling. For example, modified starches are commonly used to limit syneresis and improve texture of yogurt, sour cream, and cream cheese. Additionally, gelatin is used in yogurt formulations to improve gel structure after pumping. Other

hydrocolloids, such as carrageenan and locust bean gum, are used to prevent settling and improve mouth feel in flavored milks.

1.9.1 – Yogurt

Yogurt is a cultured dairy product produced by fermenting milk, with or without added nonfat dry milk (NFDM), with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria (Lee *et al*., 1990). Prior to fermentation, milk is usually fortified with NFDM and other stabilizers to achieve 12-14% total milk solids. Following fortification, the premix is homogenized to provide stable fat-in-water emulsions and then heated. The application of heat has three purposes: 1) to destroy pathogens or undesirable organisms; 2) to either stimulate or inhibit activity of lactic starter cultures; and 3) to denature milk proteins which provide the proper viscosity and gelation, and limit syneresis in the final product (Tamime and Robinson, 1999; Morr, 1985 and 1989). Following pasteurization, the premix is cooled to the incubation temperature of the starter culture and then inoculated. In general, yogurt is fermented at 40-45°C until the desired acidity is reached, for example pH 4.6. At this point, rapid cooling ensues to approximately 5°C to limit metabolic activity of the starter culture and to control the final acidity of the product. A more detailed outline of yogurt manufacture is found in Figure 9.

Stabilizers and emulsifiers are often added to the milk base during yogurt production to enhance and maintain the desirable characteristics of yogurt, such as body, texture, viscosity/consistency, appearance, and mouth-feel. After fermentation, the yogurt coagulum is often subjected to mechanical shear through pumping, stirring, mixing to incorporate flavors, and packing/filling. As a result, the

yogurt may become less viscous or be prone to syneresis or whey separation. The addition of stabilizers can overcome these defects. In yogurt, stabilizers have two main functions: first, the binding of water, and second, promotion of an increase in viscosity (Tamime and Robinson, 1999). Stabilizers can be used individually or as a blend. Blending of these ingredients is often used to overcome limiting properties associated with a specific ingredient. For example, one stabilizing ingredient may be suitable for regular fat yogurt, but it may not be suitable for production of a fat-free or low-fat product. Hence, blending of two or more stabilizers may be used to overcome this limitation.

According to Tamime and Robinson (1999) there are many factors to consider when choosing a yogurt stabilizer blend. First, the functional properties of the stabilizer(s) in relation to the processing conditions of the type of yogurt produced must be considered. For example, a set-style yogurt not subjected to additional shear after fermentation may not require the same type of shear-stable stabilizer as a stirred-style yogurt. Extreme processing conditions, UHT for example, could destroy stabilizer functionality. The type of yogurt produced will also govern stabilizer selection. Winterton and Meiklejohn (1978) reported that modified starch proved unsatisfactory when used in a frozen yogurt application. Later Gautneb, *et al.* (1979) found that a mixture of stabilizers and emulsifiers improved frozen yogurt quality. Stabilizer selection is also governed by federal regulations which may, or may not, allow certain substances in yogurt formulations. Selection of an optimal concentration of stabilizer can also be governed by federal regulations. Additionally, recommended usage levels are based on negative side effects such as off flavors and undesirable appearance caused by the addition of too large a quantity.

1.9.2 – Sour Cream

Sour cream, or cultured sour cream, results from the culturing of pasteurized cream with *Streptococcus lactis*, together with other flavor-producing bacteria, until the titratable acidity (TA) is at least 0.5% (Code of Federal Regulations, 2003 (b)). Sour cream must contain not less than 18% milk fat (unless nutritive sweeteners are added, in which case not less than 14.4% milk fat) and 28% total solids at a pH of approximately 4.6 (Hunt and Maynes, 1997; Code of Federal Regulations, 2003 (b)). A general process for sour cream production starts by blending skim milk and cream to the desired fat and solids levels. Stabilizers are added, and the blend is then pasteurized and homogenized. Next, the homogenized blend is inoculated with culture and incubated to the desired pH and titratable acidity. A more detailed outline of sour cream manufacture is outlined in Figure 10.

An alternative sour cream, labeled as *acidified sour cream*, results from souring pasteurized cream with safe and suitable acidifiers, with or without lactic acid-producing bacteria (Code of Federal Regulations, 2003(b)). Although sour cream prepared by acidification possesses a different flavor and consumer acceptance from sour cream prepared by culture, fundamental texture data of acidified sour cream has not been reported in the literature. If the texture of the two sour cream products is similar, it is possible that acidified sour cream acceptability might be improved with addition of characteristic flavors produced by flavor-producing culture.

Stabilizer systems for sour cream may include milk solids or any combination of thickening and gelation agents, such as starch, guar gum, carrageenan, and locust bean gum (Hunt and Maynes, 1997). A current issue in the processing and performance of sour cream is syneresis during a wide range of storage conditions. For example, the development of prepared, frozen meals has led to a demand for sour cream that can withstand the freezing and thawing environment of a packaged dinner. In this case, replacing carrageenan and locust bean gum in the stabilizer system with methylcellulose and starch can produce a suitable nonfat sour cream (Hunt and Maynes, 1997) that resists syneresis and maintains texture after freezing and thawing.

1.10 – SUMMARY

Based on literature findings and preliminary research, derivatized whey ingredients may prove to be an extremely useful ingredient in dairy food applications. Applications for the dairy-based ingredient, functioning as a thickening agent to replace, or partially replace, common food thickeners should be investigated. Replacing non-protein stabilizers with a derivatized whey protein ingredient may provide manufacturers the opportunity to tailor ingredient labels to diet conscious consumers. For example, replacing carbohydrate-based thickeners with protein thickeners would provide a more appealing label for low-carbohydrate dieters. Also, substituting stabilizers with the derivatized whey protein ingredient might give dairy manufacturers the option of claiming an “all-dairy” product.

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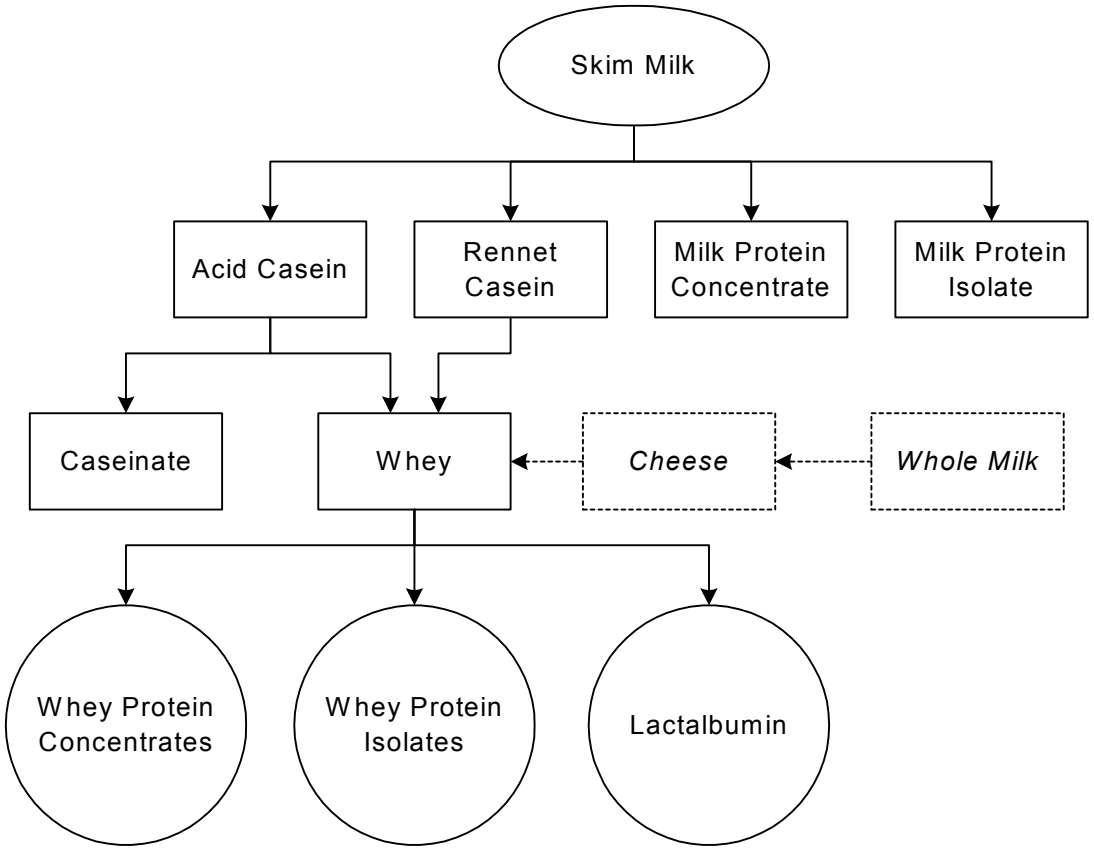

Figure 1. Protein ingredients commonly manufactured from skim or whole milk. (Huffman, 1999)

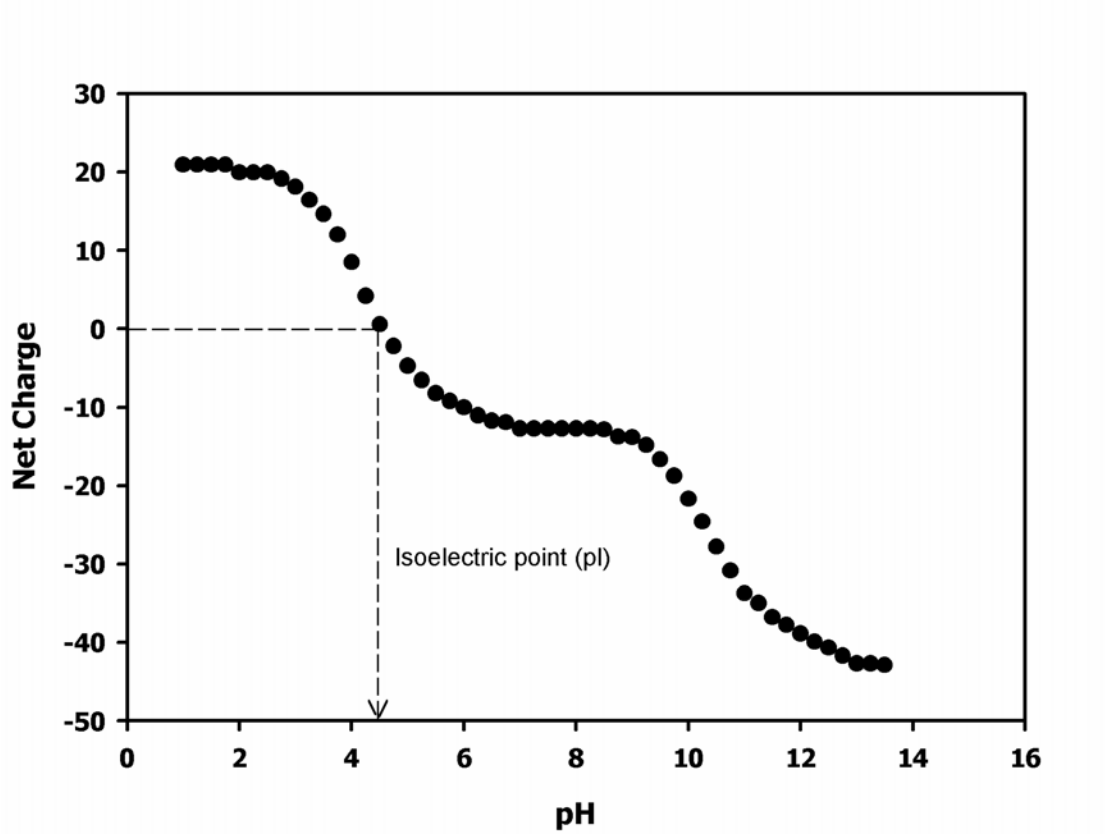

Figure 2. Estimated casein mixture net charge as a function of pH. The isolectric point (pI) is shown. (Zhong, 2003)

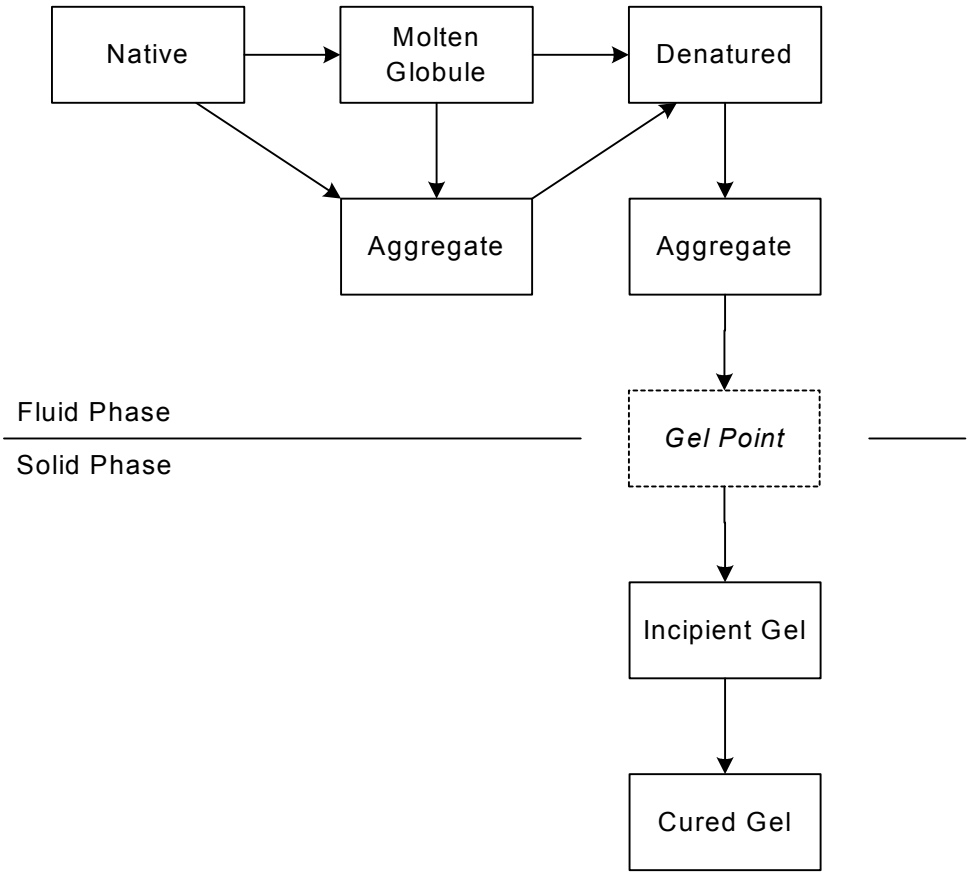

Figure 3. A generalized model for heat-induced aggregation and gelation of whey proteins. (Foegeding *et al*., 1998)

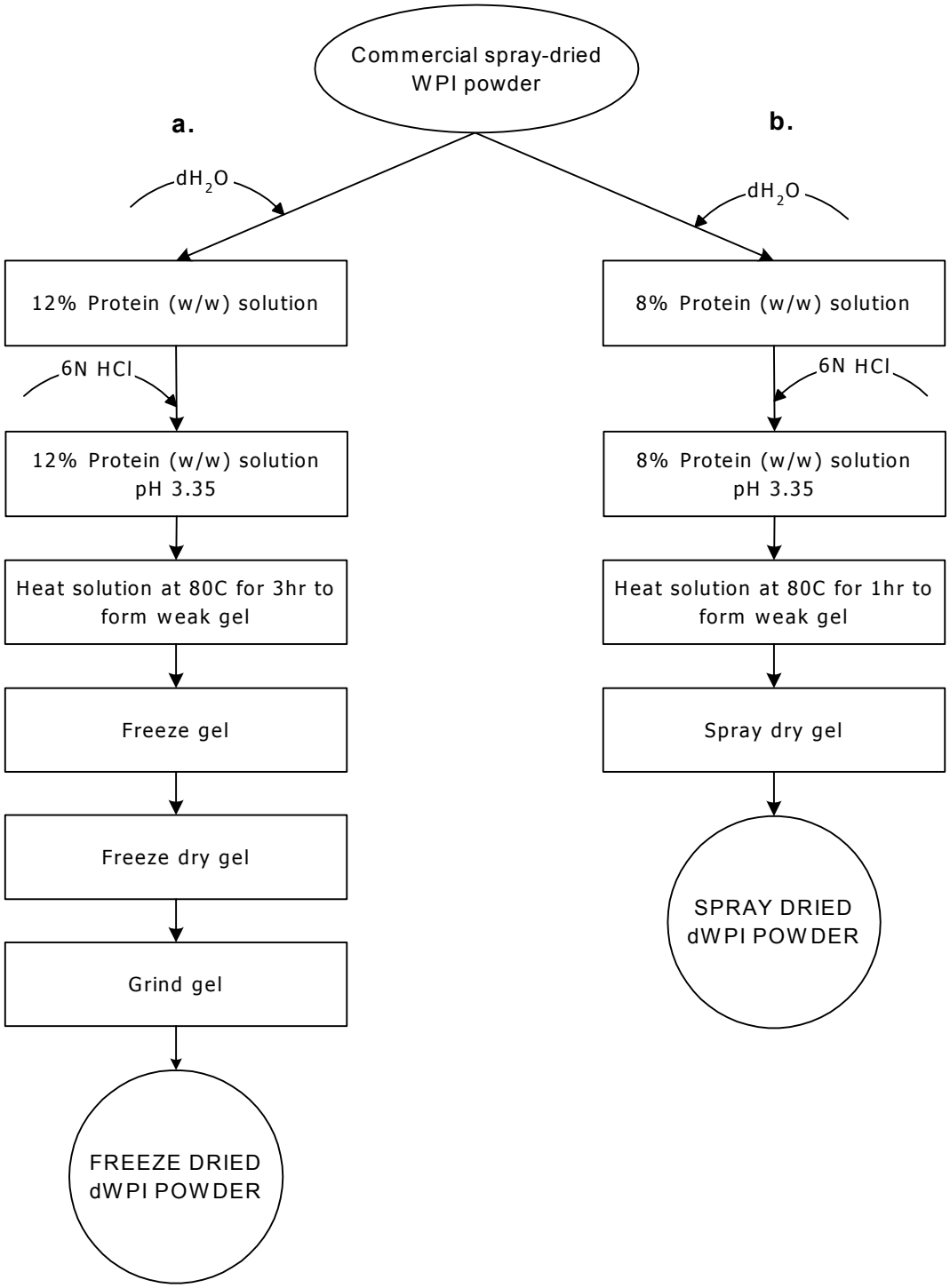

Figure 4. Block flow diagram of whey protein modification procedure.
a. freeze dry method according to Hudson *et al.* patent (2001)
b. spray dry method according to Resch and Daubert (2003)

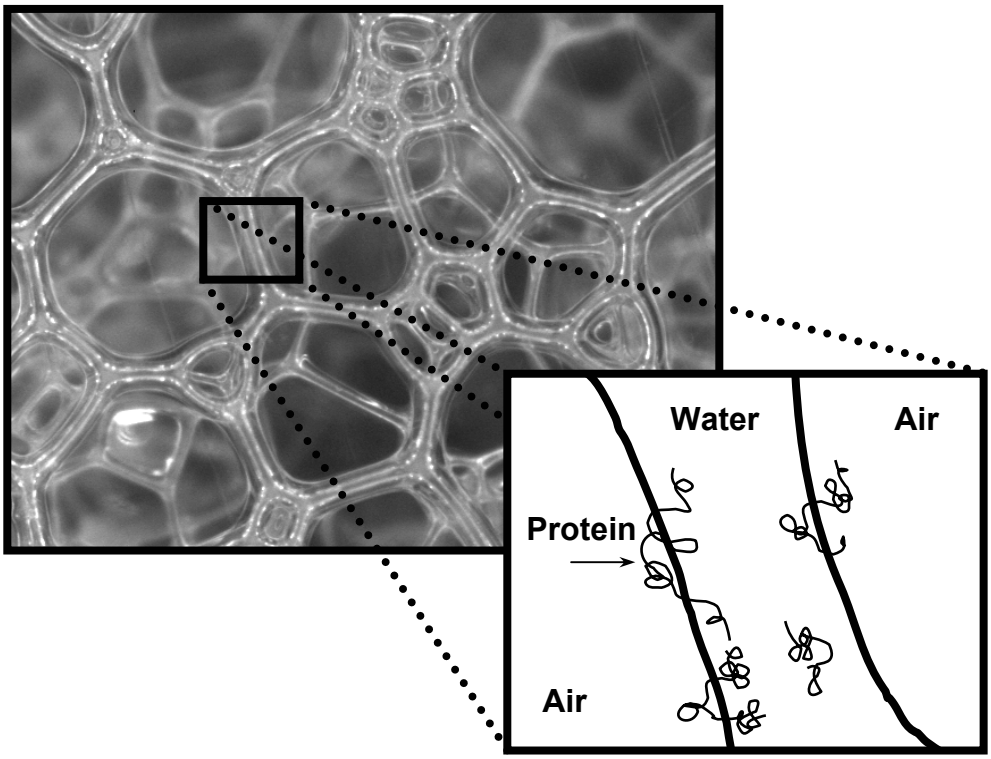

Figure 5. Schematic of foam stabilization.

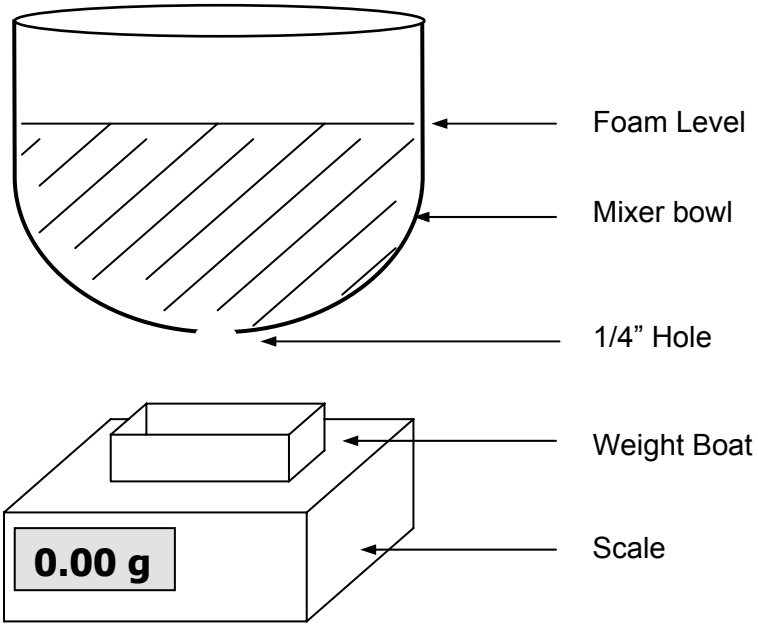

Figure 6. Schematic of foam drainage method. Weight and time are recorded as foam drains from hole in bottom of the mixer bowl.

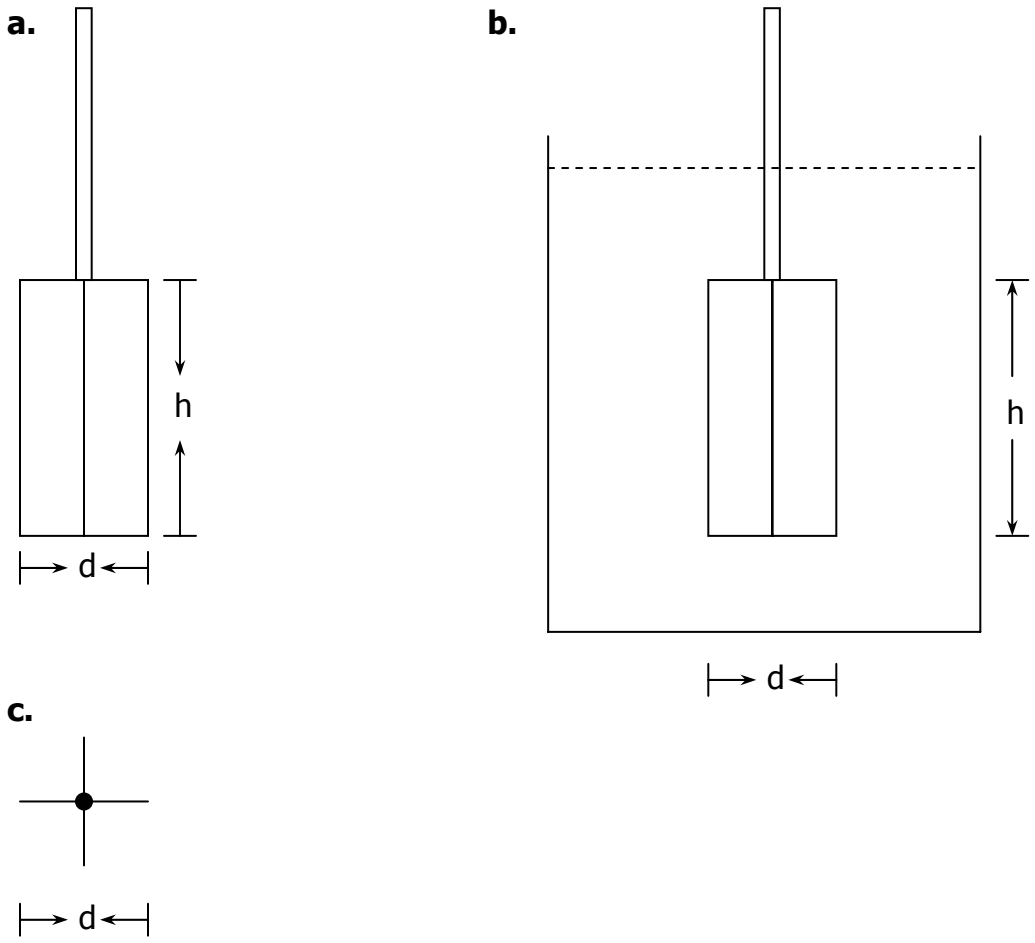

Figure 7. Four-bladed vane and vessel dimension requirements (Steffe, 1996).
a. side view of vane attachment
b. vessel dimensions
c. top view of vane

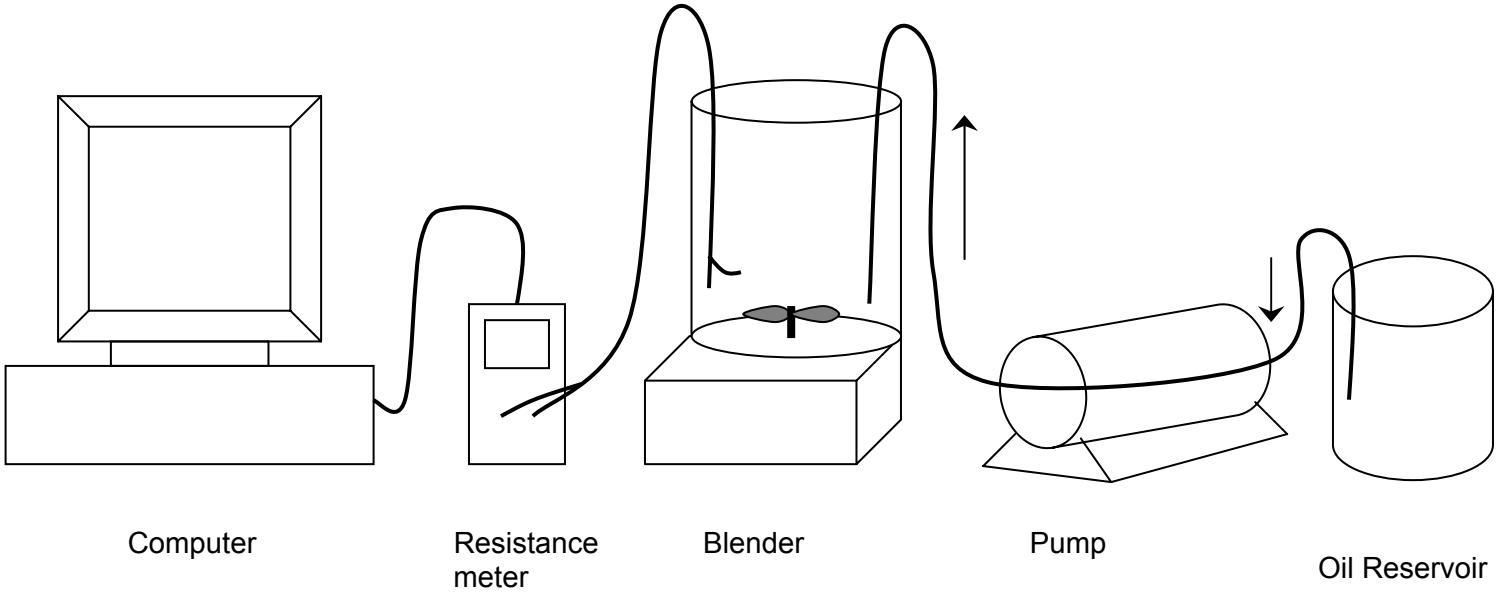

Figure 8. Schematic of emulsion forming and electrical conductivity measurement.

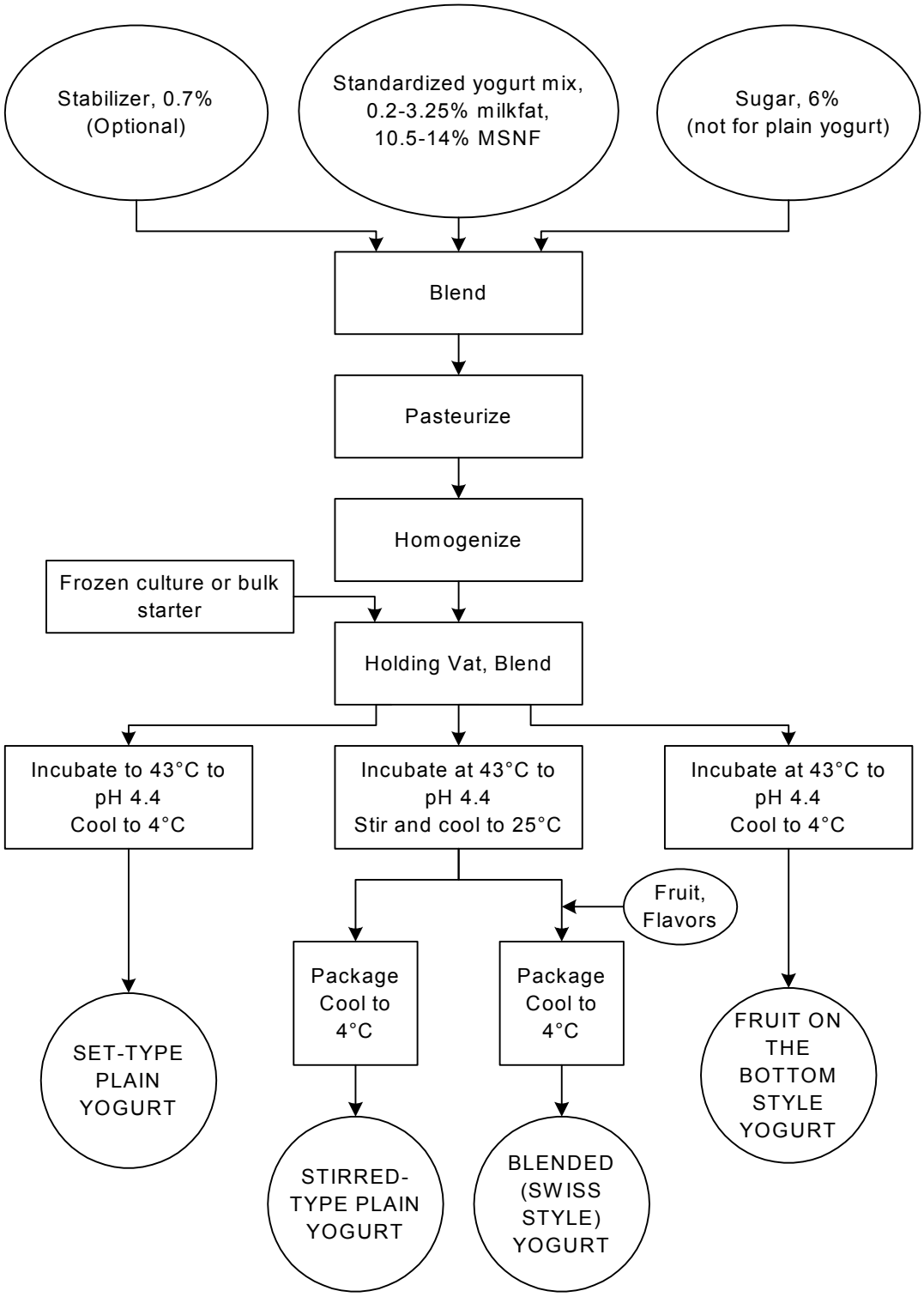

Figure 9. Process flow diagram for yogurt manufacture (Chandan, 1997).

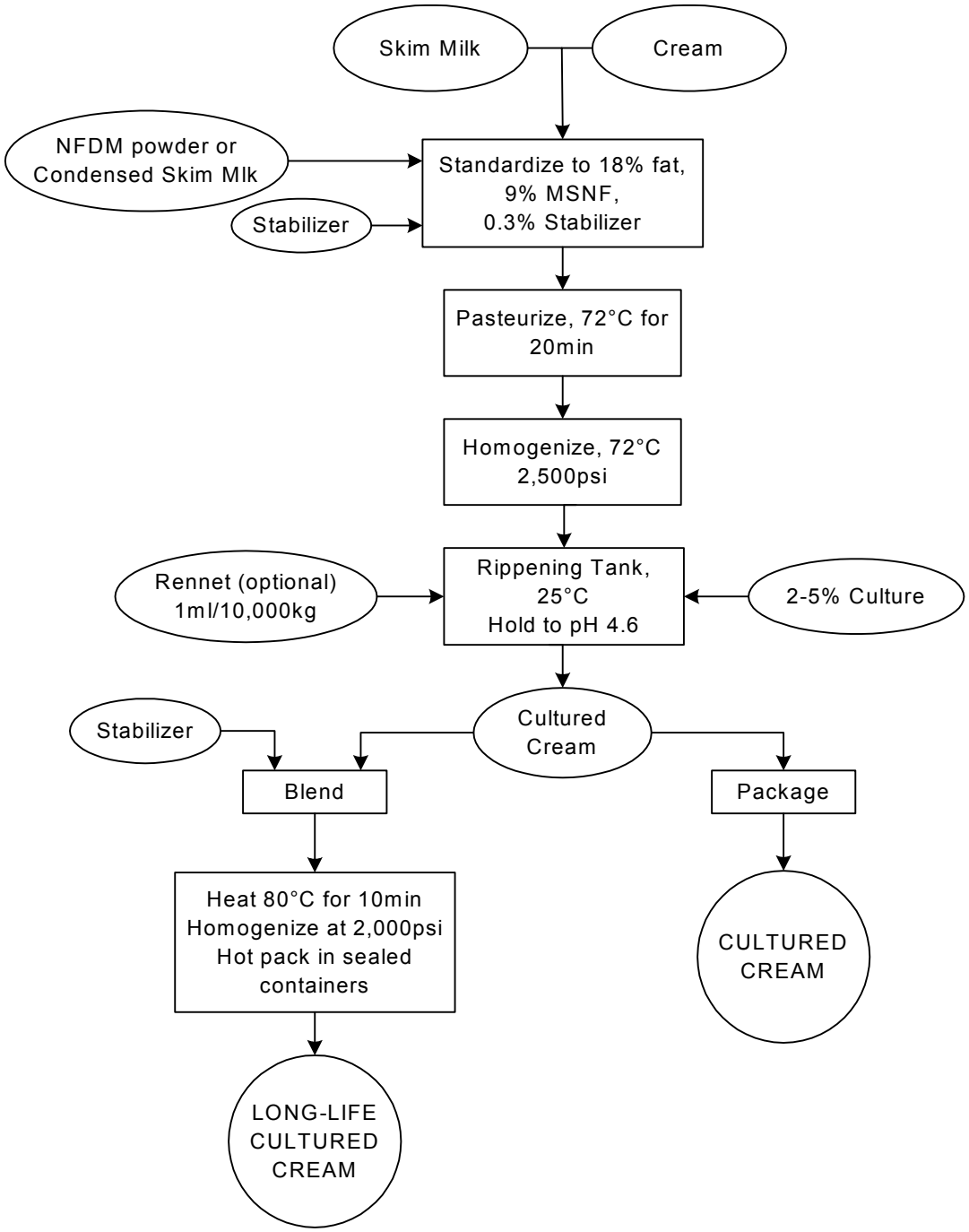

Figure 10. Process flow diagram for sour cream manufacture (Chandan, 1997).

PREPARATION AND CHARACTERIZATION OF A SPRAY DRIED DERIVATIZED WHEY PROTEIN INGREDIENT

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2.1 – INTRODUCTION

Whey proteins possess inherent functional properties such as emulsification, foaming, and gelation, which can be manipulated to meet the textural demands of many food applications (Hudson and Daubert, 2002; Kinsella and Whitehead, 1989). Specifically, heat induced gelation of whey protein is used to add texture to food products and has been researched extensively. However, heat induced gelation of whey protein has limited use. For instance, the strong gels imparted under neutral pH and salt conditions may prove incompatible with the desired texture of the food product. Additionally, for some systems, whey proteins may be unsuitable as food texturizers due to the heat treatments required to produce structure.

Gelation at ambient temperatures, termed cold-set gelation, broadens the function of whey proteins (Resch and Daubert, 2002). Cold-set gelation has been described by Barbut and Foegeding (1993), McClements and Keogh (1995), Nakamura *et al.* (1995), and Sato *et al.* (1995). These studies describe two-step processes for cold-set gelation. First, heat-denatured protein solutions were prepared at various pH and salt concentrations. Next, the heat-denatured solutions were cooled and made to thicken or gel through addition of salt (Bryant and McClements, 1998). Further studies by Thomsen (1994) and Elofsson *et al.* (1997) described a cold-gelling whey protein in dry form. This powder was produced through heat treatment during homogenization of whey protein concentrate at slightly alkaline pH, followed by drying. The dried powder then gelled upon reconstitution in a salt solution.

Hudson *et al.* (2001(a)) developed a process for creating a derivatized whey protein isolate capable of forming cold-set weak gel structures without the addition of salts or heat. The process involved preparation of a WPI solution, pH adjustment, thermal gelation, freezing, freeze-drying, and milling. While successful at producing a cold-gelling whey protein, the expense of WPI and freeze-drying limited a cost-effective scale-up. Resch and Daubert (2002) addressed the limited commercial viability of using WPI by applying the derivatization process to several lower cost whey protein concentrates (WPC). This study found that derivatized WPC powders imparted greater viscosity, water holding, and gelation at room temperature than unmodified WPC powders. Later, Resch and Daubert (2004) also developed a modification of the process of Hudson *et al.* (2001(a)) that incorporated spray drying in lieu of freeze-drying. Whey proteins modified by the new spray drying technique attained cold-set thickening and gelling behavior comparable to the powders modified by the original freeze drying method of Hudson, *et al*. (2001(a)) (Resch and Daubert, 2004).

Tomsen (1994) suggested that cold-gelling whey proteins in a dried form have potential applications in foods such as surimi, comminuted meats, dressings, and bakery products. Therefore, developing applications for the cold-gelling derivatized whey protein ingredient described by Hudson *et al.* (2001) would provide better options in formulation development. However, as Bryant and McClements (1998) have suggested, understanding the molecular basis of protein functionality is important when developing ingredients. The objectives of this study were to identify concentration dependence of derivatized whey protein isolate, and to characterize the functionality of the ingredient under various environmental conditions. Therefore,

this study served as a precursor to applications development with the goal of providing further understanding of how derivatized whey proteins responded to various protein concentrations, pH, and salt levels.

2.2 – MATERIAL AND METHODS

A commercial whey protein isolate (WPI) powder, containing approximately 93.1% (w/w) protein was used for all experiments (Bipro, produced by Davisco International Inc., Le Sueur, MN). The nitrogen content of WPI was analyzed by the Analytical Services Laboratory (Raleigh, NC) using a Perkin-Elmer PE 2400 CHN Elemental Analyzer (Perkin Elmer Corp., Norwalk, CT) and protein content was calculated from this value (N x 6.38) (Table 1). All chemicals, NaCl, NaOH, and HCl were purchased from Fisher Scientific Company (Norcross, GA).

2.2.1 – Derivatized Powder Production

Spray dried, derivatized whey protein isolate (dWPI) was produced according to the method of Resch and Daubert (2004). First, whey protein isolate powder was dispersed in deionized (DI) water to form an 8% (w/w) protein solution and allowed to stir for approximately 1hr. The pH was slowly adjusted to 3.35 with 6M HCl. The solution was transferred, for thermal treatment, to a double-jacketed steam kettle equipped with a scraped surface agitator. The solution temperature was increased to 80°C at approximately 5°C/min and held at 80°C with constant agitation for 1hr. Following heat treatment, the solution formed a semi-solid gel. The weak gel was transferred at a flow rate of 2 liter/hr by a peristaltic pump (Model 7553-80, Cole-Parmer Instrument Company, Vernon Hills, IL) to a pilot scale spray dryer (Anhydro,

Attlesboro Falls, MA) operating at inlet temperature of 88°C, outlet temperature of 32°C, and 15 psi at the nozzle.

The quality of the resulting dWPI powder was determined by subjecting a 7% (w/w) protein sample to shear rate ramps (0.1 – 100 s^{-1}) conducted on a StressTech Controlled Stress Rheometer (ReoLogica Instruments AB, Lund, Sweden). Samples with apparent viscosity less than 1 Pa s at 1 s^{-1} were deemed poor quality and discarded. Following quality analysis, all lots were combined into one stock dWPI powder, and the stock was stored in an airtight container prior to analyses.

2.2.2 – Concentration Effects

Nine concentrations (3 – 9% w/w protein) of the spray dried dWPI powder were each permitted to hydrate in DI water for ≥24 hrs at 5°C and subsequently equilibrated to room temperature prior to all rheological testing. All samples were presheared at 50 s^{-1} for 15 s followed by a 15 s equilibrium period prior to analysis in order to establish a consistent baseline strain history. All samples were analyzed at 25°C. Shear rate ramps (0.1 – 100 s^{-1}) for each sample were conducted on a Reologica StressTech Controlled Stress Rheometer and apparent viscosity of each sample at a shear rate of 50 s^{-1} was used for comparison.

2.2.3 – pH Effects

Three dWPI dispersions containing 9% (w/w) protein were prepared and the pH of each dispersion was adjusted to either 3.35, 4.0, or 5.0, prior to bringing the dispersion to final concentration with DI water. The dispersions were then permitted to hydrate in DI water for ≥24 hrs at 5°C and subsequently equilibrated to room

temperature prior to all rheological testing. Sample dilutions, 3 – 8% (w/w) protein, were prepared by diluting the 9% dispersions with DI water, and the pH of each dispersion remained relatively unchanged after dilution. Each diluted sample was subjected to shear rate ramps conducted on a StressTech Controlled Stress Rheometer using a serrated concentric cylinder geometry (CC25). Apparent viscosity was recorded at 25°C as shear rates were ramped from 0.10 to 100 s^{-1}, and sample viscosities at a shear rate of 50 s^{-1} were selected for comparison.

Additional comparisons between dWPI samples were made using oscillatory rheological methods. Prior to all oscillatory testing, a stress ramp was conducted at 0.05 Hz to define the linear viscoelastic region (LVR). Stress was ramped from 0.1 to 100 Pa while measuring the complex modulus, G* at 25°C. The stress at which linearity was lost was defined as the upper limit for the linear viscoelastic region (Figure 1).

Water holding capacity was determined by a modification of the centrifugation procedure of Fleming *et al*. (1974) in which a 30 g sample of each dispersion was centrifuged at 746 x g (2500 rpm) for 15 min. Upon removal from the centrifuge, the supernatant was carefully decanted, and the water held per gram of protein was calculated by weight difference (Equation 1.0).

$$\frac{\textit{water held}}{\textit{g protein}} = \left(\frac{\textit{pellet wt. (g) - dry powder (g)}}{\textit{dry powder (g)}} \times \textit{(\% protein in powder)}^{-1} \right) \qquad (1.0)$$

2.2.4 – Salt Effects

To determine salt effects on the physicochemical properties of the spray dried, derivatized ingredient, four 9.0% (w/w) protein dWPI dispersions were prepared by hydrating the dWPI powder in 0, 10, 50, and 100 mM solutions of NaCl. The pH of each dispersion was adjusted to 3.35 prior to bringing the dispersions to final weight with salt water. The 9.0% dispersions were hydrated overnight at 5°C and allowed to equilibrate to ambient temperature. In order to define the linear viscoelastic region, the 9.0% protein (w/w) dWPI dispersion containing no added salt was subjected to a stress sweep (0.1-100 Pa, 0.05 Hz, 25°C) using a StressTech Controlled Stress Rheometer with serrated, concentric cylinder geometry (CC25). Following this initial test, the complex viscosity of each sample was measured using a timed (300 s) oscillatory test at 0.05 Hz and 25°C.

2.3 – RESULTS AND DISCUSSION

Once the dWPI ingredient was manufactured the effects of concentration, pH, and salt were analyzed to promote understanding of performance for future food adaptation.

2.3.1 – Powder Production

Six hrs of spray drying yielded approximately 200 g of dWPI and was considered one run. At least 20 runs, or lots, were required to produce enough product for experimentation. The viscosity of each lot was compared to ensure that all samples had similar thickening ability. Figure 2 shows viscosity data of 7% protein (w/w) dispersions from various lots of dWPI, and various distribution profiles

were revealed. Samples with apparent viscosity less than 1 Pa s at $1s^{-1}$ were deemed of low thickening quality and therefore discarded. Figure 2 shows two general viscosity profiles between lots. Group 2 apparent viscosity was one order of magnitude less than group 1. Batch to batch differences were likely due to processing variations such as heating rate and time, spray drying conditions, and final moisture levels. Ideally, lots from group 2 would have been discarded, however, due to the amount of time and resources applied to each lot, all samples were combined.

After obtaining sufficient powder for experimentation, all accepted lots were sifted together into one stock powder to ensure uniformity throughout experimentation. The elemental composition of the blended derivatized whey protein ingredient was analyzed (Table 1), and total protein was calculated to be 90.4% (N x 6.38). This protein value was slightly lower than the unmodified WPI value (93.1%), most likely attributed to moisture differences between derivatized and unmodified WPI.

2.3.2 – Concentration Effects

Derivatization of WPI produces charged colloidal particles capable of instantaneous thickening and holding water (Hudson *et al.*, 2000). The protein particles are not water-soluble and therefore dispersions are created when reconstituted. Interaction of individual particles, resulting in thickening, is largely governed by concentration in solution. As expected, preliminary studies found that increasing the concentration of dWPI in dispersion increased overall viscosity (Figure 3). Increased viscosity was likely due to increased interaction, or collisions, between

colloids, as they were concentrated within solution. The dWPI dispersions display two concentration regimes: dilute and concentrated, similar to observations from starch literature (Bagley and Christianson, 1982; Rao and Tattiyakul, 1999). Additionally, settling was observed in 4% protein (w/w) dWPI dispersions but not in dWPI dispersions containing 9% protein (w/w). Given time, lower concentrations of the dispersed dWPI powder settled due to gravity. The 9% protein (w/w) dWPI dispersions most likely did not have visually noticeable settling because particles were in close contact with one another, contributing to a large increase in zero-shear viscosity, or the viscosity of the system at rest (Rao, 1999).

Figure 4 compares viscosity at 50 s^{-1} of dWPI dispersions ranging from 3% to 9% protein (w/w). This figure supports the hypothesis of a dilute and concentrated regime; dWPI dispersions from < 6.5% protein (w/w) being in the dilute regime, and dispersions > 6.5% in the concentrated regime. Effective concentration (C_o) was defined as the concentration at which a marked increase in viscosity began. More specifically, it defines the transition between dilute and concentrated regimes. To calculate C_o, two portions of each curve were identified and modeled with linear regression. The intercept of the two linear functions was quantified as C_o.

2.3.3 – pH Effects

As with all proteins, the basic structural units of dWPI are amino acids which can behave as either acid or base depending on environment (Swaisgood, 1996). The derivatization procedure produces colloids with surface groups containing amino acids capable of holding charge, and colloid surface charge is greatly affected by the pH of the suspending medium. When the pH of the suspending medium is different

from the isoelectric point (pI) of the colloids, the surface of the colloids becomes electrically charged. The charged surface attracts ions within the solution thus creating an electric double-layer that increases the effective size of the colloid (Hudson, 2001(b)) (Figure 5). Introducing an electric double layer increases the volume fraction of individual colloids, by essentially increasing their effective sizes, thus leading to an increased thickening function.

Effective concentration was measured at various pH levels to evaluate pH effects on dWPI dispersions. The effects of pH on dWPI dispersions are represented by trends in Figure 6. The effective concentration increased at pH levels closer to the isoelectric point of whey proteins (~5.2). Thus, as the surface charge of the colloids became more neutral, an overall decrease in complex viscosity was observed (Figure 7). The effective concentration for the sample at pH 5.0 was greater than 9% protein; Table 2 lists C_o values of dWPI dispersions at pH 3.35, 4.0, and 5.0.

Water holding capacity (WHC) was measured to further evaluate the effects of pH on dWPI (Figure 8). Dispersions of 6.5 and 9.0% protein (w/w) dWPI at pH 3.35 held approximately 9 and 8 grams of water per gram protein, respectively. Water holding capacity decreased slightly, yet significantly ($p < 0.05$), at pH 4.0 and 5.0. The WHC of 9.0% protein (w/w) dispersions was always lower than that of 6.5% regardless of pH, as less water was available for adsorption in the higher concentration dispersions.

2.3.4 – Salt Effects

Adding salt, or ions, to protein solutions can act to neutralize charges on the surface of proteins (Damodaran, 1996). This screening of charges can affect the solubility of the protein. Increasing salt concentration from 0 to 100mM NaCl decreased the complex viscosity of 6.5% protein (w/w) dWPI dispersions (Figure 9) approximately one order of magnitude. The increased salt concentration likely resulted in a loss of protein solubility, thus leading to an overall decrease in viscosity (Damodaran, 1996). Higher salt levels potentially favor conditions in which the proteins will salt out of solution, thus leading to aggregation and lowering viscosity.

Water holding capacity trends for salt solutions were similar to pH trends (Figure 10). As salt concentration increased, a loss in WHC was observed. A 9% protein (w/w) dWPI dispersion held approximately 9 g of water per gram protein at native pH (3.35), whereas the same solution with 100mM NaCl held only 6 g of water per gram protein.

2.4 – CONCLUSIONS

Final properties of derivatized whey protein isolate were highly dependent on processing conditions. Viscosity imparted by the spray dried dWPI ingredient was dependent on concentration, and the effective concentration of spray dried dWPI was approximately 7% protein (w/w). Effective concentration increased, and water holding and viscosity decreased as the pH of dWPI dispersions approached the pI of whey protein (~5.2). Furthermore, water holding and viscosity also decreased as salt concentration of dWPI dispersions increased. Understanding these properties

provides a basis from which to make recommendations for application of the derivatized whey ingredient.

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Table 1. Elemental analysis of spray dried dWPI.

Mineral	Amount in spray dried dWPI
N	14.17%
P	0.05%
K	0.09%
Ca	0.08%
Mg	0.01%
Na	7220ppm

* dWPI Moisture = 5.91% (±0.4)
** Nitrogen of unmodified WPI = 14.59%

Table 2. Effective concentration (C_o) of dWPI at pH 3.35, 4.0, and 5.0.

pH	Effective Concentration (% protein w/w)
3.35	6.82
4.0	7.98
5.0	> 9.00

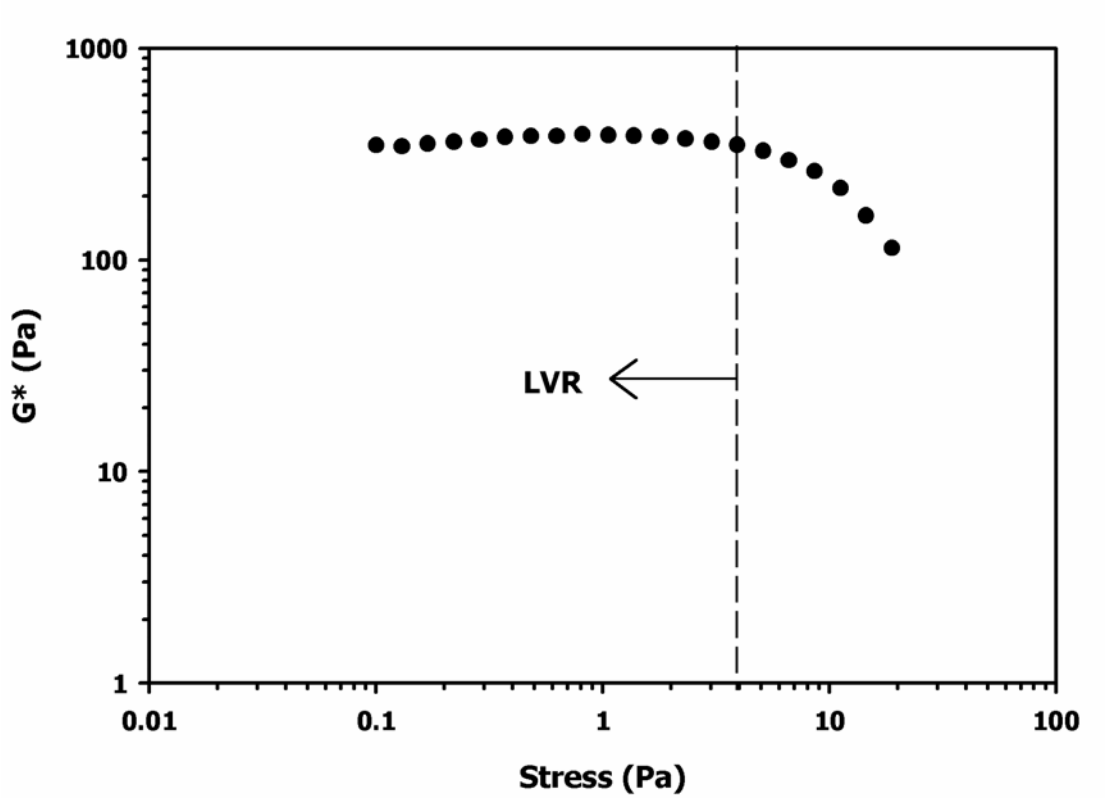

Figure 1. Complex modulus, G*, of a 9.0% protein (w/w) spray dried dWPI dispersion as stress was increased from 0.1 to 10 Pa (0.05Hz) at 25°C.

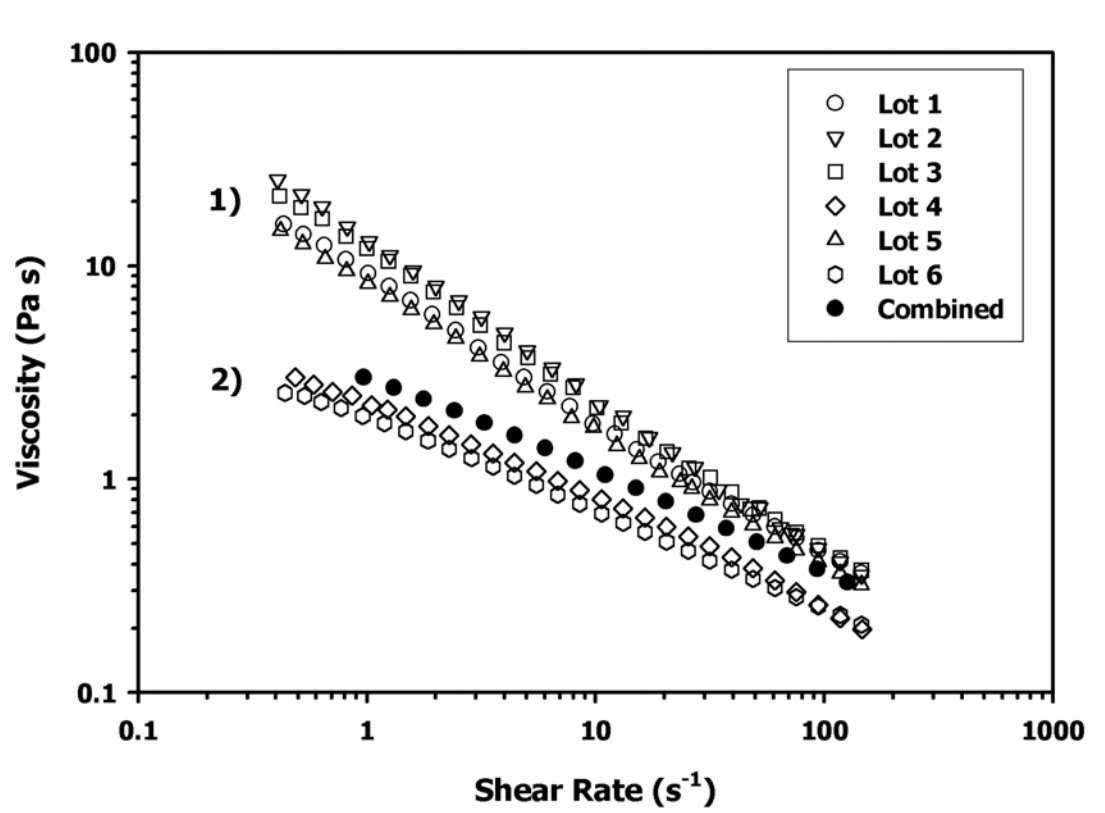

Figure 2. Apparent viscosity of various lots of 7% protein (w/w) spray dried dWPI dispersions as shear rates were ramped from 0.1 to 100 s^{-1} at 25°C.

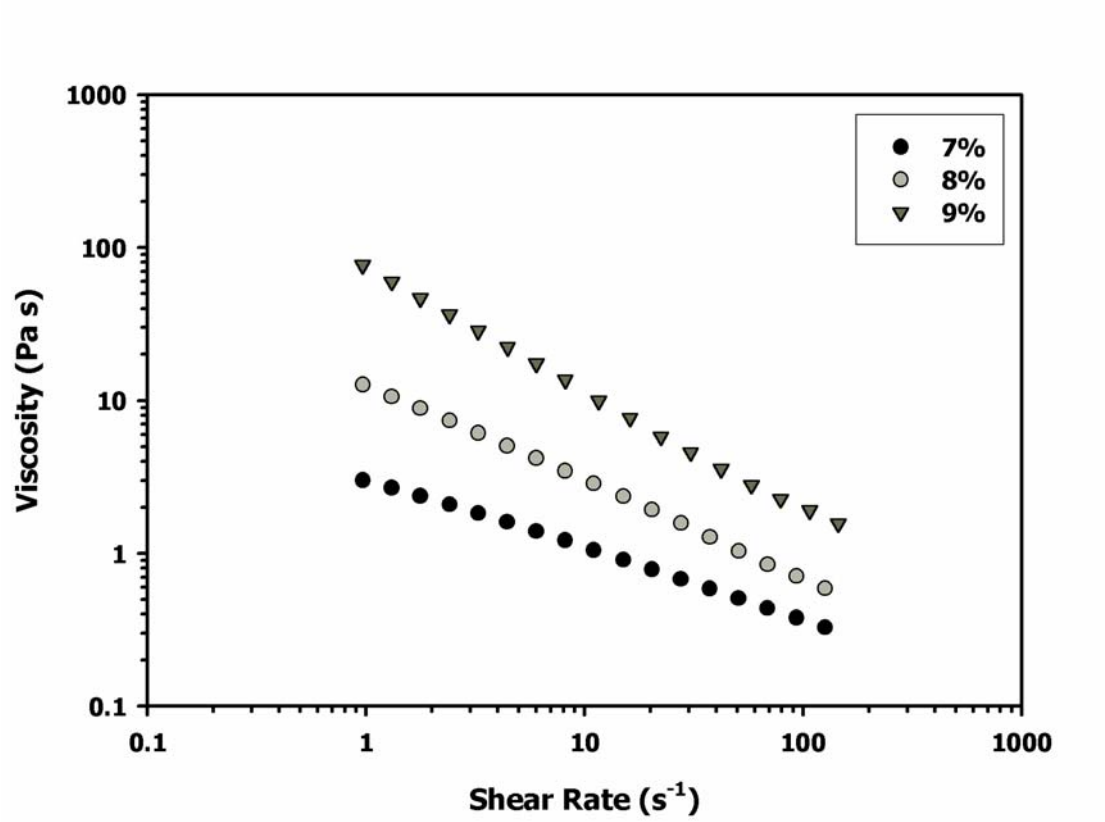

Figure 3. Apparent viscosity of dWPI at 7, 8, and 9% protein (w/w). Temperature was 25°C and pH was 3.35.

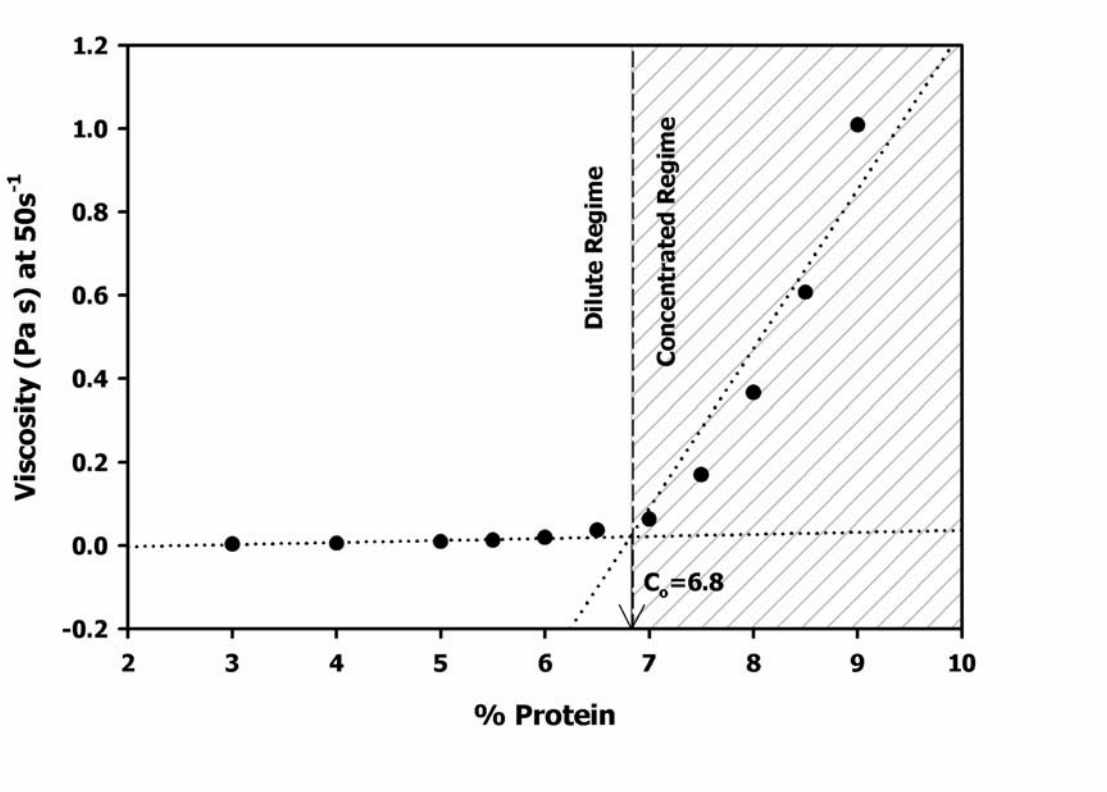

Figure 4. Apparent viscosity at 50 s^{-1} of 3 to 9% protein (w/w) spray dried dWPI dispersions at 25°C. pH was 3.35.

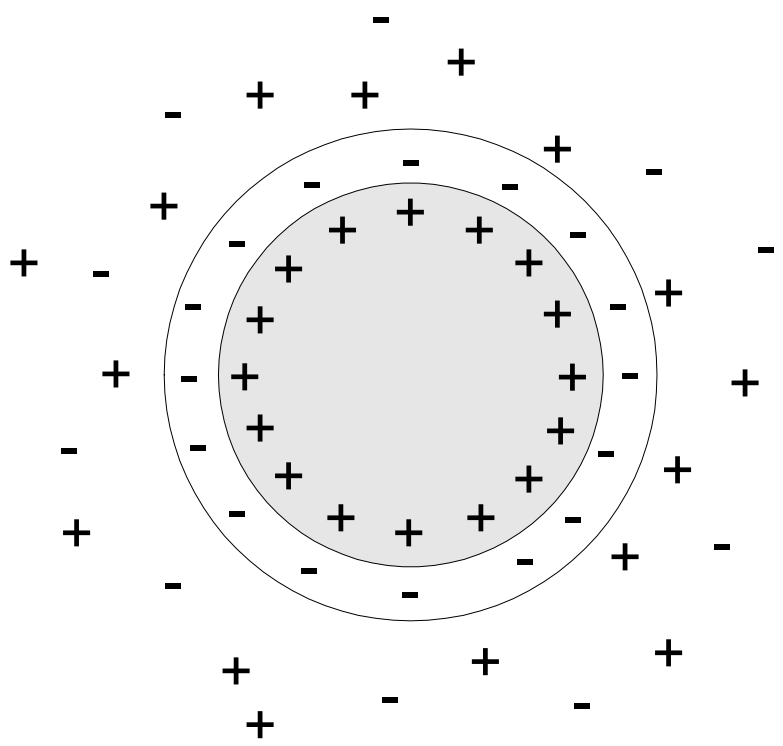

Figure 5. Illustration of the electrical double layer theory (Hudson 2001(b)).

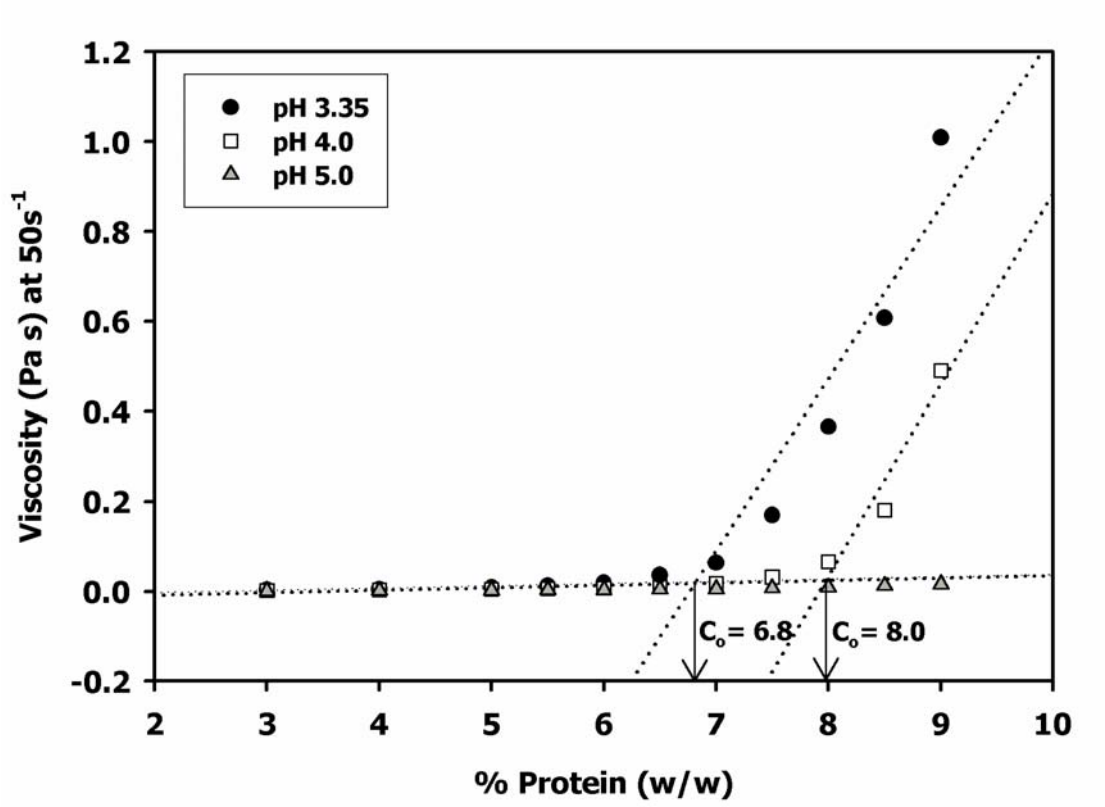

Figure 6. Apparent viscosity at 50 s^{-1} of 3 to 9% protein (w/w) spray dried dWPI dispersions at 25°C.

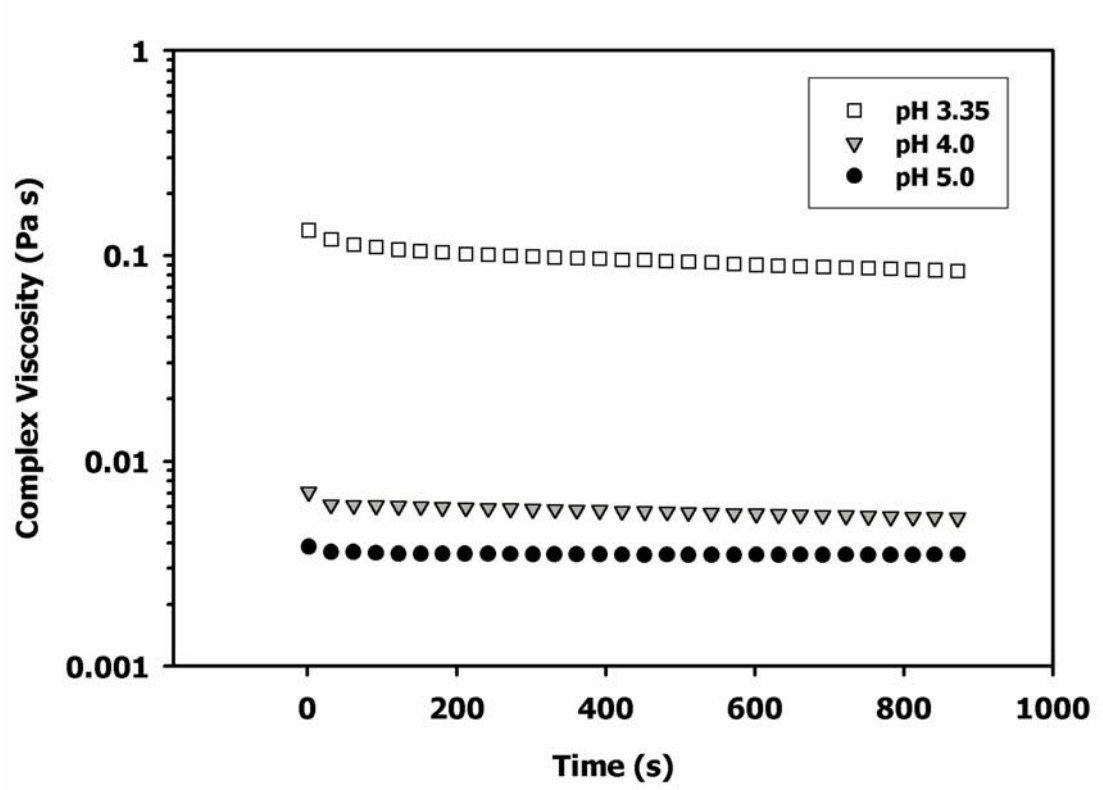

Figure 7. Complex viscosity of 6.5% protein (w/w) spray dried dWPI dispersions at 1 Pa, 0.05Hz, and 25°C.

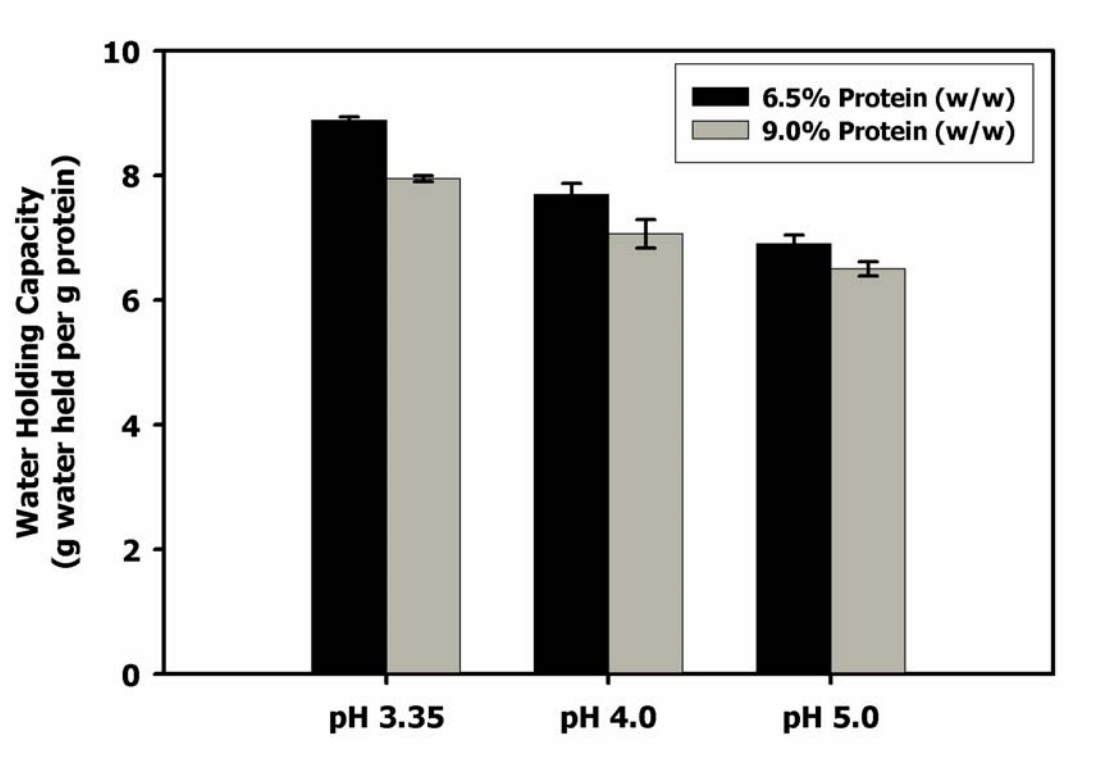

Figure 8. Water holding capacity (WHC) of 6.5 and 9.0% protein (w/w) spray dried dWPI dispersions at 5°C.

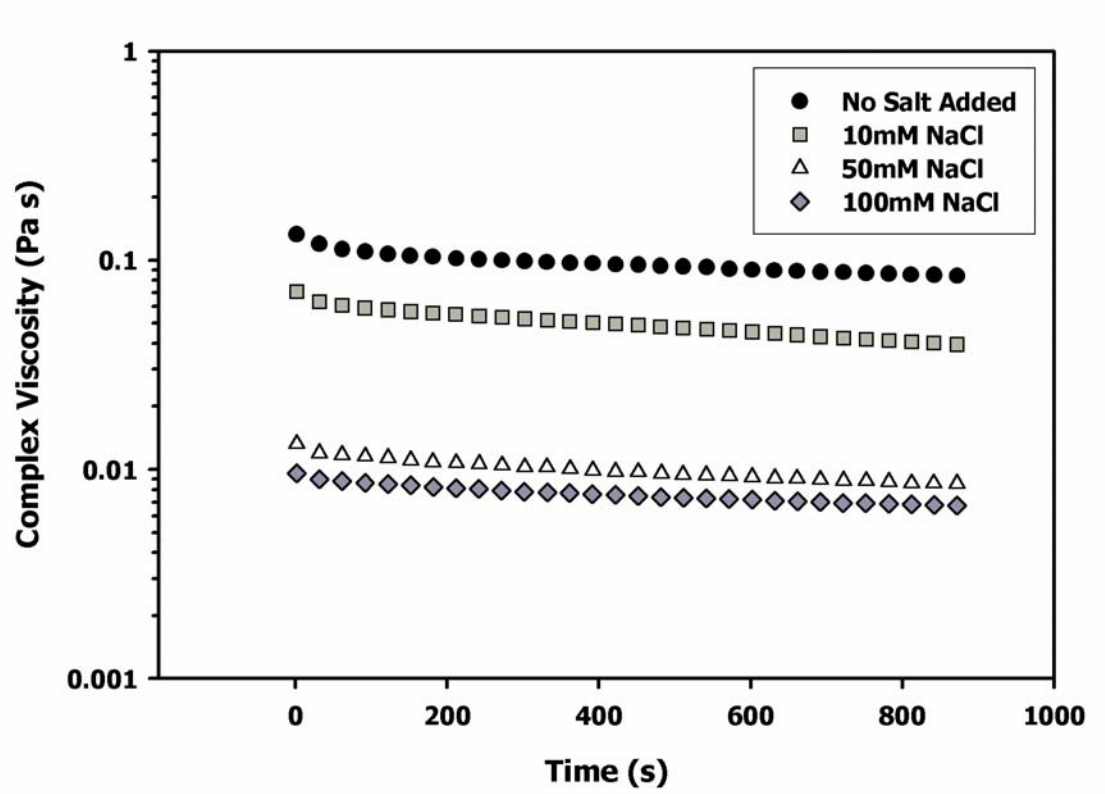

Figure 9. Complex viscosity of 6.5% protein (w/w) spray dried dWPI dispersions at 1 Pa, 0.05 Hz, 25°C, and pH 3.35.

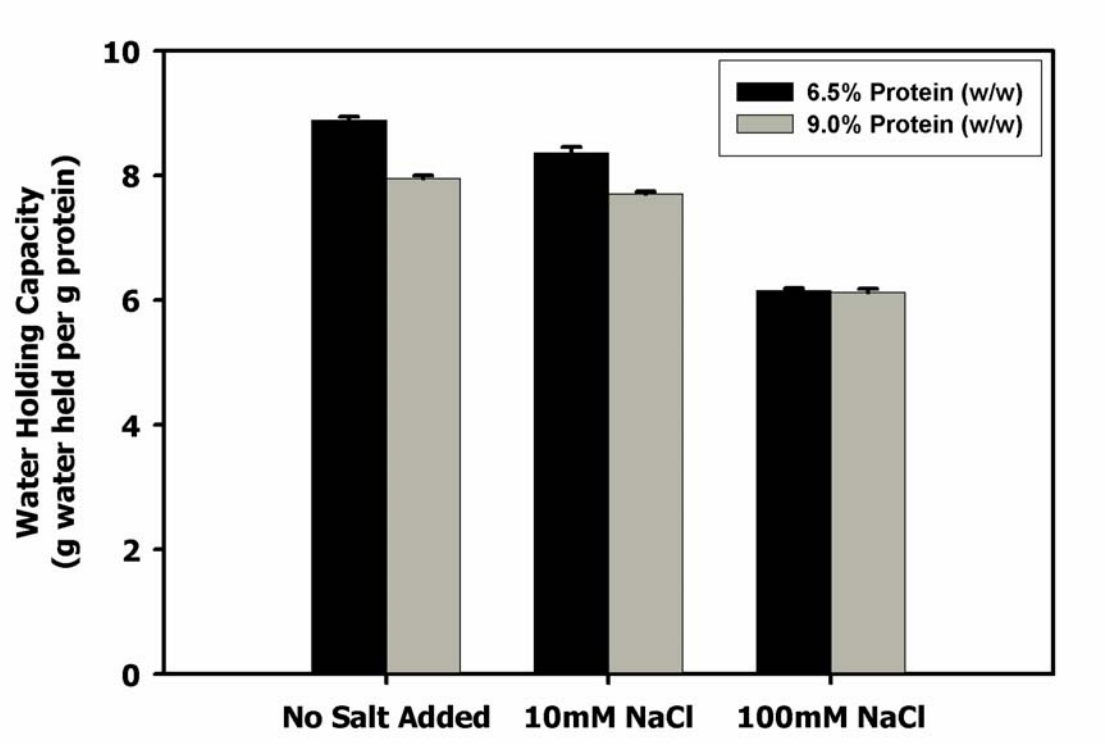

Figure 10. Water holding capacity (WHC) of 6.5 and 9.0% protein (w/w) spray dried dWPI dispersions at 5°C.

EMULSIFYING AND FOAMING PROPERTIES OF A DERIVATIZED WHEY PROTEIN INGREDIENT

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3.1 – INTRODUCTION

Protein functionality results from physico-chemical characteristics and interactions of proteins with other components in food systems (Kinsella, 1981). This collective term is related to reactions occurring in solutions, at interfaces, and during network formation (Foegeding *et al.,* 1998). For example, formation and stabilization of emulsions and foams depends on protein interactions with water and oil or water and air (Kinsella, 1981). Successful application of a protein ingredient can be optimized by a detailed characterization of functional behavior. Therefore, evaluating functionality is necessary to further expand applications for a new dairy protein ingredient.

Whey proteins are 'label-friendly' dairy ingredients widely used for the formation and stabilization of food emulsions and foams (Kinsella and Whitehead, 1989; Bryant and McClements, 1998; Dickinson, 1999). The ability of whey proteins to serve as emulsifying or foaming agents depends on their ability to adsorb at water-oil or water-air interfaces and prevent droplet coalescence (Walstra, 2003; Dalgleish, 1996). Surface-active functional performance of whey proteins is directly linked to protein structure, which varies based on several environmental factors (pH, ionic strength, and protein concentration). Separation techniques such as ultrafiltration and ion-exchange also affect the composition and functional properties of whey protein products (Huffman, 1996; Cayot and Lorient, 1997).

Emulsifying properties are important to many food applications, and several authors characterized emulsifying properties of whey proteins (McClements *et al*.,

1993; Hunt and Dalgleish, 1994; Dickinson *et al*. 1989; Dickinson, 1997; Demetriades *et al.,* 1997; Das and Kinsella, 1989). An emulsion is a suspension of two immiscible liquids. Emulsifying properties are commonly discussed in terms of emulsifying capacity (EC) and emulsion stability (ES) (Pearce and Kinsella, 1978), and various methods have been applied to measure both properties.

Emulsifying capacity is a qualitative term used to describe the amount of oil a protein can adsorb to stabilize a 2-phase system. Swift *et al.* (1961) developed an approach for evaluating EC in model food systems. The basic method consisted of blending a protein slurry with oil to form an oil-in-water emulsion. Additional oil was added to the system until the emulsion collapsed or inverted to a water-in-oil emulsion and EC was reported as the amount of oil added per 100 mg protein. Adopting this approach, Chobert *et al.* (1988) compared the emulsifying capacity of whey protein concentrate (WPC) hydrolyzed at various levels by trypsin. Emulsifying capacity of unhydrolyzed WPC was almost constant from pH 1.0-9.0, with a slight decrease in EC near the isoelectric pH (~5.2). Emulsifying capacity improved after enzymatic treatment of the proteins.

Food emulsions are thermodynamically unstable, and given enough time, will collapse as the two phases attempt to minimize contact area (Rousseau, 2000). Emulsion stability has been empirically characterized by a number of authors (Demetriades and McClements, 1998; Chobert, *et al.* 1988; Imm and Regenstein, 1997) to quantify the extent of creaming (or settling) in an emulsion. Creaming occurs due to differences in density between the two phases under the influence of gravity, leading to phase separation (Rousseau, 2000). One method described by

Demetriades and McClements (1998) requires measurement of cream and serum layer height, and relating the two values through calculation of a creaming index. The creaming index was correlated with emulsion stability to provide indirect information about the extent of droplet aggregation in an emulsion (Demetriades and McClements, 1998). Kulmyrzaev *et al.* (2000) used this method to study the influence of pH on droplet creaming of whey protein emulsions. This study found that oil-in-water emulsions were stable to creaming at low and high pH, but were less stable at a pH close to the isoelectric pH of whey protein.

Whey protein ingredients are also utilized for foam forming and stabilizing properties (Dickinson, 1999). Molecular factors affecting the ability of a protein to form and stabilize a foam are similar to those for emulsion formation (Cayot and Lorient, 1997). Protein foams are dependent on protein surface activity and film forming properties (Phillips *et al.,* 1990), which are governed by protein structure. Characterization of liquid foams is difficult because the structure is complex, delicate, and often unstable (Pernell *et al.,* 2000). Prud'homme and Khan (1996) suggested the use of a vane device for measuring yield stress of foams. A vane is a bladed, rheological device intended to minimize slip and sample disturbance. Numerous researchers applied this method to describe yield properties of foods (Yoo *et al*., 1995; Daubert *et al*., 1998; Tung *et al*., 1990). Pernell *et al.* (2000) successfully applied the vane method to relate egg white and whey protein foam yield stress to "stiffness."

Foam stability is related to drainage of liquid from the foam (Richert, 1979) and can be characterized by determining the amount of time required to attain 50%

drainage by weight (Phillips *et al*., 1990). Using this method, Yankov and Panchev (1996) found that WPC produced foams more stable than sour milk protein concentrate and "bulstartprotein." An additional property of foams is overrun. Foam overrun is determined by density and measures how much air is incorporated during foam formation. Mild heat treatment has been reported to increase the overrun of WPC foams, but excessive heating leads to a decrease in foam volume (Richert *et al.,* 1974).

Emulsifying and foaming properties of whey proteins are improved by partial protein unfolding leading to additional exposed hydrophobic regions (Kinsella and Whitehead, 1989). Processing-induced structure modifications affect protein functionality. For example, Resch and Daubert (2004) described a whey protein modification process involving a thermal treatment at low pH to form a weak gel, then spray drying the gel to a powder. The spray dried derivatized whey powders imparted greater viscosity and water holding when rehydrated at room temperature than unmodified whey powders – a functionality improvement. The goal of this research was to determine emulsifying and foaming ability of derivatized whey protein isolates (dWPI), thus further characterizing functionality of the protein ingredient. Understanding foaming and emulsifying properties of dWPI will provide insight for potential, functional roles in food formulations.

3.2 – MATERIAL AND METHODS

A commercial whey protein isolate (WPI) powder, containing approximately 93.1% (w/w) protein was used for all experiments (Bipro, Davisco International Inc.,

Le Sueur, MN). The elemental content of WPI was analyzed by the Analytical Services Laboratory (Raleigh, NC) using a Perkin-Elmer PE 2400 CHN Elemental Analyzer (Perkin Elmer Corp., Norwalk, CT). The protein content was calculated on a nitrogen basis (N x 6.38). All chemicals, NaCl, NaOH, and HCl were purchased from Fisher Scientific Company (Norcross, GA) and corn oil was purchased from a local supermarket.

3.2.1 – Derivatized Powder Production

Whey protein isolate powder was dispersed in deionized (DI) water to form an 8% (w/w) protein solution and allowed to stir for approximately 1hr. The pH was slowly adjusted to 3.4 with 6M HCl. The solution was transferred, for thermal treatment, to a double-jacketed steam kettle equipped with a scraped surface agitator. The solution temperature was increased to 80°C at approximately 5°C/min and held at 80°C with constant agitation for 1hr. Following heat treatment, the solution had formed a semi-solid gel. The gel was transferred at a flow rate of 2 liter/hr by a peristaltic pump (Model 7553-80, Cole-Parmer Instrument Company, Vernon Hills, IL) to a pilot scale spray dryer (Anhydro, Attlesboro Falls, MA) operating at inlet temperature of 88°C, outlet temperature of 32°C, and 15psi at the nozzle. Approximately 20 replications of this procedure were required to produce enough powder for experimentation.

A sample (7% w/w protein) if the dWPI powder produced during each run was subjected to a shear rate ramp (0.1 – 100 s^{-1}) conducted on a StressTech Controlled Stress Rheometer (ReoLogica Instruments AB, Lund, Sweden). Samples with apparent viscosity less than 1 Pa s at $1s^{-1}$ were of poor quality and discarded.

Following quality analysis all accepted lots were combined into one stock dWPI powder, and the stock was stored in an airtight container prior to analyses.

3.2.2 – Emulsifying Capacity

Emulsifying capacity (EC) determinations were made using a modified method described by Webb *et al.* (1979). Whey protein isolate (WPI) and dWPI solutions (0.001, 0.003, 0.005, 0.010, 0.015 % protein, w/w) were prepared by dispersing the powders in DI water. Minor pH adjustments were made prior to final dilution, to ensure a solution pH of 3.4. A 150 ml aliquot of each sample was added to a commercial Waring blender (Model 5011, Waring Products, Inc., New Hartford, CT) equipped with two electrodes placed such that each was in continuous contact with the solution during the blending operation (Figure 1). The blender was always operated on HI speed (22,000 RPM with no load). During blending, corn oil was added continuously at a rate of 1ml per second (Peristaltic Pump Model 7553-80, Cole-Parmer Instrument Company, Vernon Hills, IL) through a Tygon tube (7 mm diameter) positioned such that the dispensing end was in close proximity to the blender blades. This location prevented the accumulation of sizable quantities of oil on top of the developing emulsion. The oil temperature was maintained at approximately 23°C throughout the experiment. Emulsion formation and inversion was monitored by measuring resistance with a multimeter (Omega HHM26, Omega Engineering, Stamford, CT) equipped with RS232 output to a computer. Inversion of the emulsion was clearly defined as the point at which resistance abruptly spiked. Each sample was run in triplicate, and the amount of oil added was calculated by time and flow rate measurement. The average ml oil emulsified per 100 mg protein

was reported as emulsifying capacity. A blank containing only water was also tested to allow for correction of EC values.

3.2.3 – Emulsion Stability

Stock solutions containing DI water and 5mg/ml protein (WPI or dWPI) were prepared. Sodium azide (0.04% w/w) was added to each stock solution to prevent microbial growth, and then the pH of each solution was adjusted to 3.4 or 6.8 prior to final volume adjustment. Equal amounts of corn oil and protein solution (25 ml each) were homogenized in an Omni Mixer Homogenizer (Model 17105, Ivan Sorvall, Inc., Newtown, CT) at speed 3.5 (approx. 5,000 rpm) for 2 min.

Emulsion stability was measured according to the method of Keowmaneechai and McClements (2002(a)). Ten grams of each emulsion was placed in a glass test tube (internal diameter 16mm and height 100mm) and then stored at ambient temperature for 14 days. After storage, all of the emulsions separated into three layers, with a creamed layer at the top, a transparent serum layer at the bottom, and an emulsion layer in the middle. The total height of the emulsions (H_e) and the height of the serum (H_s) were measured in triplicate. The creaming index was reported as:

$$\textit{Creaming Index} = 100 \times \left(\frac{H_s}{H_e} \right) \qquad (1.0)$$

3.2.4 – Foaming

Stock solutions containing 6.5% (w/w) protein were prepared using unmodified WPI or the dWPI ingredient. Prior to final weight adjustment with DI water, the pH of each solution was adjusted to either 3.4 or 6.8 with 1M NaOH and 1M HCl. Solutions were stored overnight at 4°C and allowed to equilibrate at room temperature under mild agitation for at least 1 hr prior to foaming experiments.

To minimize sedimentation a Haake VT550 viscometer (Karlsruhe, Germany) equipped with a Haake pitched-paddle impeller (blade diameter = 0.04143 m, blade height = 0.02692 m) was adopted. Applying the matching viscosity method (Steffe, 1996), an apparent viscosity was calculated for each solution. Apparent viscosity readings were obtained over a 2 min interval while shearing the sample at 50 s^{-1} (100 rpm).

Foams were formed using a Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, MI) with a 4.5 qt (4.3 L) stationary bowl and rotating beaters. Solutions (200 ml) were whipped at speed setting 8 (planetary rpm of 225 and beater rpm of 737) for 20 min. At least three replications were made for each treatment.

Yield stress of each foam was measured in triplicate, using vane rheometry according to the method of Pernell *et al.* (2000). The appropriately-sized vane was used with a Brookfield YR-1 (Brookfield Engineering Laboratories, Inc. Middleboro, Massachusetts) viscometer, set to rotate at a speed of 0.3 rpm. Upon completion of whipping, the beaters were carefully removed, and the vane was lowered to the proper measurement position. The maximum torque response (M_o) was recorded for

each measurement, and these readings were used to calculate yield stress according to the single point method of Dzuy and Boger (1983 and 1985) and Steffe (1996):

$$\sigma_o = \frac{2M_o}{\pi d^3}\left(\frac{h}{d}+\frac{1}{3}\right)^{-1} \qquad (2.0)$$

where σ_o is the yield stress, and h and d are the height and diameter of the vane tool.

Overrun is a measurement of how much air is whipped into a system. For this study, a standard weigh boat of known volume was filled with the protein solution prior to whipping. The weight of this volume of solution was recorded, and the solution was whipped as outlined above. Following foam formation, the foam was gently scooped from the bowl in a circular pattern with a rubber spatula, filling the same weigh boat. Four replications were made for each foam, and the mean was used to calculate overrun according to Campbell and Mougeot (1999):

$$\%\ Overrun = \frac{(wt\ dispersion) - (wt\ foam)}{wt\ foam} \times 100 \qquad (3.0)$$

Foam drainage was measured to better understand the stability of foams over time. Foams were formed as previously outlined with slight modification to the bowl. A 0.25" hole was drilled into the underside of the bowl and sealed during whipping. After foam formation, the bowl was positioned on a ring stand with the hole directly over a scale, tared with a weigh boat. The bowl was marked so that the hole position on the ring stand was consistent throughout each replication. The weight of liquid

drained from the foam was measured every 5-10 min for 1 hr. Percent drainage was calculated as:

$$\frac{wt\ of\ solution\ drained\ from\ foam}{wt\ of\ solution\ prior\ to\ foaming} \times 100 \qquad (4.0)$$

3.3 – RESULTS AND DISCUSSION

3.3.1 – Emulsifying Capacity

Emulsifying capacity of aqueous solutions of dWPI and WPI was determined by electrical resistance. Figure 2 shows three replications of a 1mg/ml WPI solution. Continuous resistance measurement allowed a precise detection of the inversion point from an oil-in-water (o/w) emulsion to a water-in-oil (w/o) emulsion. This inversion point is seen as a spike in resistance, since water, the continuous phase in an o/w emulsion, conducts electricity better than oil. At least three replications were made, and the average of these measurements is reported as emulsifying capacity (ml oil emulsified/100 mg protein). There was not a significant difference ($p < 0.05$) between EC of dWPI and WPI (Figure 3). Emulsifying capacity was dependent on pH, showing greater emulsifying capacity at pH 6.8 than at pH 3.4.

3.3.2 – Emulsion Stability

Table 1 presents apparent viscosity of 6.5% (w/w) protein WPI and dWPI solutions. At 6.5% (w/w) protein, the derivatized whey ingredient displayed viscosities more than twice those of unmodified WPI. Prior to emulsion stability measurement, the derivatized whey ingredient was hypothesized to improve

emulsion stability by increasing the viscosity of the continuous phase, thereby limiting oil droplet flocculation. Visual inspection of emulsified samples after seven days storage at 25°C showed very little phase separation or creaming. Kulmyrzaev et al. (2000) reported similar results for WPI at pH 3.4 and 6.8. The samples were allowed to settle for an additional seven days to see if differences occurred over longer storage periods. On day 14 the height of the emulsion (H_E) and serum (H_S) layers were measured, and creaming index was calculated (Figure 4). The creaming index of WPI and dWPI was similar (approx. 25%) at pH 3.4. However, at pH 6.8 the WPI sample showed nearly twice as much creaming as dWPI. Most likely, the elevated viscosity of the dWPI solution reduced the capacity for oil droplet flocculation, there-by increasing the overall stability to creaming (Keowmaneechai and McClements, 2002(b)).

3.3.3 – Foaming

The drainage of a foam over a fixed time is used to define stability (Halling, 1981). At pH 3.4 and 6.8, foams produced from 6.5% protein (w/w) dWPI displayed greater stability than unmodified WPI foams of the same concentration (Figure 5). In the case of unmodified WPI, at least 80% of the original foam volume drained after 60 min, whereas dWPI foams showed less than 5% drainage over the same period. The pH of the foaming solution also affected stability. Unmodified WPI at pH 3.4 was less stable ($p<0.05$) than unmodified WPI at pH 6.8. Derivatized whey protein samples showed similar, less pronounced trends. Halling (1981) suggested that foam expansion is often higher for more rapidly draining foams. Increased foam stability may be due to differences in foam expansion, or overrun. Therefore, our next step measured the density, or overrun of dWPI and WPI foams.

Derivatized whey protein foams demonstrated lower overrun than WPI foams independent of pH (Figure 6). This result supported the earlier hypothesis that high foam expansion (or low density) leads to low foam stability. However, an exception to this hypothesis was seen in the dWPI sample at pH 6.8. Overrun of the dWPI sample at pH 6.8 was significantly higher ($p<0.05$) than dWPI at pH 3.4. In other words, the sample at pH 3.4 was more dense than the sample at pH 6.8. According to the hypothesis, the dWPI sample at pH 6.8 should be less stable, and drain faster. However, according to Figure 5, the dWPI sample at pH 6.8 was more stable to drainage than the dWPI sample at 3.4. A visually noticeable difference between stiffness of dWPI foams at pH 3.4 and pH 6.8 was noted during this experiment. The dWPI samples at pH 6.8 produced very stiff peaks after the 20 min of whipping required for this test. Further tests were therefore required to quantitatively characterize physical foam characteristics.

In the current study, the vane method was used to compare the physical characteristics of dWPI and WPI foams at pH 3.4 and 6.8. The yield stress of the WPI foams were hypothesized lower than dWPI foams due to overall differences in density or overrun. However, this hypothesis did not hold entirely true. Yield stress of WPI foams at pH 3.4 and 6.8 were approximately 40 Pa (Figure 7), and derivatized whey foams at pH 3.4 also had yield stress values of approximately 40 Pa. The dWPI foams at pH 6.8 had significantly higher ($p<0.001$) yield stress than all other samples. Possibly, the heat denaturation of the dWPI ingredient, along with increased disulfide-sulfide interchange at pH 6.8 improved protein-protein interactions at the air/water interface. Increased protein-protein interactions would

improve the elasticity of the continuous liquid phase of the foams, therefore increasing yield stress.

3.4 – CONCLUSIONS

Derivatized whey protein isolates are capable of stabilizing foams and emulsions. Emulsifying capacity of dWPI and WPI were similar at pH 3.4 and pH 6.8. However, dWPI creates a more stable emulsion than WPI at pH 6.8. This study showed that derivatized whey protein isolates create foams that are more stable and have lower over run than unmodified WPI foams at pH 3.4 and 6.8. Unlike WPI foams, dWPI foam stiffness can be improved through pH adjustment. Results from this study should facilitate future formulations using the derivatized whey ingredient.

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Table 1. Apparent viscosity of 6.5% (w/w) protein WPI and dWPI solutions or dispersions at 25°C.

Ingredient	pH	Apparent Viscosity[a] at 50 1/s (mPa s)
WPI	3.4	3.29 ± 0.23
	6.8	3.71 ± 0.54
dWPI	3.4	8.92 ± 0.40
	6.8	7.46 ± 0.54

[a] Mean ± standard deviation

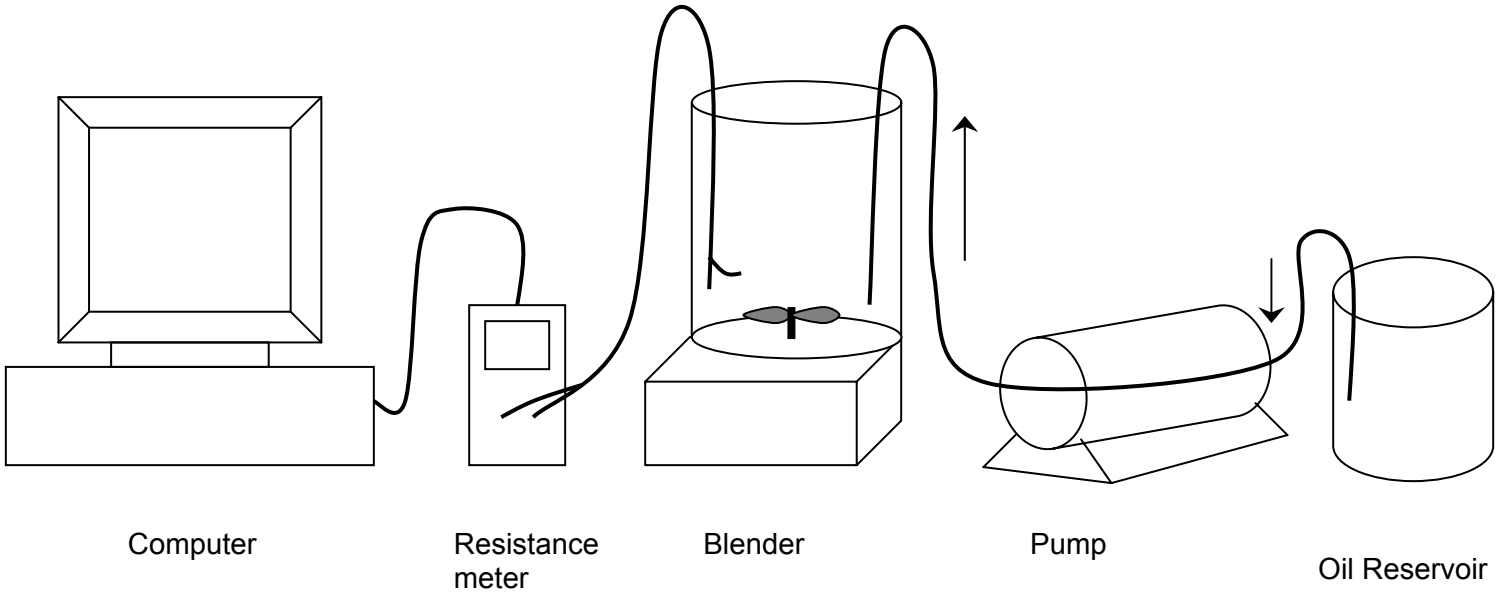

Figure 1. Schematic of emulsion forming and electrical conductivity measurement.

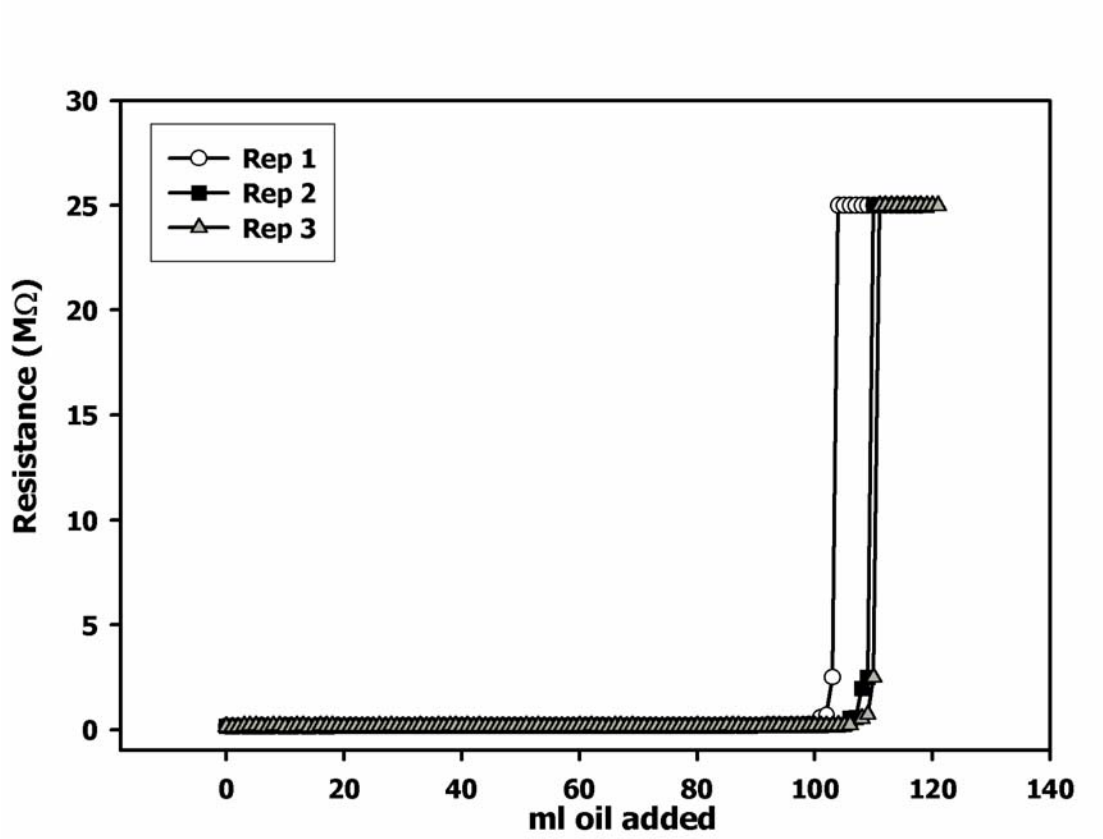

Figure 2. Emulsifying capacity of 1 mg/ml WPI solution. Temperature was 25°C and oil was added continuously at 1ml per second.

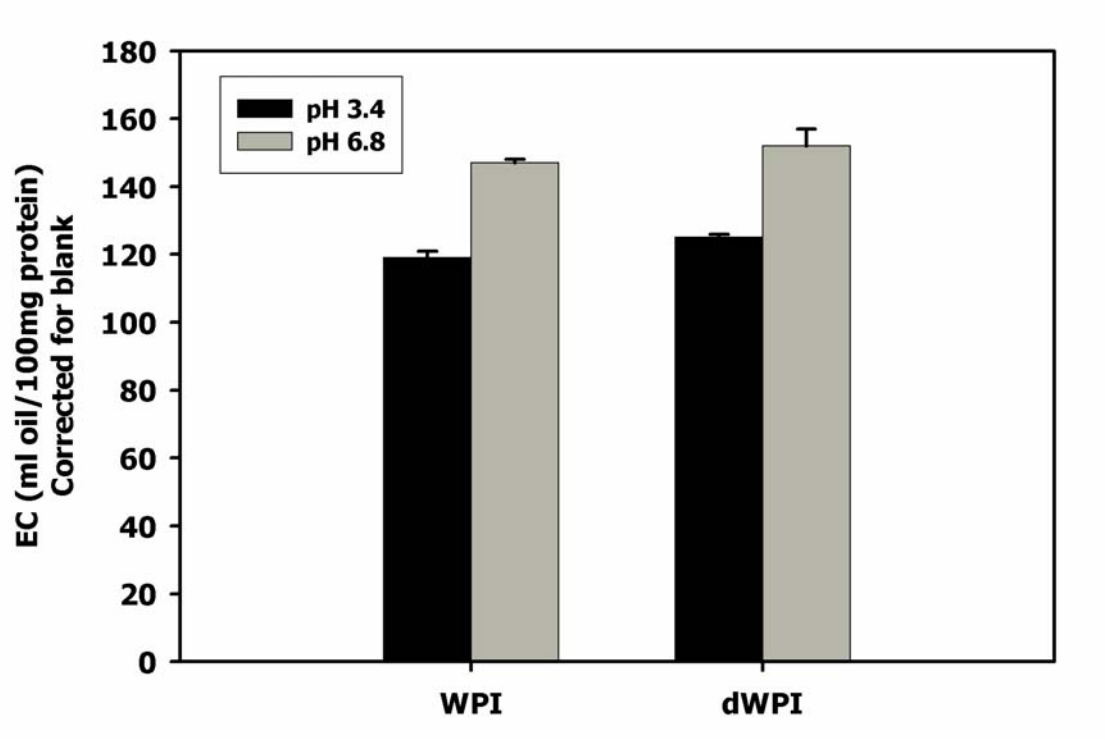

Figure 3. Emulsifying capacity of 6.5% protein (w/w) dWPI and WPI at pH 3.4 and 6.8. Error bars are one standard deviation.

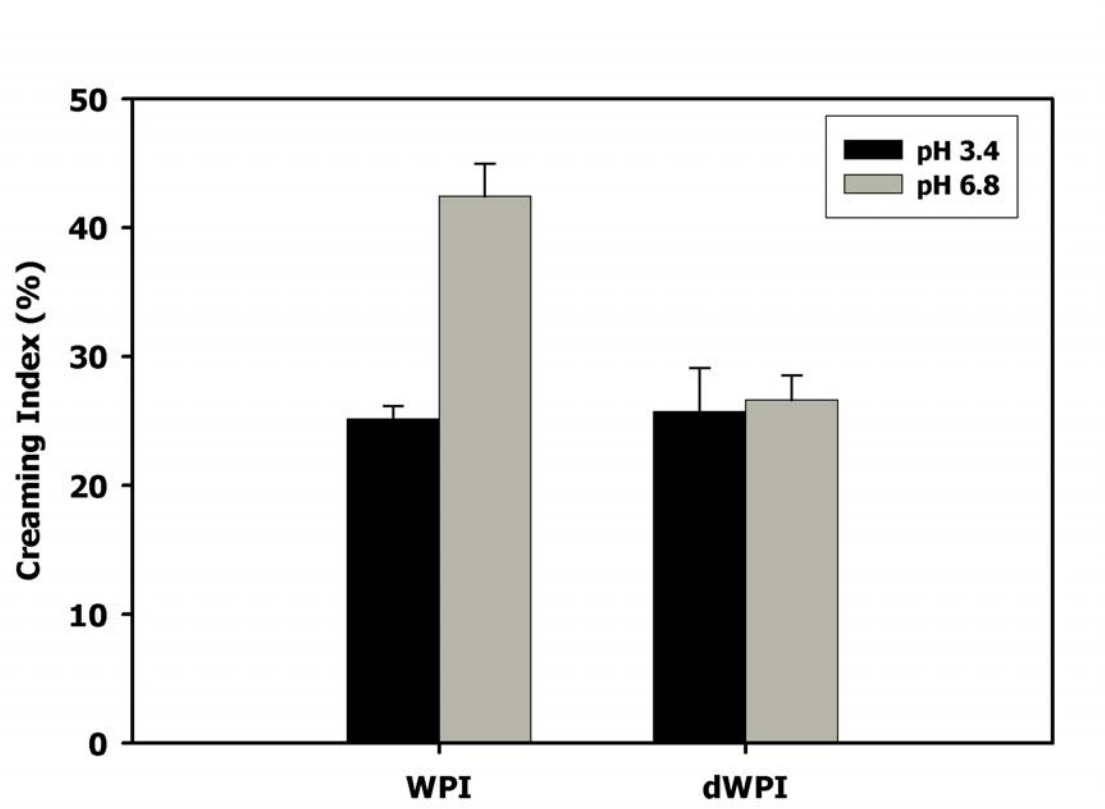

Figure 4. Creaming index of WPI and dWPI after 14 days at approx. 25°C. Error bars are one standard deviation.

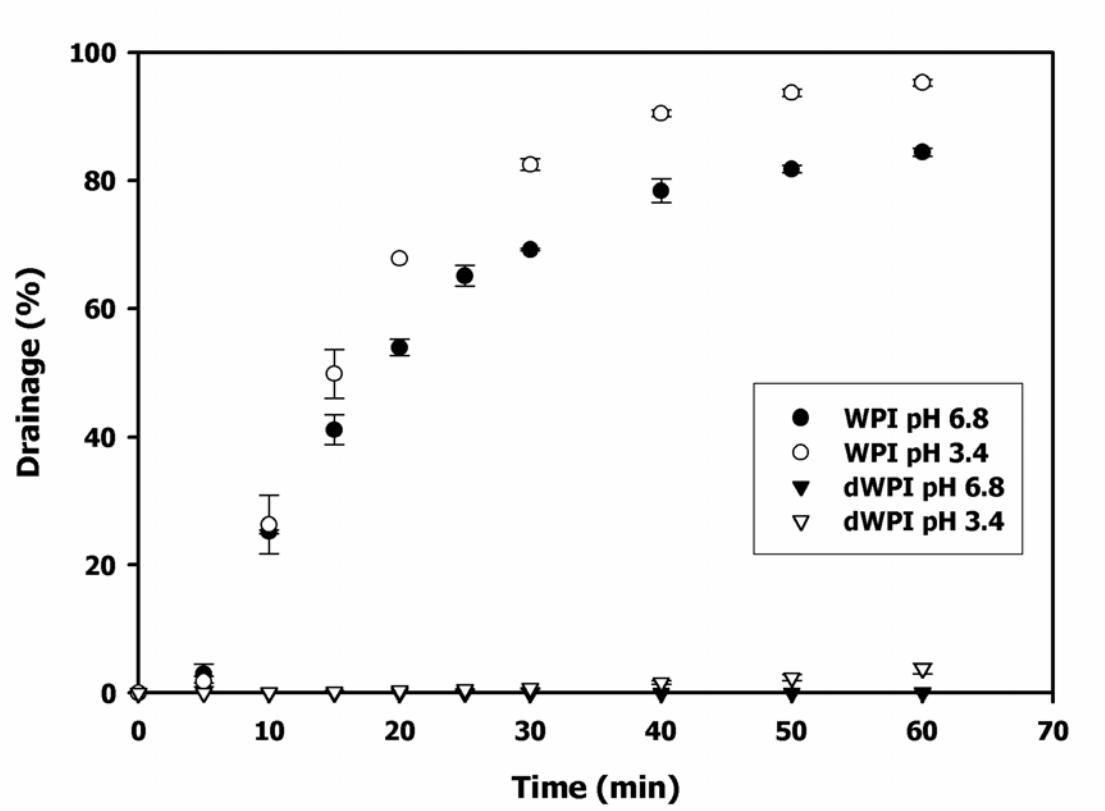

Figure 5. Foam drainage for 6.5% protein (w/w) dWPI and WPI at pH 6.8 and 3.4. Error bars are one standard deviation.

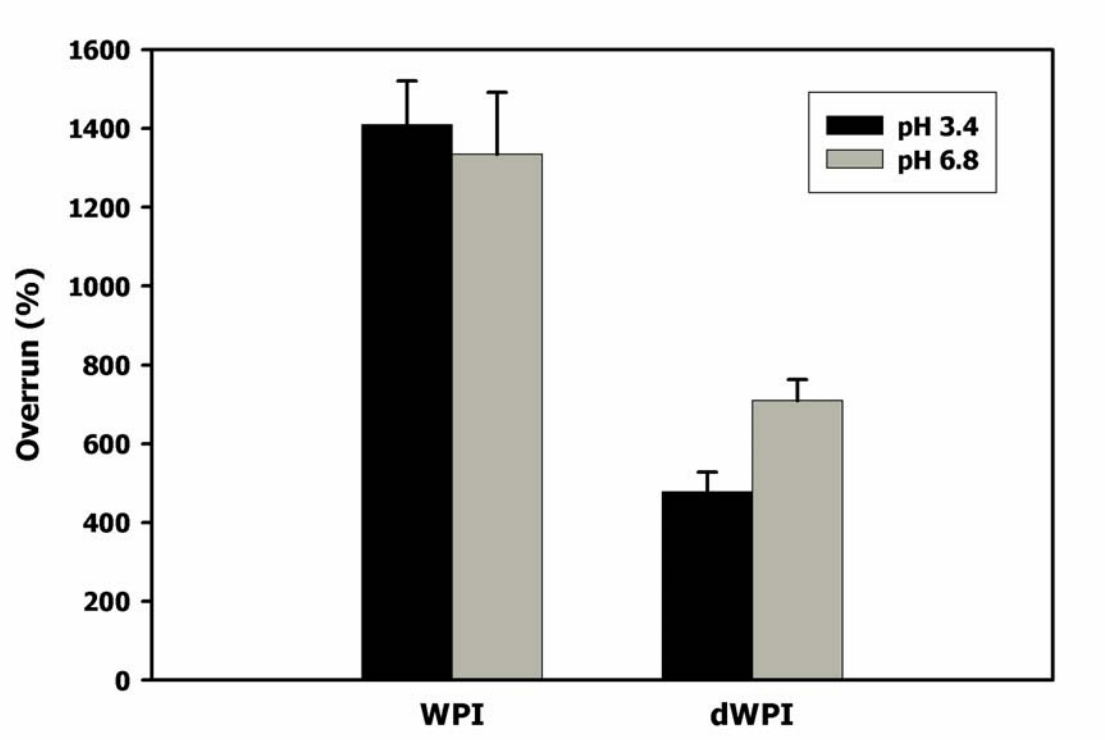

Figure 6. Overrun of 6.5% protein (w/w) dWPI and WPI foams at pH 3.4 and pH 6.8. Error bars are one standard deviation.

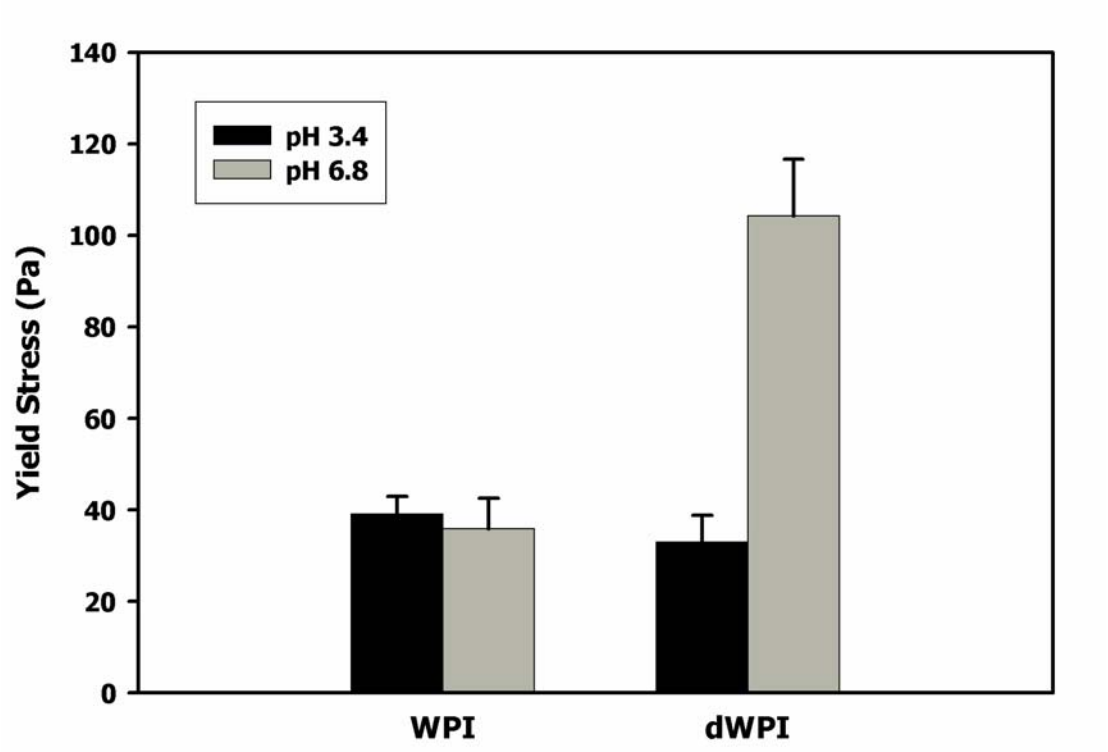

Figure 7. Yield stress of 6.5% protein (w/w) WPI and dWPI foams at pH 3.4 and 6.8. Error bars are one standard deviation.

STABILIZING DAIRY FOODS WITH DERIVATIZED WHEY PROTEIN

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4.1 – INTRODUCTION

Cultured dairy products are produced throughout the world (Lucey *et al*., 1998) and result from fermenting fortified milk inoculated with a mixed starter culture consisting of various lactic acid-producing bacteria (Schmidt *et al*., 2001). Yogurt and sour cream are similar cultured dairy products produced by fermentation with *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp. *bulgaricus* for yogurt, or *Streptococcus lactis* for sour cream. During fermentation, lactose present in milk is hydrolyzed into lactic acid, thereby decreasing the pH of the product. Once the pH reaches approximately 4.6 (perhaps pH 5.2 in combination with heat) casein micelles are destabilized and aggregate, forming a gel (Duboc and Mollet, 2001). Ultimately, the final properties of the gelled coagulum dictate texture and shelf life of the fermented product.

Various food ingredients are selected based on unique structural and functional characteristics that impart thickening behavior and provide stabilization for food products (Lindsay, 1996). In an effort to meet consumer demand, many of these ingredients are formulated in dairy products to improve quality attributes. Historically, increasing milk solids has enabled manufacturers to improve body, texture, and shelf life of yogurt and sour cream (Duboc and Mollet, 2001). The texture of both products begins to develop during pasteurization of the mix (Tamime and Robinson, 1999). Heat treatment can denature whey proteins and increase interactions between whey and κ-casein (Schmidt *et al*., 2001) – two categories of milk proteins differentiated by solubility at pH 4.6. Denaturation improves the hydrophilic properties of whey proteins and increases the bound water in the final

coagulum, thereby reducing syneresis and improving texture (Rasic and Kurmann, 1978).

Several studies report the potential for improving texture and shelf life of fermented dairy products using whey proteins (Cheng *et al*., 2000; Guinee *et al*., 1995). Dave and Shah (1998) found that adding whey protein concentrate to yogurt mix prior to pasteurization and fermentation improved firmness and viscosity of the final product. Guirguis *et al*. (1984) observed improved viscosity and reduced susceptibility to syneresis in yogurts formulated with increased total solids and/or whey protein.

Quality characteristics of yogurt and sour cream can be controlled and enhanced with stabilizing ingredients which interact with proteins, water, and other stabilizers to improve texture and limit syneresis (Duboc and Mollet, 2001; Hunt and Maynes, 1997). Typical stabilizer systems in these dairy foods are frequently starch, guar gum, carrageenan, and locust bean gum (Hunt and Maynes, 1997; Duboc and Mollet, 2001). Schmidt *et al.* (2001) found that set-style yogurt stabilized with gelatin was similar to yogurt stabilized with native wheat starch. However, the authors proposed that modified wheat starches were more shear resistant than native starches and might provide superior stability in stirred-style yogurts. Hess *et al.* (1997) studied the rheological properties of stirred-style yogurts stabilized with various commercial stabilizers and inoculated with various exopolysaccharide-producing and non-producing cultures. Rheological differences were observed between exopolylsaccharide-producing and non-producing cultures independent of

stabilizer. Therefore, both starter culture and stabilizer selection affect texture and stability of fermented dairy foods.

Various techniques are used to characterize rheological properties of yogurt and sour cream. Kovalenko and Briggs (2002) successfully applied the vane method to evaluate failure characteristics of soy-based yogurts. The vane method is used to determine yield stress, or the minimum shear stress required to initiate flow (Steffe, 1997). Yield stress has been used to predict product spreadability and scoopability of foods (Daubert *et al*., 1998). Chemical changes in yogurt and sour cream cause weak gel structures to form during storage. These weak structures were attributed to weak secondary forces (hydrogen bonding, electrostatic interactions, Van der Waal's forces) and chemical changes such as starch retrogradation. This added structure contributes to the static yield stress, which is measured using an undisturbed sample. However, these weak structures are easily disrupted, and the yield stress measured in a completely broken down sample is called the dynamic yield stress (Cheng, 1986). Additional rheological properties reported for yogurt include apparent viscosity, gel strength, and ropy behavior (extensional viscosity) (Hess *at al.,* 1997; Cheng *et al*., 2000; Guirguis *et al.,* 1984). Similar textural analytical techniques have been reported for sour cream (Lee and White, 1993).

Thixotropy, or time-dependent thinning, is another important property of foods stabilized or thickened with starch (Steffe, 1997). Thixotropy is a typical phenomenon observed in foods with significantly different dynamic and static yield stress data. When subjected to shear, structure of thixotropic materials breaks down and the materials reach a minimal thickness. This property is important in yogurt and

sour cream, as both products are often subjected to mechanical shear in the form of pumping and stirring.

Whey protein concentrates and isolates have been modified through a derivatization procedure that forms whey protein powders capable of functioning as instant thickeners, performing similarly to modified starch – a common carbohydrate based stabilizer (Hudson and Daubert, 2002; Resch and Daubert, 2004). Derivatized whey protein isolates (dWPI) are able to hold water and are relatively pH and thermally stable (Hudson and Daubert, 2002). The objective of this research was to evaluate the ability of dWPI to replace carbohydrate stabilizer functionality and limit syneresis in yogurt and sour cream. Successful replacement of non-dairy stabilizers with dWPI in fermented dairy foods may provide manufacturers greater marketing and labeling opportunities including higher protein, lower carbohydrates, and an “all-dairy” claim.

4.2 – MATERIAL AND METHODS

Whey protein isolate (WPI) powder, containing 93.1% (w/w) protein was used for all experiments (Bipro, Davisco International Inc., Le Sueur, MN). The protein content was analyzed by the Analytical Services Laboratory (Raleigh, NC) and approximated on a nitrogen basis (N x 6.38). A portion of the WPI powder was processed according to the method of Resch and Daubert (2004) to form a derivatized whey protein isolate (dWPI). For this process, an 8% (w/w) protein WPI solution was adjusted to pH 3.4 using 6M HCl and then heated at 80°C for approximately 1 hr. Following heat treatment the solution formed a semi-solid gel

that was spray dried to form the dWPI powder. The North Carolina State University Dairy Processing Plant (Raleigh, NC) provided non-fat dry milk (NFDM) and heavy cream (40% fat w/w) for sour cream production. Rhodia Food Ingredients (Madison, WI) and National Starch and Chemical (Bridgewater, NJ) donated yogurt and sour cream cultures (ABY-2C and DPL 800) and stabilizers (STRUCT-SURE™ 20 and THERMOFLO®). The following ingredients were used for sour cream production: Maltodextran-10DE (Paselli MD10, Avebe, Princeton, NJ), monoglycerides (Panalite 90 DK/DMG-40, ADM Specialty Ingredients, Decatur, IL), guar gum (TIC Gums, Belcamp, MD), sodium tripolyphosphate (STP) (Astaris, St. Louis, MO), locust bean gum (Bunge Foods, Atlanta, GA). All other chemicals were purchased from Fischer Scientific (Pittsburgh, PA).

4.2.1 – Preparation of Non-Fat Yogurt

Dry ingredients (stabilizer and NFDM) were mixed together, and dispersed in approximately 80% of the total deionized water, according to formulations in Table 1. Solids were maintained at 14% (w/w) for all yogurt formulations. As needed, stabilizer (starch or whey ingredients) was substituted for NFDM to maintain a consistent solids level. The pH of the yogurt mix was measured, and if needed, adjusted to 6.8 using 1M NaOH. The amount of NaOH added was measured and subtracted from the remaining water to be added. A water bath heated the mix to 88°C for 3 min. After heating, the mix was quickly cooled to 45°C in an ice bath. The cooled mix was inoculated (3 ml/liter) with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* starter culture, prepared by dispersing 0.25 g of freeze dried culture in 24.75 ml pasteurized skim milk (final inoculation rate was 0.030 g/liter). After inoculation, the yogurt mix was thoroughly stirred and poured into plastic

containers (400 ml) and centrifuge tubes (30 ml). The mix was incubated at 41°C for 5-6 hrs to a pH of 4.4-4.6. Following incubation, the yogurt was cooled in an ice bath and stored at 5°C for 36-48 hrs before continued testing.

4.2.2 – Preparation of 5% Fat Sour Cream

Sour cream was formulated according to Table 2. First, NFDM was dispersed at 9.2% (w/w) in water to form skim milk (Chandan, 1997). All dry ingredients were thoroughly blended, and a 5% fat sour cream base was formed by adding the dry mix and heavy cream (40% fat w/w) to the skim milk using a Tamco electric mixer (United States Plastic Corp., Lima, OH). The base was held at 5°C for 12 hrs prior to pasteurization at 82°C for 30 s (Electra High Viscosity Hybrid, Microthermics, Raleigh, NC). Next, the base was homogenized at 1,500 psi using a 2-stage, 2-piston, homgenizer (Niro/Soavi, Hudson, WI), then cooled to 25°C. The base was inoculated (2.40 ml/liter) with *Streptococcus lactis* starter culture prepared by dispersing 1.0 g of freeze dried culture in 99 ml pasteurized skim milk (final inoculation rate was 0.024 g/liter). Next, the base was thoroughly mixed and weighed (250 g) into 16 oz plastic containers (Sweetheart Cup Company, Owings Mills, MD), and the sour cream base was incubated in the containers at 25°C for approximately 16 - 18 hrs to a titratable acidity (TA) of 0.80 - 0.85% and pH 4.5 - 4.6. Finally, the sour cream was cooled to 5°C for at least 3 days prior to testing.

4.2.3 – Characterization Techniques

Similar techniques are used to quantify texture of yogurt and sour cream. However, the texture of both products is dependent on factors such as pH, temperature, and overall formulation. To insure consistency between sour cream

batches, TA, pH, total solids, and total fat were measured (Table 3). Titratable acidity was determined by titrating a 9 g sample to the phenolphthalein end point with 0.1N NaOH (Atherton, 1977). The pH was measured at 25°C using a pH meter (Model 350, Orion, Boston, MA), and total solids and fat were determined using a SmartTRAC System 5 (CEM, Matthews, NC).

4.2.3.1 – Vane Method

The vane method was performed using a 4-bladed vane (spindle 72 or 73) attached to a Brookfield YR-1 yield rheometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Yogurt and sour cream samples were refrigerated at 5°C ± 3°C and immediately tested by slowly immersing the vane into the sample using a laboratory jack to minimize sample disruption. The vane was rotated at 0.5 rpm until the material yielded, and static yield stress was measured in triplicate. Next, the sample was stirred by hand, and the dynamic yield stress was measured in triplicate.

4.2.3.2 – Water Holding Capacity and Syneresis

Yogurt water holding capacity was determined using 4 centrifuge tubes, prepared such that approximately 30 g of yogurt base was incubated, or set, in the tube (Kovalenko and Briggs, 2002). After refrigeration for 36 hrs the tubes were centrifuged (Sorvall SS-34, Dupont Company, Wilmington, DE) at 1464 x g (3,500 rpm) for 5 min at 5°C ± 3°C. After centrifugation, the supernatant was drained for 1 min, and WHC was calculated (Equation 1.1),

$$WHC = \left(\frac{m_{original} - m_{supernatant}}{m_{orinignal}} \right) \times 100 \qquad (1.0)$$

where $m_{oringinal}$ is the mass of noncentrifuged sample (g), and $m_{supernatant}$ is the mass of the drained supernatant (g).

Syneresis of sour cream samples was determined using a similar method described by Adapa and Schmidt (1998), measured over a 15-day period using a polyester mesh (PECAP Standard Polyester 7-105/52, Sefar America, Inc., Depew, NY) placed over the container without the lid. Initially, a pinhole was made in the bottom of the plastic container filled with sour cream, and the container was inverted without the lid on the mesh. Containers were held at 5°C throughout the 15-day test, and the liquid released by the sample was collected and weighed on days 2, 9, and 15. The cumulative weight of liquid collected (g) was reported as syneresis.

4.2.3.3 – Viscosity

Apparent viscosity was determined at 5°C using a StressTech controlled stress rheometer (Reologica Instruments AB, Lund, Sweden) with cone and plate attachment (diameter = 40 mm and cone angle = 4°). A small sample of yogurt or sour cream was removed from an undisturbed cup and placed on the rheometer plate. The cone was lowered and samples were presheared for 30 s at 2 s^{-1} to provide a similar baseline stress history. Next, the samples were subjected to shear rate ramps from 0.1 to 100 to 0.1 s^{-1} for yogurt and 0.1 to 100 s^{-1} for sour cream. The viscosity of each sample was tested in triplicate.

4.2.3.4 – Thixotropy

Thixotropy of yogurt samples was determined by plotting viscosity versus shear rate for increasing and decreasing shear rates. Hysteresis loops (the difference between the up and down curves) were observed for each yogurt sample (Figure 3), and the area of each loop was calculated and reported as thixotropy.

4.3 – RESULTS AND DISCUSSION

4.3.1 – Yogurt Results

Initially, yogurt mixes were prepared by mixing and dispersing dry ingredients (NFDM and stabilizer) in deionized water. Without pH adjustment, mixes containing dWPI curdled during heat treatment because the dWPI powder acidified the pH of the mix, leading to protein denaturation. To overcome this problem, the dWPI powder was first dispersed in approximately 80% of the total deionized water required by the mix. The pH of the mix was adjusted to 6.8 (approx. pH of milk), and the remaining water and NFDM were added. The pH of all mixes was always approximately 6.8 prior to pasteurization and fermentation.

Water holding capacity is a critical parameter in yogurt manufacture as it relates to syneresis, an undesirable feature (Kovalenko and Briggs, 2002). Yogurt WHC ranged from 87.4 to 98.6% and was affected by stabilizer choice. Figure 1 shows WHC for yogurt without stabilizer and yogurts stabilized with starch, dWPI, and WPI. The WPI yogurt had the highest WHC followed by starch, dWPI, and the control without stabilizer. The derivatized whey ingredient improved WHC over yogurt prepared without stabilizer.

Figure 2 shows static and dynamic yield stresses of yogurts. Yogurt formulated with 2.5% (w/w) WPI created a firm gel with a static yield stress >800 Pa. Kovalenko and Briggs (2002) studied the relationship between yield stress and WHC of soy-based yogurts and found that increasing yield stress was correlated with increasing WHC of soy yogurts. This texture is uncharacteristic of yogurt and is likely responsible for the increased WHC of the sample. Although yogurt formulated with WPI displayed improved WHC over starch and dWPI samples, the gelled texture would likely exclude WPI from use at the levels tested. Yogurt containing 2.5% (w/w) starch and the control yogurt without stabilizer had similar static yield stresses. The 2.5% (w/w) dWPI yogurt had a slightly higher static yield stress than 2.5% (w/w) starch and the unstabilized control. Yogurt with starch had a slightly higher dynamic yield stress than dWPI and the control. Dynamic yield stress is an important characteristic because yogurt must retain some level of body after mechanical shear in order to remain spoonable. After stirring, both unstabilized and dWPI yogurts became less viscous and were not easily spooned. Yogurt containing greater amounts of dWPI should be examined to observe the impact on dynamic yield stress and spoonability. Due to sample inhomogeneity, yield stress measurement for the WPI-stabilized yogurt was not measured.

Yogurt formulated with starch had a higher initial viscosity (0.1 s^{-1}) than dWPI, but after ramping shear rate to 100 s^{-1} and back to 0.1 s^{-1} the two samples displayed similar viscosity (Table 4). Time-dependent thinning is known as thixotropy and is an important property in starch stabilized products such as yogurt (Steffe, 1996). When subjected to mechanical shear (mixing or pumping), the yogurt structure is broken

down and reaches a minimal thickness. During rest, the yogurt may recover some, or all, of the original structure. Yogurt containing 2.5% (w/w) dWPI showed the least amount of thixotropy (53.7 Pa) compared to yogurt formulated without stabilizer (127.1 Pa) and yogurt formulated with 2.5% starch (201.6 Pa) (Table 4 and Figure 3). Presumably, yogurt displaying less time-dependent thinning would retain body and thickness better than highly time-dependent yogurt. Therefore, immediately after shearing, yogurt containing 2.5% dWPI would retain body and texture better than yogurt containing 2.5% starch.

Overall, dWPI can replace modified starch as a stabilizing ingredient in yogurt. However, continued optimization of dWPI usage levels in yogurt is required to mimic properties imparted by currently used stabilizers.

4.3.2 – Sour Cream Results

Sour cream stabilized with starch showed the greatest stability to syneresis (Table 5). Syneresis was not observed in any of the samples on day 1, and the sour cream stabilized with WPI was the first to express liquid, 18 g of liquid by day 9. The other three sour cream samples stabilized with starch, 2% dWPI, and 4% dWPI had not expressed liquid by day 9. However, on day 15 both dWPI samples and the WPI sample each expressed greater than 44 g of liquid, while the sour cream with starch only expressed 12.6 g. Most likely the starch interacted with the milk proteins, improving gel network, and preventing free movement of water (Lee and White, 1993).

Sour cream formulated with the derivatized ingredient had a lower yield stress than sour cream with starch or WPI (Figure 4). Protein-starch and protein-protein interactions improve gel structure (Lee and White, 1993) leading to greater yield stress. The derivatized protein ingredient was heat denatured prior to sour cream manufacture, therefore was less reactive with other proteins in the sour cream mix. However, increasing the level of dWPI from 2% (w/w) to 4% (w/w) led to a slight increase in yield stress of sour cream samples. Therefore, optimization may lead to improved yield stress of sour cream containing dWPI.

Apparent viscosity of each sour cream mix was determined after pasteurization and homogenization, but prior to fermentation (Figure 5). Addition of dWPI to sour cream premix significantly increased the viscosity of the mix. Similar viscosity profiles were seen in sour cream with 2% (w/w) starch and 2% (w/w) dWPI. However, increasing dWPI from 2% to 4% (w/w) led to an order of magnitude increase in premix viscosity. Interestingly, after fermentation both 2% and 4% dWPI sour cream samples had the lowest apparent viscosity (Figure 6).

In general, sour cream stabilized with 2% dWPI did not have similar characteristics of sour cream stabilized with 2% starch. However, increasing the level of dWPI to 4% resulted in yield stress and viscosity values closer to sour cream with 2% starch. Therefore, as the U.S. standard of identity for sour cream allows, increasing usage levels of dWPI (>4% w/w) may lead to texture and syneresis values similar to sour cream stabilized with modified starch.

4.4 – CONCLUSIONS

Substituting dWPI for starch in yogurt and sour cream formulations had significant effects on yield stress, viscosity, WHC, syneresis, and thixotropy. Substituting equal amounts of starch with dWPI resulted in yogurts with a higher static yield stress but a lower dynamic yield stress. Yogurts formulated with dWPI displayed slightly lower water holding capacity and lower thixotropy than yogurts formulated with starch. Increasing the level of dWPI from 2% protein (w/w) to 4% protein (w/w) in sour cream resulted in increased yield stress and viscosity. However, neither dWPI formulation achieved a yield stress as high as the 2% starch sample. Sour cream stabilized with the WPI was most susceptible to syneresis, followed by 2% and 4% dWPI. Sour cream stabilized with starch was least susceptible to syneresis.

Thus, using dWPI as a sour cream and yogurt stabilizer may enable manufactures to appeal to consumer labeling and nutritional demands. However, replacing starch with dWPI on an equivalent w/w basis does not provide similar stability and texture results. Therefore further optimization of yogurt and sour cream formulations including the derivatized whey ingredient is needed.

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Table 1. Formulas for non-fat yogurt stabilized with starch, dWPI, or WPI

Ingredients	Without Stablilizer	Starch	dWPI	WPI
NFDM (%)	14	11.5	11.5	11.5
Stabilizer (%)	0	2.5	2.5	2.5
Total Solids (%)	14	14	14	14

Table 2. Formula for 5% Fat Sour Cream

Ingredients	% (w/w)
Skim Milk (Formulated from NFDM)	75.00
Heavy Cream	13.00
NFDM	7.13
Stabilizer (starch, WPI, dWPI)	2.00
Maltodextran (10DE)	1.17
Monoglycerides	0.55
Guar Gum	0.35
Salt	0.30
Sodium Tripolyphosphate	0.25
Locust Bean Gum	0.25

Table 3. Titratable acidity (TA), pH, solids, and fat for sour cream batches.

Batch	TA	pH	Total Solids (%)	Total Fat (%)
2% Starch	0.82	4.6	20.24	4.39
2% WPI	0.76	4.6	21.36	4.65
2% dWPI	0.85	4.7	20.71	4.56
4% dWPI	0.74	4.6	20.94	4.75

Table 4. Yogurt viscosity and thixotropy.

Sample	Initial viscosity at $0.1s^{-1}$ (Pa s)	Final Viscosity at $0.1s^{-1}$ (Pa s)	Viscosity at $100s^{-1}$ (Pa s)	Thixotropy (Pa)
No Stabilizer	165.73	21.78	0.73	127.15
2.5% Starch	268.28	39.30	1.01	201.60
2.5% dWPI	199.31	39.25	0.46	53.67

Table 5. Sour cream syneresis.

	Liquid expressed (g)		
	Day		
Sample	1	9	15
Starch	0.0	0.0	12.6
2%WPI	0.0	18.0	>44
2% dWPI	0.0	0.0	>44
4% dWPI	0.0	0.0	>44

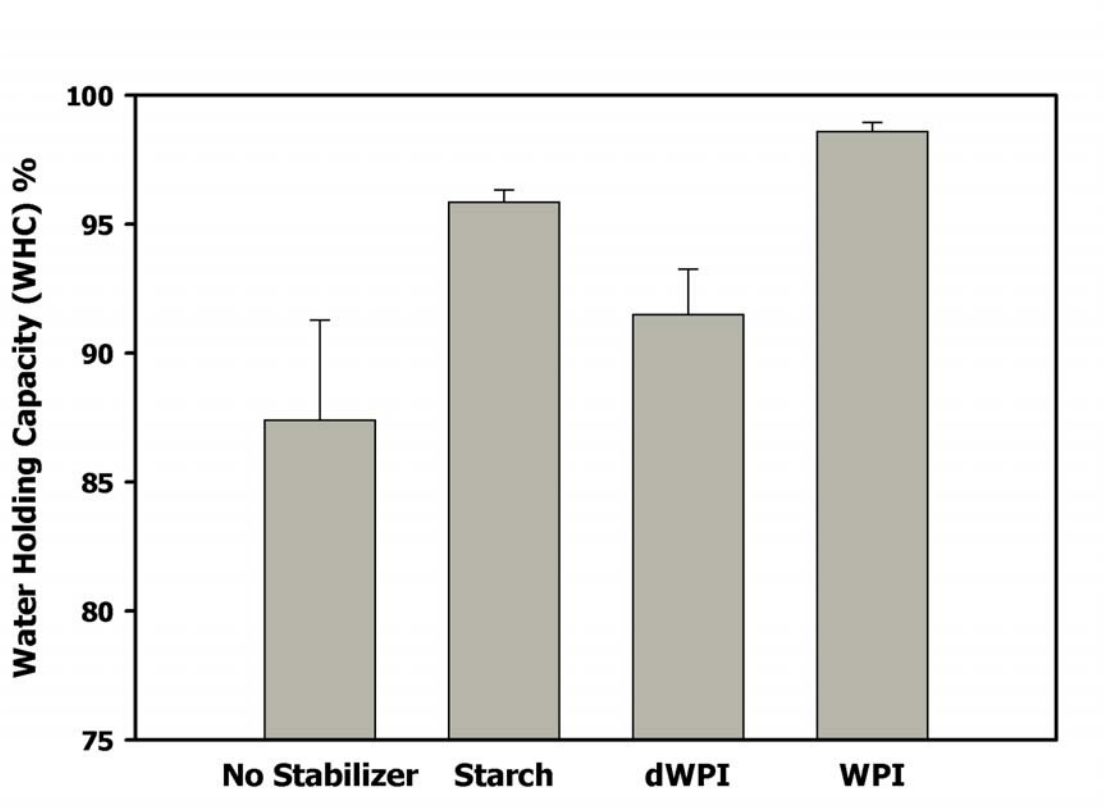

Figure 1. Water holding capacity of yogurt samples at 5°C. All stabilizers were added at 2.5% (w/w). Error bars represent one standard deviation.

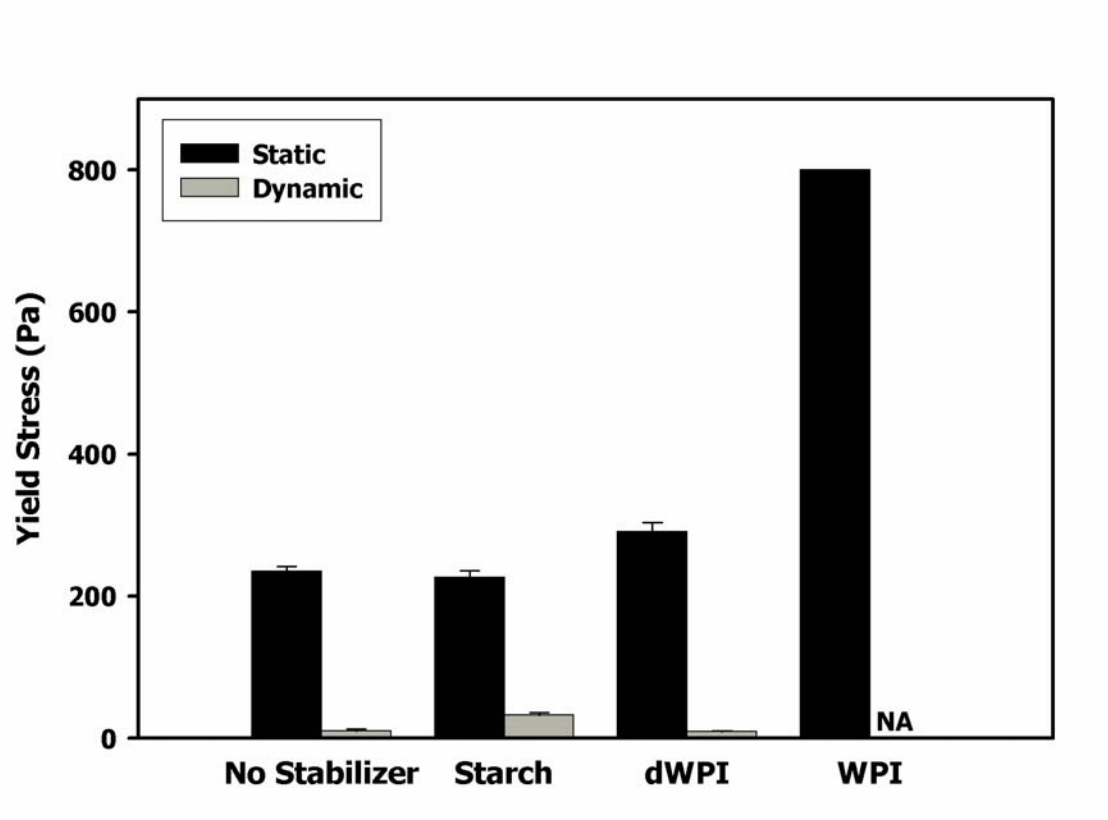

Figure 2. Static and dynamic yield stress of yogurt samples at 5°C. All stabilizers were added at 2.5% (w/w). Error bars represent one standard deviation.

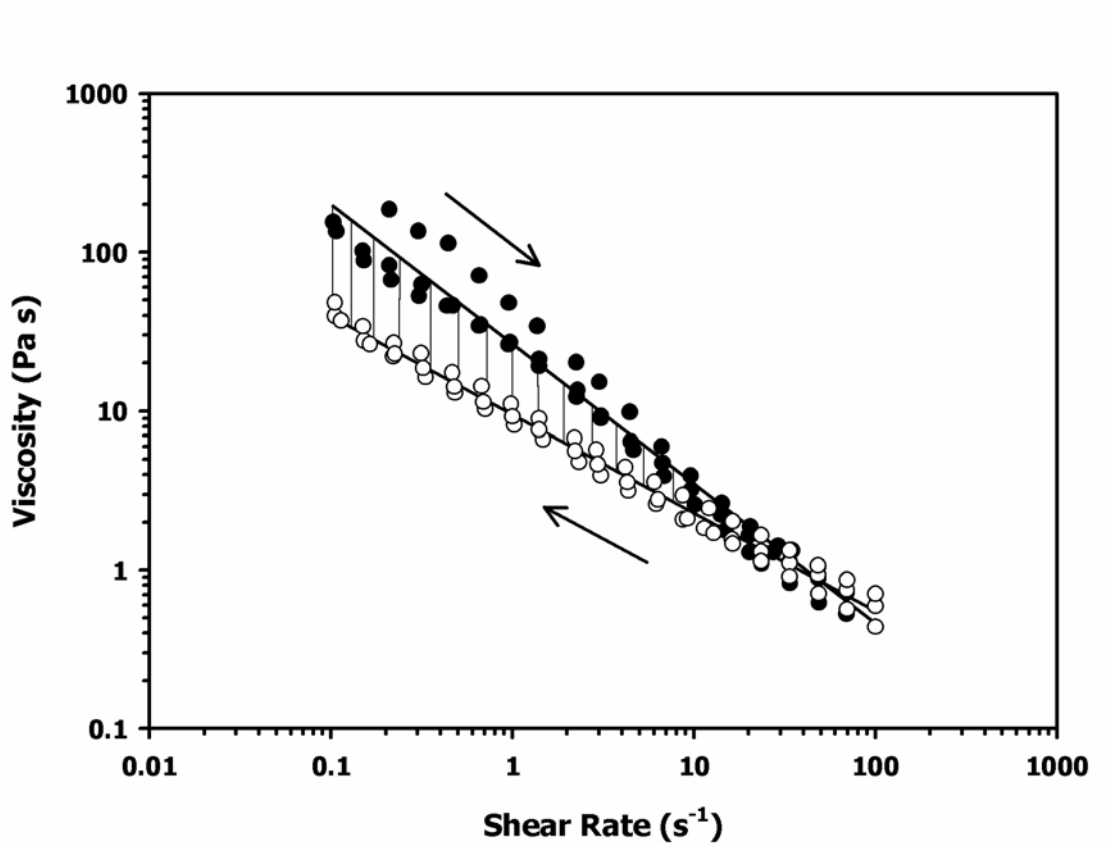

Figure 3. Hysteresis of yogurt prepared with 2% dWPI. Graph shows linear regression of three replications and the area between the up and down shear rate ramp is thixotropy. Samples were at 5°C.

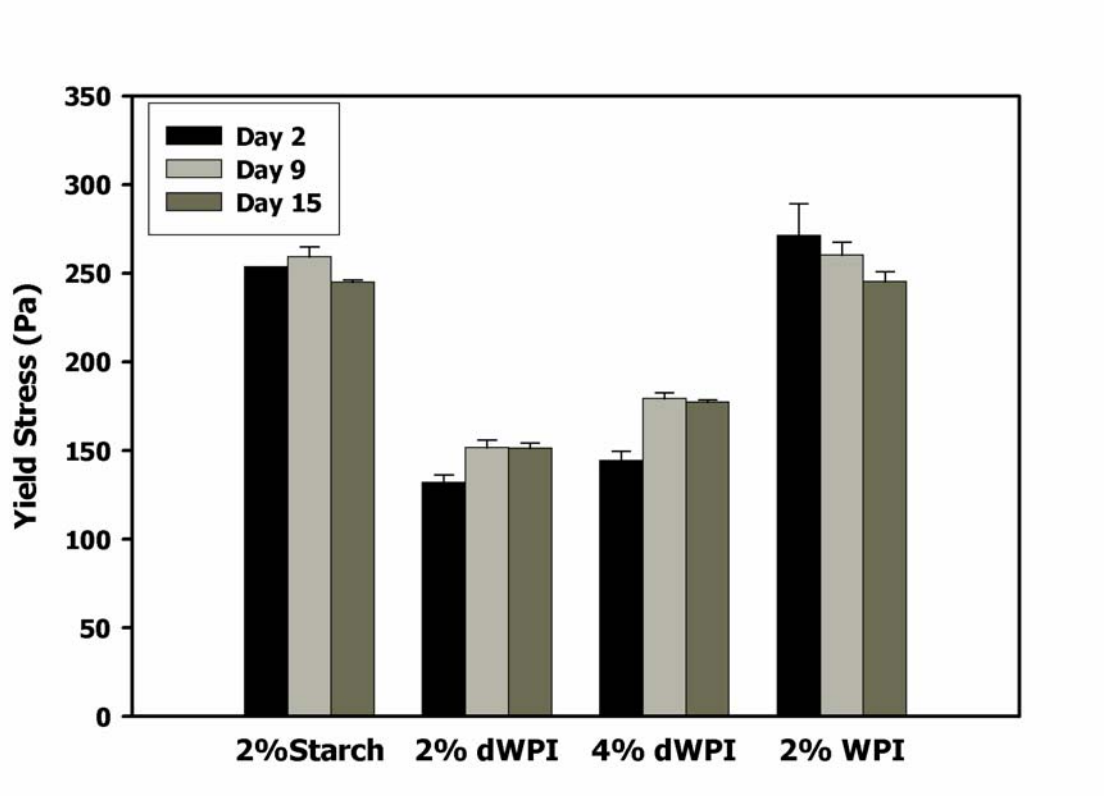

Figure 4. Static yield stress of sour cream at 5°C. Error bars are one standard deviation.

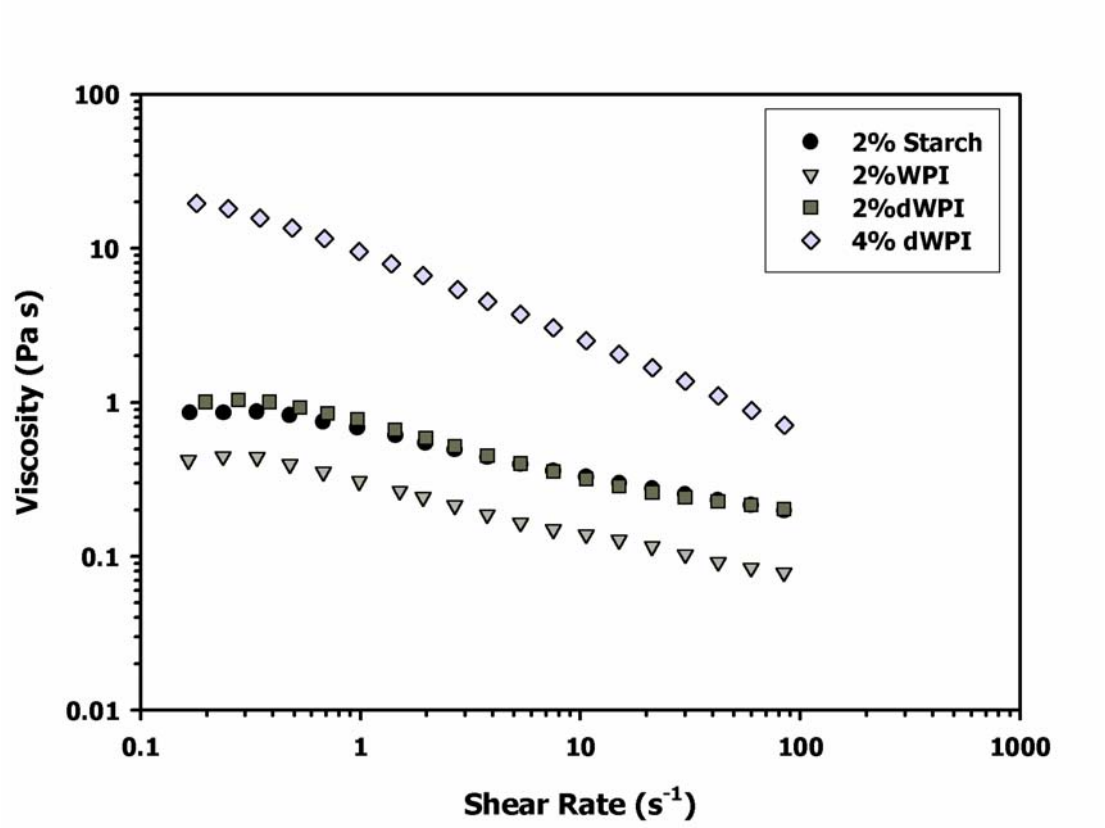

Figure 5. Sour cream premix viscosity prior to fermentation. Temperature was 5°C.

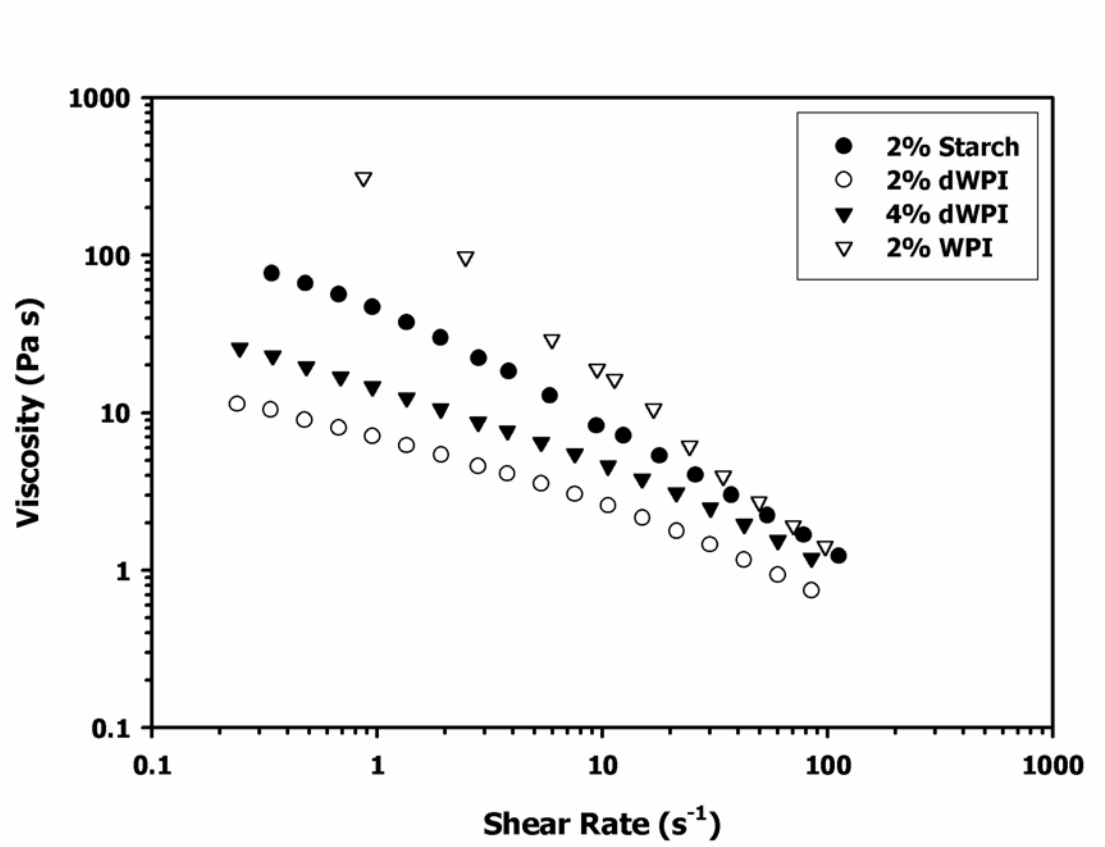

Figure 6. Sour cream viscosity at day 15. Temperature was 5°C.

APPENDIX 1.

DERIVATIZED WHEY PROTEIN ISOLATE SOLUBILITY

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A1.1 – INTRODUCTION

Studying the functionality of proteins is essential for effective use in food products (Hung and Zayas, 1992), and West (1984) identified solubility as one of the major functional properties of proteins. Commercial food proteins, including whey, are customarily spray dried and, in some cases must provide a high degree of solubility in order to be useful and functional. However, β-lactoglobulin has low solubility but good water absorption characteristics after heating, and is widely used in baked products, meats, yogurt, and processed cheese (Mann, 1971; Wingerd, 1971; Robinson et al., 1976). Therefore low protein solubility does not necessarily exclude an ingredient from use.

Major interactions that affect protein solubility are dependent on pH, ionic strength, and temperature (Damodaran, 1996). Proteins carry a net positive or negative charge at pH values below or above the isoelectric pH (pI) – the pH when the protein has a net charge of zero. When carrying a charge, proteins have increased solubility due to electrostatic repulsions between proteins. Additionally, minimum solubility typically occurs at the isoelectric pH due to limited electrostatic repulsions leading to aggregation and precipitation (Damodaran, 1996). However, some food proteins including β-lactoglobulin—a major whey protein, are highly soluble at their pI. This increased solubility is due to a large ratio of hydrophilic to non-polar residues on the surface of β-lactoglobulin. The hydrophilic regions promote protein-water interactions regardless of pH, and thus, only a slight loss of solubility is recorded at the pI of these proteins.

Heat denaturation also changes the pH-solubility profile of proteins (Zhu and Damodaran, 1994). Previous studies determined that native whey protein isolate (WPI) is highly soluble in the pH range 2-9, but when heated to denaturation temperatures, this profile changes. Heat denatured WPI solutions are less soluble than native WPI, especially at pH between 4 and 5. This change in solubility is caused by protein unfolding leading to increased hydrophobic residues on the protein surface (Damodaran, 1996).

A derivatization procedure that produced a spray dried whey protein powder (dWPI) capable of thickening solutions and holding water was outlined by Resch and Daubert (2004). The procedure involves heat denaturing whey protein isolate under acidic conditions, followed by spray drying to a powder. The powder was easily dispersed within water, however it remained unclear whether hydrating the powder created a solution or dispersion. A solution is a homogeneous mixture of at least two molecules or ions (Wertz, 1999). In order for a mixture to be classified as homogeneous, the solute must be dispersed in the solution as individual molecules or ions, each of which is surrounded by solvent molecules. Often, rather than completely dissolving, one material will be suspended as small aggregates in a liquid continuum. This type of mixture is called a dispersion or suspension since the aggregates do not exist in solution as individual molecules. Aggregates usually range between 1 nm to 1 mm, and are called colloids (Wertz, 1999). Colloidal suspensions, or dispersions, are often characterized by stability to settling. The goal of this project was to determine the solubility of dWPI in water and determine if the ingredient formed solutions or dispersions. An additional goal was to determine if pH affected the solubility of the derivatized whey ingredient. Characterizing the solubility

of the derivatized whey ingredient will aid in developing future food applications with the dairy ingredient.

A1.2 – MATERIAL AND METHODS

A commercial whey protein isolate (WPI) powder, containing approximately 93.1% (w/w) protein (Bipro, Davisco International Inc., Le Sueur, MN) was used for all experiments. The nitrogen content of WPI was analyzed by the Analytical Services Laboratory (Raleigh, NC) using a Perkin-Elmer PE 2400 CHN Elemental Analyzer (Perkin Elmer Corp., Norwalk, CT) and protein content was calculated from this value (N x 6.38). All chemicals were purchased from Fisher Scientific Company (Norcross, GA). Protein content was determined using a BCA protein assay kit (#23227) purchased from Pierce Life Science Company (Rockford, IL).

A1.2.1 – Derivatized Powder Production

Spray dried derivatized whey protein isolate was produced according to the method of Resch and Daubert (2004). First, whey protein isolate powder was dispersed in deionized (DI) water to form an 8% (w/w) protein solution and allowed to stir for approximately 1hr. The pH was slowly adjusted to 3.35 with 6M HCl. The solution was transferred, for thermal treatment, to a double-jacketed steam kettle equipped with a scraped surface agitator. The solution temperature was increased to 80°C at approximately 5°C/min and held at 80°C with constant agitation for 1hr. Following heat treatment, the solution had formed a semi-solid gel. The gel was transferred at a flow rate of 2 liter/hr by a peristaltic pump (Model 7553-80, Cole-Parmer Instrument Company, Vernon Hills, IL) to a pilot scale spray dryer (Anhydro,

Attlesboro Falls, MA) operating at inlet temperature of 88°C, outlet temperature of 32°C, and 15psi.

The quality of the resulting spray dried, derivatized whey protein (dWPI) powder was determined by subjecting a 7% (w/w) protein sample to shear rate ramps (0.1 – 100 s^{-1}) conducted on a StressTech Controlled Stress Rheometer (ReoLogica Instruments AB, Lund, Sweden). Samples with apparent viscosity less than 1 Pa s at $1s^{-1}$ were discarded. Following quality analysis, all acceptable lots were combined into one stock dWPI powder. The dWPI stock powder was stored in an airtight container prior to subsequent analyses.

A1.2.2 – Solubility Determination

Solubility was determined in duplicate by a modification of the procedure of Chobert *et al.* (1988). Stock solutions (0.1% protein, w/w) of unmodified WPI and derivatized WPI were prepared and allowed to hydrate overnight at 5°C. Stock solutions were separated into eleven equal volumes and pH of each was adjusted from 1.0 to 11.0 with 6N NaOH or HCl to limit dilution. After pH adjustment, samples were centrifuged 3615 x g (5500 rpm) for 15 min at room temperature. The protein content of the supernatant was determined using a method provided with the BCA protein assay kit. This method involved mixing 2.0 ml of a premixed working reagent with 0.1 ml of supernatant sample, incubating 30 min at 37°C, then cooling to room temperature. After cooling, absorbance of each sample was measured, in duplicate, at 562 nm within 10 min of each other. A standard curve was also prepared by measuring the absorbance of known concentrations of BSA, and this curve was used to calculate protein concentrations of the supernatants.

A1.3 – RESULTS AND DISCUSSION

The protein concentration in the supernatant of each sample was measured and calculated as % solubility. Unmodified WPI and derivatized WPI solubility was graphed against pH for comparison.

Derivatized whey protein samples were less soluble than unmodified WPI from pH 1.0 to 9.0. Unmodified whey protein was approximately 70% soluble from pH 1 to 11, with a slight loss in solubility (60% soluble) at pH 5.0. This decrease in solubility was expected, as the isoelectric point of whey protein is approximately 5.2. The derivatized whey protein isolate was least soluble at pH 5.0 (10% soluble). Derivatized whey protein solubility decreased from 30% to 10% from pH 1.0 to 5.0. At pH levels greater than 5.0, dWPI gained considerable solubility. At pH 8.0 the dWPI sample was greater than 50% soluble, and at pH 10 and 11 it matched the unmodified whey protein with 70% solubility.

A1.4 – CONCLUSIONS

Solubility results support the hypothesis that dWPI forms colloids or particles which are not readily soluble in water. The overall solubility of dWPI was less than unmodified WPI, being least soluble at pH 5.0. However, dWPI solubility can be improved with a more alkaline pH.

A1.5 – REFERENCES

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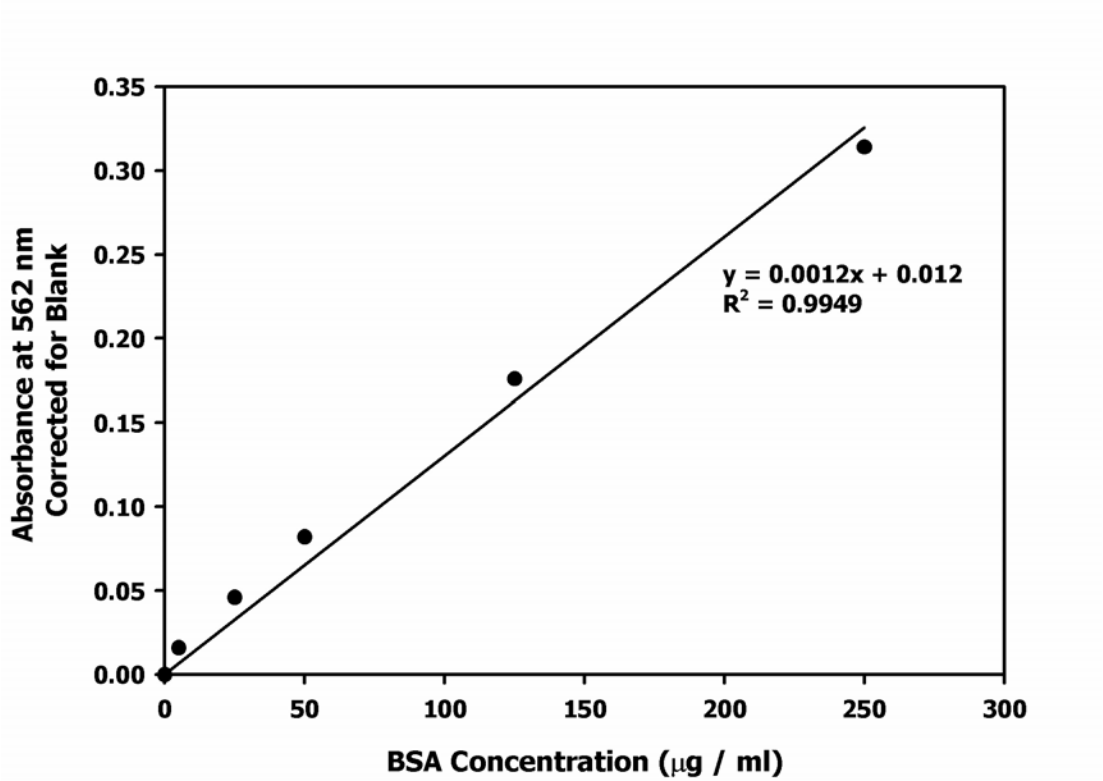

Figure 1. Standard curve for BCA protein test using known BSA concentrations. Temperature was 25°C.

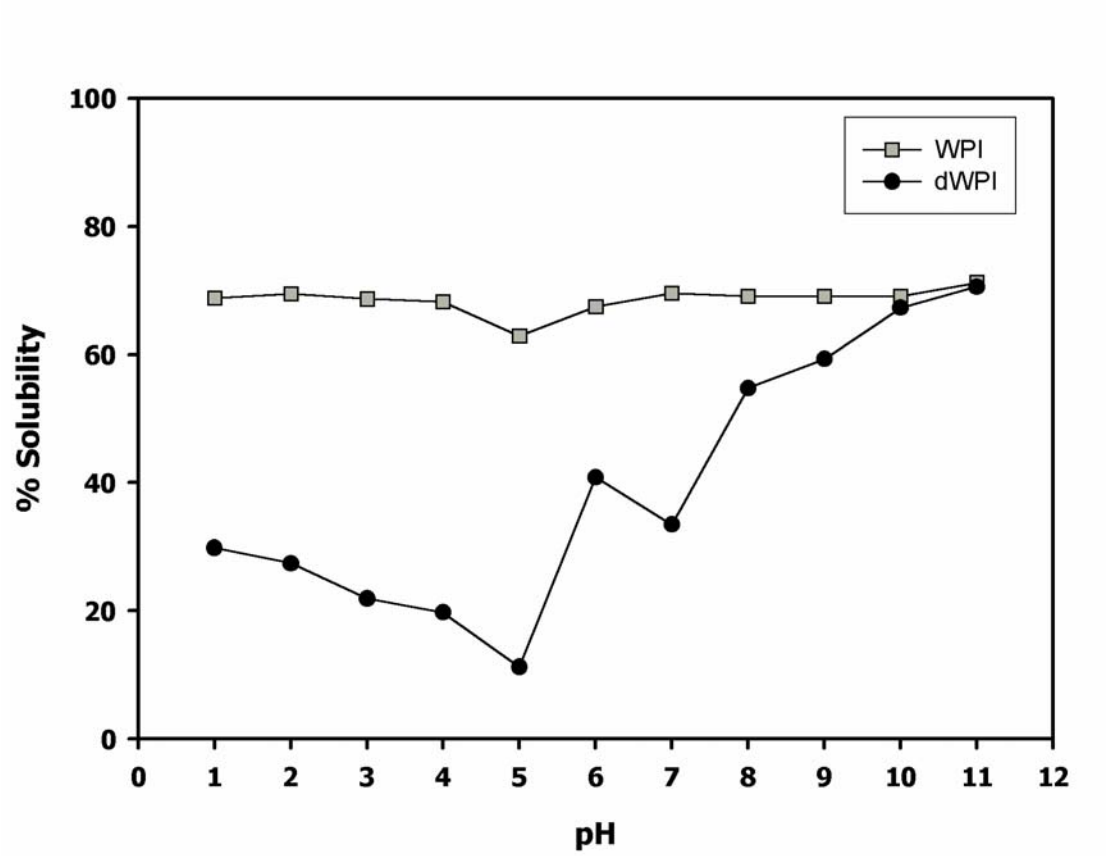

Figure 2. Solubility of 0.1% protein (w/w) solutions/dispersions at pH 1.0 to 11. Solubility is expressed as % of total protein concentration. Temperature was 25°C.

APPENDIX 2.

YIELD STRESS AND OVERRUN DATA SUPPORTING 6.5% PROTEIN (w/w) WPI AND dWPI FOAMS

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Table 1. Yield stress and overrun data for emulsifying and foaming chapter

6.5% WPI pH 3.35			
Trt	Rep	Yield Stress	Overrun
1	1	38.38	1473.12
1	1	36.66	1534.53
1	1	35.28	1477.68
1	1	.	1529.64
1	2	43.13	1359.25
1	2	39.64	1174.71
1	2	35.79	1336.15
1	2	.	1317.45
1	3	46.38	1482.27
1	3	40.94	1437.57
1	3	35.63	1302.84
1	3	.	1491.52
AVERAGE		39.09	1409.73
STDEV		3.82	110.56

6.5% WPI pH 6.80			
Trt	Rep	Yield Stress	Overrun
2	1	26.26	1464.08
2	1	25.24	1595.64
2	1	30.43	1391.23
2	1	.	1617.03
2	2	42.53	1328.61
2	2	40.93	1214.73
2	2	39.93	1267.59
2	2	.	1205.28
2	3	40.28	1363.17
2	3	38.95	1227.56
2	3	38.17	1157.04
2	3	.	1174.71
AVERAGE		35.86	1333.89
STDEV		6.67	157.50

6.5% dWPI pH 3.35			
Trt	Rep	Yield Stress	Overrun
3	1	43.62	519.93
3	1	35.25	527.80
3	1	31.56	529.25
3	1	.	517.82
3	2	38.32	495.51
3	2	31.96	496.17
3	2	27.99	490.99
3	2	.	502.77
3	3	35.40	401.66
3	3	27.32	406.80
3	3	25.86	405.86
3	3	.	440.52
AVERAGE		33.03	477.92
STDEV		5.74	49.89

6.5% dWPI pH 6.80			
Trt	Rep	Yield Stress	Overrun
4	1	119.90	737.38
4	1	106.81	738.67
4	1	93.26	728.46
4	1	.	737.38
4	2	113.23	765.34
4	2	93.83	727.20
4	2	85.76	758.52
4	2	.	754.47
4	3	119.96	620.93
4	3	108.44	643.58
4	3	96.56	634.55
4	3	.	651.80
AVERAGE		104.19	708.19
STDEV		12.38	53.72