INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
Decreasing the gelation temperature of whey protein concentrates to increase functionality

Hwang, Chien-Seng, Ph.D.
The Ohio State University, 1994
DECREASING THE GELATION TEMPERATURE OF WHEY PROTEIN CONCENTRATES TO INCREASE FUNCTIONALITY

DISSERTATION

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

By

Chien-Seng Hwang

* * * * *

The Ohio State University

1994

Dissertation Committee: Approved by

Dr. Michael E. Mangino
Dr. Grady W. Chism
Dr. Poul M.T. Hansen
Dr. Charles V. Morr

M. E. Mangino
Advisor

Food Science and Nutrition Graduate Program
DEDICATED
TO

My parents for their endless love and support
and to my wife,
Mei-Na
for her patience and encouragement
ACKNOWLEDGMENTS

I wish to express my most sincere appreciation and gratitude to my advisor, Dr. Michael E. Mangino, for his valuable guidance and inspiration during the course of this research and support throughout my graduate studies. As an advisor he has always been there for counsel and has been instrumental in teaching me a significant amount of new knowledge.

I also appreciate the reading committee, Dr. Charles V. Morr for his valuable suggestion and comments in this research, Dr. Poul Hansen for his counsel and assistant, and Dr. Grady Chism for reviewing the manuscript and for his helpful comments and discussions. Thanks also go to Dr. James Harper for his guidance and suggestion in cheese making and valuable relationship with cheese industry.

I am also grateful to all the faculty, staff and student of the Department of Food Science and Technology for their advise, friendship and kindness. I really appreciate the great assistance from Mr. A. McRoberts in the pilot plant. Thanks also go to my colleague, Linda Bruianek, Albert Chen and Korman Lee.
<table>
<thead>
<tr>
<th>Year Range</th>
<th>Education/Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 17, 1961</td>
<td>Born, Taiwan</td>
</tr>
<tr>
<td>1981 - 1985</td>
<td>B.S. in Food Science</td>
</tr>
<tr>
<td></td>
<td>Fu-Jen University, Taiwan</td>
</tr>
<tr>
<td>1987- 1988</td>
<td>Research Associate</td>
</tr>
<tr>
<td></td>
<td>Graduate Institute of Food Science and Technology</td>
</tr>
<tr>
<td></td>
<td>National Taiwan University, Taiwan</td>
</tr>
<tr>
<td>1988-1990</td>
<td>M.S. Food Science and Nutrition</td>
</tr>
<tr>
<td></td>
<td>The Ohio State University</td>
</tr>
<tr>
<td></td>
<td>Columbus, Ohio</td>
</tr>
<tr>
<td>1989- 1994</td>
<td>Graduate Research Associate</td>
</tr>
<tr>
<td></td>
<td>Department of Food Science and Technology</td>
</tr>
<tr>
<td></td>
<td>The Ohio State University</td>
</tr>
<tr>
<td></td>
<td>Columbus, Ohio</td>
</tr>
</tbody>
</table>
PUBLICATIONS


FIELD OF STUDY

Major Field: Food Science and Nutrition
TABLES OF CONTENTS

DEDICATION........................................................................................................ ii
ACKNOWLEDGMENTS....................................................................................... iii
VITA ..................................................................................................................... iv
LIST OF TABLES ................................................................................................. ix
LIST OF FIGURES ............................................................................................... x

CHAPTER

I. INTRODUCTION................................................................................................. 1

II. LITERATURE REVIEW.................................................................................... 3
  2.1. Whey ......................................................................................................... 3
  2.2. Manufacture of whey protein concentrates........................................... 6
  2.3. Structure and properties of whey proteins............................................ 7
  2.4. Functionalities of whey proteins............................................................. 9

III. EXPERIMENTAL PROCEDURES................................................................. 18
  3.1. Processing of whey protein concentrates.............................................. 18
  3.2. Determination of gel strength................................................................. 22
  3.3. Size exclusion HPLC analysis of proteins.......................................... 23
  3.4. Protein solubility..................................................................................... 23
3.5. Emulsion volume index (EVI) .................................................. 24
3.6. Measurement of viscosity ..................................................... 26
3.7. Measurement of intrinsic viscosity ........................................ 26
3.8. Effect of spray drying ......................................................... 27
3.9. Industrial scale processing of low-gelation-temperature WPC ... 28
3.10. Production of partial preheated WPC .................................. 31
3.11. Production of reduced fat smoke sausages ......................... 34
3.12. Application in angel-food cake .......................................... 36

IV. RESULTS AND DISCUSSION .................................................. 38
4.1. Effect of preheating temperature and time ......................... 38
4.2. Effect of pH ....................................................................... 42
4.3. Effect of calcium .............................................................. 45
4.4. Gelation temperature of preheated WPC ......................... 50
4.5. Size exclusion HPLC ......................................................... 52
4.6. Protein solubility .............................................................. 60
4.7. Emulsion stability ............................................................. 64
4.8. Viscosity and intrinsic viscosity of preheated WPC ............. 66
4.9. Industrial processing of preheated WPC ......................... 71
4.10. Production of WPC with different ratio of preheated WPC to unpreheated WPC ... 76
4.11. Application of preheated WPCs ........................................ 84

V. CONCLUSIONS .................................................................... 89
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Composition of sweet and acid whey</td>
<td>4</td>
</tr>
<tr>
<td>2. Concentration and properties of proteins in whey</td>
<td>5</td>
</tr>
<tr>
<td>3. Multistage cooking condition of smoked sausages</td>
<td>35</td>
</tr>
<tr>
<td>4. Effect of preheating time and temperature on the [\text{gel strength of WPCs} ]</td>
<td>40</td>
</tr>
<tr>
<td>5. The effect of [\text{pH} ] of preheating on the [\text{gel strength of WPCs} ]</td>
<td>44</td>
</tr>
<tr>
<td>6. Effect of preheating and addition of calcium chloride on the [\text{emulsion volume index of protein stabilized emulsions} ]</td>
<td>64</td>
</tr>
<tr>
<td>7. Sensory scores of normal and reduced-fat smoked sausages produced with WPC</td>
<td>84</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
</tr>
</tbody>
</table>

1. Whey protein processing procedures .................................. 20
2. Determination of gel strength of WPCs ............................... 22
3. Industrial Scale processing of low-gelation-temperature WPC ........................................................................ 29
4. Pilot plant processing of low-gelation-temperature WPC with different degree of preheating ............................ 32
5. The effect of preheating time on the gel strength of WPC heated 60°C ................................................................. 41
6. The effect of concentration of calcium ions before preheating on the gel strength of WPC heated at 60°C ........................................................................................................ 46
7. The effect of addition of CaCl₂ in the preheated WPCs on the gel strength ........................................................................ 48
8. The effect of preheating and addition of calcium chloride on the gel strength of WPCs heated at 90°C .................................................................49

9. Gelation temperature of the WPC preheated at 70°C 20 min. (40 mM CaCl2) ..................................................................................................51

10. Effect of preheating on the molecular size
distribution of proteins analysis by size exclusion HPLC .................................................................................................................................54

11. The size exclusion HPLC profile of non-preheated WPC .........................................................................................................................55

12. The size exclusion HPLC profile of preheated WPC .........................................................................................................................56

13. The size exclusion HPLC profile of WPC preheated at pH 6 .....................................................................................................................57

14. The size exclusion HPLC profile of WPC preheated at pH 4 .....................................................................................................................58

15. Influence of pH during preheating on the molecular weight distribution of WPCs ..................................................................................59

16. The effect of pH during preheating on the protein solubility of WPCs .................................................................................................62
17. The change of viscosity of WPC during preheating..67

18. The effect of concentration levels on the viscosity of WPCs.................................................................68

19. The intrinsic viscosity of unpreheated WPC..............69

20. The intrinsic viscosity of preheated WPC..................70

21. Heat transfer of whey protein solution in 11
    mm(I.D.) test tubes..................................................................................................................73

22. Proposed method for the industrial manufacture of low-gelation-temperature WPC..................................74

23. The gel strength of WPCs with different ratio of preheated to unpreheated WPC heated at 60°C........79

24. The gel strength of WPCs with different ratio of preheated to unpreheated WPC heated at 90°C.......80

25. Relationship between per cent soluble protein aggregate and gel strength at 60 °C.........................81

26. Relationship between per cent soluble aggregates and gel strength at 90°C...........................................82
CHAPTER I

INTRODUCTION

Cheese whey is the watery portion of milk that is usually obtained from the separation of cheese curd. Of the estimated 20 billion Kg (90% sweet whey and 10% acid whey) produced about 60 % was used in foods and animal feeds (Morr, 1989). An important functional property of whey proteins is their ability, under appropriate conditions, to form heat-induced gel structures capable of immobilizing large quantities of water (Hermansson and Akesson, 1975; McDonough et al., 1974; Sternberg et al., 1967). The polymer-polymer and polymer-solvent interactions are so balanced that they form tertiary network or matrix (Schmidt, 1981). Gel quality is an important factor in determination of the sales price of the WPCs. The mechanism of heat-induced gelation involves two steps: (1) an initiation step involving unfolding or dissociation of protein molecules, followed by (2) an aggregation step involving
association or aggregation, resulting in gel formation. For the formation of a highly ordered gel, it is essential that the aggregation step proceed at a slower rate than the unfolding step.

The dependence of gel strength of whey proteins on salt concentration was investigated by Schmidt et al. (1978). The maximum gel hardness was observed with his whey proteins when the CaCl$_2$ concentration was 11 mM. Removal of minerals and electrodialysis of whey protein concentrates or isolates enhanced the strength and elasticity of heat induced gels (Schmidt et al., 1984; Kuhn and Foegeding, 1991). Important variables related to gel strength include protein hydrophobicity, calcium ion concentration and the ability of the protein to unfold. Optimal gel strength in commercially available WPC products is obtained following heating to 80°C or greater. In certain applications, the formation of gels at lower temperatures would be desirable. When mixed WPC-myosin gels are formed, they are often weaker than the gels formed by either protein alone. This is due, in part, to formation of myosin gels at a much lower temperature. Substitution of WPC for egg in certain applications has been frustrated by the high temperature required to form WPC gels. The purpose of this research was to devise a method that would decrease the temperature of gelation of WPC.
CHAPTER II

LITERATURE REVIEW

2.1. Whey

The annual production of cheese whey in the United States is estimated to exceed 20 billion Kg (90% sweet whey and 10% acid whey). There are two major type of whey including sweet whey and acid whey. Sweet whey, with a pH of about 5.6 or higher, is the by-product of rennet-coagulated cheese, manufactured from whole milk, or of rennet casein, manufactured from skim milk. Acid whey, with a pH of 5.1, is produced by direct acidification of skim milk for casein manufacture or from Cottage cheese manufacture. The compositions of these types of whey were presented in table 1 (Morr, 1989). The amount and properties of whey proteins are given in Table 2.
<table>
<thead>
<tr>
<th></th>
<th>Sweet whey</th>
<th>Acid whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>4.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Protein</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Minerals</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Milk fat</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Water</td>
<td>93.4</td>
<td>93.6</td>
</tr>
</tbody>
</table>

(Morr, 1989)
Table 2. Concentration and properties of proteins in whey.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (g/liter)</th>
<th>% of total protein (%)</th>
<th>Isoelectric Point (pH)</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-lactoglobulin</td>
<td>3.0</td>
<td>50</td>
<td>5.3-5.5</td>
<td>18,300</td>
</tr>
<tr>
<td>a-lactalbumin</td>
<td>0.7</td>
<td>12</td>
<td>4.2-4.5</td>
<td>14,000</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>0.6</td>
<td>10</td>
<td>5.5-8.3</td>
<td>15x10^3-1x10^6</td>
</tr>
<tr>
<td>BSA</td>
<td>0.3</td>
<td>5</td>
<td>5.1</td>
<td>69,000</td>
</tr>
<tr>
<td>Proteose-peptone</td>
<td>1.4</td>
<td>23</td>
<td></td>
<td>4.1-40.8x10^3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6.0</strong></td>
<td><strong>100</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Morr, 19890)
2.2. Manufacture of Whey Protein Concentrates:

The annual production of whey protein concentrate in the United States had reached 173 million lbs in 1991. These WPCs contain 35 to 85% protein or even as high as 90% protein for the whey protein isolates. They are used as functional ingredients in formulated food products (Morr, 1992).

The whey drained from cheese curd containing 0.5 to 0.7% protein is pasteurized to inhibit the growth of bacteria and inactive enzymatic activity. After pasteurization whey is processed by ultrafiltration (UF) to remove water, lactose, and minerals. Following ultrafiltration the UF retentate is dialfiltered against more than 3 fold of water to remove more lactose and calcium. The retentate is further concentrated to greater than 20% total solids.

Morr and Foegeding (1990) examined the composition, physicochemical properties, flavor, and functionality of some commercial WPCs. Their results indicated the variability of commercial WPC and suggested possible processing modifications for manufacturing WPC with improved functionality. They suggested that variability was due to variations in (1) the composition of milk used for making cheese; (2) the composition of whey from plants that manufacture different kinds of cheese; (3) the composition of different lots of whey from the same plant; (4) the length and temperature of
whey storage; and (5) the processing conditions (Melachouris, 1984).

The pH of a protein solution will affect its net charge causing differences in structure. The effect of pH during processing on the gelling properties of WPI and WPC were studied by Lupano et al. (1992) and Chen (1993). They found that processing of whey proteins at acid pH (3.0 to 4.0) improved the efficiency of the membrane process and remove more calcium. Thermal gels of these calcium reduced whey proteins were higher in gel strength.

2.3. Structure and Properties of Whey Proteins:

Whey proteins are the major nitrogen containing compounds remaining in milk after precipitation of casein by acid (pH 4.6) or by rennet (pH 6.7). The whey proteins including β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), the immunoglobulins (Ig-G, Ig-A, and Ig-M), and lactotransferrin.

2.3.1. β-lactoglobulin

β-lactoglobulin is the major whey protein, representing about 50% of the whey proteins or 12% of the total milk proteins. It is a typical globular protein. The monomeric molecular weight of β-
lg is about 18,400. Between pH 5.5 and 7.0, the β-lg exist as a dimer of molecular weight of 36,700. In the pH range between 3.5 to 5.2, dimers of β-lg A or β-lg B tetramerize to form octamers of about 147,000 Da. Below pH 3.5, the dimers dissociate due to strong electrostatic repulsive forces. β-lg undergoes reversible conformational changes at pH values higher than 7.0. As a result one abnormal carboxyl group and the thiol group become more reactive.

2.3.2. α-lactalbumin

α-lactalbumin represents about 20% of the proteins of whey. It is a small molecule with a molecule weight of about 14,000 Da. Its ionic point is about pH 4.8. α-lactalbumin is a calcium-binding metalloprotein, which is also capable of binding zinc and other metals. The heat stability of α-lactalbumin is reduced by removal of calcium ions and apo-α-la does not renature following thermal denaturation. α-la may also undergo irreversible denaturation due to the formation of intermolecular disulfide bonds.

2.3.4. HPLC analysis of whey proteins:

Dairy science has developed rapidly in recent years due to the improvement in analytical techniques. High Performance
Liquid Chromatography (HPLC) with advantages of ease, speed, sensitivity and accuracy of analysis, has been used in the analysis of whey proteins during or after processing. It includes reverse phase HPLC which separates proteins on the basis of polarity and size exclusion (gel permeation chromatography) (Diosady et al., 1980; Li-Chin; 1983; Morr, 1987; Regester et al., 1992). A high degree of variability in individual proteins was found from the size exclusion HPLC results of Morr and Foegeding (1990).

2.4. Functionalities of Whey Proteins

According to De Wit (1989), functionality of whey proteins might be defined as those physico-chemical properties which contribute to desired characteristics in food products, including gelation, emulsion, foaming, viscosity, and water holding ability.

2.4.1 Gelation of Whey Proteins:

Gelation, as defined by Flory (1941), involves a pre-gel stage, a gel point, and a post-gel stage. In the first stage, protein monomers form finite soluble protein aggregates. At the gel point, for chemical gels, the transition from a viscoelastic liquid to a viscoelastic solid, the insoluble chemical gel network...
structure is suddenly established. However at the gel point, the amount of true three-dimensional gel structure is negligible; all of the aggregating material exists as monomers, dimers, and larger aggregates (Hsieh, 1993; Ferry, 1948). For the formation of a highly ordered gel, it is essential that the aggregation step processed at a slower rate than the unfolding step (Hermansson, 1978). Heat treatment of proteins weakens the bonds that maintain their secondary and tertiary structures. If thermal denaturation occurs, the proteins unfold, and bind water. Protein-protein interactions lead to the formation of a three dimensional network capable of entraining water molecules and a gel is likely to form (Mangino, 1992). If the network is too weak, the viscosity will increase but fluid flow will be possible and a true gel will not form. If, on the hand, the protein-protein interactions are too strong, the network will collapse and water will be expelled from the structure (Mangino, 1992). The gelation of whey proteins can be induced by the thermal treatment, addition of organic solvent (e.g. alcohol), or addition of urea (Xiong and Kinsella, 1990)

β-Lactoglobulin is considered to be the most important whey protein for thermal gelation. The gelling properties of α-lactalbumin were always regarded as inferior to those of β-lactoglobulin, since whey enriched with α-lactalbumin has poor heat-setting properties (Mulvihill, 1989).
Barbut and Foegeding (1993) compared the difference between thermally induced and calcium induced WPC gel by SEM. Thermally induced gels showed a particulate microstructure composed of bead-like particles attached to each other. In contrast, the calcium induced gels consisted of a fine network of protein strands.

Factors important in the gelation of WPCs include protein hydrophobicity, pH, salts concentration, calcium concentration, and free sulfhydryl concentration (Mangino, 1992). Many researches have investigated methods to improve gel strength. These include modification of proteins by thermal treatment, adjustment of ionic concentration and pH, or enzyme treatment. However, it has not been easy to reproducibly improve gel quality.

Effect of salts: The gel properties of whey proteins have been closely related to the ions in the protein solution. Gelation occurs at pH 3 - 4.2 and 7.2 - 11 only when in combination with specific salt concentrations. An excess of salt produces protein precipitate and too little salt inhibits protein-protein interactions (Paulsson et al., 1986). Schmidt (1978) reported that the best gel was observed at 11 mM CaCl2 or 200 mM NaCl. At neutral pH but not acid pH, the gel firmness appeared to be more sensitive to calcium than to disulfide interchange reactions (Shimada and
Cheftel, 1988, 1989). Dialysis of whey protein at an acid pH removed much more calcium and the increase the elasticity of the gels formed (Lupano, 1992). The hypothesis proposed by Lupano et al. (1992) state that:

At low concentrations, calcium enhances protein-protein interactions and contributes positively to the gel network. At high concentrations (above 20 mM), calcium chloride causes excessive protein aggregation (isoelectric-type) with detrimental effects on gel cohesiveness and firmness. While the mechanisms involved at acid pH are less clear.

In the recent studies of Barbut and Foegeding (1993), the addition of 20 mM CaCl2 to preheated WPI induced the gelation of whey proteins at room temperature.

**Effect of pH:** The gel strength and appearance were affected by the pH of gelation. At low pH values, the gels were rather soft and opaque. At high pH values, the gels became more elastic and transparent with greater gel strength (Schmidt, 1981). Kohnhorst and Mangino (1985) reported the dependence of sulfhydryl groups on the pH values. At pH below 7 there was little effect of sulfhydryl content on gel strength. At higher pH values, the effects of sulfhydryl groups became significant.

The viscosity transition temperature associated with unfolding/aggregation of whey proteins is pH dependent
(Hermansson, 1979). The transition temperature decreased as pH increased. Xiong (1992) described the various transitions of whey protein during thermal aggregation at pH 5.5 to 6.5. The aggregation showed a single transition (71°C) at pH 5.5, two transitions (76 and 88 °C) at pH 6.0, and no transition above 6.5. When heated at pH 6 whey proteins started to aggregate at 67°C. Jeyarajah and Allen (1994) investigated the effect of pH on the calcium-binding properties of β-lactoglobulin using an ion-selective electrode. The binding of calcium to the β-lactoglobulin was only observed at pH value above 6. The increase in negative charge of β-lactoglobulin at high pH may account for the calcium binding ability.

Effect of heat treatment: Heat treatments such as preheating, pasteurization, and sterilization affect the structure and properties of whey proteins, either reversibly or irreversibly (De Wit, 1984). Parris et al. (1991) studied the thermal denaturation of whey proteins by reverse-phase HPLC and proposed two stages of denaturation. Aggregation of whey proteins begins at 70 °C, and more aggregation was found in sweet whey than in acid whey. They also found the formation of dimers of β-Lg and a β-Lg-α-La complex having molecular weights of 36,000 and 35,000, respectively. A study of whey
protein denaturation by low resolution NMR (Lambelet at al., 1989) found that an increase in relaxation rate, $T_2$, for β-lg took place at temperature between 40 to 80 °C. Heat treatment at high pH during processing resulted in the formation of soluble denatured whey protein with improved gelation properties (De wit, 1986).

Recently some studies had focus on the behavior of preheated whey proteins. Barbut and Foegeding (1993) found that WPI preheated at temperatures higher than 70 °C would gel with the addition of 10 mM CaCl$_2$ at room temperature. However the gels were weak. Following this study, Jeyarajah and Allen (1994) look at the structure of preheated and native β-lactoglobulin after binding of calcium ions. An increase in binding affinity was observed for β-lactoglobulin after preheating. They suggested that small conformational changes after heat treatment may increase the binding affinity of calcium ions.

2.4.2. Emulsion:

Emulsions are dispersions of one liquid phase in the form of fine droplets in another immiscible liquid phase. The two immiscible phases are usually oil and water, so emulsions can be broadly classified as oil-in-water or water-in-oil emulsions depending on the dispersed phase (Narsimhan, 1992). In the formation of an emulsion, whey proteins diffuse to and are
absorbed at the oil water interface. The migration of proteins from the solution to the interface is thermodynamically favorable because some conformational and hydrational energy of the protein is lost at the interface. Once at the interface, most proteins unfold to varying extents, reorient, rearrange, and spread to form a continuous cohesive film. Hydrophobic loops orient in the apolar phase, while polar charged segments extend into the aqueous phase. Most of the molecule occupies the interface, interacts with neighboring molecules and imparts strength and viscosity to the film (Philips, 1981).

The factors affecting the emulsifying properties of proteins are adsorption kinetics, interfacial load, reduction of oil/eater interfacial tension, rheology of the interfacial film, and surface hydrophobicity of proteins (Das and Kinsella, 1990).

2.4.3. Foaming:

Foaming is defined as the creation and stabilization of gas bubbles in liquid. Essential for the formation of protein-based foams is a rapid diffusion of protein to the air-water interface to reduce surface tension, followed by partial unfolding of the protein. This will result in the encapsulation of air bubbles and in the association of protein molecules leading to an intermolecular cohesive film with a certain degree of elasticity (De wit, 1989). As the protein concentration increases the foam became more
condensed, with more uniform air bubbles of a finer texture. Generally, overrun increases with protein concentration to a maximum value after which it decreases again (De Wit, 1989).

2.4.4 Application of WPC in Bakery Products

For both nutritional and organoleptic reason, there is continuous interest in fortifying or substituting wheat flour by milk solids in bakery products. None of the milk proteins has properties close to those of gluten and attempts to replace wheat protein completely have led to unacceptable bread. Whey protein appears to contain a loaf volume depressant. Some experiments have failed to replace whole egg with WPC in the cakes. The WPC batter completely collapsed during baking with fat separation. The problem was partly solved by reduction of coagulation temperature or homogenization of the fat-WPC emulsion (De Wit, 1989).

2.4.5. Meat products

The high price of meat products and the large variations in the quality of meat proteins are important factors for using non-meat proteins. Whey proteins provide water-holding capacity and fat-
binding or emulsifying abilities are commonly in the meat products. On the other hand, today's consumers are not only interested in quality products which taste good and are convenient but are also concern about health and nutrition. With the reduction of fat, the low-fat ground beef products lost their juiciness and tenderness. Addition of whey proteins to enhance water binding has been used in some formulations. However, due to shrinkage of the whey protein network upon heating, the stability is not expected to be very high (De Wit, 1989).
CHAPTER III

EXPERIMENTAL PROCEDURES

3.1. Processing of whey protein concentrates:

Raw milk received from The Ohio State University Dairy Farm was used for making Cheddar Cheese whey. After the whey was drained, it was clarified at 55°C by centrifugation followed by high-temperature-short-time (HTST) pasteurization. Twenty grams of citric acid were added to 300 liters of whey. The pH was adjusted to 3 before concentrating five-fold by ultrafiltration (UF) with a Romicon UF-PM 50 hollow fiber membrane. The inlet pressure for the UF processing was 24 psi and outlet pressure was 12 psi at an operating temperature of about 40 °C. The UF retentate was clarified again at 55°C and further concentrated seven-fold by UF with addition of another 20 g of citric acid. Dialfiltration was performed by the addition of water to seven times the retentate volume and further concentration (seven-
fold). Following dialfiltration, one additional volume of water was added and dialfiltration continued to reduce the volume by 50% at pH 6. The retentate was adjusted to pH 7 and spray dried into whey protein concentrate powder (Figure 1) with 78.1% of protein as measured by the Kjeldahl nitrogen method (A.O.A.C, 1990). The calcium content of the WPC was 0.04%, as analyzed by Atomic Absorption methods (A.O.A.C., 1990)
Cheese Whey

\[ \downarrow \]

Separation at 55°C

\[ \downarrow \]

Pasteurization

\[ \downarrow \]

Add 20 g Citric acid, adjust pH to 3.0

\[ \downarrow \]

UF 5x

\[ \downarrow \]

Separate at 55 °C

\[ \downarrow \]

UF 7X

\[ \downarrow \]

Dialfiltration 7X at pH 3.0

\[ \downarrow \]

Add water to 2x

\[ \downarrow \]

Adjust to pH 6.0

\[ \downarrow \]

Dialfiltration to 9-10L

\[ \downarrow \]

Adjust to pH 7.0

\[ \downarrow \]

Spray Dry

Figure 1. Whey protein processing procedures
3.2. Determination of Gel Strength

To determine the gel strength of WPC, 10% protein solutions were hydrated for one hour and adjusted to pH values ranging from 2.5 to 8.0. Following hydration, 10 ml of protein solutions were placed in 11 mm (I.D.) test tubes and preheated at different temperature and pH for times ranging from 5 to 60 min. After preheating, the WPCs were cooled to room temperature in a water bath. From 50 to 200 μl of 4M CaCl₂ was added to the WPC solutions to obtain a final concentration of 10 to 80 mM CaCl₂. Gelation temperature and gel strengths were determined following heating at 40 to 60 °C for 20 min. The gel strength was measured using a 6 mm circular probe on the Instron. Gel samples heated at 90 °C were placed in 19 mm gel tubes with a cap on the bottom and heated for 20 minutes in a 90 °c water bath. After cooling for one hour, the gels were removed and cut into 20 mm long segments. The gel strength was measured with a General Instron Instrument (Model 1000) with a 6 mm circular probe. The gel were compressed using 6 mm probe for 10 mm (50%) to determine the gel strength (Figure 2).
10 % WPC solution
↓
Hydration for 1 hour
↓
Preheated at different pH, temperature for different time
↓
Cool to room temperature
↓
Addition of CaCl₂ at different levels
↓
Heating (40 to 90°C) for 20 min.
↓
Instron to measure gel strength

Figure 2. Determination of gel strength of WPCs
3.3. Size Exclusion High Performance Liquid Chromatography (SE-HPLC):

The effect of preheating on the content of α-lactalbumin, β-lactoglobulin, bovine serum albumin as well as other biopolymers in the WPCs was determined by Size Exclusion HPLC by the method of (Chen, 1993). The mobile phase was 0.1 M phosphate buffer (NaH$_2$PO$_4$) in 0.1 M sodium nitrate (NaNO$_3$) adjusted to pH 6.0 and degassed for 5 hours. A Waters 501 HPLC pump connected to a WISP 710B Sample Processor with auto injector was set at a flow rate of 0.5 ml/min. A 7.5 mm x 30 cm Beckman Spherogel TSK 3000 SW size exclusion column was preconditioned for 3 hours before use. Whey protein concentrates were prepared at 0.4% and filtered by 0.45 micron filter. Samples of 5 μl of were injected. The proteins were detected at 280 nm by a spectrophotometer detector. The HPLC System was operated by a NEC PowerMate SX Plus computer installed with a Maxima 820 Chromatography Workstation, version 3.3 software (Waters Dynamic Solutions, Millipore). Three trials were run for each protein sample.
3.4. **Protein Solubility**

The solubility of WPCs were determined by the method of Morr et al. (1985) with some modification. Ten per cent WPC solutions were hydrated for 1 hour. To observe the effect different pH condition on the protein solubility, after hydration for one hour, the WPC solutions were adjust to pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, and preheated in 19 mm (I.D.) gel tubes with a cap on one end of the tubes. After preheating at 70 °C for 20 minutes, they were cooled to room temperature in a water bath. A sorvall RC-5B refrigerated superspeed centrifuge was used to centrifuge the WPC solutions at 20,000x g for 30 minutes. The supernatant was filtered through whatman No.1 filter paper. Ten milliliters of the filtrate were used for the determination protein content by Kjeldahl method (A.O.A.C. 1980). Protein solubility was calculated as the portion of total protein recovered in the supernatant fraction.

\[
\text{% of protein solubility} = \frac{\text{% Soluble protein content (w/w)}}{\text{% Total protein content (w/w)}} \times 100 \quad (1)
\]

3.5. **Emulsion Volume Index (EVI):**

The EVI was determined by a centrifugal method (McDermott et al. 1981). In each determination, 10 % WPC was hydrated for
one hour and then stored at 5 °C overnight. The next day the solutions were preheated at 70 °C for 20 minutes and cooled to room temperature. CaCl$_2$ was added to obtain selected calcium concentrations. The following formulation was used to make the emulsions.

\[
\begin{align*}
\text{WPC} & \quad 4.1 \text{g} \\
\text{Sodium chloride} & \quad 0.1 \text{g} \\
\text{Sucrose} & \quad 13.6 \text{g} \\
\text{Lecithin} & \quad 0.4 \text{g} \\
\text{Vegetable oil} & \quad 7.2 \text{g} \\
\text{Distill water} & \quad \text{add to 100 g}
\end{align*}
\]

Before homogenization the oil with lecithin was weighed into a beaker. The other ingredients were heated to 55 or 60 °C for 0 or 20 minutes. Then they were added to the beaker with oil and lecithin. The mixture was stirred by a magnetic stirrer and homogenized by a valve homogenizer twice.

A 1 ml sample of the emulsion was placed in a porcelain spot plate to which 10 µl of Oil Red O stain was added. The sample was mixed and 10 µl of Methylene Blue was added and mixed until all particles of stain were dispersed. Microhematocrit tubes (75 mm) were filled with the sample and centrifuged in a Damon/IEC Microhematocrit Centrifuge (Model MB) for 11,5000 RPM or 13,500 X G for 30 minutes. After centrifugation, the capillary tubes were evaluated by measuring the length of the emulsion
layer(s), sediment layer(s) and total length. The following parameters are calculated:

\[(EVI) = \left( \frac{\text{Emulsion Length}}{\text{Total Length}} \right) \times 100 \] .......................................................(2)

The larger the value for EVI, the more stable the emulsion is towards gravitational creaming. Creaming is a major quality defect in this type of product.

3.6. Measurement of viscosity of WPC during preheating:

To determine the viscosity of whey protein concentrates at different time of heating, a Cannon-Fenske Routine Viscometer of sizes 100, 200, and 300 were used. After preheating at pH 7, 10 ml of 10% whey protein concentrate solutions were placed in the viscometer and immersed in 25 °C water bath for 30 minutes to measure the viscosity.

3.7. Intrinsic Viscosity

The dilutions ranged from 1% to 3% protein. A size 50, Calibrated Cannon-Fenske Routine Viscometer was used to measure the relative viscosity (relative to water) of each diluted
sample. Each viscometer was cleaned with hot nitrous acid, rinsed with distilled water, and then dried with acetone. The viscometer with sample was immersed in 25 ±0.02°C water bath 30 minutes to allow the samples to equilibrate to the bath temperature.

The specific viscosity defined as the ratio of the viscosity of diluted WPC solution ($\eta'$) to the viscosity of water ($\eta^*$).

The reduced viscosity ($\eta_{\text{red}}$) = \frac{( \eta_{sp}-1)}{C} .............................................. (3)

where $c$ = protein concentration.

By extrapolation of the reduced viscosity to the intercept at 0% protein concentration, the intrinsic viscosity, $\eta$, was determined.

3.8. Effect of spray drying

The effects of protein denaturation and aggregation caused by dehydration and heating during spraying has been extensively discussed. In order to make a spray dried WPC powder with low gelation temperature, 1000 ml of 10% WPC solution was preheated in fifty 19 mm glass tubes for 20 minutes and cooled to room temperature. The preheated WPC solutions were later spray dried to produce WPC powder. To examine the gelation temperature, the powder was rehydrated and 40 mM CaCl$_2$ was added before heating in a 60 °C water bath for 20 min.
3.9. Industrial scale processing of low-gelation temperature WPC:

If the preheated WPC could maintain the ability to gel at reduced temperature after spray drying, it would be more practical if we could preheat whey protein at the final stage of membrane processing. After separation at 55°C and pasteurization, sweet cheese whey was ultrafiltered at pH 3 to 5 fold and followed with another separation at 55°C. The retentate was concentrated 7 fold by ultrafiltration. Seven volume of water was added to perform dialfiltration. The pH of retentate was adjust to pH 7 after dialfiltration and 4 x of water was added to continue another 4 fold of dialfiltration. The concentrated whey was heated at 68.5° C for 15 min. To meet the demands of WP industries which requires that the WPC have a total solid above 15% before spray drying, the WPC was further concentrated to 15% total solid by a rotatory evaporator (Figure 3).
Cheese Whey
  ↓
Separation at 55°C
  ↓
Pasteurization
  ↓
Add 20 g Citric acid, adjust pH to 3.0
  ↓
UF 5x
  ↓
Separate at 55 °C
  ↓
UF 7X
  ↓
Dialfiltration 7X at pH 3.0
  ↓

Figure 3. Industrial scale processing of low-gelation-temperature WPC.
Add water to 4x

Adjust to pH 7.0

Dialfiltration 4 fold (Total solid 7.2%)

Heated to 68.5°C hold for 15 min.

Cooled

Concentrated to 15 % total solid by rotatory evaporator

Spray Dry (outlet temperature 70°C)

Add CaCl₂
3.10. Production of partial preheated WPC

It was found that when a portion of the WPC was preheated and mixed with non-heated proteins, the mixture was able to form gels at lower temperatures. The preheated mixtures were lower in viscosity and could be further concentrated to higher levels of total solids. After membrane processing as described in section 3.9 and adjustment of pH to 7, only half of the retentate was preheated to 68.5 °C for 15 minutes. Combinations of preheated and unpreheated retentates were made to prepared samples with 100, 75, 50, 25, and 0% of preheated WPC (Figure 4).
Cheese Whey
↓
Separation at 55°C
↓
Pasteurization
↓
Add 20 g Citric acid, adjust pH to 3.0
↓
UF 5x
↓
Separate at 55 °C
↓
UF 7X
↓
Dialfiltration 7X at pH 3.0
↓
Add water to 4x
↓
Adjust to pH 7.0
↓
Dialfiltration to 4 fold (Total solid 7.2%)
↓

Figure 4. Pilot plant processing of low-gelation-temperature WPC with different degree of preheating
Half of retentate was heated to 68.5°C hold for 15 min.
   Half of retentate was not heated
   \downarrow
   Cooled
   \downarrow
   Different combination of preheated with unpreheated
   WPC
   \(v:v=100:0, 75:25, 50:50, 25:75, 0:100\)
   \downarrow
   Concentrated to 15% total solid by rotatory evaporator
   \downarrow
   Spray Dry (outlet temperature 70°C)
   \downarrow
   Add CaCl2
   \downarrow
   Hydration for one hour
   \downarrow
   Measure gel strength after heating
   at 60°C or 90°C for 20 min.
3.11. Production of reduced fat sausages:

To determine if the low gelation temperature WPC offered an advantage in such a product, three smoked beef sausages were manufactured. The control contained 25% fat. One made with control WPC replaced some of the fat with WPC and water. For each 10% fat replaced, 1% WPC and 10% water were added. The fat was run through a coarse plate and the lean through a fine plate grinder before chopping by a bowl chopper. Whey protein solution, seasoning (salt, black pepper, coriander, nutmeg, sage, caynne, and nitrite), soy protein and fat were added. The meat mix was filled into hog casing and cooked at multi-stage cooking conditions (Table 3):
Table 3. Multi-stage cooking condition of smoked beef sausages

<table>
<thead>
<tr>
<th>Stage</th>
<th>Wet bulb (°F)</th>
<th>Time (min.)</th>
<th>Dry Bulb (°F)</th>
<th>Smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Off</td>
<td>30</td>
<td>120</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Off</td>
<td>30</td>
<td>130</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>120</td>
<td>150</td>
<td>Yes</td>
</tr>
</tbody>
</table>
The finished product contained 8.5% fat. Another product was made with WPC that had been preheated and had calcium added. The sausages were rated by a 20 member panel for a number of characteristics.

3.12. Application in Angel-Food Cake:

To replace part of the egg white in the formulation of angel food cake, WPC preheated at 70°C for 20 min. was spray dried and rehydrated to make a WPC solution with 13.3% protein to simulate liquid egg white. The following recipe was used to prepare the angel food cake.

1 1/4 cups egg white (300 ml)
1 cup plus 2 table sp. sifted cake flour
1 1/2 cup sifted granulated sugar
1/4 teasp. salt
1 1/4 teasp. cream of tartar
1 teasp. vanilla extract
1/4 teasp. almond extract

1. set out egg whites about 1 hr. ahead.
2. When ready to make, start heating oven to 375 °F. Sift flour with 1/2 cup sugar 4 times.

3. In large bowl, combine egg whites, salt, cream of tartar, extracts. With electric mixer at high speed, beat egg whites until stiff enough to hold soft, moist peaks.

4. With mixer at the same speed, beat in 1 cup sugar, sprinkling 1/4 cup at a time over egg whites. Beat until sugar is just blended.

5. Stop mixer. Shift in flour mixture by fourths, folding in each addition with 15 complete fold over strokes of rubber spatula and turning bowl often. After all flour has been folded in, give batter 10 to 20 extra strokes.

6. Gently push batter into ungreased 4"-deep 10" tube pan. With spatula, cut through batter once without lifting spatula out of batter.

7. Bake 30 to 35 min. or until cake tester, inserted in center, come out clean. Put the pan upside-down on a glass bottle and cool for one and a half hour.
CHAPTER IV

RESULTS AND DISCUSSION

4.1. Effect of preheating temperature and time

The effect of preheating conditions are presented in table 4. To decrease the gelation temperature to below 60 °C, whey proteins must be preheated at above 65 °C at pH 7. Preheating at 70°C increased gel strength to above 1 Newton when gelation occurred at 60 °C. After preheating at different temperature, the calcium concentration of the WPC solutions was adjusted to 40 mM by addition of concentrated CaCl2. Control WPC will not gel at temperatures below 78°C. Preheating at temperatures above 75 °C for 20 minutes caused the formation of gel during preheating. Barbut and Foegeding (1993), reported that WPI could be preheated at a temperature above 75°C without gel formation. In our experiments WPC with reduced calcium (< 0.04%) formed a gel at temperatures above 75 °C. Whey protein
isolate contains almost no calcium for the formation of crosslinks and was stable at temperatures above 75°C. Gel formation occurred only after the addition of calcium at room temperature. Proteins were heated to above 70°C for 0 to 30 minutes and then calcium added to give a final concentration of 40 mM. Figure 5 demonstrates that to form a good gel the WPCs must be preheated about 20 minutes. Heating more than 30 minutes, the viscosity of the whey protein increased. The preheated whey proteins were cooled to room temperature. After addition of calcium chloride, the viscosity of the WPC increased, however, no gel formation was observed. This is different from the observation of Barbut and Foegeding (1993). In their research, the preheated WPI solution could gel at room temperature after addition of calcium chloride in the solution. We did observe that when WPC was preheated at 75 °C for five minutes and addition calcium chloride (40 mM) was added, a gel was formed at 60 °C. The differences can probably be explained by the much lower concentration of calcium in WPI compared to our reduced calcium WPC. Further work in this area is warranted.
Table 4. Effect of preheating time and temperature on the gel strength of WPCs at 60°C. (40 mM of CaCl₂ in the final WPC solutions)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time (min)</th>
<th>After preheating</th>
<th>Gel strength (NT) After 2nd heating</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>20</td>
<td>No gel</td>
<td>No gel</td>
</tr>
<tr>
<td>65</td>
<td>20</td>
<td>No gel</td>
<td>0.07</td>
</tr>
<tr>
<td>70</td>
<td>20</td>
<td>No gel</td>
<td>1.6</td>
</tr>
<tr>
<td>75</td>
<td>20</td>
<td>Formed gel</td>
<td>-----</td>
</tr>
<tr>
<td>75</td>
<td>5</td>
<td>No gel</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Figure 5 - The effect of preheating time on the gel strength of WPC heated at 60 °C
4.2. Effect of pH

WPC at 10 % protein were adjusted to pH values of 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, and 8.0 and were preheated at 70 °C (Table 5). After preheating at these pH values, the samples' pH were adjusted back to 7.0. Forty mM of CaCl₂ was added prior to heating at 60°C to determine their ability to gel at reduced temperatures. Preheating at pH 2.5 to 6.0 increased the viscosity of the protein solution. The sample preheated at pH 3 had a more translucent color than the sample preheated at pH 5.0. The samples preheated at pH 3 and 4 became very viscous and almost formed a gel. Since these pH were very close to the isoelectric pH of β-lactoglobulin and α-lactalbumin, both proteins had reduced net charge. The intermolecular interaction of hydrophobic and van der Waals bonds were increased. The sample preheated at pH 6.0 had a lower viscosity than those preheated at pH 3 to 5. No gel was formed when these samples were adjusted to pH 7 and heated at 60 °C for 20 min. At pH values 7.5 and 8.0, the preheated WPC solutions formed gels during preheating.

Hermansson (1979) had reported a sharp increase in viscosity of 10 % WPC solution at about 70°C and the transition temperature decreased as pH increased. When the preheating temperature was increased to 75 °C, samples preheated for 5 minutes formed gels at 60 °C. In other studies (Parris et al., 1991 and Xiong, 1992),
infrared spectroscopy and optical density also showed that about 68°C is the transition temperature at which whey proteins begin to unfold and aggregate.

On the other hand, if the WPC were preheated at pH above 8.0 for 20 minutes, formation of gels took place during the preheating stage. To make a low-gelation-temperature WPC preheated at pH 8, the WPC solution was heated at 65°C for 20 minutes. When heated at this temperature, the WPC did not gel during preheating. Thus, the solution could be heated again after addition of calcium to form the gel at 60 °C.
Table 5. The effect of pH of preheating on the gel strength of WPCs. (WPCs were heated at 70 °C for 20 min and 40 mM of CaCl$_2$ was added before heating)

<table>
<thead>
<tr>
<th>pH</th>
<th>Preheating Temperature(°C)</th>
<th>After Preheating</th>
<th>Gel strength (NT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>3.5</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>5.5</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>Viscosity increased</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>No gel</td>
<td>1.6</td>
</tr>
<tr>
<td>7.5</td>
<td>70</td>
<td>Form Gel</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>Form Gel</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>No gel</td>
<td>0.73</td>
</tr>
<tr>
<td>8.5</td>
<td>70</td>
<td>Form Gel</td>
<td></td>
</tr>
</tbody>
</table>
4.3. Effect of calcium

Calcium ions serve as crosslinking agents during gel formation. Excessive calcium causes too much crosslinking and protein precipitation occurs before a strong gel network can become established (Kuhn and Foegeding, 1991). Figure 6 demonstrates that the addition of calcium to WPC prior to preheating decreased the gel strength at 60 °C. With calcium addition higher than 0.065%, gel formation at 60 °C was impossible. This suggest that the calcium of the WPC must be removed during ultrafiltration and diafiltration process of whey. Preheating at the early stage of ultrafiltration or before ultrafiltration would not be effective in reducing gelation temperature in a large scale processing.

To form a gel at reduced temperature, the calcium ions must be added following preheating. After preheating, the WPCs were cooled to room temperature. The addition of less than 20 mM CaCl₂ was inadequate for gel formation at 60 °C (Figure 7). The best concentration for the formation of firm gel was about 40 mM CaCl₂. The proper combination of preheating and addition of CaCl₂ not only caused a decrease in gelation temperature but also increased gel strength upon heating to 90 °C (Figure 8). With only preheating or addition of calcium ions, the gel strength was not improved. With both preheating at 70°C for 20 minutes and the addition of 40 mM CaCl₂, the gel strength was about twice as high
when heated to 90°C. This improvement in gel strength would be very desirable in the application of WPC in surimi industries. It was estimated that almost half of the high-gel-strength WPC produced in the United States was exported to Japan. Their surimi industries require a whey protein concentrate with a gel strength above 2 Newton or more. The higher the gel strength, the better the price.
Figure 6 - The effect of concentration of calcium chloride before preheating (70 °C, 20 min) on the gel strength of whey protein concentrate heated at 60 °C.
Figure 7 - The effect of addition of CaCl2 in the preheated WPCs on the gel strength (preheated at 70 °C for 20 min).
Figure 8- The effect of preheating (70°C, 20 min) and addition of calcium chloride on the gel strength of WPCs heated at 90 °C in 19 mm tubes.
4.4. Gelation temperature of the preheated WPC:

Under normal conditions the gelation temperature of 10% whey protein concentrate is about 75 °C. Figure 9 demonstrates that with preheating at 70°C for 20 minutes and addition of 40 mM CaCl₂ the gelation temperature had decrease to below 40°C. After addition of CaCl₂ into the preheated WPC solution, just mild heating could result in the formation of protein gel. This differs from whey protein isolate (Barbut and Foegeding, 1993) which formed a gel right after addition of CaCl₂ at room temperature.
Figure 9. Gelation temperature of the WPC preheated 70°C, 20 min. and with addition of 40 mM CaCl2.
4.5. Size Exclusion HPLC

The size exclusion HPLC elution patterns (Figure 10, 11 and 12) showed very different results for the preheated and unpreheated WPCs. Following heating at pH 7 for 20 minutes, substantial quantities of high molecular weight aggregates were detected, while α-lactalbumin and β-lactoglobulin were reduced. Figures 13, 14 and 15 demonstrate significant quantities of high molecular weight proteins were only formed at pH 7.0. β-lactoglobulin and α-lactalbumin deceased with the formation of large aggregates. WPC heated at pH values below 6 had very small amount of proteins with high molecular weights.

The structure of whey protein is related to the pH condition. Between pH 5.5 and 7.0, the β-lg exists as dimers of molecular weight of 36,700. In the pH range between 3.5 to 5.2, dimers of β-lg A or β-lg B tetramerize to form octamers of about 147,000 Da. Below pH 3.5, the dimers dissociate. Lambelet et al. (1989) studied the denaturation of whey proteins by the low resolution NMR spectroscopy. The temperature range at which relaxation rate (T2) increased was closely related to the pH of the solution. At high pH of 8.0, the range would begin from 40 to 65°C. At low pH of 3, the range increased to 60-80°C.
With addition of calcium chloride either before or after preheating, the HPLC patterns were essentially the same as those without addition of calcium.
Figure 10. Effect of preheating on the molecular distribution of proteins analyzed by size exclusion HPLC.
Figure 11. The size exclusion HPLC profile of unpreheated WPC.
Figure 12. The size exclusion HPLC profile of preheated (70°C 20 min) WPC.
Figure 13. The size exclusion HPLC profile of WPC preheated at pH 6.
Figure 14. The size exclusion HPLC profile of WPC preheated at pH 4.
Figure 15. Influence of pH during preheating on the molecular weight distribution of WPCs (preheated at 70 °C, 20 min).
4.6. Protein Solubility:

Protein solubility is closely related to the functionality of whey proteins. Protein solubility of whey protein concentrates preheated at different pH condition is presented in figure 16. WPC solutions heated at pH values below 6 had lower protein solubilities than those not preheated or that were preheated at pH 7.0. Preheating at pH 7.0 for twenty minutes, did not result in a large decrease in solubility. These results and the HPLC patterns indicated that during preheating (70°C, 20 min.) the β-lactoglobulin and α-lactalbumin aggregated to form a soluble complex with an apparent molecular weight greater than 150,000 Da. When preheated at pH values below 6, the amount of β-lactoglobulin and α-lactalbumin decreased, but little of the soluble aggregates were formed. They probably aggregated to form insoluble aggregates that were removed during the filtration procedure.

These data and the HPLC profiles for the pH during preheating suggested that in the industrial production of low-gelation-temperature WPC, after ultrafiltration, pH of whey must be adjusted to 7.0 before preheating. Preheating at pH values below 6.0, do not allow the formation of enough partially aggregated large proteins to produce low-gelation-temperature WPC. On the other hand, it might be possible to preheat at alkaline pH values,
but the concentration of the protein must be below 10% to prevent formation of gel at this stage. The preheating time probably would be less under the alkaline pH conditions. Further research on the behavior of proteins preheated in alkaline environments is needed.

These data also suggest that the rate limiting step in the gelation of WPC is the formation of soluble aggregates. These probably represent whey proteins that have been thermally unfolded, but have not been cross linked to a significant extent. When too much calcium is present during heating or the pH is too low to prevent significant charge repulsion, the formation of these soluble aggregates does not occur. WPC preheated under conditions that do not favor soluble aggregate formation do not form gels at reduced temperatures. Preheating at pH 7.0 at 70 °C causes a decrease in the amount of native β-lactoglobulin and α-lactalbumin present with a concomitant increase in the formation of soluble aggregates. Proteins heated under these conditions still are relatively soluble (95%). They are capable of forming gels with only a relatively minor input of thermal energy when calcium has been added to about 40 mM. This suggests that the aggregation process requires much less energy than does the unfolding step. The enhancement of gel strength of preheated WPC when gel formation occurs at 90° C probably relates to the existence of a more optimal concentration of
soluble aggregates. When gel formation occurs at 90 °C without preheating, the rate of protein unfolding compared to cross link formation must be less than optimal. Thus, the formation of an adequate number of soluble aggregates allows for the formation of relatively weak gels at low temperatures (40°C) and an enhancement of gel formation at higher temperatures (90°C). Jeyarajah and Allen (1994) suggested that the conformational changes after heat treatment may increase the binding affinity of calcium ions, which may affect the formation of crosslinks.
Figure 16 - The effect of pH during preheating on the protein solubility of WPCs (*: unpreheated)
4.7. Emulsion stability:

Emulsion stability has been reported to be increased by the heating of protein solutions prior to homogenization. In this study (Table 6), preheating at 70 °C for 20 minutes and addition of 40 mM of calcium chloride to the emulsion formula caused the formation of gel before homogenization could be completed. This was probably due to the unfolding and aggregation of the proteins. To prevent gel formation, the CaCl₂ content and preheating temperature were reduced. Reduction of CaCl₂ to 10 mM or temperature to 66 °C allowed emulsion formation without formation of a gel. The EVI of the emulsion was 80% higher than the control sample indicating that preheating and addition of CaCl₂ could increase emulsion stability. Thus, proteins with decreased gelation temperature may be capable of increasing the stability of emulsions towards gravitational creaming.
Table 6. Effect of preheating and addition of calcium chloride on the emulsion volume index (EVI) of protein stabilized emulsion.

<table>
<thead>
<tr>
<th>Preheating temperature</th>
<th>CaCl2 (mM)</th>
<th>EVI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No preheating 0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>No preheating 40</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>70 °C</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>70 °C</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>70 °C</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>70 °C</td>
<td>20</td>
<td>Gel</td>
</tr>
<tr>
<td>70 °C</td>
<td>40</td>
<td>Gel</td>
</tr>
<tr>
<td>66</td>
<td>40</td>
<td>17</td>
</tr>
</tbody>
</table>
4.8. Viscosity and intrinsic viscosity of preheated WPC

The total solids of retentates to be spray dried should be around 30% to reduce the energy cost of drying. It is undesirable to have very high viscosity or gel formation in the final retentate. The viscosity change during preheating was measured and the data are presented in figure 17. The viscosity of the preheated WPC was more than three times the viscosity of the unheated WPC. When concentrated to above 25% total solid, the viscosity was as high as 25 centipoise (Figure. 18). Therefore, during preheating procedure, we must keep an eye on the viscosity change. Too much heating may cause problem when we spray dry the whey protein at high total solid.

Intrinsic viscosity is a measurement of protein's effective hydrodynamic volume per unit of mass, determined by its resistance to flow. It is related to the radius of gyration and the mean end-to-end distance (Creighton, 1984). Figures 19 and 20 present the intrinsic viscosity of preheated and non-preheated WPC. The intrinsic viscosity of the unpreheated and preheated WPC were 0.148 and 0.175 dl/g respectively. After preheating, the hydrodynamic volume of the protein molecules have increased due to unfolding. This stable unfolded state is capable of gel formation at relatively low temperatures.
Figure 17 - The change of viscosity of WPC during preheating (70°C).
Figure 18. The effect of concentration levels on the viscosity of WPCs
Figure 19. The intrinsic viscosity of unpreheated WPC.
Figure 20. The Intrinsic viscosity of preheated WPC.

\[ y = 0.011x + 0.175 \quad r^2 = 0.985 \]
4.9. Industrial processing of preheated WPC

The final purpose of this research was to produce whey protein concentrate powder having a low gelation temperature under conditions that are suitable for industrial scale. During processing of WPC, the heat treatment and dehydration during spray drying have the potential to alter the functionality of WPC. At the test tube level we have found preheated WPC has reduced gelation temperature when heated with addition of proper amount of calcium chloride. It would be more convenient if the WPC could be preheated during membrane processing. To achieve this goal, preheated whey proteins must retain their ability to gel at temperatures below 60°C after spray drying. In experiment 3.7 preheated and spray dried WPC powder formed a good gel at 60°C with addition of 40 mM calcium chloride. This result suggested that it was possible to produce preheated WPC powder which could be spray dried and still gel at temperatures below 50.

To simulate the heating condition in the test tube (11 mm I.D). The heat transfer of the WPC solution was measured and recorded every 2 minutes (Figure 21). The temperature of the WPC after 20 minutes of heating was 68.5°C. These data suggest that proper heating for the WPC in large scale to attain enough unfolding and partial aggregation would be heating to 68.5°C and hold for 15 minutes. The concentration of protein must be
maintain below 10%. Too much heating could result in the formation of gel before spray drying or impair the ability to form gels at reduced temperature. Figure 22 represents the procedure for the production of low-gelation-temperature WPC on an industrial scale. The preheated WPC produced by this procedure had the ability to form gels at 60°C and formed a stronger gel at 90°C. The retentate was preheated at 68.5°C for 15 minutes at pH 7.0 in the final stage of ultrafiltration. Since the previous data showed that addition of calcium before preheating was detrimental, the preheating step must be performed after membrane processing in order to remove calcium from the whey. At this stage the protein content of the retentate was below 8%, which could prevent the formation of gel. Preliminary experiments indicated that preheating at protein levels higher than 12% may cause the formation of gel. In other trials it was observed that if whey was preheated before ultrafiltration or immediately after ultrafiltration to 5x, gel strength was diminished.

On the other hand, CaCl2 was added in the form of powder into the preheated WPC powder. After rehydration, the WPC solution containing 40 mM CaCl2 had the same gelation quality as that of WPC with calcium added after preheating. By this procedure, the low-gelation-WPC could be supplied in a powder from the WPC industries.
Figure 21. Heat transfer of whey protein in a 11 mm I.D. test tubes.
Figure 22. Proposed method for the industrial manufacture of low-gelation-temperature WPC.
Figure 22. Continued

↓
Dialfiltration 4X (Total solid 7.2%)
↓
Preheated to 68.5°C hold for 15 min.
↓
Cooled
↓
Concentrated to 20% Total solid
↓
Spray Drying
↓
Add CaCl₂
↓
Hydration
↓
Heating and measure gel strength
4.10. Production of WPC with different ratio of preheated WPC to non-preheated WPC

Spray drying of preheated WPC solutions was possible at a total solids content of below 10%. To be commercially viable, spray drying will probably have to be possible at total solids contents of at least 20%. The increase in viscosity after preheating may cause the formation of gel or cause difficulty during spray drying of the retentate. Preliminary results suggested that if equal volumes of preheated and control retentate were mixed, spray drying was possible at a higher solids levels. A number of pilot scale runs were made by preheating a portion of WPC to 68.5 °C and holding for 15 minutes. The WPC that was preheated was obtained by performing ultrafiltration at pH 3.0 until the volume was reduced to 20% of the original. The pH of a portion of the WPC was adjusted to 7.0 and the sample was preheated at 68.5 °C for 15 minutes. Various ratios of preheated to unheated WPC were made (100:0, 75:25, 50:50, 25:75, and 0:100). Ultrafiltration and diafiltration of the combined WPC were continued as shown in section 3.10. The results showed that with 25% of preheated WPC the WPC solution was able to form a weak gel at 60 °C. When heated to 90 °C for 20 minutes, the gel was stronger than those without preheating.
Preheating occurred at total solid below 10% to prevent gelation during membrane processing. After preheating and mixing of preheated and non-preheated WPC, the WPCs were able to be further concentrated to higher levels of total solid without gel formation. With only 25% of the preheated WPC in the WPC, the WPC was able to form a weak gel at 60 °C (Figure 23). When heated at 90°C, it's gel strength was 40% more than the control sample (Figure 24). With preheated WPC above 50% the gel strengths were much higher than the control. Thus, with preheating of only a portion of the whey, it was possible to not only reduce the gelation temperature but also tremendously increase the gel strength of WPCs. The HPLC profiles of WPC with different ratios of preheated WPC are presented in table 6. With a high ratio of preheated WPC, the peak height of α-lactalbumin and β-lactoglobulin decreased and those of large proteins increased (Table 6).

To determine the affect of soluble aggregate content on gel strength, the per cent of soluble aggregates of the total proteins reported in table 6 were plotted against gel strength. These data are presented in figures 25 and 26. Figure 25 demonstrates a linear relationship between the percentage of soluble aggregates in the WPC and gel strength at 60 °C. This line is significant at $p = 0.008$. These data support the previous speculation that the formation of soluble aggregates is a necessary prerequisite for low
temperature gelation. The data for gel strength at 90 °C is not linear as seen in figure 26. In this case, it appears that the formation of soluble aggregates prior to high temperature heating tends to increase gel strength to a point. Further aggregate formation either does not increase gel strength or may even cause a slight decrease. It is possible that once a certain threshold of aggregates is formed, further formation does not significantly contribute to increased gel strength. It is also possible that with increased thermal treatment, losses in protein solubility are beginning to have a negative effect that offsets the gains obtained by aggregate formation. Processors who wish to employ this method to increase gel strength will probably have to optimize the amount of aggregate formation that yields optimal gel strength under their specific processing conditions.

In addition to differences in gelation properties, these WPCs with different percentages of preheated WPC may have differing functionality's in emulsion and foaming systems. It may be possible to adjust the percentage of preheated WPC to provide different functionality for the application of WPC in different food systems. More work in this area is wanted.
Figure 23. The gel strength of WPCs with different ratio of preheated to unpreheated WPC heated at 60°C.
Figure 24. The gel strength of WPCs with different ratio of preheated to unpreheated WPC heated at 90 °C.
Figure 25. Relationship between per cent soluble aggregate content and gel strength at 60 °C.
Figure 26. Relationship between per cent soluble aggregates and gel strength at 90°C.
Table 6. Peak high (micro-volt) of HPLC profile of the WPC with different percentage of preheated WPC.

<table>
<thead>
<tr>
<th>PH:NH</th>
<th>Large proteins</th>
<th>β-lg</th>
<th>α-la</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>12000</td>
<td>7332</td>
<td>0</td>
</tr>
<tr>
<td>75:25</td>
<td>9300</td>
<td>7800</td>
<td>2600</td>
</tr>
<tr>
<td>50:50</td>
<td>6500</td>
<td>8000</td>
<td>3100</td>
</tr>
<tr>
<td>25:75</td>
<td>4800</td>
<td>9300</td>
<td>4300</td>
</tr>
<tr>
<td>0:100</td>
<td>4800</td>
<td>12000</td>
<td>8100</td>
</tr>
</tbody>
</table>
4.11. Application of preheated WPCs

4.11.1. Fat reduction in meat products

The USDA policy for low fat products was established in 1991. This policy states that, "The finished product may contain no more than 30% of a combination of fat and added substances and no more than 10% fat. If a product is not low-fat it must be labeled as imitation." Table 6 presents the results of sensory analysis of a full fat product, a reduced fat product produced with normal WPC and a reduced fat product produced with WPC with a reduced temperature of gelation. These results show that regular whey can be used to produce a low-fat product with a similar overall acceptability of the full fat product. The texture and appearance scores of the whey product were less than the control product, but not significantly so. The problem with appearance was due to increased shrinkage of the control WPC sausage, probably due to differences in the gel points of the meat and whey proteins. The product made with the preheated WPC scored better than the control sausage or the normal WPC sausage in all categories. It was significantly better than the full fat product in flavor and texture and significantly better than the normal WPC product in flavor, texture and appearance. These data suggests that there may be functional advantages to WPC that can form gels at reduced temperatures. The low
temperature WPC used in the above studies was produced at a maximum total solids content of 12%. Research will be required to optimize and scale up production. Spray drying of the product at a reasonable bulk density may well provide the greatest technical challenge.
Table 7. Sensory scores of normal and reduced fat smoked sausages produced with WPC.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Control</th>
<th>Whey</th>
<th>Preheated Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptability</td>
<td>6.2</td>
<td>6.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Flavor</td>
<td>5.6</td>
<td>5.6</td>
<td>6.4*</td>
</tr>
<tr>
<td>Appearance</td>
<td>6.9</td>
<td>6.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Texture</td>
<td>5.1</td>
<td>4.8</td>
<td>5.8*</td>
</tr>
</tbody>
</table>

* = significantly different than the control at P = 0.05
4.11.2. Replacement of egg white in angel food cake

In formulations of an angel food cake, if the egg white was replaced with WPC, problems with delayed onset of gelation generally lead to product failure. It is possible to obtain acceptable overruns with WPC. However, WPC foams continue to expand with increasing temperature and rupture prior to protein gelation. It was assumed that if the temperature of gelation could be reduced, the film of the foam would be able to gel and form a strong foam before rupture. In this research, sprayed dried preheated WPC powder was used to replaced 25 and 50 % of egg white in the formulation of angel food cake. However, the cakes produced were not improved in cake volume when compared to those with non-preheated WPC. In another test, the foam of preheated WPC with CaCl2 was heated in a microwave to observe the expansion of foaming. In comparison to the heated foam of egg white, which would retain a continuous network of foaming during expansion, the foam of preheated WPC aggregate and water exudes, failing to form a continuous sponge-like network. The foaming ability was decrease due to the increase in viscosity of the whey protein solution. De Wit (1989) demonstrated that the maximum concentration for WPC foams was observed between 8 to 12% (m/v) whey proteins. More time was need to initiate the
foaming. The heated foam of egg white has elasticity while the foam of WPC is weak. The heated foam of egg white bound water more tightly than WPC did, so the WPC foam had some water expelled. The cakes with addition of WPC or preheated WPC had less volume and were more moist.
CHAPTER V

CONCLUSIONS

Cheese whey was membrane processed at pH 3 to produce a WPC containing 75% protein with reduced calcium content. WPC solutions containing 8 to 12% protein were preheated at temperatures ranging from 50°C to 80°C for times ranging from 5 to 50 minutes. The purpose of this preheating was to partially unfold the proteins and make them more sensitive to calcium mediated thermal gelation. Calcium chloride at a final concentration of from 0 to 80 mM was added after cooling of the WPC solutions. The temperature of gel formation and the strength of the gels formed were determined. Samples preheated at 70°C for 20 minutes formed gels after heating at 60 °C for 20 minutes when greater than 15 mM CaCl₂ was added. The preheated WPC with the addition of 40 mM CaCl₂ and heating to 90°C for 20 minutes, produced gels that were 1.8 times as strong as the control samples. No gel formation
occurred with samples that were preheated at pH values below 6.0. On the other hand, when the pH was higher than 7.5, the WPC formed gels during preheating. The protein contents examined by HPLC and protein solubility showed the differences in composition and proteins solubility after preheating at different pH values. By appropriate combinations of preheating times and temperatures, it was possible to produce WPC that formed gels at below 50°C. With the addition of a preheating step during final stages of ultrafiltration, WPC with a low-gelation-temperature could be produced at an industrial scale. WPC produced in this manner were shown to form more stable emulsions than did the control samples. Possibly, the formation of a weak gel at the lipid-water interface increased the stability of the emulsions. It was concluded that with proper pre-treatment, it was possible to lower the temperature of WPC gelation to values similar to myosin and egg albumin.
CHAPTER VI

SUMMARY

1. By appropriate manipulation of the initial calcium content, the pH and temperature of preheating and the final calcium content, it was possible to produce WPC that formed gels at temperatures more than 20°C below their normal gelation temperature.

2. The gels formed at reduced temperatures were as strong as those normally produce at 90°C and those produced at the normal temperature of gelation had increased gel strengths.

3. The preheating temperature was very critical. Whey protein concentrates must be heated to 70°C for 20 minutes to attain enough unfolding and partial aggregation of protein to result in a lower gelation temperature. Preheating at pH values below 7.0 were ineffective.
4. Size exclusion HPLC demonstrated that significant amounts of soluble aggregate formation were required in order to decrease the gelation temperature. Excessive heating resulted in either decreased gel strength or the formation of a gel at inappropriate points during processing.

5. Emulsions produced with preheated proteins were twice as stable as those produced with control products.

6. WPC with a reduced temperature of gelation could be used to produce reduced fat sausages that were rated by a sensory panel as superior to the full fat control.

7. More research needs to be conducted to scale the process from a laboratory or small pilot scale to one that can be used in a full size production facility.

8. With above 25% of preheated WPC in the WPC, the protein solution was able to gel at 60°C.
REFERENCES


