EFFECT OF PROCESSING ON THE COMPOSITION, MICROSTRUCTURE AND FUNCTIONAL PROPERTIES OF CHEESE WHEY PROTEIN CONCENTRATE

A Thesis

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To My Parents
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CHAPTER 1
INTRODUCTION

Whey protein concentrates (WPC) that contain 35-80% protein are Generally Recognized As Safe (GRAS) food ingredients used for nutritional and/or functional purposes (Morr and Foegeding, 1990). The selection of WPC for food product applications is generally made on the basis of their compositional and functional properties (Schmidt et al., 1984; Morr, 1987).

An important functional property of whey protein concentrates is their ability to form heat-induced gel structures capable of immobilizing large amounts of water in food systems (Hermansson and Akesson, 1975; Holley, 1990). This heat-induced gel structure is critical to the formation of desired texture in certain foods, such as structured meat and fish products. Protein gelation in these products yields various texture levels of hardness, cohesiveness, springiness and chewiness (Haxann, 1987; Holley, 1990; Brandenberg et al., 1992).

Whey protein gel is defined as a three-dimensional, ordered, protein network capable of entrapping a large quantity of water with a low degree of syneresis (Morr, 1979; Mulvihill and Kinsella, 1987). An equilibrium between intermolecular attractive and repulsive forces which is governed by many factors such as protein concentration, heating kinetics, pH and ionic conditions is needed (Gault and Koroleczak, 1992). Wide differences
in composition and functional properties have been observed for WPC's prepared by
differing separation and purification methods (Morr et al, 1973). Gelling properties also
greatly depend on this composition as well as other non-protein components, such as lipid
and minerals.

This study was done to investigate the gelling properties of WPC made by several
different membrane fractionation processes. The whey was adjusted to acid pH prior to
ultrafiltration (UF) to produce WPC with greatly reduced lipid and calcium (Ca) content.
The composition of WPCs as well as the rheology (shear stress and shear strain) and
microstructure of their heat-induced gels were analyzed.
CHAPTER II
OBJECTIVES

1. To produce delipidized and least-calcium-content WPC by different membrane processing methods.

2. To compare the compositional and functional properties of these WPC with commercial WPC.

3. To determine the rheological properties, i.e., shear stress and strain at failure, of heat-induced gels formed by these WPC.

4. To investigate the microstructure of WPC gels by scanning electron microscopy (SEM) and relate this property to their rheological properties.
CHAPTER III
LITERATURE REVIEW

3.1. WPC manufacture

3.1.1. WPC processing

The utilization of cheese whey is being improved by the manufacturing of un-denatured whey protein concentrates (Morr et al., 1973). Procedures for the manufacture of whey protein products are based on the known behavior of whey components under defined conditions (Matthews, 1984). Morr et al., (1973) and Richert et al., (1974) reported several processes for producing WPC that have been developed and are available to the industry. These whey fractionation processes include: ultrafiltration (UF), ion exchange gel adsorption, electrodialysis protein-metaphosphate complexation and lactose crystallization, have also been reported (Mathews, 1984; Zall, 1984).

3.1.2. Ultrafiltration and membrane fouling

UF and subsequent diafiltration (DF), with its low cost and high recovery of un-denatured protein (de Wit and de Boer, 1975), are the most generally used processes for the separation and purification of whey proteins (Morr, 1992; Matthews, 1984). The principle for manufacture of WPC by UF is based on molecular size differences (Matthews, 1984). The UF process is shown in Figure 1 (Marshall, 1982; Holley, 1990).
Figure 1. Schematic representation of ultrafiltration

Source: Marshall, 1982
A major limiting factor in UF processes is the decline of membrane flux with time due to fouling (Kuo and Cheryan, 1983; Patocka and Jelen, 1987). Fouling is described as a condition in which a membrane undergoes plugging and coating by components in the feed stream in such a way that its output (flux) is reduced (Patocka and Jelen, 1987; Maubois, 1980). For cottage cheese whey, UF membrane fouling can be caused by interaction with calcium and phosphate ions and proteins (Lee and Merson, 1976a; Muller and Harper, 1979). Membrane compaction and chemical deterioration (Kuo and Cheryan, 1983), is also involved in membrane fouling.

3.1.3. Improvement of WPC manufacture

The improvement of UF membrane flux is possible by decreasing the pH of whey to solubilize its calcium phosphate (Kuo and Cheryan, 1983). The lower pH may also improve UF membrane flux by inducing changes in the state of β-lactoglobulin (β-lg), the major protein in whey. The effect of pH on the association/dissociation of β-lg is well known. β-lg dissociates to a monomeric form below pH 3.5, and associates to an octameric form between pH 3.5 and 5.4. This protein undergoes rapid ionization-linked transitions accompanied by increasing dissociation at pH ≥ 5.4 (Lee et al., 1975; Lee and Merson, 1976a). This association/dissociation behavior has been implicated as a factor responsible for the reduction of UF membrane fouling at pH 3.

Previous studies demonstrated that certain UF operating parameters (transmembrane pressure, recirculation flow rate and temperature) and processing conditions could be optimized (Kuo and Cheryan, 1983). Operating the UF at 50°C
instead of 30 or 40°C is preferred. Although high recirculation flow rates resulted in
increased UF membrane fouling, higher recirculation flow rates with low pressure (≤ 50
psi) are beneficial (Kuo and Cheryan, 1983). Prefiltration (Lee and Merson, 1976b) and
conventional centrifugal clarification (Kuo and Cheryan, 1983) of whey at low pH
significantly improved UF flux. Modification of specific protein side chains by adding
acid, calcium-sequestering agents resulted in increased UF membrane flux (Breslau et al.,
1975; Lee and Merson, 1976a; Kuo and Cheryan, 1983; Patocka and Jelen, 1987).

Breslau et al. (1975) suggested a process scheme for cheddar or cottage cheese
wheys to obtain a crystal clear, bland protein solution which was both acid and heat
stable. pH of pasteurized whey was adjusted to 3.0 and it was concentrated by UF to a
trichloroacetic acid (TCA) precipitable protein content of 4 percent. The whey was then
demineralized by DF and the resulting retentate was adjusted to pH 4.6 and held to allow
precipitated protein to gravity settle. The pH of the supernatant was then readjusted to 3.0
and further concentrated by UF/DF. About 80% of the high quality retentate protein was
recovered in the resulting WPC.

3.1.4. WPC composition

The composition and functional properties of WPC strongly depend on whether
the whey was sweet or acid, the cheese source, and the nature of processing used to
manufacture them (Kosikowski, 1979). The general composition of WPC is shown in
Table 1 (Morr and Foegeing, 1990; Morr and Ha, 1992). An advantage of WPC is that
it can be prepared with a wide range of functional properties (Mawhah and Kennedy,
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<th>Moisture&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Normal UF WPC</td>
<td>6.3</td>
<td>55.8</td>
<td>3.0</td>
<td>4.8</td>
<td>0.22</td>
<td>26.4</td>
<td>4.2</td>
<td>7.0</td>
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<td>Neutral UF/DF WPC</td>
<td>6.7</td>
<td>73.9</td>
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<td>46.1</td>
<td>2.4</td>
<td>7.0</td>
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<sup>a</sup> Oven-drying method  
<sup>b</sup> Rose-Gottlieb extraction  
Source: de Wit and Klarenbeek, 1984
Whey proteins are the primary reason for interest in WPC utilization since many desirable attributes of foods may be directly or indirectly related to the functionality of their protein components (Mangino, 1984).

Cheese whey proteins include: β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), immunoglobulins (Ig), and other minor proteins. β-lg and α-la, exhibit the highest concentrations of proteins in whey at about 50 and 12% of the total proteins, respectively (Modler, 1985) probably the most important in the physicochemical properties of whey protein products (Schmidt and Morris, 1984). β-lg exists as a dimer of two identical subunits at the pH of milk. Each monomer contains one sulfhydryl (SH) group and two disulfide bonds (Modler, 1985; Ribadeau-Dumas and Grappin, 1989). About 90% of the free-SH groups in milk are accounted for by β-lg (Schmidt and Morris, 1984). α-lactalbumin differs from β-lg in that it is a monomer with four disulfide linkages and no free-SH groups. The presence of SH and disulfide groups in the whey proteins contributes to disulfide interchange and formation of complexes among the whey proteins when heated as well as interaction of the whey proteins with κ-casein, which contains two SH groups when milk is heated (Modler, 1985).

3.2. WPC functionality

Functional properties are "those physical and chemical properties which affect the behavior of proteins in food systems during processing, storage, preparation and consumption" (Kinsella, 1976). Morr and Ha (1992) defined protein functional properties as those physicochemical properties that influence the structure, appearance, texture,
viscosity, mouthfeel, or flavor retention of the product. A list of those key physicochemical and functional properties of food proteins is given in Table 2. Functional properties are also defined as "any property of a food or food ingredient except its nutritional ones that affects its utilization" (Pour-El, 1981).

Protein functional properties are greatly influenced by compositional and processing factors (Kinsella, 1982) and this relationship is still poorly understood (Morr, 1985). WPC utilization has not reached its full potential due in part to the sample-to-sample variability in functionality (Harper, 1984; Kim et al., 1997). In food systems, a protein also displays functionality by interacting with non-protein components (Mangino, 1984; de Wit and de Boer, 1973). Kinsella (1976) suggested that flavor, gelation, binding, water binding and viscosity are among the most important properties to consider in discussing proteins. Heat treatment has been shown to affect foaming (Richert et al., 1974), emulsion (Aoki et al., 1981), and gelation properties of WPC and other protein ingredients (Hermansson, 1979; Mangino et al., 1987).

3.2.1. Solubility

Solubility makes an important contribution to emulsion, foaming, and gelation properties of proteins because it reflects the proportion of native and denatured proteins percent (Kinsella, 1982).

Morr and Ha (1992) postulated that a series of reactions are involved in the protein resolubilization phenomenon: 1. dispersion of the powder into the aqueous phase to promote particle hydration and swelling; 2. dissociation of low molecular solutes and
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Source: Morr and Ha, 1992
protein molecular complexes; and 3. rearrangements of protein molecular structures to allow the hydrophilic residues to project into the aqueous phase and the hydrophobic residues to remain within the interior of the protein molecular complexes.

Protein solubility depends on the physicochemical state of the protein molecules, which are favorably or adversely affected by heating, drying and other processing treatments during their manufacture and storage (Morr et al., 1985). Heat treatment during processing of WPC preparations generally is expected to have a negative effect on protein solubility. Protein solubility is influenced by certain intrinsic properties, i.e., amino acid sequence and composition, protein conformation, and surface polarity/hydrophobicity, as well as environmental conditions such as ionic composition, pH and temperature (Kinsella, 1982). Since the latter parameters also affect protein functionality, solubility should be a reliable predictor of protein functionality (Morr and Ha, 1992). Solubility of WPC and WPI at pH 6.5 and 4.6 has been investigated by de Wit et al. (1988), and the solubility at pH 6.5 was directly related to the degree of protein aggregation. The same study also pointed out that solubility at pH 4.6 provides a more sensitive method for evaluating protein denaturation.

Several methods have been utilized for recovering and quantitating soluble proteins, such as water-soluble nitrogen, nitrogen solubility index, water-soluble protein or protein dispersibility index (Wolf and Cowan, 1975). A collaborative study was done to develop a rapid, standard food protein solubility procedure (Morr et al., 1985). This method can be used to determine the percentage of 'soluble' whey protein in WPC that is dispersed in 0.1 M NaCl solution under controlled experimental conditions.
3.2.2. Foaming

A foam is generally described as a two-phase system in which a distinct gas bubble phase is surrounded by a continuous liquid lamellar phase. Surface activity and film forming properties of specific protein components, even at low concentration, could have great influence on the foaming properties of a protein ingredient in a food system (Phillips et al., 1990).

Protein contributer to foam formation by lowering interfacial tension (Leman and Kinsella, 1989; Morr and Ha, 1992). Foam formation depends on the ability of the protein to disperse and diffuse rapidly to the interfacial film which is sufficiently cohesive to entrap and retain air, hold moisture, and withstand mechanical shocks and shearing during formation and aging changes (Halling, 1981; Phillips et al., 1990; Morr and Ha, 1992).

Processes that increase protein hydrophobicity, such as 'partial' heat denaturation (thermal unfolding), enhance its interfacial activity and foaming properties, but further denaturation decrease these properties (Richert et al., 1974; Leman and Kinsella, 1989). Kinsella and Whitehead (1988) found a curvilinear relationship between protein surface hydrophobicity and foam stability. This suggests that heat denaturation enhances foam capacity by increasing the surface activity of proteins possessing low surface hydrophobicities. Flexibility (Townsend and Nakai, 1983; Meste et al., 1990) and total hydrophobicity rather than surface hydrophobicity have been proposed as major contributing factors for desirable foaming characteristics of a protein (Kinsella and Whitehead, 1988).

Increasing the ionic strength of the protein solution reduces the electrostatic
repulsion between the adsorbed protein film and arriving protein molecules and thereby increases the rate of protein adsorption. Phillips et al. (1991) reported that the addition of 0.1-2 M sodium sulfate depressed the maximum foam expansion properties of 5% whey protein isolate (WPI) solutions at pH 5. The effects of these high mineral ion concentrations were thought to be due to ion binding and inhibition of protein hydrophobic interactions that impair their ability to form the protective membrane around the foam cells during whipping. Such high ionic strengths might weaken intermolecular electrostatic bonding, thus impairing their film-forming properties (Morr and Ha, 1992). Kinsella and Whitehead (1989) observed that calcium has a negative effect on foaming property by decreasing the thickness of the electrical double layer, thereby inducing coalescence of the protein-coated air bubbles.

The adsorption of whey protein components is influenced by pH (Lema and Kinsella, 1989). De wit et al. (1988) and Meste et al. (1990) reported that the foaming properties of WPC exhibit maximum foam expansion and stability between pH 4 and 5. This is probably because the isoelectric points of α-La and β-Lg are 4.5 and 5.4, respectively. At this pH, the repulsive forces between these proteins are low, allowing rapid protein adsorption and association to form an interfacial film (Lema and Kinsella, 1989). The contribution of individual whey proteins to the formation of an adsorbed layer has been studied (Yamauchi et al., 1980; Oortwijn et al., 1979): At neutral pH, β-Lg was the predominant protein in the adsorbed layer; however, adsorption of β-Lg was retarded at lower pH (Joos, 1975; Lema and Kinsella, 1989).

Several methods including whipping, air injection, air sparging and shaking have
been used to incorporate air into protein solutions (Waniska and Kinsella, 1979; Phillips et al., 1990; Cooney and Morr, 1972), but the most useful method for comparing foaming properties is whipping because this method relates best to industrial processes (Halling, 1981). A standard method of overrun and foam stability was established in a collaborative study (Phillips et al., 1990).

3.2.3. Gelation

Protein gels may be defined as three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water with a low degree of syneresis (Morr, 1979; Mulvihill and Kinsella, 1987; Gault and Korolczuk, 1992). According to Ferry (1948), "gels are differentiated from other structured, network systems in which small proportions of solid are dispersed in relatively large proportions of liquid by the property of mechanical rigidity or the ability to support shearing stress at rest". Mangino (1984) defined protein gelation as a protein aggregation during which protein-protein and protein-solvent interactions occur in which attractive forces (ionic bridges, hydrophobic interactions, and disulfide bonds) and repulsive forces (electrostatic protein repulsion and protein-water interactions) are balanced.

The mechanisms of protein gel formation are not fully understood and have not been systematically studied (Mulvihill and Kinsella, 1987). Ferry (1948) proposed that the final gel state corresponds to aggregates of partly denatured protein and involves the following mechanism:
xPN → xPD → (PD)x

where x = the number of protein molecules,
PN = native protein,
PD = denatured protein.

Thus, gelation is a two-stage process involving an initial denaturation or conformational alteration (unfolding) of the native protein induced by heat (Mulvihill and Kinsella, 1987), followed by subsequent protein-protein aggregation (Mangino, 1984).

The steps involved in heat-induced protein gelation can be divided into four stages: (1) protein unfolding, (2) water binding, (3) protein-protein interactions, and (4) water immobilization (Mangino, 1984). When a protein is heated, the bonds which maintain its secondary and tertiary structures, i.e., hydrogen and disulfide bonds (Morr and Ha, 1992), are weakened, and, at some temperature, they are broken. The breaking of these noncovalent bonds with its resulting alteration of protein structure is denaturation (Mangino, 1984). In the early stages of thermal denaturation, most protein molecules begin to unfold (Whitaker, 1977). At higher temperature, attractive forces will have been weakened enough to allow for extensive water-ion interactions (Mangino, 1984), and leads to a slight increase of the amount of water tightly bound to the protein (Fennema, 1977). If a three-dimensional network formed by the protein-protein interaction is able to entrap water molecules, a gel will form (Mangino, 1984).

A balance between attractive forces necessary to form a network and repulsive
forces necessary to prevent its collapse is required for gel formation (Mangino, 1984). If the network is too weak, a fluid flow is possible, and a true gel will not form. If the protein-protein interactions are too strong, the network will collapse, and water will be expelled from the structure (Mangino, 1984).

The major compositional factors important to protein gel formation include free sulfhydryl content, protein hydrophobicity, and calcium content (Schmidt and Morris, 1984; Fligner and Mangino, 1991). The ratios of carbohydrate, nonprotein nitrogen and lipid to protein content and the amount and type of whey protein utilized also influence gelation (Hernansson, 1982a; Schmidt et al., 1984; Schmidt and Morris, 1984; Morr and Foegeding, 1996; Brandenburg et al., 1992). Protein concentration, pH, ionic composition, and heating rate and temperature must be properly controlled to obtain a desired gel structure (Morr and Ha, 1992).

**Protein concentration**

Both the concentration of WPC as a whole and the concentration of individual protein components determine the likelihood of gel formation and its characteristics (Mangino, 1984). When protein concentration is too low, intramolecular protein-protein interactions tend to occur rather than intermolecular interactions between molecules, and a protein network is difficult to establish. As protein content increases, the intermolecular crosslinks (Mangino, 1984) as well as gelling time (Hillier et al., 1979; Mulvihil and Kinsella, 1987) increase. A rigid opaque gel is usually obtained when the protein concentration is further increased and more water is tightly bound to the protein molecules.
Langley and Green (1989) examined the effect of protein content and composition on the properties of whey protein gels. Compressive strength, elastic modules, impact strength, and gel opacity increased with β-lg content.

pH

The denaturation of proteins (Schmidt et al., 1978b) as well as appearance and strength of WPC gels is dependent on the pH of the solution (Hillier et al., 1980; Schmidt et al., 1978b; Schmidt and Morris, 1984; Gault and Korolczuk, 1992). Gels formed at pH 6.0 or lower were described as more coagulated and less elastic than were gels formed at pH 7.0 to 9.0 (Stemberg et al., 1976; Hillier et al., 1980). Increasing pH from 7 to 10 generally decreased the gel strength of whey protein concentrate (Schmidt et al., 1978a).

In the pH range close to the isoelectric point (pI), white and opaque gels are obtained (Gault and Korolczuk, 1992).

Ionic strength

The role of the ionic environment is particularly significant to WPC gelation (Schmidt and Morris, 1984). A change in mineral composition alters the overall ionic balance and strength of a WPC system and could be expected to influence its functional properties (Morr, 1979). Previous studies (Schmidt et al., 1984; Mulvihiil and Kissella, 1987; de Wit et al., 1988; Morr and Forgeding, 1990) have reported that addition of electrolytes, such as calcium and sodium salts to a protein solution, can affect the strength
and texture of WPC gels.

Mineral ions affect the structure of protein molecules as well as the nature of protein-water interactions (Mangino, 1984). The appearance of gels formed in solutions with added salts is more aggregated and opaque than those formed without added salts (Schmidt et al., 1978b). Johns and Ennis (1981) observed that replacement of calcium ions with sodium ions in acid whey prior to manufacture of WPC yielded a produce with higher solubility and increased gelation time at 70°C, and the resulting gels were more elastic and translucent. The addition of sodium chloride (NaCl) at 0.1-0.3 M or addition of calcium chloride (CaCl₂) at 5-20 mM to dialyzed WPC increased gel strength (Schmidt et al., 1978b). The addition of greater than 25 mM CaCl₂ or 0.4 M NaCl caused a decrease in gel strength. Schmidt et al. (1979) predicted maximum gel hardness occurred at 11.1 mM CaCl₂.

Calcium reportedly affects both the rate of whey protein denaturation and the loss of solubility of the denatured protein molecules (Richert, 1975), and is also an effective protein crosslinking agent (Mangino, 1984). Schmidt et al. (1979) demonstrated significant interaction between calcium and concentration of free sulfhydryl groups in whey protein system. The nature of the calcium effect on properties of whey protein gels deserves further study (Mangino, 1984).

Sulfhydryl content

The concentration of sulfhydryl (SH) groups has been related to the strength and textural characteristics of heat-induced WPC gels (Langley and Green, 1989). Low SH
concentrations resulted in enhanced gelation, while higher SH concentrations, impaired gel formation. However, the study with commercial WPC by Kohnhorst and Mangino (1985) failed to correlate SH concentration with gel strength. Gel texture is not strictly related to the total number of disulfide bonds that form during gelation (Shimada and Cheftel, 1988). Hydrophobic interactions and intermolecular disulfide bonds caused by disulfide interchange reactions in the WPI gel network are probably responsible for the firmer gel structure at increasing protein concentration (Shimada and Cheftel, 1988).

Gel appearance is directly related to the total sulfhydryl content in the WPC (Hillier et al., 1980). Clear gels were formed by low SH concentration whey products, whereas gels formed from high SH concentration whey products, are more opaque.

Disulfide bond formation in heated proteins is dependent upon pH. Differential Scanning Calorimetry (DSC) results for β-lg and BSA show greater protein thermostability at pH 3.0 than at pH 6.0-7.5. These differences are probably due to increased disulfide interchange caused by proton dissociated sulfhydryl (SH) groups at alkaline pH (de Wit and Klarenbeek, 1984). Shimada and Cheftel (1988) demonstrated that at neutral and alkaline pH values, gel firmness and total SH group concentration decreased with increasing pH (6.5-9.5), whereas gel elasticity, transmittance, and protein solubility are increased at these latter pH values.

Other components

Residual milkfat globules and phospholipoprotein complex particles may inhibit the ability of the protein molecules to form a continuous gel structure by competing with
hydrophobic protein interaction sites (Morr, 1992).

Mulvihill and Kinsella (1987) observed that lactose impairs WPC gelation. Removal of lactose and salts by dialysis improved WPC gelation (Schmidt et al., 1978b). Lactose affects primarily the gelation temperature and this may be avoided by performing the gelation test at higher temperatures (de Wit et al., 1988).

3.2.3.1. Water holding capacity

An essential property of milk proteins is their ability to interact with water and especially to hold it (Hermansson and Luciano, 1982). Water holding capacity (WHC), water holding ability (WHA), or 'compressible water' have been used as a parameter for evaluating the gelling properties of milk proteins (schmidt et al., 1979; Foegeding and Ramsey, 1987; Gault and Korolczuk, 1992). The "compressible water" was measured by increased weight of filter paper due to water uptake during compression on the Instron (Schmidt et al., 1979).

Relatively high WHC and water retention have been reported for WPC gels (Sternberg et al., 1976). Hermansson (1982b) found that the elasticity and water binding properties of gels decreased with an increasing degree of random protein aggregation, resulting in a coarser blood plasma gel network with larger pores.

The addition of NaCl to neutral pH WPC solutions gave higher WHC values compared to the addition of NaCl to lower pH (5.0-6.0) WPC solutions (Gault and Korolczuk, 1992). However, the WHC of gels decreased continuously as the concentration of added NaCl was increased. Schmidt et al. (1979) observed that the addition of CaCl₂
and cysteine resulted in WPC gels with increased compressible water in the dialyzed WPC gels.

Electron microscopic data confirm a close relationship between protein aggregation and WHC of WPC gels, but there is no relationship between WHC and gel firmness (Hermansson, 1982b; Gault and Korolczuk, 1992).

3.2.3.2. Rheological analysis

Food rheology is the study of the deformation, fracture, and flow of food materials in terms of the fundamental units of physics. Stress and strain conditions at failure relate to textural properties determined by sensory techniques. Failure means breaking the structural bonds of the food on the macroscopic level (Hamann, 1983). Shear stress, the amount of force required to rupture a sample, is associated with sensory perception of hardness. Shear strain, the amount of deformation required to rupture a sample, is associated with the sensory perception of cohesiveness (Holley, 1990). Montejano et al. (1985) reported that among the instrumental parameters, true shear strain at failure was the most frequent and significant predictor of sensory notes.

Uniaxial compression is commonly used as a quantitative method for testing the texture-building and structure-building components in various food products (Holley, 1990). The theory and calculation procedures of shear stress and shear strain have been reviewed by Holley (1990).
3.2.3.3. Microstructure

Microstructural evaluation involves monitoring reactions and observing physical changes of structures at a level that is not observable by the human eye. The data required for this evaluation should be directly related to molecular transformations and interactions (Davis and Gordon, 1984). Simple model systems can be used to build up to the final gel system. The development of a three-dimensional gel network can thus be evaluated by judging random and ordered solute-solute interactions as well as solute-solvent interactions (Davis and Gordon, 1984).

The smallest particle size that can be resolved by the scanning electron microscope (SEM) is 150 - 200 Å. By electron scattering, globular protein and other structures can be interpreted. Researchers have examined protein gels and dairy products by SEM (Davis and Gorden, 1984). Hermansson (1982b) related textural properties by SEM and water-binding characteristics of blood plasma gels. SEM data showed egg white and WPC gels to consist of a network of spherical particles apparently adhering together (Beveridge et al., 1984). Yost and Kinsella (1992) found no notable change in the overall microstructure of gels by SEM upon addition of fat globules. WPC gels formed by heating solutions to which salts had been added appeared more aggregated than those formed in distilled water WPC solutions (Hermansson, 1979). Bottcher and Foegeging (1992) demonstrated that addition of calcium to WPC solutions resulted in gels consisting of spheres, whereas gels formed by heating WPC solutions with Na ions or no added ions formed a fibrous network. Further studies are needed in this area of research to confirm these results and to relate them to the rheological properties of the gels.
CHAPTER IV
MATERIALS AND METHODS

4.1. Manufacture of whey protein concentrates

Commercial swiss cheese whey from Holmes Cheese Co., (Millersburg, OH) was used in this study. The raw milk was HTST pasteurized at 72°C before adding to the cheese vat. Whey was passed through a Westfalia milk clarifier/sePARATOR and cooled to 0-5°C at the cheese factory. The cooled whey was placed in 10 gal milk cans and hauled to the Vivian Hall pilot plant where it was adjusted to pH 6.0 with HCl to solubilize calcium phosphate, pasteurized at 72°C, 15 seconds, and stored at 0-5°C.

The experimental conditions used for making the three different WPCs (WPC Control, WPC A, and WPC B) are summarized in Figure 2. All whey protein concentrates were made in duplicate.

WPC Control was made by the most conventional UF/DF process. One hundred twenty liters (L) of pasteurized whey was heated to 45°C in a 65°C water bath and concentrated to a VCR of 20 by ultrafiltration (UF). The UF retentate was diafiltered with 5 volumes of distilled water. The final UF/DF retentate (about 5 L) was stored at 0-5°C, adjusted to pH 6.5, and spray dried on the next day.

WPC A was made using the same procedure as for WPC Control, but the whey was adjusted to pH 3.0 prior to UF/DF.
WPC B was produced according to the procedure of Breslau et al. (1975). One hundred forty liters of pasteurized whey was adjusted to pH 3.0, warmed to 45°C and UF to a VCR of 6.5. The UF retentate was diafiltered with 3 volumes of distilled water, adjusted to pH 4.6, and held overnight at 0.5°C to allow the precipitated material to settle. The supernatant was collected by siphoning and/or centrifuging the precipitate at 1,000 x g, readjusted to pH 3.0, warmed to 45°C and UF to VCRfinal = 20. After diafiltration with 5 volumes of distilled water, the final UF/DF retentate was adjusted back to pH 6.5 prior and spray dried.

Two commercial WPC products, WPC P (Calpro 75) and WPC F (Foremost), were used as controls. Manufacturing procedures for these commercial WPCs allegedly included UF/DF steps but no further details are known.

4.1.1. Ultrafiltration

UF/DF was performed with a Romicon PM10 (MW cut-off 10,000 daltens), 2.3 m² tubular polysulfone membrane (Romicon Inc., Woburn, MA). Inlet and outlet pressures were set at 16 and 12 psi, respectively. Concentration was expressed as volume concentration ratio (VCR = initial feed volume / retentate volume).

UF/DF membrane flux was determined by collecting permeate in a graduated cylinder for 1 minute at the initial time and again after each 10 L of permeate had been collected. Membrane flux was computed and expressed as L/m²/hr.

A cleaning procedure for the UF membrane is provided in Appendix A.
4.1.2. Spray-drying

UF retentates were spray dried with a Lab S-1 spray dryer, 1968 model (Anhydro Inc., Attleboro Falls, MA) equipped with a rotary atomizer nozzle operated at a wheel speed setting of 150 V. Inlet and outlet air temperatures were 190°C and 80-84°C, respectively.
Pasteurized Swiss Cheese Whey

pH 6.0 $\downarrow$ pH 3 $\downarrow$ pH 3 $\downarrow$
$\downarrow$ warm to 45°C $\downarrow$ warm to 45°C $\downarrow$
$\downarrow$ UF to VCR 20 $\downarrow$ UF to VCR 20 $\downarrow$
$\downarrow$ UF to VCR 5 $\downarrow$
$\downarrow$ DF with 5V water $\downarrow$ DF with 5V water $\downarrow$
$\downarrow$ DF with 3V water $\downarrow$
$\downarrow$ pH 6.5 $\downarrow$ pH 6.5 $\downarrow$
$\downarrow$ pH 4.6 $\downarrow$
$\downarrow$ Spray Dry $\downarrow$ Spray Dry $\downarrow$
$\downarrow$ centrifuge at 1,000 x g $\downarrow$
$\downarrow$ supernatant to pH 3 $\downarrow$
$\downarrow$ UF to VCR 20 $\downarrow$
$\downarrow$ DF with 3V water $\downarrow$
$\downarrow$ pH 6.5 $\downarrow$
$\downarrow$ Spray Dry $\downarrow$
$\downarrow$ (WPC Control) $\downarrow$ (WPC A) $\downarrow$ (WPC B)

Figure 2. Processing flow-chart for making the WPCs
4.2. Composition analysis

4.2.1. WPC composition analysis

The composition of experimental and commercial WPCs were determined by the following procedures:

**Total solids**

Total solids was determined by a procedure adapted from AOAC (1984). Duplicate two gram samples of WPC powder were weighed into separate 57 mm aluminum pans and dried in an convection air oven for 16-18 hours at 100-105°C.

**True protein content**

True protein content was determined in triplicate according to Barbano et al. (1991). Samples were weighed 0.2 g into separate 50 ml beakers and 10 ml of distilled water was added. The solutions were transferred to separate 80 ml Kjeldahl digestion flasks. Forty ml of 15% TCA solution was added to each flask and they were mixed occasionally for 5 minutes to precipitate the proteins. The precipitated protein mixtures were then filtered through 15 cm Whatman No.1, N-free filters. Two additional 10 ml aliquots of 15% TCA solution were used to rinse the Kjeldahl flask and poured through the filters. The protein precipitate and filter paper were then return to their respective Kjeldahl flasks and their protein contents were determined by macro-Kjeldahl using a N conversion factor of 6.28 (AOAC, 1984).
Total lipid and phospholipid contents of WPC were determined by the method of Morr & Seo (1988). Two gram aliquots of each WPC powder were accurately weighed in triplicate into separate 250 ml Erlenmeyer flasks. Thirty-two ml of 0.05 N NaCl solution was added in increments with stirring to form smooth dispersions. Lipids were extracted with 80 ml of methanol and 40 ml of chloroform. After stirring for 15 minutes, additional 40 ml of distilled water and chloroform were added and the solution was stirred for another 15 minutes. The mixture was then centrifuged for 20 minutes at 1,500 x g and the top clear layer was carefully removed and decanted. The chloroform layer was separated from the insoluble residue by filtering through a 15 cm Whatman No.1 filter paper. An equal volume of a 2:2:1.8 v/v mixture of chloroform, methanol and distilled water was added to the filtrate, after which they were mixed momentarily and recentrifuged. The top layer and insoluble residue were carefully removed and decanted as before. The bottom layer was transferred into a washed, oven dried and preweighed 125 ml flat bottom boiling and solvent was removed with a rotary evaporator. The flasks were dried in a vacuum desiccator overnight and reweighed to determine total lipid contents.

Phospholipid content was determined by redissolving the dried total lipid fraction in 2-3 ml of chloroform and fractionating it with a PrepSep-Si sep-pak column (Fisher Scientific, Fairlawn, NJ) which had been attached to a vacuum flask and preconditioned by passing 20 ml of chloroform. The neutral lipid fraction was eluted with 20 ml of chloroform and the phospholipid fraction was subsequently eluted with 20 ml of methanol.
Fractions were placed in a preweighed flat bottom boiling flask and evaporated to dryness with a vacuum rotoevapor. Phospholipid content was determined by reweighing the dried boiling flasks.

Lactose

Residual lactose was quantified by ion-exchange HPLC according to the method of Lee et al. (1990) and Bouzas et al. (1991). Ten microliters (µl) of microfiltered sample solution was injected onto a 30 cm x 7.8 mm i.d. Aminex HPX-87H cation exchange HPLC column and a H⁺ cation microguard cartridge (Bio-Rad Laboratories, Richmond, CA). A HP1047A (Hewlett-Packard, Palo Alto, CA) refractive index detector was used to quantitate lactose in the effluent stream. Isocratic separation was done at 65°C with a 0.6 ml/min flow rate. Mobile phase was 0.008 N H₂SO₄, prepared by diluting reagent grade H₂SO₄ in HPLC grade water, and filtering through a 0.45 µm membrane filter (Alltech Associates Inc., Deerfield, IL), and degassing thoroughly under vacuum. The concentration of lactose was obtained from peak area with an auto integrator HP3396A using reference lactose solutions. Reference lactose was obtained from Sigma Chemicals (St. Louis, MO).

Total ash

Total ash contents were determined by a procedure adapted from AOAC (1984). Triplicate one gram WPC samples were weighed accurately into separate clean, tared crucibles and ignited in an electric muffle furnace at 600°C for 16-18 hours.
Calcium and Sodium

Calcium (Ca) and sodium (Na) contents were obtained by the Atomic Absorption/Flame Emission spectroscopy method of Pollman (1991). A two channel double beam IL 951 Video II Atomic Absorption Spectrophotometer (Instrumentation Laboratory Inc., Wilmington, MA) equipped with a microcomputer and screen readout was used in this study. Ashed samples were dissolved in 1 ml of concentrated nitric acid and transferred to separate 100 ml volumetric flasks. Sample solutions were then diluted with 0.1% Lanthanum (La) solution (Sigma Chemical Co., St Louis, MO) for calcium determination, or with 0.2% potassium chloride (KCl) solution (Fisher Scientific) for Na determination. Further dilutions were made, if necessary, to obtain proper spectrophotometer readings. Ca and Na were determined at 422.7 and 589.0 nm, respectively, with an air-acetylene flame light source.

4.2.2. Protein composition analysis

Size-exclusion HPLC

Individual proteins were quantified by size-exclusion HPLC (SE-HPLC) using a procedure adapted from Morr (1987). Ten μl of whey and WPC solutions were analyzed with a Hewlett Packard 1050 HPLC, equipped with an auto-sampler, a Beckman spherogel TSK 3000 SW column (7.5 mm x 30 cm), a spherogel guard column (7.5 mm x 7.5 cm), and a UV-detector set at 280 nm. Phosphate buffer (0.1 M) (NaH₂PO₄) containing NaN₃ was used as the mobile phase. Separation was isocratic with a flow rate of 0.5 ml/min. Peak areas were automatically integrated and converted to protein
concentrations using a standard curve developed with reference proteins (Sigma Chemical Co., St. Louis, MO).

**Gel electrophoresis**

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) method, as suggested by Laemmli (1970), was conducted to fractionate and compare the protein in the resulting WPCs. One ml of each 0.8% protein solution was diluted with 4 ml of sample buffer (SDS reducing buffer). All sample solutions were then heated in a boiling water bath for 7 minutes, and cooled to room temperature. Ten μl of each sample was loaded with a Hamilton syringe into a 12% acrylamide pre-cast mini protein II gel (Bio-Rad Laboratories). Gels were assembled in a Bio-Rad Mini-Protein II unit containing electrode running buffer containing SDS in both the upper and lower chambers. The electrophoresis was started immediately and operated at 200 volts for approximately 45 min. The gels were removed and stained with coomassie Brilliant Blue R-250 for 1/2 hr and destained in 10% Methanol/7.5% Acetic Acid for about 4 hours. Low molecular weight standards from Bio-Rad Laboratories (Richmond, CA) were run simultaneously and used to identify whey proteins present in WPC powders.

4.2.3. **Particle size distribution of WPC powders**

WPC powders were size fractionated with six sieves with opening sizes from 212 to 53 μm (ATM Sonic sifter, ATM Corp., Milwaukee, WI) at an amplitude of 6 pulse dial for 3 minutes. The fractions were recovered and weighed. Particle size distribution was
computed from these data.

4.2.4. Microstructure of WPC powders

WPC fractions were mounted on double sided tape, sputter coated with gold platinum and examined by scanning electron microscopy (SEM) (Jeol JSM 820, Tokyo, Japan) in the scanning EM facility, Orton Hall.

4.3. Protein functionality

4.3.1. Solubility

WPC protein solubility was determined by the method of Morr et al (1985) using the Bradford reagent (Bio-Rad Chemical Division, Richmond, CA) modification to determine their total and soluble protein contents.

Triplicate 500 mg samples of dry WPC were accurately weighed into separate 150 ml standard beakers and small aliquots of 0.1M NaCl solution were added with gentle stirring to form a smooth paste. Additional 0.1M NaCl solution was then added to bring the total volume of the dispersion to about 40 ml. The beaker was placed on a magnetic stirrer that had been covered with a 5 cm x 5 cm square of cloth mat to insulate the beaker and prevent heating during the subsequent stirring period. A 2.5 cm smooth, plastic-coated stir bar was added and the dispersion was stirred at a rate that just failed to form a vortex. The pH of the dispersion was immediately determined and adjusted to 4.6 or 7.0 with 0.1 N HCl or NaOH solution. The dispersion was stirred for a total of 1 hour under these conditions and the pH was intermittently monitored and maintained
at the prescribed value throughout the stirring period. The dispersion was then transferred into a 50 ml volumetric flask, diluted to the mark with additional 0.1 M NaCl solution and mixed by inverting and swirling. One tenth ml of the flask content was pipetted into a test tube for nitrogen content analysis of the original sample. The rest of the dispersion was then centrifuged for 30 minutes at 20,000 x g and the resulting supernatant was filtered with Whatman No.1 filter paper. The protein content of the filtrate was determined by the coomassie blue dye binding method (Bradford, 1976) according to the Bio-Rad protein assay (Bio-Rad Chemical Division, Richmond, CA). Triplicate 0.1 ml sample solution aliquots were placed in separate dry test tubes and 5.0 ml diluted dye reagent was added. The solutions were then mixed for 5 minutes with a vortex machine, transferred to duplicate standard 3.5 ml cuvettes (Bio-Rad Laboratories) and their absorbencies were read at 595 nm with a spectrophotometer (spectronic 1201, Milton Roy Company, Rochester, NY).

4.3.2. Foaming properties

The foaming properties of the WPC were determined in triplicate according to the standardized procedure of Phillips et al (1990). One commercial egg white powder (EWP) (egg white solids, Type P-20, HENNINGSEN FOODS, INC., NY) was used for comparison. The proteins were dispersed in demineralized water by the procedure of Morr et al. (1985). WPC powder weighed to contain 10.0 g of protein into a 250 ml beaker and distilled water was added while stirring with a small glass rod to form a smooth paste. Enough water was added to bring the total volume to approximately 130 ml. Dispersions
were stirred for 30 minutes on a magnetic stirrer and the pH was adjusted to 7.0 with 0.1 N HCl or NaOH solution. After another 30 minutes stirring, the pH was readjusted and the total volume of each WPC solution was brought to 200 ml with distilled water, yielding a 5% protein solution (w/v).

The WPC solutions were whipped in a double beater, Deluxe Mixmaster (Sunbeam Appliance Co., Chicago, IL). One hundred ml protein dispersion was poured into the 1.5 L stainless steel bowl and whipped at speed setting 6 for 2 minutes and setting 8 for 13 minutes, which from our preliminary studies provided stable foams. After whipping 15 minutes, the mixer was stopped and the mixer head was carefully lifted from the bowl to minimize destruction of the foam. Samples of foam were quickly but gently scooped out with a stainless steel spatula and transferred to two previously tared 100ml weighing boats, taking care to avoid forming entrapped air pockets. The excess foam was scraped off the top of the weighing boat with the metal spatula to level the top of the foam even with the top of the weighing boat. The weight of the boat filled with foam was recorded.

The overrun was calculated by the following equation:

\[
\text{% Overrun} = \frac{(\text{wt 100 ml WPC solution})-(\text{wt 100 ml foam})}{(\text{wt 100 ml foam})} \times 100
\]

Foam stability

Foam stability was measured by determining the weight of the liquid drainage at ambient temperature from each WPC dispersion after 15 minutes whipping. To facilitate
continuous drainage, a 2 mm hole was drilled in the bottom of the weighing boat, near one of its corners. The hole was sealed with a small strip of plastic tape placed over them on the outside of the weighing boat during weighing. After the WPC foam had been weighed, the tape was immediately removed and the weighing boat was placed in a 10 cm funnel at a 30° angle, and with the hole positioned at the lowest level to facilitate constant drainage. The drained liquid was collected in a tared graduated cylinder and weighed 15 minutes after whipping stopped. This weight was used as an index of foam stability. Preliminary studies revealed no adverse effects of temperature or humidity fluctuations on the foaming process during normal laboratory operating conditions (Phillips et al., 1990).

Foam stability = \( \frac{\text{wt of 100ml foam} - \text{wt of drainage}}{\text{wt of 100ml foam}} \) \times 100

4.3.3. Gelation

The procedure for WPC gel preparation and rheological analysis were done according to the standard Failure-Compression Test for Protein Gels (Lee, 1992). One commercial egg white powder (EWP) (egg white solids, Type P-20, HENNINGSSEN FGODS, INC., NY) was used for comparison. Protein solutions were prepared with distilled water by the procedure of Morr et al. (1985).
4.3.3.1. Gel preparation

Ten percent true protein solutions (w/w) were made by incrementally adding double distilled water to accurately weighed WPC samples while stirring with a glass rod to form a smooth paste (Figure 3). The slurries were mixed gently on a magnetic stirrer using a 2 cm stir bar for a total time of 1 hour. The pH of the slurries was readjusted and their final volume was achieved with double distilled water. The protein dispersions were transferred into separate vacuum flasks and degassed with a water aspirator. After degassing, each of the slurries was poured into several 85 mm length Pyrex brand glass tubes (22 mm O.D., 19 mm I.D.), one end of which was sealed with a heat resistant, plastic cap. The inside surface of these tubes were sprayed with Sigmaflote (Sigma Chemical Co., St. Louis, MO) prior to use to prevent sticking. The tubes and their contents were heated in a 90°C water bath for 15 minutes and immediately transferred to a 10°C ice-chilled water bath for 20 minutes. The tubes were tightly covered with parafilm and held at room temperature overnight. The gels were tested for rheological properties and sectioned for microstructure examination the following day after they were prepared.
10% WPC solution in double distilled water

↓

stir for 1 hour on magnetic stirrer

↓

degas and pour into pyrex tubes

↓

heat 15 min at 90°C

↓

cool 20 min in 10°C water bath

↓

store overnight at room temperature

↓

rheological and microstructural analysis

Figure 3. Gel preparation procedure
4.3.3.2 Rheological analysis

The gel plug was removed from the pyrex tube with minimum distortion and cut into 19 mm length sections with a wire-type cheese cutter (G&S Metal Products Company Inc., Cleveland, OH) to give an even, perpendicular cut with uniform geometry. The failure-compression test for protein gels was done with a rheometer Rheotex 305 (Tesco International Inc., San Mateo, CA) with a 5 mm compression adapter and 80% deformation setting at 60 mm/min crosshead speed and 60 mm/min chart speed. Rheological properties, i.e., true shear stress and shear strain, were calculated from force deformation curves as described by Hamann (1983). A poisson's ratio of 0.49 was used in calculations (Appendix B) (Montejano et al., 1984).

4.3.3.3 Water holding capacity

Water holding capacity (WHC) of WPC gels was determined by the microcentrifugation method of Kocher and Foegeding (1992). Gel sections were cut with the wire-type cheese cutter and a straw to give uniform cylindrical pieces (0.44 mm O.D. x 1.0 cm length). These were placed in a centrifuge microfilter tube fitted with a 0.45 μm pore size nylon mesh screen at the bottom and spun in a COSTAR mini centrifuge Model 10MVSS (COSTAR Corporation, Cambridge, MA) at 4,000 x g. WHC was defined as:

\[
\frac{\text{total water in the gel (g)} - \text{released water (g)}}{\text{total protein in the gel (g)}}
\]
4.3.3.4. Microstructure of whey protein gels

Specimen preparation for scanning electron microscopic (SEM) examination is shown in Figure 4. The 19 mm O.D. whey protein gel plugs were cut with the wire-type cheese cutter to give a 1 mm thick slice and these were then sectioned with a razor blade to make several 2 x 1 x 1 mm specimen cubes. Several specimens of the same sample were placed in a single microporous specimen capsule (SPI Supplies Division of Structure Probe, INC., West Chester, PA). Five percent glutaraldehyde (GA) and 1% osmium tetroxide (OsO₄) was used for fixation for 4 hours and 1 hour, respectively, at 4°C. After each treatment, the specimens were rinsed 2-3 times for at least 15-30 min each with distilled water. Specimens were then dehydrated with 30 min treatments in aqueous ethanol solutions that progressively increased in concentration from 30% v/v to 100% ETOH and then with two 30 min treatments with propylene oxide. Dried specimens were fractured with a razor blade and mounted on stubs, and sputter coated with gold/platinum before observation by SEM.

4.4. Statistical analysis

Data were analyzed by ANOVA procedure of SAS (1988). Mean separation was conducted using the technique of Duncan (1955), where alpha = 0.05.
prepare 2 x 1 x 1 mm gel sections
↓
4 hours with 5% GA and 1 hour with 1% OsO₄
↓
Dehydrate by sequential exposure to 30% v/v to absolute (100%) ethanol
↓
Propylene oxide treatment
↓
Dry fracture and mount on stubs
↓
Sputter coat with gold/platinum
↓
Observation

Figure 4. Sample preparation for SEM
5.1. Whey processing

**Initial whey composition**

Commercial swiss cheese whey used in the study had a pH of 6.7 and contained 17 mM calcium. The whey contained 0.8% protein and 0.07% lipid (Table 3).

**UF flux**

Membrane flux and flux changes during manufacturing of the 3 experimental WPC products are shown in Figures 5 and 6. Although initial flux values were somewhat different for each of the processes, pH 3.0 processes, i.e., WPC A #1, #2 (A1, A2) and WPC B #2 (B2), exhibited higher values compared to those for WPC Control (C1 and C2). The two processes with lowest flux reduction were B2 and A1 (Figure 6). WPC B #1 (B1), however, displayed low initial flux (Figure 5) and relatively high flux reduction (Figure 6). B2 and A1 at 50% volume reduction exhibited the highest membrane flux, followed by A1 > C1 > C2 > B1 (Figure 5). The lowest flux reduction at the same point (50% volume reduction) was also observed for B2, followed by A1, C1, A2, B1, and C2 (Figure 6). The inconsistency of flux and flux reduction during UF processing of these
Table 3. Composition of commercial Swiss cheese whey* used in this study

<table>
<thead>
<tr>
<th>pH</th>
<th>True protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7</td>
<td>0.8</td>
<td>0.07</td>
<td>0.51</td>
<td>17</td>
</tr>
</tbody>
</table>

* Commercial Swiss cheese whey was provided by Holmes Cheese Co., Millersburg, OH.

Table 4. Initial volumes of whey and WPC product yields

<table>
<thead>
<tr>
<th></th>
<th>WPC Control</th>
<th>WPC A</th>
<th>WPC B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#1</td>
<td>#2</td>
<td>#1</td>
</tr>
<tr>
<td>Initial volume (L)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Product yield (g)</td>
<td>654</td>
<td>565</td>
<td>521</td>
</tr>
</tbody>
</table>
Figure 5. UF flux for manufacturing the three experimental WPCs
Figure 6. Percent UF flux reduction for manufacturing the three WPCs.
whey and UF retentates was probably due to uncontrolled differences in whey and also to unknown variations in whey handling.

**WPC powders**

Final product yields of the experimental WPCs are provided in Table 4. The initial volumes of whey used for UF processing were 120 L for Control and WPC A, and 140 L for WPC B.

Particle size distribution of WPC powders were determined by an ATM sonic sifter (ATM Corp., Milwaukee, WI). Results in Table 5 indicate that 6 out of 8 WPCs contained from 33.66% to 67.92% fine powders (< 53μm), whereas WPC A #2 and WPC B #1 contained about 40% large particles (> 212μm) and about 27% fine particles.

Spray dried WPC powders observed by SEM (Figure 7a - 7h) revealed a mixture of spherical shaped particles as well as some broken powder fragments. By comparing pictures with the same magnification, all particle sizes are within a certain range but the amounts of damaged particle fragments are different, i.e., WPC B #2 (Figure 7h) contained more fragments than WPC B #1 (Figure 7g). This agrees with the particle size distribution data shown in Table 5. However, the information obtained from these pictures may not explain the entire data obtained from particle size distribution. For example, it is difficult to identify the difference between WPC A #1 (Fig 7e) and WPC A #2 (Fig 7f) which presented different particle size distributions in Table 5.

The particle fragments in these experimental WPC powders might be caused by the poor condition of the lab scale atomizer and spray dryer. These results indicate that
Table 5. Particle size distribution of WPC powders

<table>
<thead>
<tr>
<th>Sieve opening size (µm)</th>
<th>Commercial WPC</th>
<th>WPC Control</th>
<th>WPC A</th>
<th>WPC B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>F</td>
<td>#1</td>
<td>#2</td>
</tr>
<tr>
<td>212</td>
<td>1.80</td>
<td>6.60</td>
<td>7.77</td>
<td>20.79</td>
</tr>
<tr>
<td>180</td>
<td>0.90</td>
<td>0.94</td>
<td>0.00</td>
<td>1.98</td>
</tr>
<tr>
<td>150</td>
<td>0.90</td>
<td>2.83</td>
<td>0.97</td>
<td>1.98</td>
</tr>
<tr>
<td>106</td>
<td>1.80</td>
<td>0.94</td>
<td>4.85</td>
<td>2.97</td>
</tr>
<tr>
<td>75</td>
<td>9.91</td>
<td>4.72</td>
<td>20.39</td>
<td>20.79</td>
</tr>
<tr>
<td>53</td>
<td>24.32</td>
<td>16.04</td>
<td>25.24</td>
<td>17.82</td>
</tr>
<tr>
<td>Fines (&lt;53)</td>
<td>61.26</td>
<td>67.92</td>
<td>40.78</td>
<td>33.66</td>
</tr>
</tbody>
</table>

All values are expressed in percent of powder retained in the sieves.
Figure 7. Microstructure of WPC powder by SEM
a. Microstructure of Commercial WPC P powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
b. Microstructure of Commercial WPC F powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
c. Microstructure of WPC Control #1 powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
d. Microstructure of WPC Control #2 powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
Figure 7 (continued)

Microstructure of WPC A #1 powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
f. Microstructure of WPC A #2 powder by SEM
Magnification = 250 x (upper) and 590 x (lower)
g. Microstructure of WPC B #1 powder by SEM
Magnification = 250 x (upper) and 500 x (lower)
Figure 7 (continued)

h. Microstructure of WPC B #2 powder by SEM
   Magnification = 250 x (upper), and 500 x (lower)
WPC powder particles consist of very thin and fragile walls that are easily broken during spray drying.

5.2. Composition

WPC composition

The composition of different WPC products are presented in Table 6. Data of total lipids and ionic contents are also graphically shown in Fig 8. True protein contents of the different WPCs ranged from about 70% (commercial WPC) to 83% (WPC B). All WPCs were examined for total lipid and phospholipid contents. As expected, WPC B, prepared by the pH precipitation procedure, contained the lowest total lipid (19.45 - 21.45 mg/g) and phospholipid contents (1.11 - 1.88 mg/g). Commercial WPCs used in this study gave the highest total lipid contents: 64 mg/g (6.4%) for WPC P and 81 mg/g (8.1%) for WPC F. Total lipid contents of WPC A are considered slightly lower than what they show in the table, this is because the high Na content caused a gel-type precipitate during chloroform/methanol extraction of lipids. This gel-type precipitate prevented removal of the chloroform/methanol through the filter paper, thereby decreasing total lipid recovery. Experimental WPCs generally displayed lower lactose contents (0.26-1.64%) than commercial WPCs (8.30 and 11.15%). WPC B, with more diafiltration treatments, gave lowest lactose concentrations (0.26-0.38%). WPC prepared from whey by the low pH treatment during UF/DF processing, i.e., WPC A and B, contained higher ash and Na concentrations than control WPC. The Ca contents of WPC A and B are 32-56 and 43-80 µg/g, respectively, which are extremely low compared to the other WPCs in this study.
Table 6. Composition of WPC products

<table>
<thead>
<tr>
<th></th>
<th>Commercial WPC</th>
<th>WPC Control</th>
<th>WPC A</th>
<th>WPC B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>F</td>
<td>#1</td>
<td>#2</td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>96.6±0.11</td>
<td>96.04±0.19</td>
<td>95.85±0.03</td>
<td>95.97±0.06</td>
</tr>
<tr>
<td>True Protein (%)</td>
<td>70.59±0.49</td>
<td>70.35±0.56</td>
<td>73.85±0.48</td>
<td>76.27±0.25</td>
</tr>
<tr>
<td>Total Lipid (mg/g)</td>
<td>64.33±0.11</td>
<td>81.15±0.20</td>
<td>48.39±0.48</td>
<td>57.80±0.17</td>
</tr>
<tr>
<td>Phospholipid (mg/g)</td>
<td>4.22±0.28</td>
<td>7.93±0.37</td>
<td>4.88±0.31</td>
<td>6.32±0.30</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>11.15±0.23</td>
<td>8.30±0.06</td>
<td>1.36±0.03</td>
<td>0.82±0.00</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.77±0.02</td>
<td>2.86±0.01</td>
<td>2.47±0.00</td>
<td>2.92±0.01</td>
</tr>
<tr>
<td>Ca (µg/g)</td>
<td>3830±106</td>
<td>4188±102</td>
<td>2654±64</td>
<td>2907±63</td>
</tr>
<tr>
<td>Na (mg/g)</td>
<td>2.28±0.07</td>
<td>2.07±0.04</td>
<td>2.86±0.09</td>
<td>4.55±0.17</td>
</tr>
</tbody>
</table>

\* Mean of duplicate determinations
\*\* Mean of triplicate determinations
\*\*\* Mean of quadruplicate determinations
(>2600 μg/g). The high Na contents of WPC A and B resulted from using NaOH neutralize their UF retentates prior to spray drying.

**Protein composition**

SE-HPLC data in Table 7 indicated that the different WPC manufacturing processes affected their protein compositions. WPC B exhibited much higher concentrations of β-Lg (82-90% of total protein content) compared to WPC A and WPC Control which ranged from 55 to 62%, the normal range for WPC. Commercial WPCs, with only 36-43% of β-Lg, were slightly lower than the average in this regard. The change of protein distribution in WPC B maybe related to pI precipitation of the denatured proteins.

SDS-PAGE results (Figure 9) revealed that the different pH treatments caused only minor changes in the concentrations of major whey proteins in their resulting WPCs.

5.3. **Functional properties**

**Solubility**

Solubilities for the different WPCs are in Table 8. Commercial WPC F and both trials of WPC B were completely soluble at both pH 4.6 and pH 7.0, whereas other WPCs exhibited solubilities ranging from 93.6% (C1) to 96.1% (C2) at pH 7.0 and from 76.9% (A2) to 95.9% (C1, P) at pH 4.6. The low solubilities of WPC A1 (79.9%) and A2 (76.9%) at pH 4.6 indicated that part of the protein was denatured (de Wit et al., 1988) and may have inhibited their functional properties. The high solubilities of WPC B (B1
Table 7. Protein composition of WPC products (% of total protein)*

<table>
<thead>
<tr>
<th></th>
<th>Commercial WPC</th>
<th>WPC Control</th>
<th>WPC A</th>
<th>WPC B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>F</td>
<td>#1</td>
<td>#2</td>
</tr>
<tr>
<td>β-Lg</td>
<td>42.79±0.14</td>
<td>35.99±0.74</td>
<td>54.57±0.08</td>
<td>55.46±0.42</td>
</tr>
<tr>
<td>α-La</td>
<td>10.18±0.08</td>
<td>11.24±0.11</td>
<td>11.06±0.00</td>
<td>8.24±0.25</td>
</tr>
</tbody>
</table>

* Mean of quadruplicate determinations
<table>
<thead>
<tr>
<th></th>
<th>Solubility</th>
<th>Foam Overrun&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Foam Stability&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.0</td>
<td>pH 4.6</td>
<td></td>
</tr>
<tr>
<td><strong>Commercial WPC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>95.2±0.2</td>
<td>95.9±0.1</td>
<td>391±4</td>
</tr>
<tr>
<td>F</td>
<td>99.9±0.0</td>
<td>99.9±0.0</td>
<td>N</td>
</tr>
<tr>
<td><strong>WPC Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>96.1±0.1</td>
<td>95.9±0.1</td>
<td>438±21</td>
</tr>
<tr>
<td>#2</td>
<td>93.6±0.4</td>
<td>91.6±0.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>WPC A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>94.9±0.1</td>
<td>79.9±0.5</td>
<td>596±37</td>
</tr>
<tr>
<td>#2</td>
<td>94.7±0.2</td>
<td>76.9±0.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>WPC B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>99.4±0.4</td>
<td>99.9±0.0</td>
<td>N</td>
</tr>
<tr>
<td>#2</td>
<td>99.9±0.0</td>
<td>99.8±0.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>E WP</strong></td>
<td>ND</td>
<td>ND</td>
<td>1088±20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean of duplicate determinations on 5% protein solutions

N: No stable foam was formed

ND: Not determined
and B2) at both pHs were expected because denatured protein would have been removed during processing.

**Foaming**

WPC P, C1, and A1 exhibited only poor foaming properties, whereas none of the other WPCs produced a stable foam (Table 8). Five percent egg white protein solution were whipped by the same procedure for comparison. Although WPC P, C1, and A1 gave 391-596% overrun, foam stability of these WPCs was still low (6.1-32.7%) compared to egg white protein with 1088% overrun and 96.5% stability.

High lipid contents might explain the poor foaming properties in WPC P, F, Control and maybe also for WPC A (Table 6, Fig 8). But for WPC B, which contained only about 20 mg/g total lipid, some other reasons must be identified. High mineral ion concentrations reportedly depressed the maximum foam expansion properties of 5% WPI solutions at pH 5 (Phillips et al., 1991). These inhibitory effects were thought to be due to ion binding that impair their ability to form a protective membrane around the foam cells during whipping. This could help us to understand why no stable foam was formed in WPC A and WPC B. It was well demonstrated that partial heat denaturation (thermal unfolding) of the protein could enhance its foaming properties (Richert et al., 1974). However, this step was not included in the standard method we used. Due to the rigid structure of β-Lg, partial heat denaturation might contribute to the protein unfolding and thereby improve the foaming properties of WPC products. In our study, WPC B contained a high percentage of β-Lg (Table 7), which at neutral pH was the predominant
protein in the absorbed layer (Joos, 1975). However, the high Na ion concentration may still have been responsible for the poor foaming properties of their WPC.

**Gelation**

Water content, WHC, pH, shear strain (SN) and shear stress (ST) of WPC gels are in Table 9. Shear stress of WPC gels are also graphically shown in Fig 10. Mean water content values varied from 86.4% (WPC F) to 88.3% (WPC B2). Mean values of WHC (g water/g protein) ranged from 1.95 (WPC P) to 6.21 (WPC B2) for different WPCs. WPC B and WPC A had better WHC, whereas both commercial WPCs gave the lowest WHC. The pH of WPC solutions were also examined. For 10% true protein WPC solutions made with double distilled water, except WPC P (pH = 6.17), all WPC solutions ranged from pH 6.35 to 6.49.

Values of shear strain (SN) at failure of gels formed from WPC produced using a low pH treatment (WPC A) were significantly higher than the others (Table 9). WPC B1 presented the lowest SN (0.79 m/m) and the highest ST (91.94 kPa). Both WPC A and B gels, made by low pH and pl precipitation processing procedures, displayed higher ST than the others. Shear stress data of commercial WPC, experimental control WPC and egg white protein gels were not significantly different (Table 9).

For WPC B, high percentage of β-Lg (Table 7) would be expected to form more disulfide bonds that would result in stronger gels (Table 9, Fig 10). Residual milkfat and phospho-lipoprotein complex materials may inhibit the ability of the protein molecules to form a continuous gel structure by competing with hydrophobic protein interaction sites.
Table 9. Gel properties of 10% protein WPC and EW solutions in double distilled water

<table>
<thead>
<tr>
<th></th>
<th>Water Content of 10% gel(^1) (%</th>
<th>WHC(^2) (g water / g protein)</th>
<th>pH</th>
<th>Shear Strain(^3) (m/m)</th>
<th>Shear Stress(^3) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercial WPC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>86.5±0.0</td>
<td>1.95(^f)</td>
<td>6.17</td>
<td>1.06(^d)</td>
<td>8.71(^e)</td>
</tr>
<tr>
<td>F</td>
<td>86.4±0.1</td>
<td>3.55(^e)</td>
<td>6.49</td>
<td>1.09(^de)</td>
<td>10.63(^e)</td>
</tr>
<tr>
<td><strong>WPC Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>87.0±0.0</td>
<td>5.55(^bc)</td>
<td>6.44</td>
<td>1.34(^b)</td>
<td>8.87(^e)</td>
</tr>
<tr>
<td>#2</td>
<td>87.5±0.0</td>
<td>4.42(^d)</td>
<td>6.42</td>
<td>1.22(^bcd)</td>
<td>8.72(^e)</td>
</tr>
<tr>
<td><strong>WPC A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>86.9±0.1</td>
<td>5.90(^ab)</td>
<td>6.36</td>
<td>1.55(^a)</td>
<td>35.78(^c)</td>
</tr>
<tr>
<td>#2</td>
<td>86.8±0.1</td>
<td>5.22(^c)</td>
<td>6.41</td>
<td>1.66(^a)</td>
<td>26.49(^d)</td>
</tr>
<tr>
<td><strong>WPC B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>88.1±0.1</td>
<td>6.11(^a)</td>
<td>6.46</td>
<td>0.79(^e)</td>
<td>91.94(^e)</td>
</tr>
<tr>
<td>#2</td>
<td>88.3±0.0</td>
<td>6.21(^e)</td>
<td>6.35</td>
<td>1.23(^bc)</td>
<td>67.82(^b)</td>
</tr>
<tr>
<td><strong>EW P</strong></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.34(^b)</td>
<td>8.78(^e)</td>
</tr>
</tbody>
</table>

\(^{\text{abcd}}\) Values with different superscripts differ (P<0.05)

\(^1\) Mean of six replicate determinations

\(^2\) Mean of triplicate determinations

\(^3\) Mean of six replicate determinations

ND Not determined
Figure 10. Shear Stress of WPC Gels
(10% true protein in double distilled water)
(Morr, 1992). In our study, WPC B, with least total lipid and phospholipid content (Table 6), exhibited the highest ST value, giving further support to this concept. Lactose impaired WPC gelation was reported by Mulvihill and Kinsella (1987). Both commercial WPC contained high lactose compared to the three experimental WPC products (Table 6). Higher lactose in WPC A could also explain its lower gel ST value compared to that of WPC B.

The role of the ionic environment is particularly significant to WPC gelation (Schmidt and Morris, 1984). The addition of NaCl or CaCl₂ to dialyzed WPC increased gel strength (Schmidt et al., 1978b). However, too much Ca caused a decrease in gel strength (Brandenburg et al., 1992). In our study, Ca was successfully removed by low pH treatment in WPC A and WPC B (Table 6), and the resulting ST values of these WPC gels were also significantly high.

Microstructure of WPC gels (Fig 11a-11h) confirmed that the gels with more compact structures (WPC A and WPC B, Fig 11e-11h) actually exhibited higher ST values and better WHC, whereas commercial WPC (Fig 11a, 11b) and WPC Control (Fig 11c, 11d) gels displayed loose structures, lower ST values and lower WHC as well (Table 9). The compact structure and the resulting high ST values of WPC A and B gels could also be related to their high Na concentrations (Table 6, Fig 8). Further study is needed to fully understand the effects of ion composition on WPC gel structure and gel strength (ST value).

The appearances of these WPC gels were observed. Only WPC B gels exhibited a translucent appearance, whereas all the other gels were opaque. Translucent appearance
Figure 11. Microstructure of WPC Gel by SEM
a. Microstructure of Commercial WPC P Gel by SEM
   (10% true protein in double distilled water)
   Magnification = 1,000 x (upper), and 25,000 x (lower)
b. Microstructure of Commercial WPC F Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
c. Microstructure of WPC Control #1 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
Figure 11 (continued)

d. Microstructure of WPC Control #2 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
Figure 11 (continued)

e. Microstructure of WPC A #1 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
f. Microstructure of WPC A #2 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
g. Microstructure of WPC B #1 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
h. Microstructure of WPC B #2 Gel by SEM
(10% true protein in double distilled water)
Magnification = 1,000 x (upper), and 25,000 x (lower)
would likely be related to the ionic composition. Replacement of Ca with Na in acid whey formed more translucent gels (Johns and Ennis, 1981). This was found in WPC B but not in WPC A, for both WPC with similar high Na and low Ca content. The reason for the opacity of WPC A gel may be the presence of denatured protein. Solubility data confirmed that this WPC had only 76-79% solubility at pH 4.6 (Table 8).
CHAPTER VI
CONCLUSIONS

The use of low pH and pH precipitation through UF processing resulted in WPC products with low Ca and low lipid/phospholipid contents that exhibited improved gel properties. However, these low Ca low lipid WPC products failed to form stable foam structures, due possibly to their high Na concentrations. Further investigations would be needed to confirm this latter assumption.

The microstructure of WPC powders and their heat-induced gels was examined by SEM. The decomposed fragments in experimental WPC powders may have been caused by the poor atomization and powder collection conditions of the lab scale spray dryer. Relationships between these broken pieces of the WPC powders and their compositions as well as functionalities were not fully evident. The more compact WPC gels exhibited higher shear stress values and better water holding capacities.
APPENDIX A

CLEANING PROCEDURE FOR UF MEMBRANE
CLEANING PROCEDURE

After each UF/DF processing of whey, the membrane system was cleaned using the following procedure:

I. Drain and rinse
1. Fully open the retentate outlet valve (valve R) and the permeate outlet valve (valve P).
2. Drain the process tank and system.
3. Rinse the process tank and system several times with distilled water.
4. Recirculate with distilled water for 10 min.

II. Chlorine cycle
1. Fully open valve R and valve P.
2. Drain the process tank and system.
3. Recirculate with 200 ppm chlorine solution until the membrane is free of proteins.
   (This is achieved by monitoring the chlorine concentration with test paper and keep it at 200 ppm. At the clean point the chlorine level stays stable.)
4. Drain and rinse the whole system with distilled water for 5 min (without recirculation).
5. Recirculate with distilled water for 10 min.
III. Acid cycle
1. Fully open valve R and valve P.
2. Drain the process tank and system.
3. Recirculate\textsuperscript{b} with 10 L, 45°C phosphoric acid solution\textsuperscript{a} for 10 min.
4. Close valve P, and continue recirculation for 20 min.
5. Drain and rinse the whole system with distilled water for 5 min (without recirculation).
6. Recirculate\textsuperscript{a} with distilled water for 10 min.

IV. Caustic cycle
1. Fully open valve R and valve P.
2. Drain the process tank and system.
3. Recirculate\textsuperscript{b} with 10 L, 45°C, 1% NaOH\textsuperscript{a} for 10 min.
4. Close valve P, and continue recirculation for 20 min.
5. Drain and rinse the whole system with distilled water for 5 min (without recirculation).
6. Recirculate\textsuperscript{a} with distilled water for 10 min.

V. Sanitization cycle
1. Fully open valve R and valve P.
2. Drain the process tank and system.
3. Recirculate\textsuperscript{b} with 200 ppm chlorine solution\textsuperscript{c} for 10 min.
4. Drain and rinse the whole system with distilled water for 10 min (without recirculation).
a Recirculate with only the retentate outlet tube in the process tank

b Recirculate with both retentate and permeate tubes in the process tank

c 30-35 ml DIBAC in 10 L of distilled water

d 30-35 ml phosphoric acid in 10 L of distilled water

e 200 ml, 50 % NaOH in 10 L of distilled water
APPENDIX B

SAMPLE CALCULATION OF TRUE MAXIMUM SHEAR STRESS AND STRAIN
True maximum shear stress and strain values are obtained from the voltage vs time plots from uniaxial compression testing with an Rheotex 305 machine as described in Chapter III. Figure A-1 is a such a plot. Peak 1 and 2 are used to calculate the stresses and strains. Peak 1 corresponds to the point of failure in the compressed gel; peak 2 corresponds to the completion of the first cycle of uniaxial compression by the Rheotex machine. Values from the voltage vs time plots are substituted into equations to yield the true maximum shear stress and strain. A sample calculation is given on the following page.
original gel height = 19 mm
distance of adapter travelled to 80% deformation of the gel = 15.2 mm
diameter of adapter = 5 mm
force applied at the point of failure (correspond to the first peak height, a) = 200 g
distance travelled to peak corresponding to failure (x) = 20 mm
distance travelled to peak corresponding to completion of first cycle (y) = 25 mm

\[ \Delta L = \text{change in height of the gel at the point of failure} \]
\[ = 19 - 15.2 \left( \frac{x}{y} \right) \]
\[ = 19 - 15.2 \left( \frac{20}{25} \right) \]
\[ = 6.84 \text{ mm} \]

\[ F = \text{force in newton required for failure} \]
\[ = \frac{a \text{ grams}}{1000} \times 9.807 \text{ m/s}^2 \]
\[ = \frac{200}{1000} \times 9.807 \]
\[ = 1.96 \text{ Newton.} \]

\[ \varepsilon_{\text{max}} = \text{maximum normal strain (m/m)} \]
\[ = \frac{\Delta L}{L} \]
\[ = \frac{6.84}{19} \]
\[ = 0.36 \text{ m/m.} \]

\[ \varepsilon_{\text{true max}} = \text{true maximum normal strain (m/m)} \]
\[ = -\ln \left( 1 - \varepsilon_{\text{max}} \right) \]
\[ = -\ln \left( 1 - 0.36 \right) \]
\[ = 0.45 \text{ m/m.} \]
\( \sigma_{\text{max}} \) = maximum normal stress (kilopascals = kPa)  
\[
= \frac{F}{\pi r^2} / 1000 \\
= \frac{1.96}{3.14} \times (2.5 \times 10^{-3})^2 / 1000 \\
= 99872.61 / 1000 \\
= 99.87 \text{ kPa.}
\]

\( \sigma_{\text{max}} \) = true maximum normal stress (kPa)  
\[
= \frac{\sigma_{\text{m}} (1)}{1 + 0.49 (e_{\text{m}})^2} \\
= \frac{(99.87) (1)}{1 + 0.49 (0.45)^2} \\
= 90.85 \text{ kPa.}
\]

SN = true maximum shear strain (m/m)  
\[
= \frac{\varepsilon_{\text{m}} (1.49)}{1.49} \\
= (0.45) (1.49) \\
= 0.67 \text{ m/m.}
\]

ST = true maximum shear stress (kPa)  
\[
= \frac{\sigma_{\text{max}}}{2} \\
= 90.85 / 2 \\
= 45.43 \text{ kPa}
\]
REFERENCES


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Lee, C. 1992. NE-123 Participants to the standardization of failure gel testing method. UR!


