USE OF INGREDIENTS AND PROCESSING TO CONTROL THE STABILITY OF HIGH WHEY PROTEIN CONCENTRATION RETORT STERILIZED BEVERAGES

A Dissertation

by

GABRIELA PEREZ HERNANDEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Food Science and Technology

USE OF INGREDIENTS AND PROCESSING TO CONTROL THE STABILITY OF HIGH WHEY PROTEIN CONCENTRATION RETORT STERILIZED BEVERAGES

A Dissertation

by

GABRIELA PEREZ HERNANDEZ

Submitted to Texas A&M University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Approved as to style and content by:

Ronald L. Richter (Chair of Committee)	Alejandro Castillo (Member)
Lloyd W. Rooney (Member)	Luis Cisneros-Zevallos (Member)
Rhonda K. Miller (Chair of Food Science and Technology Faculty)	Gary R. Acuff (Head of Department)

May 2005

Major Subject: Food Science and Technology

ABSTRACT

Use of Ingredients and Processing to Control the Stability of High Whey Protein Concentration Retort Sterilized Beverages. (May 2005)

Gabriela Perez Hernandez, B.S., University of Guanajuato, Mexico;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Ronald L. Richter

Stable retorted whey protein beverages with 5% protein concentration were prepared. The effect of protein concentration, fat concentration and homogenization pressure on the heat stability and the stability of emulsions of sterilized whey protein beverages was determined. Beverages containing >1% protein formed aggregates during the heat treatment. Food grade additives were added to the beverages with >1% protein to determine if the heat stability could be improved. Lecithins and polyphosphates improved the heat stability while hydrocolloids decreased the heat stability. Lecithins improved the heat stability of emulsions better than polyphosphates but polyphosphates were more effective in beverages without fat. Lecithins modified by acetylation or hydrolysis provided more protection against heat denaturation of proteins than regular lecithin. Acetylated lecithin created the emulsions most stable against creaming. Improvement in the emulsion stability by the use of phospholipids was associated with a more negative charge at the interface of the fat droplets. The effect of polyphosphates on the heat stability was related to the chain length of the polyphosphates. Short chain polyphosphates (dp~4) were more effective than other polyphosphates. Polyphosphates probably improved the heat stability of the systems by changing the structure of water and this prevented aggregation of whey proteins. Hydrocolloids decreased heat stability most probably through thermodynamic incompatibility that locally increased the concentration of proteins and promoted aggregation during the heat treatment.

The effect of homogenization pressure, concentration of acetylated lecithin, and the concentration of short chain polyphosphate on the storage stability of retorted whey protein beverages containing 5% protein and 3% fat was determined. The creaming index and particle size index changed over 28 d of storage and indicated creaming of the emulsions. The use of homogenization pressures of 55 and 90 MPa compared to 20 MPa reduced the magnitude of the change of the particle size index and creaming index during storage. Inclusion of polyphosphates reduced the storage stability of the emulsions.

Optimization of parameters showed that emulsions formulated with 5% protein, 3% fat and 0.3% lecithin without polyphosphates and homogenized at 90 MPa had the best stability after 28 d of storage.

DEDICATION

To God who makes all possible,

To my parents, Manuel and Gloria,
My sister, Gloria Alicia and my brother, Juan Manuel

ACKNOWLEDGMENTS

I would like to thank Dr. Ron Richter, my advisor, for his invaluable support, guidance and friendship during my years at College Station. I will miss him.

I would like to thank my committee, Dr. Lloyd Rooney, Dr. Alejandro Castillo, and Dr. Luis Cisneros for their advice, and help provided to me whenever it was needed.

I want to thank Luis Fernando Reyes and Lisa Lucia for their help with the sterilization process; they were always willing to help.

Especial thanks to Anita Gerung for her kind help and enthusiasm, Ben Davis for his great assistance in this research project, Alexander Lin, Sachin Bhatia, Rudy Suhareli, Yvonne Suhareli, Lei Ungkuraphinunt , Lorena de la Torre, and Carolina Bauab who helped me doing my research and for the great times together in the Dairy Lab.

I want to thank Veronica Rosas for her kind assistance during my years in College Station. I am also grateful to Mary Morales and Linda Rice.

I want to thank Jeff Pfaff from BK Giulini, Eric Bastian from Glanbia Inc., Marcelo Mitre from Continental Custom Ingredients, Bill Monagle from the Solae Company for providing me with samples.

I would like to thank the Dairy Management Inc. for funding this research and the National Council for Science and Technology of Mexico (CONACYT) for supporting part of my studies at Texas A&M University.

Finally, I would like to thank my friends and roommates, Christian Garcia, Rafael Mateo, Carmen Gomes, Paulo Cesar de Silva, Laura Silva, Gabriel Krishnamoorthy, Javier Bueso, and Odette Mendez, for making my life at College Station very happy.

TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

TABLE Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

CHAPTER I
INTRODUCTION

Whey proteins have excellent nutritional and functional properties (Wit 1998), and new research reveals that whey proteins contain bioactive peptides that may have positive effects in weight management, body composition, satiety and other biomarkers of health (Zernel 2003). With all these advantages whey proteins have become part of sports beverage, infant formulae, geriatric formulae, liquid meal replacements and other similar products. These products are frequently sterilized in a retort and stored for extended periods of time. Some of the problems to manufacture whey protein retort process beverages are the poor stability of the whey proteins during the heat treatment especially when these beverages contain high concentration of proteins and the inherent thermodynamic instability of emulsions especially when these products are store for extended periods of time. Shelf life of retorted products can be almost a year. However, the use of selected additives and changes of the processing conditions can improve the heat stability and emulsion stability of the retort process whey protein beverages. Additives such as emulsifiers, hydrocolloids and phosphates can interact with whey proteins and modify the functional properties of the whey proteins. The mechanisms by which these additives modify the characteristics of the systems are very complex and diverse. Emulsifiers can displace protein from the interface of fat globules or interact with proteins in the bulk phase and/or proteins attached to fat globules changing the protein conformations and subsequently the heat stability and emulsion stability of the system. Phosphates can interact with proteins or change the structure of water changing the heat stability of the proteins. Hydrocolloids can interact with proteins, increase viscosity of the bulk phase and affect heat stability and emulsion stability. Changes on processing conditions such as homogenization pressure can cause changes in the structure and size of fat droplets affecting emulsion and heat stability of the beverages.

The dissertation follows the style and format of Journal of Food Science.

The purpose of this research is to make whey protein beverages with high protein content that can withstand the heat treatment applied in commercial retort sterilization and study their stability during storage.

1.1 Objectives

The following is a list of the objectives for part 1 to 5.

Part 1**:** To determine the effect of protein concentration, fat concentration, and homogenization pressure to find a combination of ingredients that can withstand the heat treatment and establish a baseline for further experiments.

Part 2: To determine the effect of pH on the heat stability of whey protein solutions and emulsions.

Part 3: To determine the effect of phospholipids, phosphates with different chain lengths, selected emulsifiers and hydrocolloids on the properties of whey solutions and emulsions and to select those that improve heat stability and emulsion stability.

Part 4: To determine the effect of the concentration of selected additives and homogenization pressure on storage stability of retorted whey protein stabilized emulsions.

Part 5: To optimize parameters to create emulsions with the best storage stability after retort processing.

CHAPTER II
LITERATURE REVIEW

2. 1 Milk proteins

Milk proteins are classified into two groups: caseins and whey proteins. Caseins are found in micelles, which are high order molecular structures of aggregated individual caseins, with their structure still unclear. Caseins in the form of micelles are poor emulsifiers but if they are converted to caseinates they become excellent emulsifiers. Individual casein molecules have a disordered structure; they have no tertiary structure and limited secondary structure. This makes them insensitive to heat treatment. However, they precipitate at their isoelectric point of about pH 4.6. Whey proteins are the milk proteins that remain soluble when the pH of milk is lowered to 4.6. Whey proteins have a compact globular structured held together by hydrophobic, covalent and hydrogen bonding. Because of this highly ordered structure they denature, aggregate or form gels when they are heated above their denaturation temperature (Fox 2003). Their use is limited in applications that require extensive heat treatments unless gels are required. The use of whey proteins in retorted sterilized products is limited by two problems: emulsion stability which refers to stability against creaming, coalescence, depletion flocculation, and other emulsion related problems, and the ability of whey proteins to withstand the required heat treatment without precipitation or gelation.

The major proteins in whey are β-lactoglobulin (β-lg; 50%), α-lactalbumin (α-la; 20%) and bovine serum albumin (BSA; 5%) with minor amounts of protease-peptone, immunoglobulins G1, G2, A, M and numerous enzymes (Table 1). The physical properties of the major whey proteins are shown in Table 2. The forms of whey proteins used in the food industry are whey protein concentrates (WPC) and whey protein isolates (WPI). Whey protein concentrates range in concentration of protein from 34 to 85%. Whey protein isolates contain more than 90% protein. WPI can be prepared either by membrane processes or by ion exchange. WPC and WPI contain different amounts of the individual whey proteins depending upon the source of the whey and the

manufacturing process (Table 3). The ratio of the major whey proteins (β-lg:α-la:BSA) in a commercial WPC is about 10:4:1 and is generally believed that the functional properties of the major protein (β-lg) will dominate the behavior of the WPC and WPI (Havea and others 2001). Differences in the composition of WPC and WPI caused by variable sources of whey and manufacturing processes will affect the functional properties of the ingredients.

Table 1. Approximate composition of whey proteins in milk. Adapted from Walstra and others 1999.

	Mmol/m^3 milk	g/kg milk	g/100g protein
Whey proteins	~320	6.3	19
β-lactoglobulin	180	3.2	9.8
α-lactalbumin	90	1.2	3.7
Serum albumin	6	0.4	1.2
Protease-peptone	~40	0.8	2.4
Immunoglobulins	~4	0.8	2.4
IgG1, IgG2		0.65	1.8
IgA		0.14	0.4
IgM		0.05	0.2

Table 2. Properties of whey proteins. Adapted from Walstra and others 1999.

Property	β-lactoglobulin (B)	α-lactalbumin (B)	Serum albumin
Molar mass	18,283	14,176	66,267
Amino acid residues/molecule	162	123	582
Phosphoserine (res./mol.)	0	0	0
Cysteine (res./mol.)	5	8	35
-S-S- linkages/mol	2	4	17
Hexoses (res./mol.)	0[1]	0[2]	0
Hydrophobicity[3] (kJ/res.)	5.1	4.7	4.3
α-Helix (approx. %)	11	30	46
Charged residues (mol %)	30	28	34
Net charge/residue	-0.04	-0.02	-0.02
Distribution of charge	Even	Even	
Isoelectric pH	5.2	~4.3	4.7
Association tendency	Dimer	No	No
Ca^{2+} binding	-	([4])	-

[1] 8 in a rare variant (Dr).
[2] A small fraction of the molecules has carbohydrates residues
[3] Tangford-Bigelow scale
[4] Binds 1 mole Ca^{2+} per mole; very strong bond.

Table 3. Major whey proteins as percentage of the total protein in whey protein products. from Hufman and Harper 1999.

	WPC		WPI	
	Cheese whey	Casein whey	MF	IE
β-lactoglobulin(%)	52	65	60	80
α-lactalbumin(%)	15	21	22	14
Bovine serum albumin(%)	2	4	2	3
Immunoglobulins(%)	5	10	5	3
Glycomacropeptide(%)	26	0	21	0

MF Micro filtration, IE Ion exchange

2.2 Principal proteins in whey

β-Lg is the principal whey protein in the milk of the cow representing approximately 50% of the total whey proteins. It is rich in sulphur amino acids and it contains 2 intramolecular disulphide bonds and 1 mole of cysteine per monomer of 18 kDa. β-Lg is a small protein with 162 aminoacid residues (MW ~18,400). It is soluble in dilute salt solutions, folds into an 8-stranded, antiparallel β-barrel with a 3-turn α-helix on the outer surface and a ninth β-strand flanking the first strand. β-lg forms dimmers at neutral pH. At pH 2 it is present in the monomeric form and at pH 3.5-6.5 forms octomers (4 dimers). β-lg belongs to a family of proteins called lipocalins which bind small hydrophobic ligands in their structural β-barrel or calyx. This hydrophobic binding site is open at high pH allowing the ligands to bind and closes at low pH (Kontopidis and others 2004, Sawyer 2003). There is extensive information concerning the mechanism of the heat denaturation of β-lg. However, the process is not completely understood. It is clear that the mechanism depends on the pH, the ionic strength and the nature of ions, concentration and purity of the protein, dielectric constant, temperature and genetic variants (Sawyer 2003). The mechanism of β-lg denaturation in vitro suggested that there is a dissociation of the dimmer between 30 and 55 °C. Increasing

these temperatures caused monomer unfolding and increased thiol reactivity that lead to disulphide interchange and aggregation. However hydrophobic mechanisms are also involved and aggregation can occur without the involvement of the sulphydryl groups. The presence of other components during heating also affects the denaturation characteristics of β-lg. Lactose and palmitate have been shown to stabilize β-lg against heat denaturation (Sawyer 2003).

α-Lactalbumin (α-La) represents approximately 20% of the protein in bovine whey. It has a molecular weight of ~18,000 Da, is rich in tryptophan, contains 4 intramolecular disulphide bonds per mole, but it does not contain cysteine (sulphydryl groups). α-La binds one Ca^{2+} per mole in a pocket that contains 4 Asp residues. α-la is the most heat stable of the principal whey proteins, and it renatures following heat denaturation. However at pH below 5, the Asp residues of α-la become protonated and cannot bind Ca^{2+}, in this form α-la can be denatured at low temperatures (Fox 2003).

2.3 Heat stability of whey proteins

One of the most important functional properties of whey proteins is their ability to form gels upon heating but this is not desired in retort beverages. The ability to form gels or resist heat aggregation depends on many factors such as protein concentration, protein purity, heating temperature and time, heating rate, pH, ionic strength and the presence of other ingredients (Demetriades and others 1997; Hunt and Dalgleish 1995). Singh and Havea (2003) reported that the behavior of commercial whey proteins upon heating is not uniform due to the inconsistency of the product in quality and composition as is supported by data in Table 3. In aqueous solutions whey proteins exist in compact, organized, globular conformations held together by hydrophobic interactions, hydrogen bonding, ion-par interactions and van der Waals' interactions. Heating causes proteins to denature and loose their native structure, the compact structure unfolds into a random, disordered structure, exposing the buried hydrophobic amino acid residues. Sulfhydryl groups can also undergo oxidation to disulphide (S-S) or cysteic acid ($-SO_3H$) groups or sulphydryl-disulphide interchange reactions. Through sulphydryl-disulphide interchange

and hydrophobic interactions the unfolded proteins may associate and form aggregates (Singh and Havea, 2003).

During the formation of an emulsion, whey proteins are removed from the bulk phase and become part of the adsorbed layer of protein at the oil and water interface. Adsorption of whey proteins at the interface can alter the native structure of the proteins, expose some amino acid residues that previously were buried within the protein structure and make them available to interact at the interface. Consequently, the behavior of whey proteins upon heating in emulsions or solutions might be different. In emulsions prepared with whey proteins, proteins might interact in the bulk phase and also interact with proteins adsorbed to the fat droplets.

Monahan and others (1996) studied the effect of heating 1% WPI emulsions at temperatures between 30 – 90 °C. Heating at temperatures below 70 °C did not cause protein aggregation, but significant aggregation was observed between 75 – 80 °C. This aggregation decreased as the temperature was raised above 80 °C, which suggested that interdroplet interactions are higher in the range of 75 - 80 °C and intradroplets predominate above 80°C, Monahan and others (1996) also showed that adding sulfhydryl blocking agents to the emulsions prior to heating did not inhibit the aggregation of emulsions. This data indicated that non-covalent interactions such as hydrophobic interactions and van der Waals forces are responsible of the initial formation of aggregates and later disulphide bonds strengthen the already formed aggregates. Euston and others (2000) studied the heat induced aggregation kinetics of whey protein stabilized emulsions (WPC 0.2-2% wt%, soya oil 20%) at boiling temperature. They reported the aggregation mechanism reaction was of the order 1.5, the same as the reaction order found for β-lg stabilized emulsions (Dannenberg and Kessler 1988). Euston and others (2000) also reported that increased protein content increased the rate of aggregation and the removal of unadsorbed protein (bulk phase protein) drastically decreased the rate of aggregation. Hunt and Dalgleish (1995) reported that whey protein emulsions (2% protein, 20% soya oil) remain fluid after heating if the non-adsorbed whey protein is removed regardless of the total protein content. Euston and

others (2000) concluded that the mechanism for aggregation of whey protein stabilized emulsions consists of protein-protein, droplet-droplet and protein-droplet interactions and the last interaction apparently is the dominant aggregation mechanism of the heated emulsions. A possible mechanism is the increase of hydrophobicity of the proteins during heating, proteins can then rearrange at the interface of the fat globules with their hydrophobic aminoacids into the oil phase, which lowers their hydrophobicity. Those proteins in solution have higher hydrophobicity and this hydrophobicity promotes the first stages of heat aggregation.

The composition and structure of the proteins adsorbed at the o/w interface in emulsions dictate the behavior of the emulsion and its stability against heat. This structure can be manipulated by the use of ingredients and different processing conditions such as homogenization pressures, heat treatments and cooling rates. The effects of low molecular weight emulsifiers at the interface of protein emulsions are well known. Emulsifiers may remove protein from the interface or may form complexes with protein at the interface. Most commonly, the competitive adsorption of proteins and surfactants during or after emulsification reduces the protein surface coverage at the oil-water interface (Dickinson 1997). Emulsifiers also have an effect on the heat stability of whey protein stabilized emulsions. Perez-Hernandez (2001) reported that lecithin and monoglycerides had a protective effect against heat stress on emulsions stabilized with 3% whey protein. However, the mechanism by which these two emulsifiers protect the emulsion is different. Lecithin did not displace whey proteins from the interface of the oil droplets; however, lecithins modified the structure of the interface forming a thick layer which indicated association of lecithins with the protein at the interface. Monoglycerides displace whey proteins from the lipid interface. Lecithins have been reported to improve the heat stability of milk. McCrae (1999) suggested that lecithin changes the heat stability of milk by targeting the membrane proteins but the mechanism by which lecithin improves the heat stability of milk is not completely understood. Euston and others (2001) studied the heat aggregation kinetics of whey protein stabilized emulsions (1% whey protein, 20% soya oil) that were stabilized with different

emulsifiers. Water soluble emulsifiers displaced more proteins from the interface compared to the oil soluble emulsifiers and improved the heat stability of the emulsions. These data suggested that if the surface coverage of the emulsion droplets with proteins is reduced there will be less chance of aggregation of the particles as there will be fewer molecules on the surface that can participate in aggregation. Ingredients other than emulsifiers can improve the heat stability of emulsions. Perez-Hernandez and others (2002) reported that phosphates with long chains (n~100) improved the heat stability of whey protein stabilized emulsions even at high protein concentration while medium chain polyphosphates (n~20) promote gelation. Most of the research on heat stability and emulsion stability of whey protein stabilized emulsion has been conducted in systems containing very low concentrations of protein (0.2- 2%) and high amounts of fat (20%). However this research will focus on increased concentrations of protein and low fat concentration emulsions where almost no research has been reported. Using high pressures for homogenization of the emulsions might reduce the amount of whey protein in the bulk phase due to the formation of smaller droplets and more surface area where proteins can attach and ultimately improve the heat stability of the emulsions. Selection of ideal combination of emulsifiers and phosphates might improve the heat stability of the emulsions due to changes at the oil and water interface of the emulsion and ionic changes in the emulsions.

2.4 Emulsion stability

Emulsions are dispersions of one liquid into another, most commonly oil-in-water or water-in-oil. The interfacial area in emulsions is very large and is associated with positive free energy (the interfacial tension) making emulsions thermodynamically unstable systems that tend with time towards the lowest free energy state and eventually, failure of the emulsion. To create emulsions with long-term stability it is necessary to use emulsifiers that will accumulate at the interface and create an energy barrier toward flocculation and coalescence. The emulsifiers can be surfactants, proteins, amphiphilic

polymers, or combination of polymers and surfactants (Claesson and others 2004, Angelo 1989, Friberg 1997)

Emulsion droplets are in constant motion in an emulsion and collide with each other frequently (Danner and Schubert 2001). The droplets may separate again in stable emulsions, or they may stick together with a thin film between them in a process known as flocculation, or they might merge in an irreversible process known as coalescence (Fig 1) (Danner and Schubert 2001). Preventing droplets from closely approaching each other to prevent coalescence will help to create emulsions with increased shelf life.

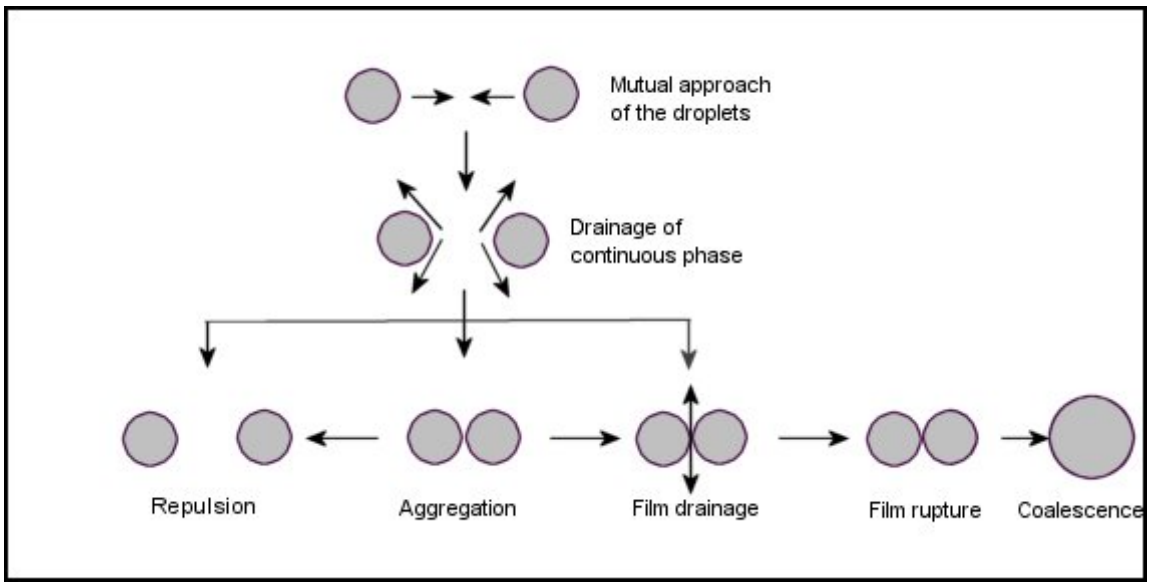


Fig. 1. Process of coalescence. Adapted from Danner and Schubert 2001.

Emulsions can become unstable by: phase segregation, aggregation, coalescence and spontaneous size disproportionation (Berg 2004).

Phase segregation applies to systems that are unstable with respect to the spatial distribution of the dispersed phase. Emulsions are generally in the gravitational field, if the dispersed phase particles are denser than in the medium, they will tend to settle toward the bottom of the container (sedimentation), and if they are lighter, they will tend to rise toward the top (creaming). The Stoke's law can be useful to predict the behavior of suspended particles in dilute solutions:

$$v = \frac{2r^2(\Delta\rho)g}{9\mu} \qquad \text{(Eq. 1)}$$

where the velocity (v) of the fat globules is determined by the acceleration force (g) the difference in density (Δρ) between the dispersed and continuous phases, the radius of the particles (r) and the viscosity (μ) of the continuous phase. Creaming promotes flocculation and coalescence (Berg 2004).

Flocculation or aggregation is the process in which two or more droplets collide to form an aggregate, but the droplets retain their individuality. Smoulochowski (1917) derived expressions for collisions frequency and assuming each collision results in the particles sticking together, aggregations models were developed. Aggregation by Brownian motion alone is perikinetic aggregation and is given by:

$$-\frac{dn}{dt} = k_r n^2 \quad \text{with} \quad k_r = \frac{4kT}{3\mu} \qquad \text{(Eq. 2)}$$

And the half-life of the dispersion is:

$$t_{1/2} = \frac{3\mu}{4kTn_0} \qquad \text{(Eq. 3)}$$

where n is the particle number density and k_r is the rate constant for aggregation, k is the Boltzmann constant, T is the temperature and μ is viscosity.

Aggregation assisted by shearing is orthokinetic aggregation and the aggregation rate is given by:

$$-\frac{dn}{dt} = \frac{16}{3}Ga^3n^2 \qquad \text{(Eq. 4)}$$

where G is the shear rate, a is the particle radius.

In orthokinetic aggregation the rate of aggregation depends strongly in the particle size while in perikinetic aggregation, aggregation is independent of particle size. The total rate of aggregation is taken as the sum of perikinetic and orthokinetic aggregation. While temperature and viscosity are important in Brownian aggregation, they cannot compare to the sensitivity of orthokinetic aggregation rate to particle size. (Berg 2004, Vanapalli and Coupland 2004).

Coalescence occurs when the liquid droplets join together to form a larger droplet with simultaneous destruction of the interfacial area. In order to coalesce, fat globules need to approach each other under the influence of long range van der Waals force often assisted through sedimentation and creaming (Berg 2004). When two droplets approach there is a liquid film between them, the liquid film must drain to some critical thickness, remain there for a finite period of time and suddenly break. And the rate of coalescence is governed by the time required to drain (t_{drain}) this film to a critical thickness (h_c) and it follows Reynolds equation for two rigid discs spaced by the interfacial film:

$$t_{drain} = \frac{3\mu A}{4\pi p}\left[\frac{1}{h_c^2} - \frac{1}{h_0^2}\right] \qquad \text{(Eq. 5)}$$

where μ is the medium viscosity, A is the area of the circle of contact, p is the effective pressure pushing the surfaces together, and h_0 is the distance of separation which exist when the first flattening occurs (Van Aken 2004, Berg 2004). Coalescence-stable emulsions leading to long-term stability are most often produced by macromolecules or polymers that may form a gel in the film, avoiding drainage (Berg 2004).

Processes that counteract droplet aggregation and/ or coalescence include: 1) Steric repulsion which occurs when the size and geometry of the molecules adsorbed at the interfaces prevent droplets from approaching each other; 2) Electrostatic repulsion, the DLVO theory describes the balance between attractive (Van der Waals) and repulsive (electrostatic) forces; 3) Born hydration repulsion refers to the solvation layer surrounding molecules. The solvation layer has to be dispersed to allow contact between droplets; 4) The Gibbs-Marangoni effect where interfacial surfactants resist displacement and leads to osmotic retention of liquid from the continuous phase and 5) Viscoelastic properties refers to the viscoelastic behavior of the droplets films that resist mechanical rupture. The stability mechanisms of protein emulsions are mainly steric and electrostatic repulsion and viscoelastic properties of the adsorbed layers (Wabel 1998).

2.5 Emulsion stability of whey proteins

Proteins are large amphiphatic molecules containing combinations of ionic, polar and non polar regions that cause proteins to have surface active properties. Proteins will adsorb at the surface of the fat globules when emulsions are formed. The adsorbed layer of protein at the interface of the fat globules will dictate the behavior of emulsions. However, the composition and structure of the protein layers can be modified by the composition of the system, the use of emulsifiers and hydrocolloids, and environmental factors such as pH and ionic strength. Whey proteins adsorb at the interface of oil in water emulsions forming a pseudo two-dimensional system of densely packed deformable particles (Dickinson 1997). During emulsification the molecules that adsorb first might not have time to unfold properly before other proteins surround them in a congested packed monolayer. β-lg partially unfolds at the interface exposing the free sulfhydryl group, which leads to slow polymerization of the protein in the adsorbed layer via the interchange between sulfhydryl and disulphide groups (Monahan and others 1993, Dickinson and Matsumura 1991, Dickinson 1997). This allows whey proteins to form secondary layers at the surface of the oil droplets. Hunt and Dagleish (1994) reported that the minimum amount of whey protein needed at the interface to make stable WPI-stabilized (20% oil) emulsions was 1.5 mg of protein/m^2 of lipid surface. A maximum surface concentration of ~3.25 mg/m^2 was reached at protein concentrations of 2.5 wt%. Euston and others (2000) reported that the protein at the interface increases almost linearly with protein content and shows no sign of reaching a plateau as a result of extensive multilayer formation. Demetriades and others (1997) studied whey protein stabilized emulsions (2% WPI and 20% oil) over a range of pH (3-7) and ionic strength (0-100 mM NaCl). Emulsions prepared at pH 4 to 6 showed low stability, droplet aggregation and high viscosity and this effect was increased at higher ionic strength. The poor stability of whey proteins at these conditions was explained by the low charge of proteins near their isoelectric point diminishing the electrostatic repulsion. As ionic strength was increased the electrostatic repulsion between droplets was decreased due to counter-ions in the aqueous phase, which shielded the charges in droplet surfaces.

Yamauchi and others (1980) reported low stability, high viscosity and a maximum concentration of protein adsorbed at the interface of whey protein stabilized emulsions at pH 5. They did not find differences in the properties of the emulsions when the ionic strength was increased (20% oil, 1-8% wt dry whey). Bhatia (2001) found that the source of whey, acid or rennet, had an effect on the stability of whey protein emulsions prepared with 3% protein and 3% fat, emulsions prepared with rennet whey were more stable than emulsions prepared with acid whey. The presence of the glycomacropeptide in the rennet whey might have had a positive effect on the stability of the emulsions. However, the results were inconclusive. Perez-Hernandez (2001) reported that the emulsions stabilized with 3 % whey protein and 3% butter oil were significantly improved by increased homogenization pressure and by the addition of lecithin and monoglycerides to the emulsions. The mixture of lecithin and monoglycerides had a synergistic effect on the stability of the system. Most of the research on the stability of whey protein stabilized emulsions has been conducted in emulsions containing a high concentration of lipid and a low concentration of protein where most of the protein is adsorbed to the fat globules. The purpose of this research is to use emulsions with high concentration of whey proteins and reduced concentration of lipids.

2.6 Phospholipids

Lecithin is a mixture of several phospholipids present in the cell membrane of animals and plants and is one of the most common emulsifiers used in food systems. Phospholipids have an amphiphilic molecular structure with a lipophilic segment consisting of two fatty acids and a hydrophilic group provided by a phosphoric acid ester. Phospholipids are classified by the type of substance linked to the phosphate group (Fig 2). The presence of the hydrophilic and lipophilic part in phospholipid molecules make them surface-active. Crude lecithins are a mixture of oil and phospholipids with minor amounts of other substances prepared from the de-gumming process in the manufacture of oil. Commercial lecithins are usually extracted from soybeans. Soybean oil contains 1.5 – 3.0% phosphatides. Crude lecithin contains

approximately 30% oil, 16% phosphatidylcoline (PC), 14% phosphatidylethanolamine (PE), and intermediate levels of inositol phosphatides (PI), phosphatidic acid (PA), and low levels of phosphatidyl serine (PS) and lysophosphatides (LPC and LPE). Other nonphosphatides include steroids, vitamin E and free fatty acids (Szujah and Sipos 1989, Bergenståhl 1997).

Lecithins can be modified either chemically or enzymatically to produce lecithins with different hydrophilic/lipophilic balance to modify performance of lecithin as an emulsifier. Some modified lecithins include acetylated, hydroxylated, hydrolyzed and hydrogenated lecithin (Szujah and Sipos 1989). Acetylation occurs primarily on the amino group of phosphatidylethanolamine. The acetylated amino group of PE introduces a substituent on the positively charge portion of the zwitterionic phosphatidylcholine and converts it to a negatively charged lecithin and the effective hydrophilicity of the PE is increased by making the polar head larger through acetylation. Acetylated lecithin has better solubility than non-modified lecithin and improved oil-in-water emulsifying properties. In hydroxylated lecithins hydrogen peroxide not only bleaches the lecithin but also hydroxylates lecithin imparting hydrophilic properties to the lecithin. This improves moisture retention and contributes to the formation of stable oil-in-water emulsions. Partually hydrolyzed lecithins produced either chemically or enzymatically have increased hydrophilic properties and greatly enhanced oil-in –water emulsifying properties compared to lecithin and they do not lose their emulsifying characteristics in the presence of calcium and magnesium ions as rapidly as do the unmodified types. Better control of the hydrolysis is obtained by using enzymes for selective cleavage of the alpha or beta fatty acids or the polar end groups which can be hydrolyzed to give phosphatidic acid. Hydrogenated lecithin has a greater oxidative stability and is less hygroscopic than non modified lecithin but has reduced solubility in the usual solvents (Bergenståhl 1997). Recent reviews on modification of phospholipids with final functional properties have been done by Hossen (2005) and Doig and Diks (2003).

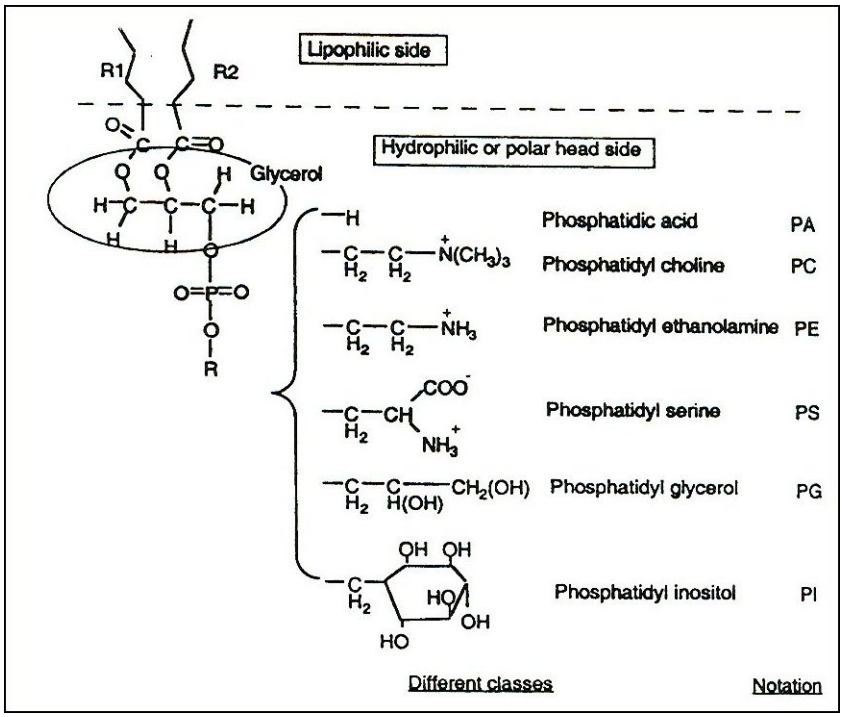


Fig. 2. Structure of phospholipids.R1 and R2 are fatty acids attach to phospholipids (From Garti 2002).

2.7 Phosphates

Polyphosphates have the general formula $M_{(n+2)}P_nO_{(3n+1)}$ where M is a hydrogen or monovalent metal. Their anions are composed of chains in which each phosphorus atom is linked to its neighbors through two oxygen atoms, forming linear, unbranched structures (Fig. 3). The degree of polymerization, n, can take values from 2 to 10^6 and as n increases the cation to phosphorus ratio approximates unity (Kuleav and others 2004). Polyphosphates with degrees of polymerization from 2 to 5 can be obtained in pure form but polyphosphates with higher degrees of n are obtained as mixtures of polyphosphates with different chain lengths to produce a size distribution. As a general rule as the P_20_5 content increases so does the average chain length. Polyphosphates are salts of acids that, in solution, contain two types of hydroxyl groups that differ in their tendency to dissociate. The terminal hydroxyl groups (two per molecule of polyphosphoric acid) are

weakly acidic, whereas the intermediate hydroxyl groups, of which there are a number equal to the number of phosphouros atoms in the molecule, are strongly acidic.

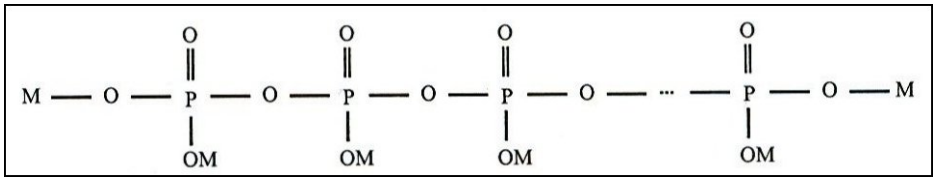


Fig. 3. Structure of polyphosphate (From Kuleav and others 2004).

Phosphates interact with proteins due to characteristic polyelectrolytical nature of both proteins and phosphates. In systems with a pH below the isoelectric point of the proteins they acquire a net positive charge; the opposite occurs at pH values above the isoelectric point. Binding of phosphates (strongly anionic) to proteins should increase as the pH of the medium decreases. Phosphates can bind to proteins at pH values above the isoelectric point because of stronger interactions between the increasingly ionized phosphates and cations and other positively charged groups that might exist bound to protein molecules. The phosphate-protein interactions are primarily electrostatic and are strongly influenced by changes in the net charge of proteins caused by changes in pH, the addition of cations, or both, and to the degree of polymerization which determines the degree of ionization of phosphates. Phosphates influence the solubility of proteins by altering their net charge, by influencing the pH of the solution, and by increasing the ionic strength of solutions (Molins 1991). Polyanionic phosphates will interact with positively charged groups on protein molecules, to increase the net negative charge of the proteins making them more water soluble. These interactions between proteins and phosphates have been explored in the dairy industry and some uses have been reported (Molins 1991).

Polyphosphates were used to remove whey proteins from effluents before the development of membrane separation technologies. Wingard (1972) patented a whey

protein separation process in which the pH of the solution is adjusted below the pI of the whey protein and potassium polymetaphosphate and sodium phosphate are added to the solution to bind and remove the positively charged protein. Melachouris (1977) patented a process for recovering whey protein with phosphates with improved solubility and clarity that could be used in the fortification of acidic beverages. Al-Mashikh and Nakai (1987) used sodium hexametaphosphate (SHMP) to selectively remove more than 80% of β-lactoglobulin from Cheddar cheese whey without removing the other whey proteins to make a product with a higher ratio of α-la to β-lg that could be used in infant formula. Dybing and others (1982) increased the yield of cottage cheese by 1.25 or 2.45% by adding 0.05 or 0.2% (w/w) phosphates to milk before making cottage cheese by the short set culture procedure. Phosphates precipitated some of the whey proteins allowing them to remain in the cheese curd. These whey proteins normally remained in the whey during the manufacture of cottage cheese and were removed with the whey. The binding of β-lg with polyphosphates at a pH below the pI of the proteins increased as the phosphate chain length and concentration increased. However, at high protein concentrations, the binding became independent of phosphate chain length (Melachouris 1972). Phosphates are used in the dairy industry to inhibit protein gelation in condensed milk and in milk sterilized by ultra-high temperature (UHT) processing where gelation occurs during extended storage time. Evidence indicates that inhibition of gelation during storage depends upon the chain length of the polyphosphates. Kocak and Zadow (1986) reported that the addition of 0.5 or 1.0 g/kg of sodium hexamethaphosphate to UHT-processed milk resulted in at least a six fold extension of the shelf life of UHT milk. However SHMP from a different supplier did not have the same protective effect and this might be explained by the different chain lengths of the product although it was marketed as SHMP. Harwalkar and Vreeman (1978) studied the effect of phosphates in sterilized concentrated milk and conclude that 1.5 g of sodium orthophosphates/kg of product accelerated gelation and the same concentration of SHMP delayed gelation. Kepacka and Pijanowski (1970) add phosphates in concentrated milk before and after sterilization

and concluded that polyphophates exert their beneficial effect by protecting the milk during sterilization and ortho- and pyrophosphates predominated after the heat treatment.

Complete hydrolysis of polyphosphates during milk sterilization was also reported by Glandfor and Thomasow (1977). Phosphates have also been useful to improve the heat stability of milk. UHT processed goat milk which is less heat stable than cow milk was heat stable with 0.3 or 0.5 g phosphates/l of milk (Montilla and Calvo 1997). Khan and others 1992 studied the heat stability of buffaloes' milk and concluded that sodium dihydrogen phosphate improved the heat stability of raw, concentrated, reconstituted and concentrated sterilized buffaloes' milk. The use of phosphates to prevent precipitation and gel formation of Ca fortified milk and dairy products have been reported recently (Sher and others 2002). Phosphates can form soluble complexes with Ca without precipitation.

2.8 Hydrocolloids

The use of hydrocolloids in dairy beverages is very common. Hydrocolloids help to provide the final texture or viscosity to beverages, suspend solid particles in a liquid system such as cocoa powders in milk and stabilize emulsions by decreasing the creaming of fat droplets. Polysaccharides can stabilize emulsions mainly by increasing the viscosity of the bulk phase and hence decreasing the rate of creaming as explained by the Stokes Law (Eq. 1). However, mixtures of biopolymers (protein – hydrocolloids) are also known to form incompatible systems. Thermodynamically, the mixing of biopolymers in the system is an unfavored process, since the interpenetration of macromolecules decreases the entropy of the system (Syrbe and others 1998). Mixtures of biopolymers segregate into two phases, by two mechanisms: thermodynamic incompatibility; when the interaction between different biopolymers is more repulsive than the average interaction between like biopolymers which causes segregation of phases with each phase mainly loaded with one biopolymer species and complex coacervation; when the net attraction between different biopolymer molecules causes polymer complex formation (Syrbe and others 1998). The complex usually has a density

different from the surrounding medium and forms a separate phase at the top or bottom of the system (Bryant and McClements 2000). One of the most common causes of coacervation is electrostatic attraction between oppositely charged biopolymers (Syrbe and others 1998). A system in which the polymers do not separate into two phases has miscibility, the contact between different biopolymers is similar to contact between like biopolymer species and spontaneous mixing occur. However, high molecular weight polymers tend to exhibit complex coacervation and incompatibility.

A polymer as an immobilized layer in protein stabilized emulsions affects the balance between configurational restrictions and enthalpic effects which determines how close the soluble biopolymer can approach the adsorbed polymer. Incompatibility, miscibility and coacervation transforms into non-adsorption, weak adsorption and strong adsorption (Dickinson and Euston 1991, Syrbe and others 1998).

Syrbe and others (1998) reported that nonionic hydrocolloids such as long chain maltodextrins, dextrans and methylcellulose in whey protein hydrocolloid systems showed incompatibility in systems with a pH range of 5 to 7. Whey protein solutions with the anionic hydrocolloids high methoxyl pectin, sodium alginate or sodium carboxymethylcellulose did not present phase separation. However, if whey protein was denatured by thermal or high pressure treatment segregation occurred.

The mixture of whey proteins with polysaccharides exposed to retort temperatures has not been reported. However some studies of the heat aggregation kinetics of whey protein and denaturation of ß-lg have shown that some polysaccharides exert a beneficial effect whereas others have a detrimental effect on the heat aggregation of whey proteins (Euston and others 2002, Zhang and Foegeding 2003, Croguennoc and others 2001, Capron and others 1999, Syrbe and others 1998).

Carrageenans are high molecular weight polysaccharides comprised of repeating galactose units and 3,6-anhydrogalactose, both sulfated and non-sulfated, joined by alternating α-(1,3) and ß-(1,4) glycosidic linkages. The main carrageenan types are λ, κ and ι-carrageenan and can be prepared in pure form by selective extraction techniques.

The types of carrageenan differ in their structure. The ester sulfate and 3,6-anhydrogalactose content is 25% and 34% for κ, 32% and 30% for ι, and λ contains 35% ester sulfate with little or no 3,6-anhydrogalactose content (Imeson 2000). Xanthan gum is an extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. Xanthan gum is a linear (1→4) linked ß-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1→4) to a terminal mannose unit and (1→2) to a second mannose than connects to the backbone (Sworn 2000). At retort temperatures, xanthan viscosity is temporarily reduced, ensuring good thermal penetration in retorted foods. Xanthan gum recovers its viscosity upon cooling. Carob bean gum or locust bean gum structure consist of linear chains of mannose units linked by 1→ 4-ß-D-glycosidic bonds at which the hydrogen atom of several primary hydroxyl groups on C6 are substituted by single α-D-galactose units by 1→ 6 linkages. The galactose content of carob bean gum is 17-26% wt (Wielinga 2000).

CHAPTER III
MATERIALS

3.1 Part 1: Effect of protein and fat content and homogenization pressure on physical properties of whey protein stabilized emulsions

Emulsions and solutions were prepared by dissolving the desired amount of WPI Provon 190 (Glanbia Inc., Idaho, USA) in distilled water and storing the solutions at 4 °C overnight. The pH of the solutions was adjusted to pH 7. The desired amount of butter oil (Level Valley, West Bend, Wis. USA) was added. The samples were heated to 65°C and mixed prior to homogenization at 20 or 90 MPa with a Rannie Lab 2000 homogenizer (APV System, Wilmington Mass. USA). The composition of the samples was 1, 3, 5, 7 or 9% protein with 0, 1 or 3% fat. Samples without fat were not homogenized.

3.2 Part 2: Effect of pH on whey protein solutions and emulsions

Emulsions and solutions were prepared by dissolving WPI Provon 190 (Glanbia, Idaho, USA) in distilled water and storing the solutions at 4 °C overnight. The desired amount of butter oil (Level Valley, West Bend, Wis. USA) was added. The samples were heated to 65 °C in a water bath and mixed prior to homogenization at 20 MPa. Samples without fat were not homogenized. After homogenization the pH was adjusted with NaOH or HCL from pH = 3 to pH = 8 prior to heat treatment. The composition was 3% whey protein and 0 or 3% milk fat for the whey protein solutions and emulsions respectively.

3.3 Part 3: Effect of selected additives on whey protein solutions and emulsions

3.3.1 Phospholipids

Emulsions and solutions were prepared by dissolving WPI Provon 190 (Glanbia, Idaho, USA) in distilled water and storing the solutions at 4 °C overnight. The pH was adjusted to 7. Lecithins (The Solae Company, Mo. USA) were added and dissolved

followed by butter oil (Level Valley, West Bend, Wis. USA). The samples were heated to 65 °C and mixed before homogenization at 20 MPa. The composition of the samples was 3, 5 and 7% protein with 0 and 3% fat and 0.5% lecithin. Three types of lecithin were used (Table 4).

Table 4. Experimental phospholipids.

Lecithin	Commercial name	HLB values
Deoiled soy lecithin	Centrolex F	7
Acetylated deoiled	Precept 8140	8
Hydrolyzed deoiled	Precept 8160	9

3.3.2 Polyphosphates

Whey protein solutions and emulsions were prepared by dissolving WPI Provon 190 (Glanbia, Idaho, USA) in distilled water and the solutions stored overnight at 4 °C. The pH of the mixture was adjusted to 7 and selected polyphosphates (Table 5) (BK Giulini Corp., Simi Valley, Calif. USA) and butteroil (Level Valley, West Bend, Wis. USA) were added. The samples were heated to 65 °C and mixed before homogenization at 20 MPa. The composition of the samples was 3 or 5% protein with 0 or 3% fat and 0.2% phosphates. Before sterilization the samples were divided into two batches, the pH of one batch was not adjusted and the pH of the other batch was adjusted to pH 7.

Table 5. Properties of experimental polyphosphates.

Brand name	Acronym	Description	P_2O_5 content	Degree of polymerization
JOHA S9	Short chain (SC)	Na polyphosphate, Na phosphate	59.7% +/- 1%	N=4 80-90% : 10-15%
JOHA C NEW	Medium chain (MC)	Na polyphospate	68.5% +/- 1%	N=20-21
JOHA IMP	Very long chain (VLC)	Insoluble Na Metaphosphate	68.7 – 70%	N=100, VLC
JOHA KM2	Mixture short chain (MixSC)	Na phosphate Na polyphosphate Na citrate	40.4%	
JOHA C Special	Mixture Medium chain (MixMC)	Na polyphosphate Na phosphate	65.7%	
JOHA BT1	Mixture Long chain (MixVLC)	Na polyphosphate K polyphosphate	NA	

3.3.3 Emulsifiers

Emulsions and solutions were prepared by dissolving WPI Provon 190 (Glanbia, Idaho, USA) in distilled water and storing the solutions overnight at 4 °C. The pH of the solutions was adjusted to 7. Different emulsifiers (Table 6) (Continental Custom Ingredients, West Chicago, Ill. USA) and butteroil (Level Valley, West Bend, Wis. USA) were added to the solutions. The samples were heated to 65 °C and mixed before homogenization at 20 MPa. The composition of the samples was 3 or 5% protein with 0 or 3% fat and 0.2% emulsifiers.

Table 6. Experimental emulsifiers.

Emulsifiers	Acronym	Characteristics
Monoglycerides	MAG	Non ionic
Sodium Stearoyl Lactylate	SSL	Ionic
Mono & Diglycerides & Polysorbate 65	MAG &POLY	Non ionic

3.3.4 Hydrocolloids

Emulsions and solutions were prepared by dissolving WPI Provon 190 (Glanbia, Idaho, USA) in distilled water and storing the solutions overnight at 4 °C. The pH of the solutions was adjusted to 7. Different hydrocolloids (Table 7) (Continental Custom Ingredients, West Chicago, Ill. USA) and butteroil (Level Valley, West Bend, Wis. USA) were added. The samples were heated to 65 °C and mixed before homogenization at 20 MPa. Samples without fat were not homogenized. The composition of the samples was 3 or 5% protein with 0 or 3% fat and 0.05% hydrocolloids.

Table 7. Experimental hydrocolloids.

Hydrocolloids	Acronym	Characteristics
κ-carrageenan	κ-CGN	Ionic
ι-carrageenan	ι-CGN	Ionic
Locust bean gum	LBG	Non ionic
Xanthan gum	XG	ionic

3.4 Part 4: Storage stability of retorted whey protein emulsions.

The emulsions for storage stability after retort processing contained 5% protein and 3% fat. Acetylated lecithin and the polyphosphate MixSC were chosen because data indicated they improved the stability of heated emulsions. Whey protein emulsions were prepared by dissolving WPI Provon 190 (Glanbia Inc., Idaho, USA) in distilled water and were stored overnight at 4 °C. Phosphates, lecithin and butter oil were added to create formulations for a Surface Response Experimental Design (Table 8) generated by JMP software (SAS Institute, Inc., Cary, N.C. USA). The samples were heated to 65 °C, mixed and homogenized. The samples were canned and retort-sterilized immediately after homogenization. The sterilized samples were stored at room temperature and analyzed for particle size, fat content, pH, and viscosity after 1, 14 an 28 days of storage.

Table 8. Response surface design of experiment in part 4 and 5.

Treatment	HP[1] (MPa)	ACET[2] (%)	MixSC[3] (%)
1	20	0.3	0.1
2	20	0.5	0
3	20	0.5	0.2
4	20	0.7	0.1
5	55	0.3	0
6	55	0.3	0.2
7	55	0.5	0.1
8	55	0.5	0.1
9	55	0.5	0.1
10	55	0.7	0
11	55	0.7	0.2
12	90	0.3	0.1
13	90	0.5	0
14	90	0.5	0.2
15	90	0.7	0.1

[1]HP homogenization pressure (MPa)
[2]ACET acetylated lecithin
[3]MixSC Mixture short chain polyphosphate

CHAPTER IV
METHODS

4.1 Particle size analysis

The size of the milk fat globules was determined after homogenization of the samples using a Beckman-Coulter LS 230 Light Scattering instrument (Coulter Corporation, Miami, Fla.) Samples were diluted with water, using a real refractive index of 1.33 for the water and 1.54 for the protein stabilized fat globules. The volume-surface average diameter, dvs (μm) and specific surface area (cm^2/ml) were determined.

4.2 Apparent viscosity

Apparent viscosity was measured with a Brookfield RV DV-III rheometer (Brookfield Engineering Laboratories, Stoughton, Mass. USA) using a low viscosity adaptor. The spindle speed was 60 rpm. Viscosity was recorded at 10 seconds intervals during 1 minute of shearing at 22 ± 1 °C. Apparent viscosity was reported as the average of the last three readings. When samples had aggregates or formed gels viscosity was not measured.

4.3 Heat stability

Part 1, 2 and 3

Samples (5 ml) were placed in borosilicate tubes (16x100mm) and heated in an autoclave to 121 °C/10min. The heated samples were cooled to room temperature and visually inspected for aggregation.

4.4 Emulsion stability (fat layer thickness)

Part 1, 2 and 3

Emulsions (4 ml) were placed in borosilicate tubes (12 x 17 mm) and centrifuged at 1000 x g for 30 min at 20 °C. The depth of the layer of fat on the top was measured and used to calculate the emulsion stability.

4.5 Emulsion stability (Fat content)

Part 3 emulsions with phospholipids

A secondary test was used to measure the stability of emulsions containing phospholipids. The emulsion stability was calculated as (Fs/Fo) x 100, where Fo was fat in the original milk sample and Fs was the concentration of fat in the serum after centrifugation at 1400 x g for 30 min at 20 °C.

4.6 Zeta potential (ζ)

Part 3 emulsions with phospholipids

The zeta potential was measured using a Zeta Potential Analyzer Zeta PALS (Brookhaven Instruments Corporation, Holtsville, N.Y. USA). Samples were diluted in water. The pH of the samples was 7.

4.7 Retort sterilization

Aluminum cans (16 oz) were filled with 450 g of sample and sealed using a closing machine. Sealed cans were sterilized in a pilot rotary retort (Hermannstock Maschf., W. Germany) at 250 °F for an F_o value of 5 minutes. The retort was operated in rotary mode at 7 rpm. The temperature in the can was monitored using thermocouples. Sterilized samples were cooled immediately to 110 °F by running water.

4.8 Creaming index

One can at each storage period was opened carefully to not disturb the sample and 30 ml were separately removed from the top and bottom of the can. The concentration of lipid in the top (T) and bottom (B) samples was determined by the Mojonnier method (AOAC, 1984). The creaming index (CI) was calculated as CI= (% fat in T) / (% fat in B)

4.9 Particle size index

The particle size distribution of the top (T) and bottom (B) samples were analyzed using an LS 230 particle size analyzer (Beckman-Coulter Co., Miami, FL). The mean particle size (MPS), expressed by D(3,2) of both samples was determined from particle size distribution data and the particle size index (PI) was calculated as the ratio of MPS in top sample (MPS-T) and MPS in bottom sample (MPS-B).

4.10 Statistical analysis

Part 1, 2, and 3

The data was analyzed using the GLM procedure (SPSS 12.0.1, SPSS Inc. Chicago, Ill. USA). Estimated marginal means were computed and compared using the Bonferroni test.

Part 4

The data was analyzed using the GLM and repeated measures procedure in SPSS (SPSS 12.0.1, SPSS Inc. Chicago, Ill. USA)

Part 5

Analysis of the response surface model was analyzed with JMP 5.1 software (SAS Institute, Inc., Cary, N.C. USA).

CHAPTER V
RESULTS AND DISCUSSIONS

5.1 Part 1: Effect of composition and homogenization pressure

Particle size distribution and dvs

Probability values for the effect of experimental parameters on the physical properties of the whey protein stabilized emulsions are in Table 9. Particle size (dvs) was significantly affected by the interaction of homogenization pressure (HP) and protein (p=0.04). However the interaction was an orderly interaction and an increase in homogenization pressure caused a decrease in the size of the fat globules at all concentrations of protein (Fig. 4). The dvs mean for samples homogenized at 20 MPa was 0.852 μm compared to 0.366 μm for samples homogenized at 90 MPa (Table 10). An increase in the homogenization pressure from 20 to 90 caused the particle size distribution of the emulsions to change. The distribution of the fat droplets was multimodal for all treatments. Typical distributions for emulsions containing 3% fat and 3% protein homogenized at 20 and 90 MPa are shown in Fig. 5. Homogenization at 20 MPa caused two distinctive peaks at particle sizes of 0.4 and 2 μm. Two peaks were also found after homogenization at 90 MPa with the peaks at sizes of approximately 0.4 and 1.8 μm. However, the predominant peak in emulsions homogenized at 20 MPa was at 2 μm which means most of the fat was present in globules with a diameter of 2 μm while at 90 MPa the most predominant peak was about 0.4 μm.

The smallest particle size when emulsions were homogenized at 20 MPa was in emulsions containing 5% protein (Fig. 4). However after homogenization at 90 MPa the size of the fat globules continued to decrease as the protein content increased. The concentration of fat did not significantly affect the size of the fat globules (Table 9). Particle size distributions of emulsions with different concentrations of protein are shown in Fig. 6. It is apparent that the distribution of the particle sizes shifted to a higher proportion of smaller particles as the concentration of protein increased (p=0.001, Table 9) after homogenization at 20 and 90 MPa. Figure 7 shows the particle size distribution

of emulsions that contained 1 or 3% milkfat and 3% protein after homogenization at 20 and 90 MPa. The fat concentration in the emulsions did not significantly affect the particle size distribution. The particle size distribution and diameter of the fat droplets strongly depended on the homogenization pressure (p=0.001, Table 9) used to form the emulsions rather than on composition of the system at the concentrations used in this experiment.

Surface area

A decrease in particle size is concomitant with an increase in the surface area of the fat globules (Fig. 4 and 8). Surface area of the fat droplets was increased as the concentration of protein was increased (Fig. 8). Homogenization at 20 compared to homogenization at 90 MPa caused the surface area of the fat globules to increase from 70,781 cm^2/ml at 20 MPa to 167,524 cm^2/ml at 90 MPa. The concentration of milk fat did not affect the surface area (Table 9) but when the protein concentration was increased, there was a slight increase in the surface area of the fat globules. These results reflect the strong dependence of changes in dvs and surface area on the pressure used for homogenization rather than composition of the samples.

Table 9. Probability values for the effect of homogenization pressure, fat and protein concentration determined by ANOVA.

Model	Dvs (μm)	Surface Area (cm^2/ml)	Fat layer (mm)
HP	.000	.000	.000
Protein	.001	.015	.000
Fat	.077	.290	.000
HP x Protein	.040	.042	.000
HP x Fat	.406	.700	.049
Protein x Fat	.145	.450	.123
R^2	.969	.913	.772

Table 10. Effect of homogenization pressure, fat and protein concentration on physical properties of whey protein stabilized emulsions.

Variable	Dvs[1] (μm)	Surface Area[1] (cm^2/ml)	Fat layer[1] (mm)
Pressure (MPa)			
20	0.852a	70,781a	1.894a
90	0.366b	167,524b	1.272b
Fat (%)			
1	0.596a	121,864a	1.172a
3	0.623a	116,440a	1.994b
Protein (%)			
1	0.672b	104,837a	1.806a
3	0.623ab	114,799ab	1.875a
5	0.576a	118,787ab	1.500b
7	0.598a	124,289ab	1.514b
9	0.577a	133,049b	1.222c

[1]Means within columns for each variable followed by the same letter are not significantly different (α=0.05).

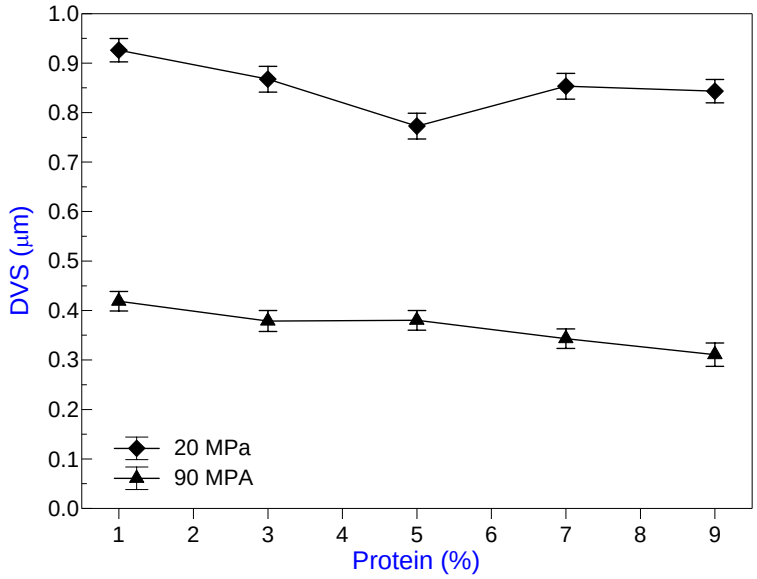


Fig. 4. Effect of homogenization pressure and protein content on estimated means of dvs. Error bars show the mean +/- 1.0 SE.

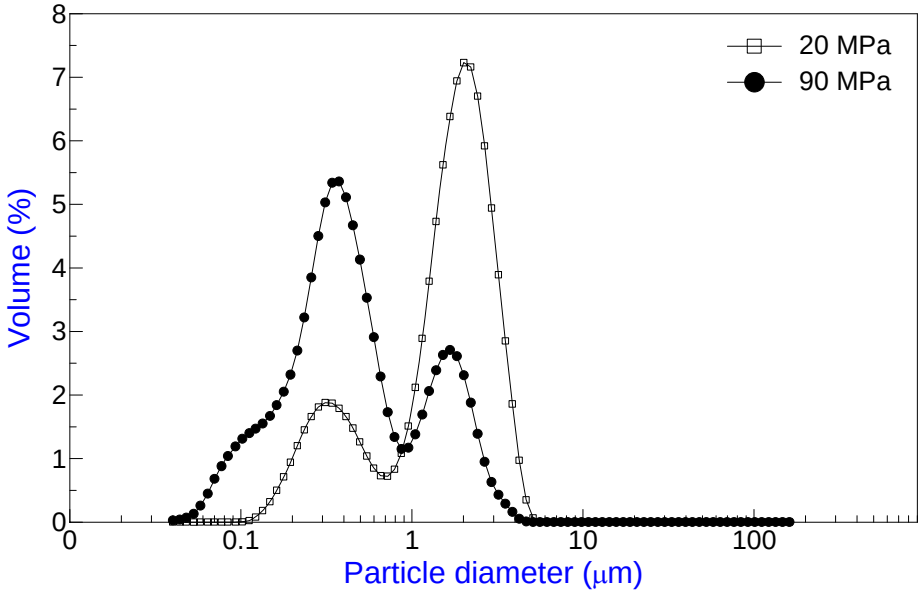


Fig. 5. Effect of homogenization pressure on particle size distribution of whey protein emulsions (3% protein, 3% fat).

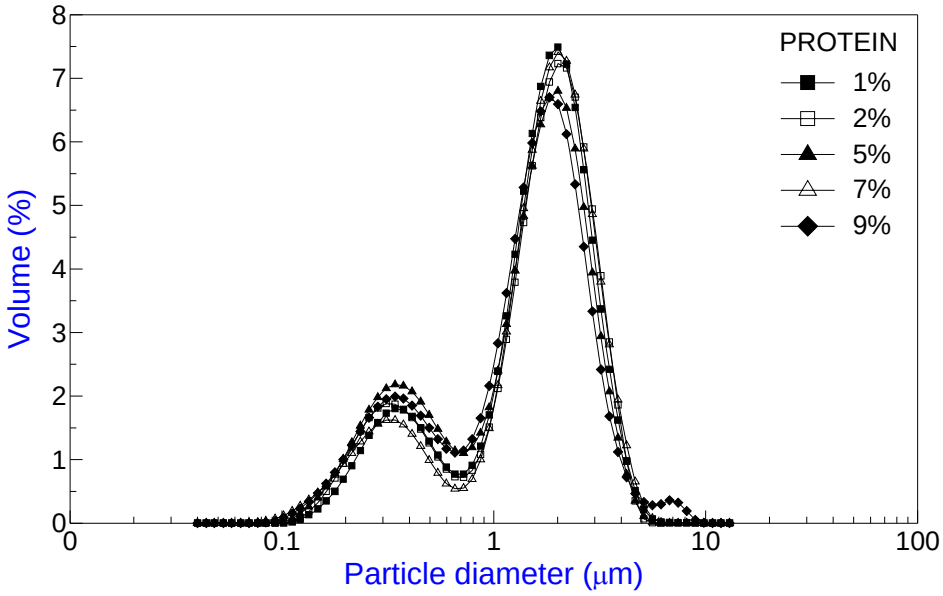


Fig. 6. Effect of protein content on particle size distribution of whey protein emulsions with 3% fat homogenized at 20 MPa.

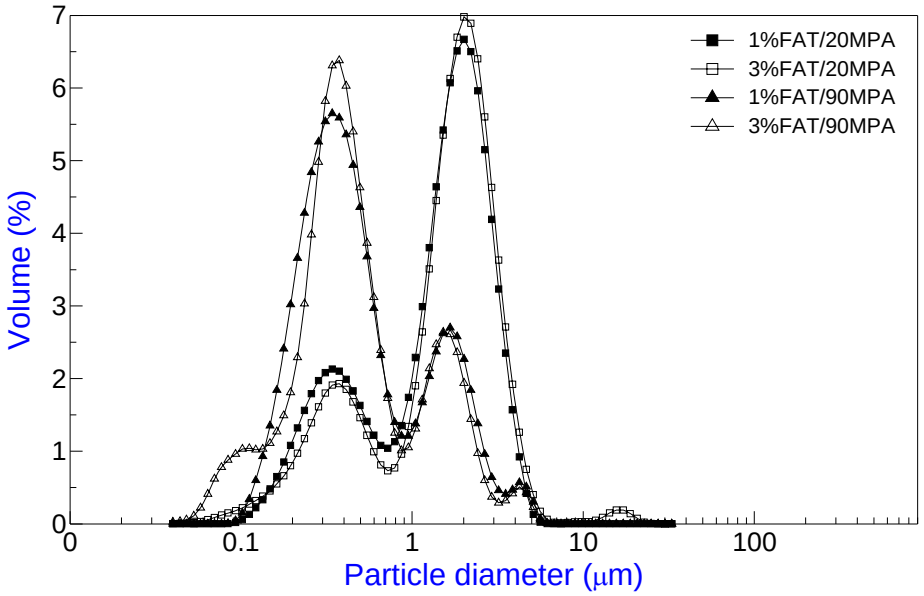


Fig. 7. Effect of fat content on particle size distribution of whey protein emulsions with 3% protein homogenized at 20 MPa and 90 MPa.

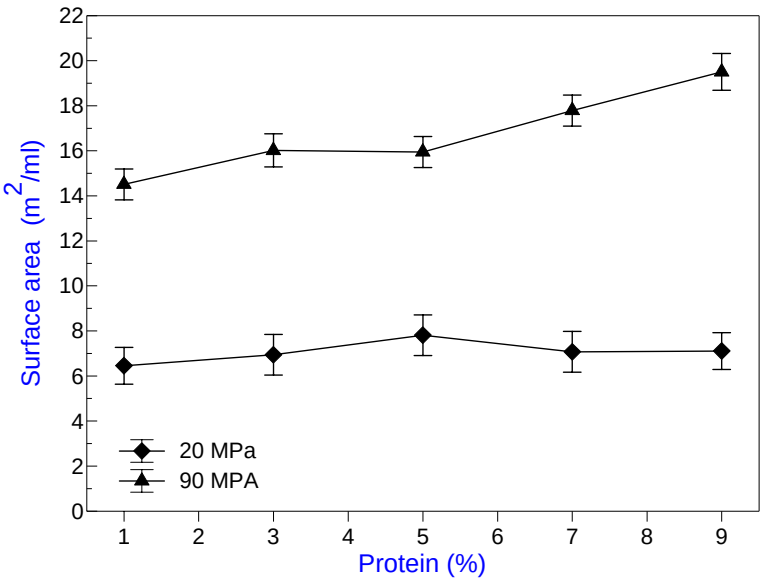


Fig. 8. Effect of homogenization pressure and protein content on estimated means of surface area. Error bars show the mean +/- 1.0 SE.

Emulsion stability

The length of the fat layer separated by centrifugation was used as an indirect method to measure the stability of the emulsions. A longer fat layer was associated with reduce stability of the emulsions relative to creaming. However, results from this method must be interpreted carefully to predict long term stability since other mechanisms can be involved in the stability of emulsions. The size of the fat layer was affected by the interaction of homogenization pressure (HP) and protein (p=0.000), and the interaction of HP and fat concentration (p=0.049) (Table 9). Data in Fig. 9 shows the interaction effect of HP x protein on the length of the fat layer. The length of the fat layer decreased when the homogenization pressure was increased regardless of protein concentration which indicated increased emulsion stability. An increase in protein content decreased the size of the fat layer in emulsions homogenized at 20 MPa but the effect of the protein

content on emulsion stability was not as important for emulsion stability when the samples were homogenized at 90MPa. However, emulsions that contained 9% protein were the most stable. The interaction between homogenization pressure and fat concentration is depicted in figure 10. Examination of this data revealed that emulsion stability is higher when samples are homogenized at 90 MPa compared to 20 MPa irrespective of fat concentration.

The decreased size of the fat globules and the increased stability of the emulsions associated with the use of high homogenization pressure on protein-stabilized emulsions has been previously reported (Lin 2002, Bhatia 2001, Perez-Hernandez 2001, Cano-Ruiz 1996). Stoke's law (Eq. 1) can be used to explain the increased stability of emulsions associated with smaller fat globules. The rate of creaming is directly proportional to the size of the fat globule and the density difference between the dispersed and the aqueous phase. An increase in the homogenization pressure not only decreased the particle size but also decreased the difference in densities between the fat globules and bulk phase because protein adsorbs at the interface of the fat globules. Lin (2002) reported that homogenization of recombined sterilized milk at 55 rather than 20 MPa decreased the size of the fat globules and increased the emulsion stability. However an additional increase in the homogenization pressure from 55 MPa to 90 MPa did not improve the stability of the emulsion. This might be related to the increased difficulty to decrease the size of the lipid droplets further. The smaller fat globules generated by homogenization at 55 MPa would be more difficult to disrupt than the fat globules generated by homogenization at 20 MPa. The pressures differences created during homogenization can disrupt a droplet if this pressure, which depends on the power density, surpasses the Young Laplace pressure of the droplet (Walstra 1999).

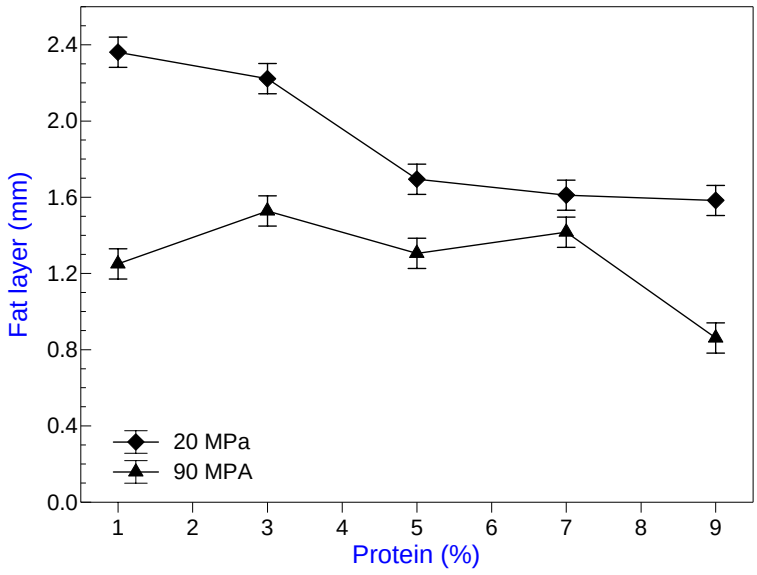


Fig. 9. Effect of homogenization pressure and protein content on estimated means of the fat layer. Error bars show the mean +/- 1.0 SE.

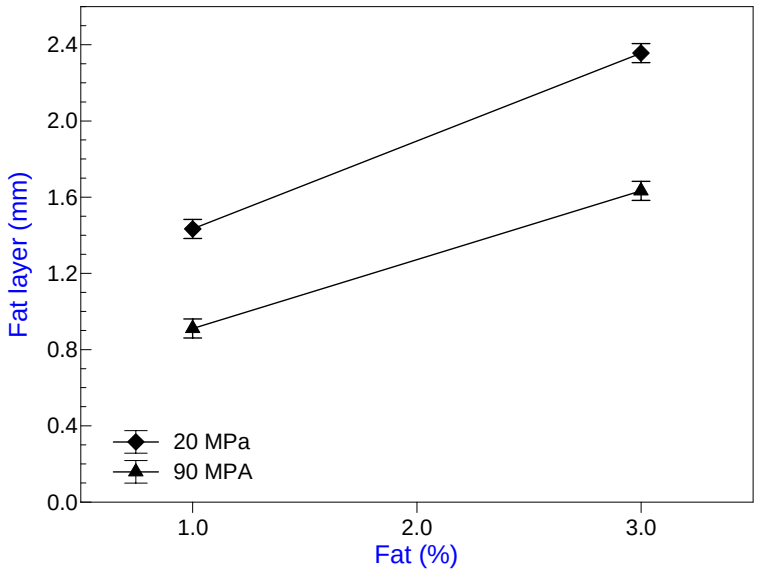


Fig. 10. Effect of homogenization pressure and fat content on estimated means of the fat layer. Error bars show the mean +/- 1.0 SE.

Heat stability

Samples that did not display aggregates or a gel when inspected visually after heating were considered to be heat stable. The heat stability of the emulsions was not affected by homogenization pressure (Table 11 and 12). Samples that formed aggregates or gelled after homogenization at 20 MPa also gelled after homogenization at 90 MPa. Some authors (Euston and others 2000) have reported that the removal of protein from the bulk phase in whey protein stabilized emulsions improved the heat stability of the emulsions. Applying higher homogenization pressures could have depleted the protein concentration in the bulk phase because the surface area of the fat droplets doubled when the emulsions were homogenized at 90 MPa compared to homogenization at 20 MPa (table 10), and therefore, improve heat stability. However, the amount of protein displaced from the bulk phase was small compared to the amount of protein in the bulk phase and no changes in heat stability were observed. The amount of whey protein adsorbed to the fat globules interfaces is approximately 3.20 -3.30 mg of protein / m^2 of lipid surface area (Perez-Hernandez 2001 and Hunt and Dalgleish 1994). The percentage of protein removed from the bulk phase calculated using a protein load of 3.20 mg of protein / m^2 of lipid surface area and the surface area values reported in table 10 would be approximately 0.07% and 0.16% for emulsions homogenized at 20 and 90 MPa, respectively. This concentration of protein displaced from the bulk phase was too small to cause changes in the heat stability.

The properties of gels that formed after emulsions were homogenized at 20 and 90 MPa were different. The gels formed from emulsions homogenized at 90 MPa appeared to be harder than the gels that formed from emulsions homogenized at 20 MPa. However, no attempt was made to characterize the gel properties. Suhareli (2003) characterized heat set whey protein gels following homogenization at several pressures. He reported that gels from systems homogenized at the higher pressures were stronger than those produced by using low homogenization pressure.

Emulsions and solutions containing 1% whey protein remained stable after heating. Samples made with 3% protein were heat stable only when they did not contain

fat, but those that had 1 and 3% fat exhibited some aggregation. Samples that had more than 5% protein formed gels. It is well known that heat aggregation of whey proteins is increased when the protein concentration is increased (Euston and others 2000, Ju and Kilara 1998). The increased protein concentration causes the number of possible sites for interactions to increase. A possible explanation for the greater stability in samples without fat may be explained if whey protein adsorbed at the interfaces changed their conformation to a conformation more favorable for the formation of heat aggregates. When globular proteins adsorb at the interface hydrophobic amino acid residues and free sulfhydryl groups might be exposed to the water phase and when layers adsorbed on adjacent droplets collide, hydrophobic and disulphide bonds might be formed between these layers strongly increasing the attractive interaction forces (Van Aken 2004). Hydrophobic interactions are the initial reactions in the heat aggregation of emulsions and disulfide bonds help reinforce the interactions (Singh and Havea 2003). Another cause of the formation of aggregates in samples containing fat might be the stronger attractive forces between droplets rather than between molecules (Israelachvili 2003).

Results from Part I show that in order to make retort processed whey protein beverages with a protein concentration $\geq$ 3%, the use of additives will be required to improve heat stability. Homogenization pressure did not appear to affect the heat stability (Table 11 and 12). However, use of high homogenization pressure had a great benefit on the stability of emulsions against creaming (Table 9).

Table 11. Heat stability of oil-in-water emulsions homogenized at 20 MPa.

	Protein (%)				
Fat (%)	1	3	5	7	9
0	OK	OK	G	G	G
1	OK	X	G	G	G
3	OK	G, soft	G	G	G

OK: no visible aggregates or gelation; X: some aggregation; G: formation of gel.

Table 12. Heat stability of oil-in-water emulsions homogenized at 90 MPa.

	Protein (%)				
Fat (%)	1	3	5	7	9
0	OK	OK	G	G	G
1	OK	X	G	G	G
3	OK	G	G	G	G

OK: no visible aggregates or gelation; X: some aggregation; G: formation of gel.

5.2 Part 2: Effect of pH

Physical properties

Whey protein emulsions were unstable at pH 4 and 5 and separated into two phases after homogenization (Fig. 11). Whey proteins are known for their solubility at low pH. However, this is not true when they are used to prepare emulsions. Data in Fig. 12 show the increase in particle size that occurred when the emulsions were heated at pH 4 and 5 compared to other pH values. The dominant diameter of fat globules in emulsions at pH 3, 6, 7 and 8 was about 2 μm while at pH 4 and 5 the diameters increased to 4 and 6 μm, respectively.

One of the mechanisms in protein-stabilized emulsions is the electrostatic repulsion between proteins adsorbed at the droplets surface. When the pH of the whey protein emulsions approached the isoelectric point of the whey proteins, pH 5.2 and ~ 4 for β-lactoglobulin and α-lactalbumin (Walstra 1999), respectively, the net charge of the adsorbed layers should be near zero. This would reduce the electrostatic repulsive interaction potential, and promote flocculation and aggregation of the fat globules to induce separation of the emulsion.

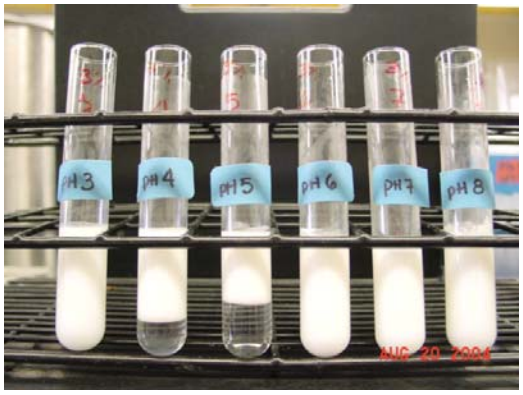


Fig. 11. Effect of pH on whey protein stabilized emulsions before heat treatment.

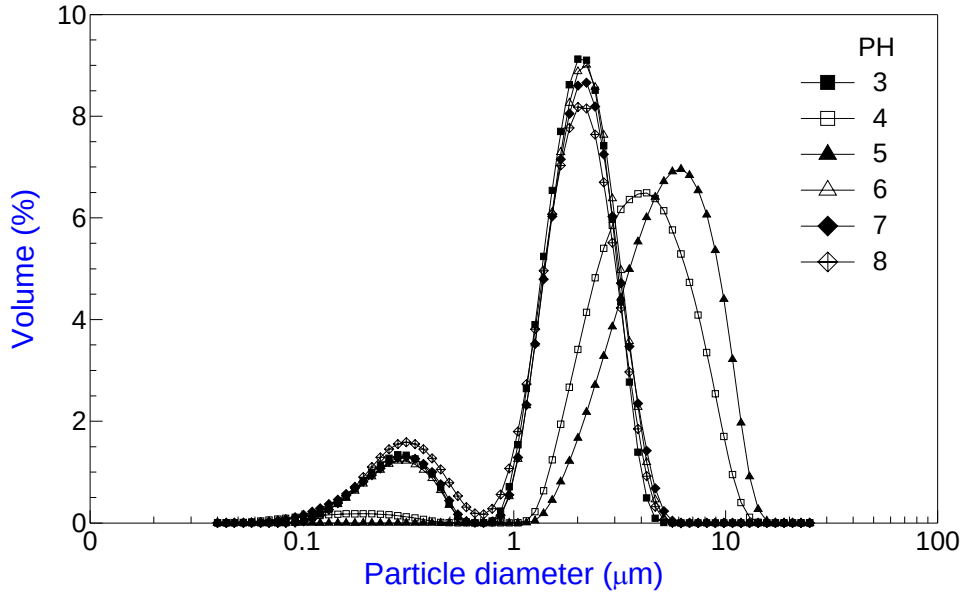


Fig. 12. Effect of pH on particle size distribution of whey protein stabilized emulsions containing 3% protein and 3% fat homogenized at 20 MPa.

The properties of protein coated emulsion droplets usually resemble those of isolated proteins in solution. In spite of this, emulsion droplets stabilized by whey proteins aggregate at their isoelectric point whereas individual protein molecules do not. Demetriades and others 1997 had similar findings in a different system which was 2% WPI and 20% oil emulsions. They explained that aggregation of the fat globules was due to the stronger van der Waals attractions between emulsion droplets than those between individual protein molecules in solution.

Dickinson and Matsumura 1994 proposed that the difference in the stability of whey proteins at different pH values might arise from changes on the relative amounts of adsorbed β-lactoglobulin and α-lactalbumin. At pH 7 the whey proteins adsorb with equal affinity while at lower pH α-lactalbumin adsorbs more readily. Hunt and Dalgleish

1995 also reported more adsorbed α-lactalbumin in emulsions made at pH 3 than in those emulsions made at pH 7.

Heat stability

Whey protein solutions containing (3% protein) were completely clear and soluble previous to the heat treatment at all the pH values studied. The solutions at pH 4, 5 and 6 formed gels with syneresis after the heat treatment while the samples at pH 3, 7 and 8 remained fluid. Solutions at pH 3 and 8 were clear whereas solution at pH 7 changed to white color (Fig 13 and table 13). Whey protein emulsions remained fluid after the heat treatment at pH 3 and 8 but emulsions at pH 7 had some aggregates (Fig 14 and table 13). The heat denaturation properties of whey proteins have been extensively studied and it is known that pH, ionic strength and type of ions have a significant effect on the denaturation and gelling of whey proteins (Sawyer 2003). Kella and Kinsella (1988b) suggested that the increase in stability of ß-lg at low pH could be attributed by additional hydrogen bonding in the protein due to titration of carboxy groups in the pH range 3.0-1.5 preventing denaturation.

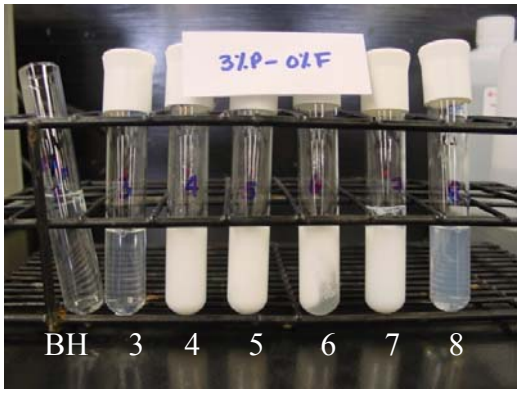


Fig. 13. Whey protein solutions after heat treatment at pH 3, 4, 5, 6, 7 and 8. BH before heat treatment.

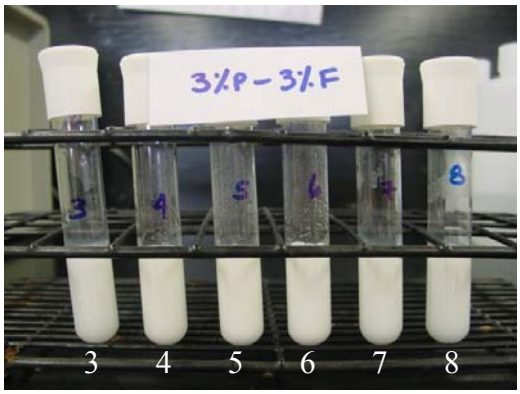


Fig. 14. Whey protein emulsions after heat treatment at pH 3, 4, 5, 6, 7 and 8.

Table 13. Effect of pH on physical properties of whey solutions and emulsions after heat treatment 121°C/10 min.

	Whey protein solutions (0% fat)		Whey protein emulsions (3% fat)	
PH	Observations	Color	Observations	Color
3	OK	clear	OK	white
4	G w/syneresis	white	G	white
5	G w/syneresis	white	G	white
6	G w/syneresis	white	G	white
7	OK	white	X	white
8	OK	clear , whitish	OK	white

OK: no visible formation of aggregates; X: some aggregation; G: formation of gel.

5.3 Part 3: Effect of additives

5.3.1 Effect of phospholipids

Particle size distribution and dvs

Three types of lecithins (Table 4) were used to study the properties of whey protein stabilized emulsions and solutions with 3, 5, 7% of protein. Probabilities values for the main effects and interactions are shown in Table 14. The interaction between the type of lecithin and protein concentration on the physical properties of the emulsions was not significant showing that the phospholipids have the same effect at each concentration of protein. The size of fat globules was affected by the type of lecithin added (p=.001) (Table 14). The size of the fat globules increased when regular deoiled lecithin was added to the protein stabilized emulsions but decreased when acetylated lecithin or hydrolyzed lecithin were added to the emulsions (Fig 15 and Table 15). Fig 16, 17 and 18 show the typical particle size distribution of emulsions with phospholipids containing 3, 5 and 7% protein, respectively. Particle size distributions are affected by the type of lecithin added. Regular lecithin increased the volume percent of fat that corresponds to droplets of approximately 2 μm compared to the other lecithins and this change is more notable in emulsions that contained 3 and 7% protein.

Perez-Hernandez (2001) reported a slight increase in the size of fat globules when deoiled regular lecithin was added in WPC stabilized emulsions containing 3% protein and 3% butter oil. The dvs increased from 0.800 μm to 0.850 μm. Sünder and others (2001) reported an increased in the particle size of emulsions stabilized with WPI and WPC (1-2% protein, 10% vegetable oil) when deoiled lecithin and hydrolysed lecithin were added to the emulsions compared to a control. However, when the concentration of lecithin increased from 0.39% to 0.78% a small reduction of the single droplet size was noted. This effect was more pronounced for the hydrolysed lecithins compared to other lecithins. Perez-Hernandez and others (2004) studied the effect of different types of phospholipids on the physical properties of whey protein stabilized emulsions (2% protein, 10% vegetable oil) and found that the particle size of the

emulsions was significantly smaller when the phospholipids contained a higher content of lysophospholipids.

Table 14. Probability values for the effect of protein concentration and lecithin determined by ANOVA.

Model	Dvs (μm)	Surface Area (cm^2/ml)	Fat layer (mm)	ES (%)
Protein	.004	.004	.000	.000
Lecithin	.001	.002	.001	.001
Prot x Leci	.201	.202	.336	.052
R^2	.589	.571	.466	0.924

Table 15. Effect of protein concentration and lecithin on physical properties of whey protein stabilized emulsions.

	Dvs[1] (μm)	Surface Area[1] (cm^2/ml)	Fat layer[1] (mm)	ES[1] (%)
Lecithin				
Control	0.870ab	72770ab	2.417a	33.07a
Regular	1.002b	60593a	1.972b	41.58b
Acetylated	0.84a	73943ab	1.889b	50.32c
Hydrolyzed	0.749a	81843b	2.083ab	42.24b
Protein (%)				
3	0.954a	64291a	2.33a	36.83a
5	0.862ab	72134ab	2.14a	46.78b
7	0.780b	80437b	1.79b	

[1]Means within columns for each variable followed by the same letter are not significantly different (α=0.05).

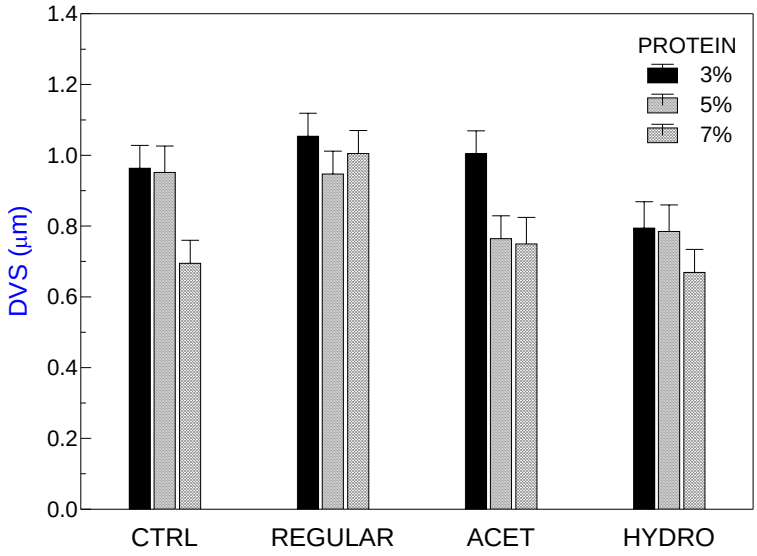


Fig. 15. Effect of lecithins on dvs. Bars show the mean +/1 SE.

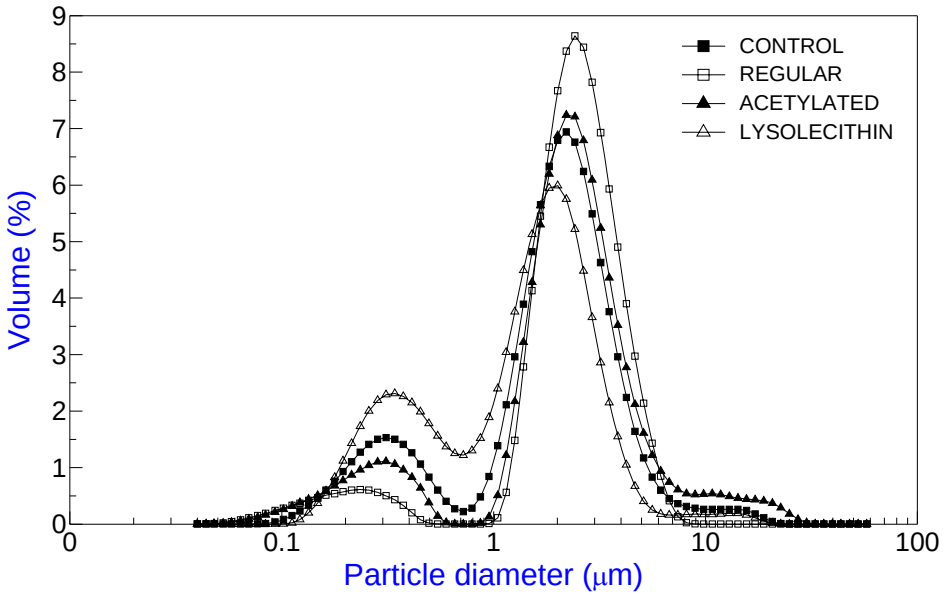


Fig. 16. Effect of lecithin on the particle size distribution of emulsions with a concentration of 3 % protein.

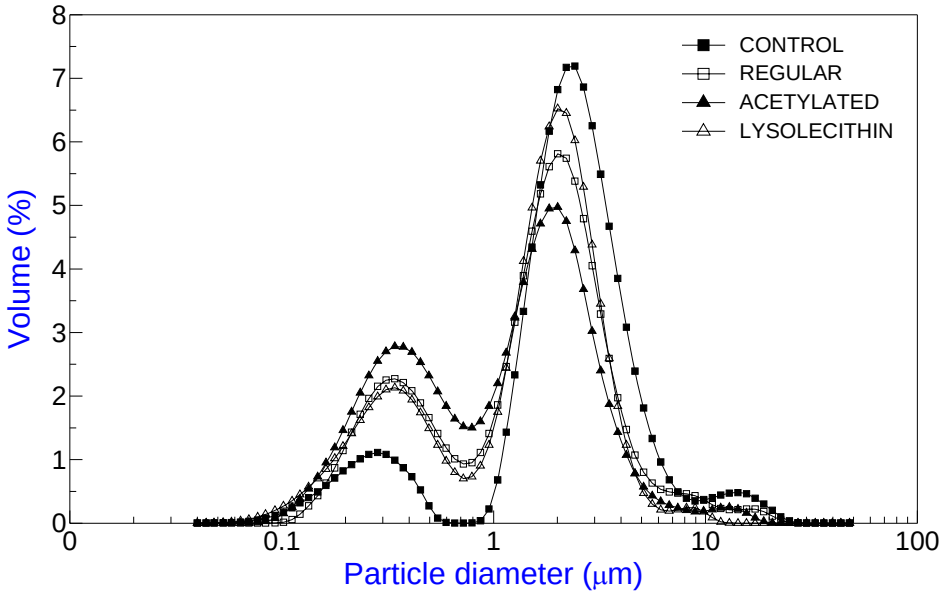


Fig. 17. Effect of lecithin on the particle size distribution of emulsions with a concentration of 5% protein.

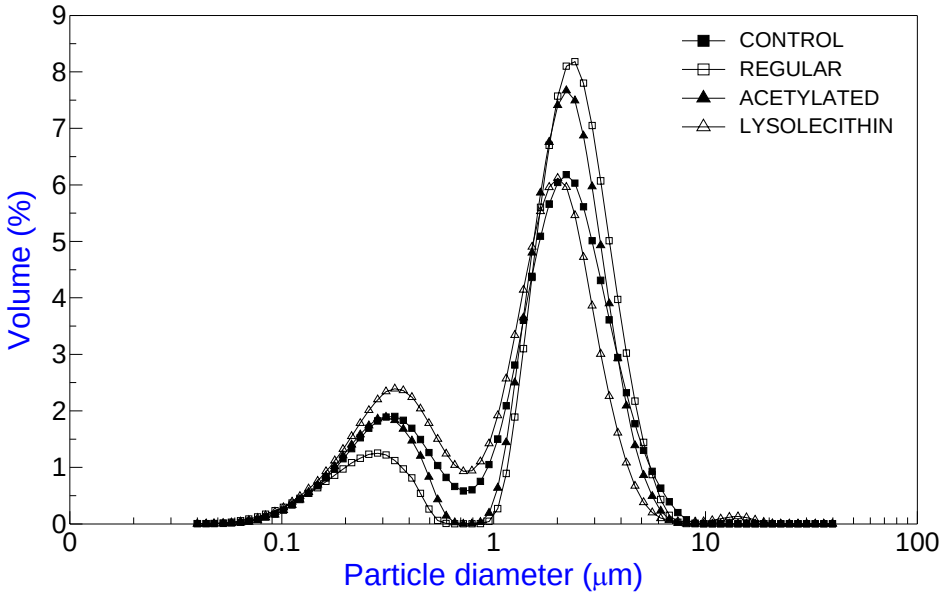


Fig. 18. Effect of lecithin on the particle size distribution of emulsions with a concentration of 7 % protein.

It is evident that there is an interaction between phospholipids and whey proteins and this interaction affects the properties of the emulsions. ß-lg has been reported to bind specific ligands such as free fatty acids, tryglicerides, retinol and phospholipids (Kontopidis and others 2004, Sawyer 2003). The mechanisms by which ß-lg binds phospholipids is not clear, Kristensen and others (1997) studied the complex formed between phospholipids and ß-lg using differential scanning calorimetry (DSC) and reported that ß-lg only binds negatively charge phospholipids like phosphatidic acid (PA), however charge is not the only constraint since neither PA with unsaturated fatty acids nor distearoylphosphatidylglycerol interacted with ß-lg suggesting that the interaction between ß-lg and phospholipids depends not only on charge, but also the acyl chain and polar head of the phospholipids. Brown (1983) reported that phosphatidylcholine (PC) was unable to bind to native ß-lg, but modifying the α-helix of ß-lg permitted binding of PC. Brown therefore suggested that the interaction site was the hydrophobic part of the α-helix with the hydrocarbon chain of the lipid, while the polar head group is likely to interact with the hydrophilic exterior of the protein. Specific interactions between ß-lg and lysophosphatidylcholine (lysoPC) have also been reported (Sarker and others 1995, Wüstneck and others 2000). Sarker studied the interaction between ß-lg and lysoPC at the interfaces of foams and reported that ß-lg bound only one molecule of lysoPC per protein molecule and lysoPC decreased the surface tension. Wüstneck and others (2000) reported that ß-lg and lysoPC form complexes with lower interfacial activity and increasing the concentration of lysoPC decreased both the interfacial elasticity and viscosity.

The hydrolyzed lecithin used in this research was 45 to 55% hydrolyzed thus the lysophospholipids might have formed a complex with ß-lg and reduced the interfacial tension, allowing the formation of smaller fat globules as explained by the Young Laplace pressure (Becher 2001).

Emulsion stability

The emulsion stability measured as mm of fat layer separated from the emulsions were in the order: acetylated lecithin > regular lecithin > hydrolyzed lecithin > control. Emulsions containing acetylated lecithin show the highest emulsion stability (Table 15, Fig. 19). However the use of the depth of the fat layer to measure the emulsion stability in emulsions containing phospholipids did not produce an acceptable model (R^2=0.46) (Table 14) for estimating emulsion stability. This might have been caused if lecithin altered the packing of the fat globules in the fat layer and created high variation in the results; thus a secondary method using fat concentration rather than thickness of the fat layer to measure emulsion stability was determined to corroborate the results. This method was used only for emulsions containing 3 and 5% protein. This method produced a better statistical model (R^2=0.92) than the method based on the fat layer (R^2=0.46). The emulsion stability for best to worst was in the order: acetylated lecithin > hydrolyzed lecithin > regular lecithin > control as shown in Table 15 and Figure 20. Acetylated lecithin showed the best emulsion stability (Fig. 20). The methods based on thickness of the fat layer and concentration of fat were significantly correlated (p-value = 0.04, Pearson correlation) but the second method caused less variation of the data and better separation of the means. Using the thickness of the fat layer to measure emulsion stability provides a fast, inexpensive and easy approach to predict the emulsion stability compared to the method based on the concentration of fat. However the method based on fat concentration is more sensitive to differences in emulsion stability.

Smaller fat globules were found when hydrolyzed lecithins rather than acetylated lecithins were used to make the emulsions. But the acetylated lecithin produced more stable emulsions. Creaming is strongly dependent upon droplet size (Stokes's law) but other mechanisms can contribute to the improved stability against creaming in phospholipids and protein stabilized emulsions. Acetylated lecithin has the highest HLB, indicating a more hydrophilic nature, and a more bulky polar head group compared to non-acetylated lecithin. This modification might account for the improved stability of emulsions which contained acetylated lecithin compared to other lecithins.

Stability of emulsions is also a function of the electrostatic properties of the interface. Zeta potential (ζ) was measured in emulsions containing 5% whey protein (Table 16) and the particles in the emulsions with more negative charge were those containing acetylated lecithin and hydrolysed lecithin. A strong correlation between the emulsion stability and zeta potential was found (p-value = 0.001, Pearson correlation). There is a general agreement that increasing Zeta potential away from zero gives rise to enhanced stability as predicted by DLVO-theory (Becher 2001, Friberg 1997).

These results indicate that charge repulsion is a more important mechanism for stabilizing whey protein emulsions containing lecithin than is particle size. These results are supported by Wabel (1998) who reported that the stability of parenteral emulsions stabilized with phospholipids was strongly dependent on zeta potential.

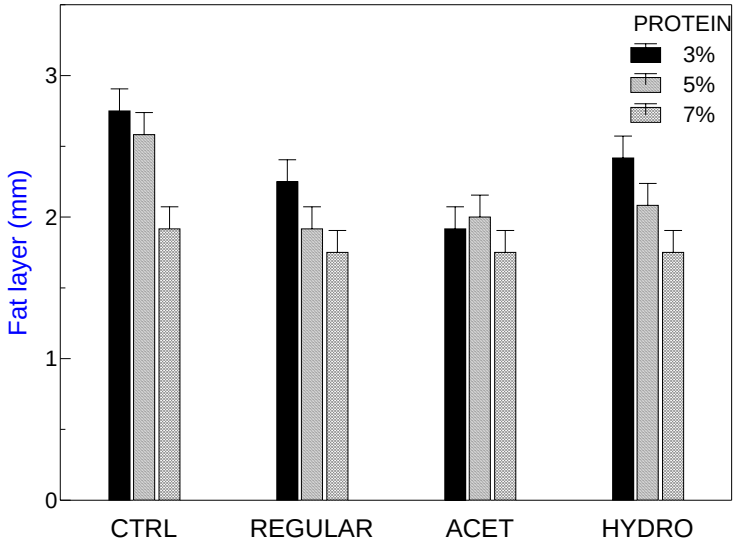


Fig. 19. Effect of lecithin type on depth of fat layer. Error bars show the mean +/- 1.0 SE.

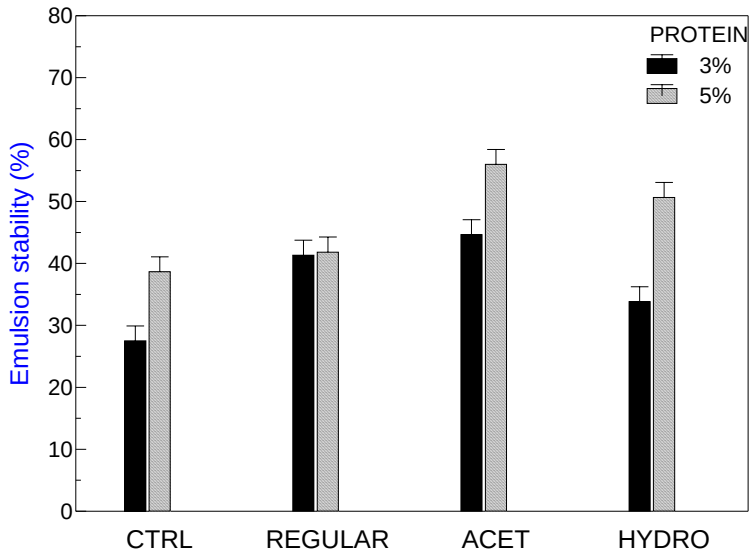


Fig. 20. Effect of type of lecithin on emulsion stability. Error bars show the mean +/- 1.0 SE.

Table 16. Zeta Potential (mV) of particles in emulsions containing 5% protein and 3% fat after homogenization at 20 MPa.

Lecithin	Zeta Potential (mV)	Standard error
Control	-45.50	1.06
Regular	-48.18	0.48
Lysolecithin	-53.97	3.17
Acetylated	-55.31	0.92

Effect of heat treatment

The heat stability of the samples increased when lecithins where added to the formulation (Table 17 and 18). The improvement was more notable when the samples contained fat. Whey protein stabilized emulsions remained fluid, without aggregation even at 5% protein when lecithins were included in the formulations. Emulsions that contained 5% protein and hydrolyzed lecithin or acetylated lecithin had the lowest viscosity (Table 18) which might be attributed to the formation of less heat aggregates. Whey protein solutions without fat contained heat generated aggregates at 5% protein regardless of the type of lecithin. Fat apparently has an important function in the heat stability of the whey protein emulsions. Heat stability studies with evaporated milks showed similar results. Milk with added lecithin was more heat stable when fat was present in the products (Hardy and others 1985, Singh and others 1992). McCrae and others (1999) considered two possible mechanisms by which lecithin improves heat stability in homogenized milk : a) that lecithin increases heat stability by displacement of or by interaction with surface-adsorbed protein and b) that lecithin interacts with free protein in solution thereby influencing heat-induced free protein and bound protein interactions. McCrae and others (1999) concluded that lecithin does not require fat to affect heat stability, and lecithin does not exercise its effect on heat stability by interaction with protein in the bulk phase but it affected heat stability by targeting membrane proteins. However, the mechanism by which lecithins improved the heat stability in whey protein in solution is not clear. As previously discussed phospholipids can interact with proteins and affect properties such as stability against heat aggregation. Kristensen and others (1997) using DSC demonstrated that the interaction between phospholipids and ß-lg can lead to a stabilization of the protein against thermally-induce folding. The effect was found to be dependent on the type of polar head group on the phospholipids and steric factors. A negatively-charged group seemed to be necessary for the interaction (Kristensen and others 1997). However, the reason phospholipids appear to be more beneficial to protect whey proteins against heat stability when fat is present is not clear. Possibly, changes in the conformation of ß-lg during adsorption at the interface

of the fat droplets made it easier for the phospholipids to interact with the protein, and this change in conformation does not occur when whey proteins are in solution. The apparent viscosities of emulsions with modified lecithins after sterilization were lower than the apparent viscosity of emulsions with regular lecithin (Table 18) in emulsions that contained 5% protein which indicates that there was less formation of heat aggregates when the modified lecithins where present. Modified lecithins might be able to form complexes more readily with the whey protein than unmodified lecithin and this complex could protect the emulsion against the heat stress. Perez-Hernandez and others (2004) studied the properties of emulsions stabilized with whey protein and different phospholipids. They reported that the phospholipids with a higher concentration of lysophospholipids provided the best protection for the emulsion against heat during autoclaving. Wabel (1998) studied the stability of parenteral emulsions stabilized with lecithins during autoclaving and found emulsions with a more negative zeta potential produced by charged phospholipids had better stability against heat stress. Also Wabel reported that hydrolysis of the lecithins occurred during autoclaving, and the hydrolysis products do not move from the oil-water interfacial layer. Consequently, lecithin hydrolysis increases the zeta potential of the oil droplets. Phospholipids contained in liposomes hydrolyse faster than phospholipids at the interface of fat droplets.

Table 17. Heat stability and apparent viscosity of whey protein solutions with lecithins.

	3% protein		5% protein		7% protein	
		μ(cp)		μ (cp)		μ (cp)
Control	OK/x	-	G	-	G	-
Regular	OK	1.52a	X	-	G	-
Hydrolyzed	OK	1.65a	X	-	G	-
Acetylated	OK	1.68a	X	-	G	-

OK: No visible aggregates or gelation; X: some aggregation; G: Gel

Table 18. Heat stability and apparent viscosity of whey protein emulsions with lecithins.

	3% protein		5% protein		7% protein	
		μ (cp)		μ (cp)		μ (cp)
Control	OK/x	-	X	-	G	-
Regular	OK	1.65a	OK	3.36a	G	-
Hydrolyzed	OK	1.72a	OK	2.38b	X	-
Acetylated	OK	1.72a	OK	2.52b	G	-

OK: No visible aggregates or gelation; X: some aggregation; G: Gel

5.3.2 Effect of phosphates

Particle size distribution and dvs

The addition of polyphosphates (Table 5) and protein concentration did not affect the particle size distributions and diameters of the fat globules (Table 19 and 20). The mean dvs of the emulsions containing 3 and 5% protein with different polyphosphates are shown in Fig. 21. No significant differences were found among the control and the different types of polyphosphates (Table 19). Figure 22 and 23, show the particle size distribution in emulsions containing different types of polyphosphates and containing 3 or 5% protein respectively. Bimodal distributions were found for all the emulsions and no visual differences were observed among the control and the emulsions containing the different types of polyphosphates. Differences in surface area caused by polyphosphates were not found because surface area depends on the size of the fat globules (Table 20).

Table 19. Probability values for the effect of protein concentration and phosphate type determined by ANOVA.

Model	Dvs (μm)	Surface Area (cm^2/ml)	Fat layer (mm)
Protein	.651	.829	.238
Phosphates	.601	.635	.000
Prot x Phosphates	.393	.319	.247
R^2	.455	.466	.477

Table 20. Effect of protein concentration and phosphate type on physical properties of whey protein stabilized emulsions.

Phosphates	Dvs[1] (μm)	Surface Area[1] (cm^2/ml)	Fat layer[1] (mm)
Phosphates			
Control	0.863a	69643a	2.52ac
SC	0.871a	69319a	2.77bc
MC	0.912a	67371a	2.54ac
VLC	0.877a	69606a	2.92b
MixSC	0.788a	77549a	2.42a
MixMC	0.879a	68356a	2.96bc
MixVLC	0.838a	72736a	2.85b
Protein (%)			
3 %	0.853a	70991a	2.68a
5 %	0.869a	70317a	2.74a

[1]Means within columns for each variable followed by the same letter are not significantly different (α=0.05).

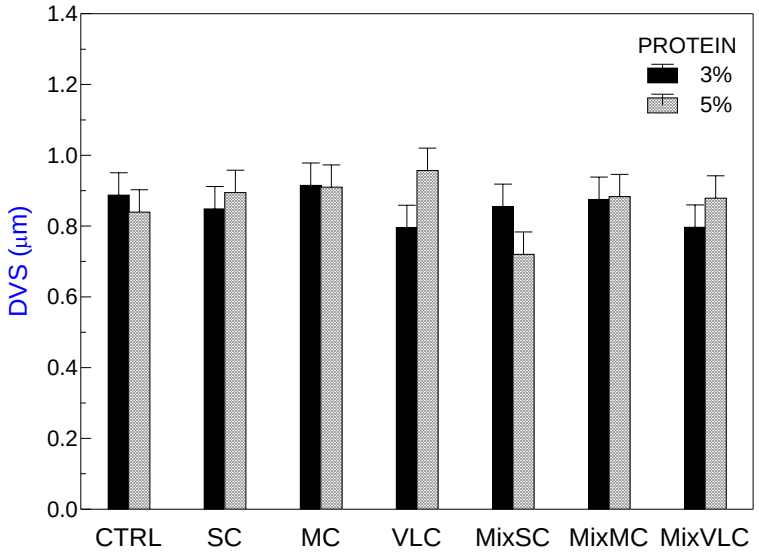


Fig. 21. Effect of different types of polyphosphates and protein on dvs. CTRL: control, SC: short chain, MC: medium chain, VLC: very long chain, MixSC: mixture short chain, MixMC: mixture medium chain, MixVLC: mixture very long chain.

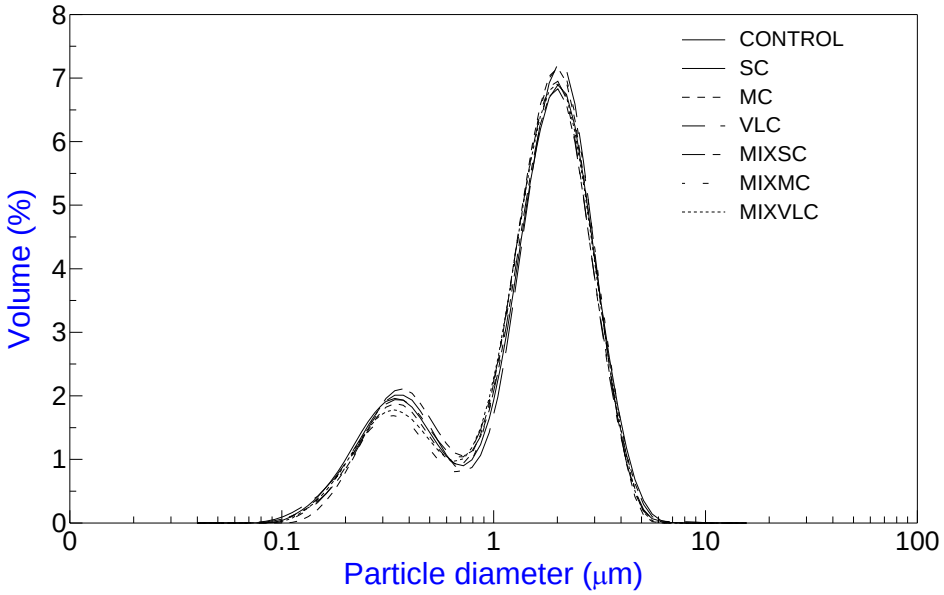


Fig. 22. Emulsions containing 3% protein and 3% fat, homogenized 20 MPa.

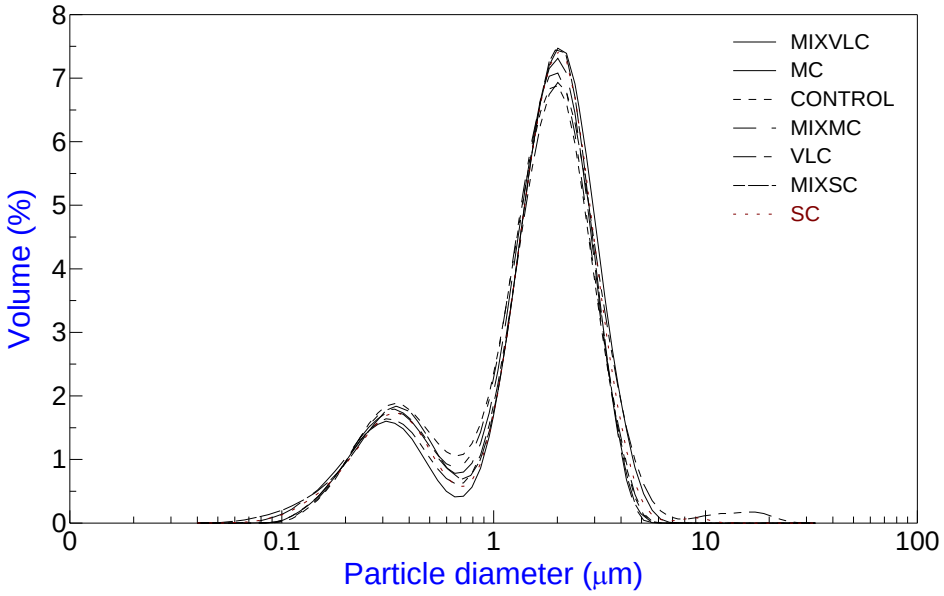


Fig. 23. Emulsions containing 5% protein and 3% fat, homogenized 20 MPa.

.

Creaming stability

Polyphosphates had a significant effect on the stability of the emulsions measured as the depth of the fat layer separated from the emulsions after centrifugation. However, the protein concentration did not have a significant effect on the creaming of the emulsions (Table 19). The addition of polyphosphates changed the pH of the emulsions (Table 21), MixSC and SC caused an increase in the pH while MC caused the pH to decrease. The magnitude of the change in pH depended on the concentration of protein. Samples with 3 % protein had a greater change in pH than samples with 5% protein likely due to the buffering properties of the proteins. An increased pH in the system would cause a more negative charge on the amino acid residues which would cause more electrostatic repulsion and affect the creaming stability of the emulsion. This change in pH which was not accounted for in the statistical model, and it might explain why the model only explained 47% of the variability. Emulsions that contained polyphosphates that caused an increase in the pH (MixSC an SC) were more stable when the protein concentration was 3% protein rather than 5% (Fig. 24). The emulsions that contained 3% protein had a higher pH than emulsions that contained 5% protein. Therefore, the change in pH might be responsible for the better stability of the emulsions with 3% protein. The emulsions with MixSC had the shortest cream layer depth (Table 20) and a pH higher than the control but the fat layer length was not significantly different from the control.

Table 21. pH change after addition of polyphosphates.

Polyphosphates	pH change	
	3 % Protein	5 % Protein
SC	+ 0.26	+0.12
MC	- 0.19	-0.15
VLC	NC	NC
MixSC	+0.63	+0.39
MixMC	NC	NC
MixVLC	NC	NC

NC No change. CTRL: control, SC: short chain, MC: medium chain, VLC: very long chain, MixSC: mixture short chain, MixMC: mixture medium chain, MixVLC: mixture very long chain.

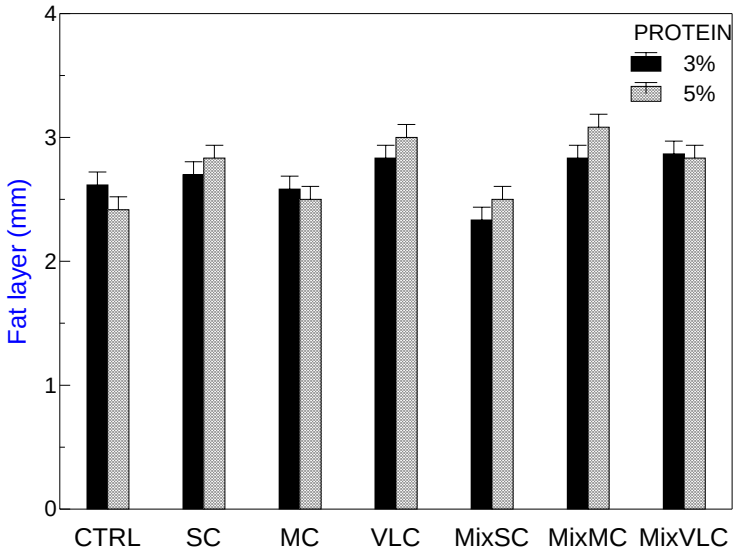


Fig. 24. Effect of polyphosphates and protein on fat layer. CTRL: control, SC: short chain, MC: medium chain, VLC: very long chain, MixSC: mixture short chain, MixMC: mixture medium chain, MixVLC: mixture very long chain.

Heat stability

Heat stability was measured in samples that had the pH adjusted to 7 before heating as well in samples without adjustment of the pH. Data in Table 22 are the effect of heat treatment on whey protein solutions prior to adjustment of the pH. The control and all samples that contained 3% protein and polyphosphates were heat stable. All solutions were clear (Fig. 25 a) prior to heating but after the heat treatment, the color of the solutions changed to white but where fluid without the presence of visible aggregates (Fig. 25 b). However, the solutions containing SC and MixSC remained clear. Solutions containing phosphates with long chains (VLC and MixVLC) had some sediment before and after heat treatment which is explained by the very large degree of polymerization which affected the solubility of this phosphate. Solutions containing 5% protein were clear before heat treatment (Fig. 25c) but after heat treatment only solutions containing SC and MixSC phosphates were stable (Fig 25d). Solutions containing long chain polyphosphates (VLC and MixVLC) did not form gels but aggregates were present. An attempt was made to eliminate the effect of pH so the pH was adjusted to 7 before the heat treatment and the solutions that contained MixSC and SC were also the more heat stable (Table 23 and Fig. 26) after pH adjustment. The apparent viscosity in solutions containing SC and MixSC was higher when the pH was adjusted to 7 compare to no adjustment in pH (Table 22 and 23). It is apparent from these results that the increase in pH is not the only factor responsible for the effect of polyphosphates during heat treatment. Polyphosphates with a degree of polymerization around 4 (SC and MixSC) were more heat stable than phosphates with a higher degree of polymerization. Data from the effect of heat treatment on whey protein emulsions is in Table 24. Emulsions made with 3% protein but without polyphosphates had some visible aggregation. Emulsions with polyphosphates were heat stable although some sedimentation was observed in samples containing VLC and MixVLC. Apparent viscosity after heat treatment was significantly lower in emulsions that contained small chain length phosphates (MixSC and SC). Emulsions made with 5% protein were heat stable only when they contained MixSC and SC. Phosphates with chain lengths of approximately

20 units (MC and MixMC) formed gels but the phosphates with long chains (VLC and MixVLC) did not form gels. However, the emulsion with long chains had some aggregation.

MixSC had lower viscosity than SC after heat treatment (Table 24). Data representing the effect of pH adjustment before heat treatment is in table 25. The results were consistent with the results obtained for the emulsions that did not have the pH adjusted. However apparent viscosity was higher for the sample containing MixSC compared to emulsion containing SC when the concentration of protein was 5%.

In summary phosphates with short chains (MixSC and SC) were found to provide better protection against heat stress for whey protein solutions and whey protein stabilized emulsions. This protective effect is due not only to the increase in pH caused in the samples because samples with adjusted pH containing MixSC and SC were still more heat stable than the long chain phosphates. Medium chain phosphates were prone to form gels with the whey proteins and long chain phosphates did not form gels but some sedimentation due to the insolubility of the phosphates and small amounts of aggregation were noted. Kella and Kinsella (1988a) reported that phosphates and citrates increased the heat stability of ß-lactoglobulin by delaying the dissociation of ß-lactoglobulin dimmers which is the initial step in the heat denaturation of ß-lactoglobulin, this protective effect on the protein is probably caused by changes in the structure of water.

Heat treatment caused a pH decrease in all samples containing phosphates (Tables 22, 23, 24 and 25). This change in pH might have been caused by the hydrolysis of the polyphosphates. Glandorf and Thomasow (1977) have reported that polyphosphates added in milk were totally hydrolyzed to mono- and diphosphates when milk was sterilized at 115 °C for 20 min.

Table 22. Heat stability and apparent viscosity of whey protein solutions with polyphosphates. Without pH adjustment.

	3 % Protein				5 % Protein			
	PH *	PH**	OBS	μ (cP)+	PH *	PH**	OBS	μ (cP)+
CTRL	7.05	7.01	X,S / white	-	7.05	7.01	G - white	-
SC	7.31	6.83	OK / clear	1.60 [ab]	7.12	6.76	OK/ clear	4.09[a]
MixSC	7.70	7.10	OK / clear	1.51 [a]	7.38	6.96	OK / clear	3.17[a]
MC	6.81	6.38	OK / white	1.84 [b]	6.83	6.37	G	-
MixMC	7.01	6.54	OK / white	1.87 [b]	6.93	6.51	G	-
VLC	7.04	6.84	OK, S /white	1.47 [a]	7.01	6.82	X, S / white	-
MixVLC	6.97	6.66	OK,S /white	1.55 [a]	6.96	6.68	X, S / white	-

Table 23. Heat stability and apparent viscosity of whey protein solutions with polyphosphates. With pH adjustment.

	3 % Protein				5 % Protein			
	PH *	PH**	OBS	μ (cP)+	PH *	PH**	OBS	μ (cP)+
CTRL	7.05	7.05	X,S / white	-	7.05	7.00	G - white	-
SC	7.05	6.68	OK / clear	1.65 [a]	7.05	6.72	OK / clear	5.41[a]
MixSC	7.05	6.76	OK / clear	1.68 [a]	7.05	6.80	OK / clear	11.33[b]
MC	7.05	6.45	OK / white	1.89 [b]	7.05	6.49	G	-
MixMC	7.05	6.55	OK / white	1.96 [b]	7.05	6.57	G	-
VLC	7.05	6.66	OK,S /white	1.56 [a]	7.05	6.77	X, S / white	-
MixVLC	7.05	6.57	OK,S /white	1.61 [a]	7.05	6.65	X, S / white	-

* pH before heating, ** pH after heating, + Apparent viscosity @ 60 rpm ($74s^{-1}$), T= 22°C OK: No visible aggregates or gelation; X: Some aggregation; G: Gels

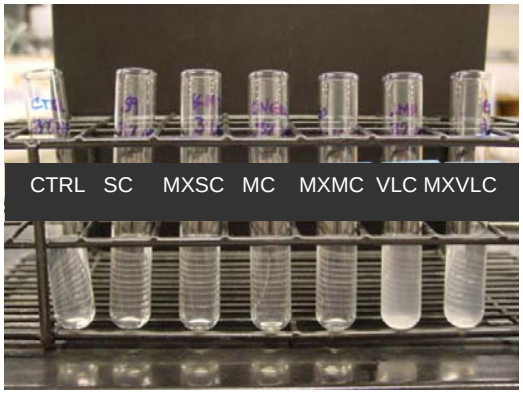
Table 24. Heat stability and apparent viscosity of whey protein emulsions with polyphosphates. Without pH adjustment.

	3 % Protein				5 % Protein			
	PH *	PH**	OBS	μ (cP)+	PH *	PH**	OBS	μ (cP)+
CTRL	7.05	7.09	X, S /white	-	7.05	7.02	G / white	-
SC	7.31	6.85	OK/ white	1.64 [a]	7.17	6.75	OK / white	3.85 [a]
MixSC	7.68	7.12	OK / white	1.51 [a]	7.44	6.96	OK / white	3.03 [b]
MC	6.86	6.41	OK / white	2.01 [b]	6.90	6.42	G / white	-
MixMC	7.03	6.58	OK / white	1.94 [b]	7.00	6.54	G	-
VLC	7.06	6.84	X, S / white	-	7.07	6.94	X, S / white	-
MixVLC	7.00	6.68	X, S / white	-	7.01	6.75	X, S / white	-

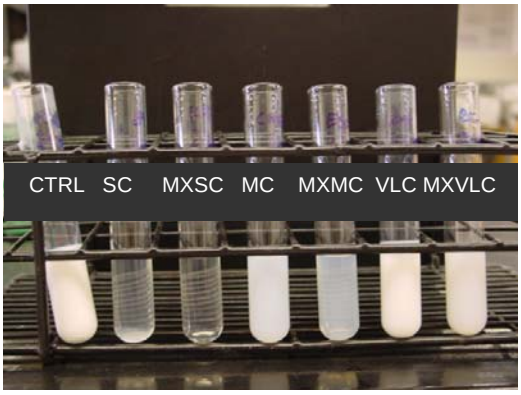
Table 25. Heat stability and apparent viscosity of whey protein emulsions with polyphosphates. With pH adjustment.

	3 % Protein				5 % Protein			
	PH *	PH**	OBS	μ (cP)+	PH *	PH**	OBS	μ (cP)+
CTRL	7.05	7.08	X, S /white	-	7.05	7.03	G - white	-
SC	7.05	6.73	OK / white	1.68 [a]	7.05	6.73	OK / white	6.56 [a]
MixSC	7.05	6.74	OK/ white	1.70 [a]	7.05	6.78	OK / white	13.28 [b]
MC	7.05	6.49	OK / white	1.98 [b]	7.05	6.49	G / white	-
MixMC	7.05	6.58	OK / white	2.11 [b]	7.05	6.55	G	-
VLC	7.05	6.85	X,S / white	-	7.05		X, S / white	-
MixVLC	7.05	6.68	X,S / white	-	7.05	6.75	X, S / white	-

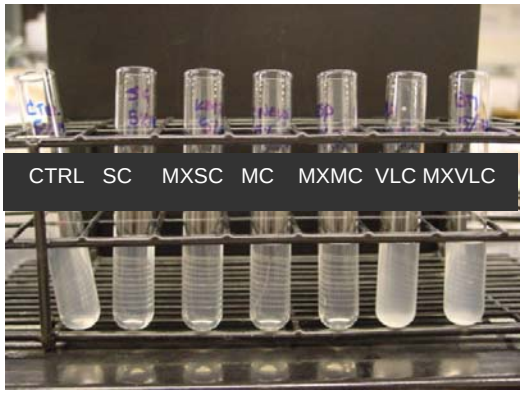
* pH before heating, ** pH after heating, + Apparent viscosity @ 60 rpm ($74s^{-1}$), T= 22°C OK: No visible aggregates or gelation; X: Some aggregation; G: Gels



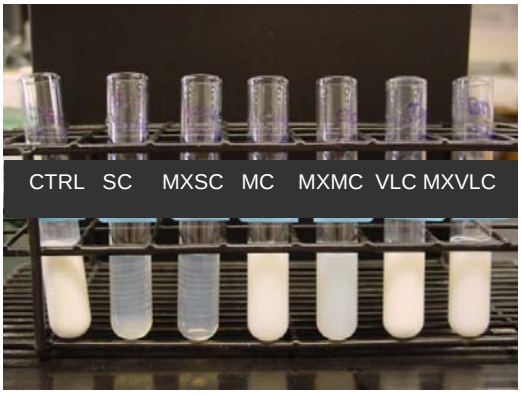
a. Solutions 3% protein before heating.



b. Solutions 3% protein after heating.



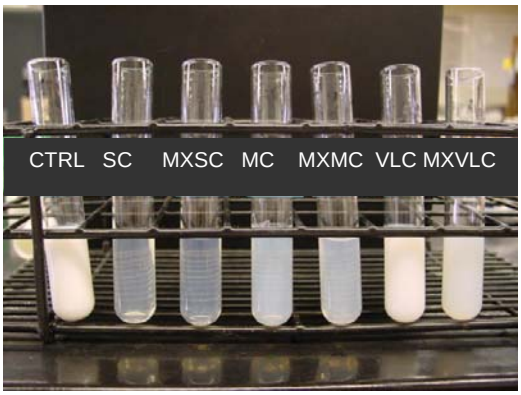
c. Solutions 5% protein before heating.



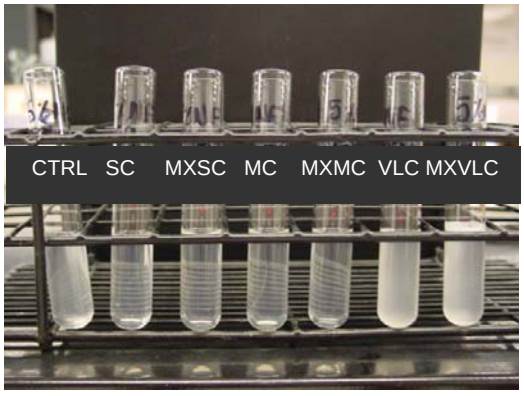
d. Solutions 5% protein after heating.

Fig. 25. Whey protein solutions before and after heat treatment. CTRL: control, SC: short chain, MC: medium chain, VLC: very long chain, MixSC: mixture short chain, MixMC: mixture medium chain, MixVLC: mixture very long chain.

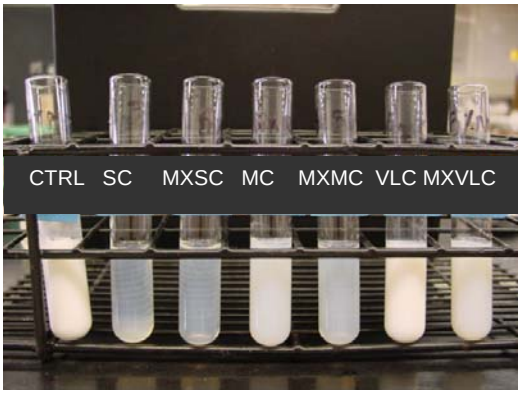
a. Solutions 3% protein before heating, pH adjusted to 7 before heating.



b. Solutions 3% protein after heating, pH adjusted to 7 before heating.



c. Solutions 5% protein before heating, pH adjusted to 7 before heating.



d. Solutions 5% protein after heating, pH adjusted to 7 before heating.

Fig. 26. Whey protein solutions before and after heat treatment. PH was adjusted to 7 prior to heating. CTRL: control, SC: short chain, MC: medium chain, VLC: very long chain, MixSC: mixture short chain, MixMC: mixture medium chain, MixVLC: mixture very long chain.

5.3.3 Effect of emulsifiers

Particle size distributions

Emulsions prepared with the emulsifiers were unstable and separated into two phases after homogenization. Particle size distributions were measured in the top and bottom of the emulsions to evaluate possible mechanisms that lead to instability. The particle size distribution of an emulsion containing monoglycerides is shown in Fig. 27. It is evident that emulsions containing monoglycerides had particles of smaller size compared to the control. Low molecular weight emulsifiers are very effective for reducing interfacial tension and therefore can be used to create emulsions with small particles. The bottom part of the emulsion with monoglycerides had a bimodal distribution; one peak corresponded to particles with a diameter of approximately 0.33 μm while the second peak where the majority of the particles had a diameter of approximately 2 μm. The particle size distribution from the upper part of the emulsion was also bimodal but the peak corresponding to 2 μm was more dominant. The rapid segregation of particles in the emulsions containing monoglycerides might be explained by decreased steric and electrostatic repulsion. Monoglycerides have been reported to displace proteins from the interface of fat droplets stabilized with proteins (Perez-Hernandez 2001). Displacement of proteins from the interface would decrease the negative charge of the fat droplets because they would no longer be covered with protein. The absence of protein from the surface would also reduce steric repulsion associated with protein polymers attached to the interface. Emulsions containing 3 and 5% protein and monoglycerides aggregated after heat treatment. It seems that monoglycerides did not improve the heat stability of the emulsions as much as lecithins. However, separation of the emulsion was already in progress prior to heat treatment. This might have increased local concentrations of proteins which could have promoted heat aggregation.

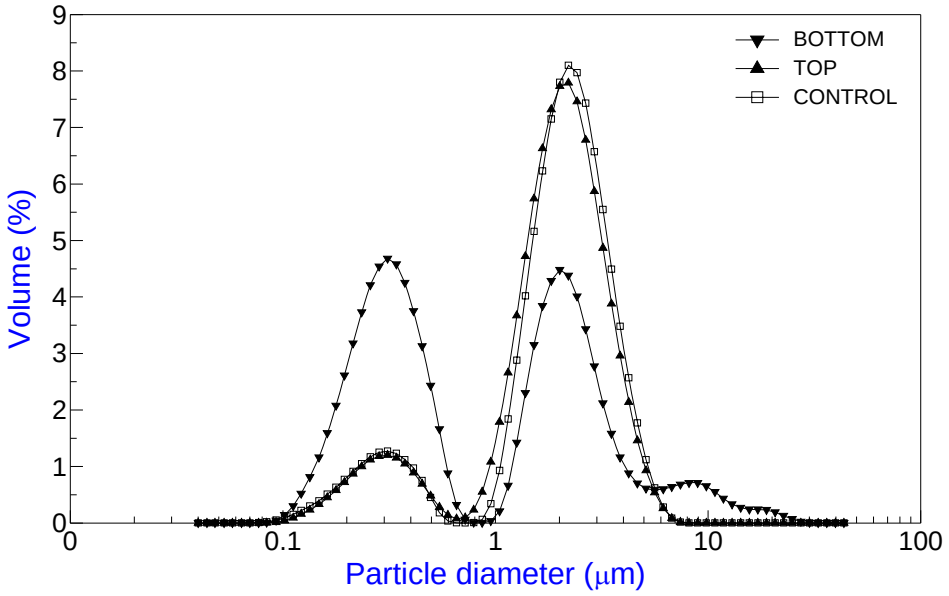


Fig. 27. Particle size distribution of emulsions containing monoglycerides with 3% protein and homogenized at 20 MPa.

Data for the particle size distribution of emulsions containing sodium stearoyl lactylate (SSL) is in Fig. 28. SSL is very hydrophilic with an HLB value of 21 and it has a negative charge (Krog and Van Sparso 2004). The particle size distribution was very dispersed when the emulsions contained SSL compared to the control. Emulsions containing SSL were also unstable and tended to separate into two phases. However, the mechanism by which SSL caused emulsions to be unstable might be different from the mechanisms associated with the effect of monoglycerides since the particle size distributions in these emulsions was different. Emulsions created using SSL had trimodal particle size distributions in the top and bottom of the emulsions. The major peaks were for particles with diameters of approximately 0.35, 1.7 and 15 μm in the top and bottom of the emulsions. This distribution indicates that SSL promoted some aggregation or flocculation of the fat droplets or caused protein aggregation since big particles were also found in the bottom of the emulsions. SSL might have interacted with

proteins at the interface of fat droplets and probably changed their conformations or promote bridging flocculation. Anionic surfactants have been shown to interact with proteins and to alter their structure. A model explaining the mechanism by which anionic surfactants interact with globular whey proteins has been suggested (Jones 1975). This mechanism proposed that the surfactant initially bind with specific sites on the surface of the protein, followed by the formation of ionic bonds between the negatively charged groups of the surfactants and the cationic amino acid residues of the proteins. The tertiary structure of the protein then unfolds, either as a result of electrostatic repulsion between the negative charges of the surfactants bound to the proteins or because the hydrophobic chains of the surfactants penetrate the apolar regions of the protein. The second stage of the proposed mechanism requires hydrophobic interactions between the chains of surfactant and the newly exposed non-polar residues of the protein until all sites become saturated. An increase in heat stability was expected since anionic emulsifiers including SSL have been reported to increase the denaturation temperature and decreased the size of heat-induced whey protein polymers (Giroux and Britten 2004). However the emulsions containing SSL and 3 or 5% protein formed aggregates during the heating process.

Particle size distributions of emulsions containing polysorbates (Fig. 29) showed extensive segregation of the fat droplets. Particles in the bottom of the emulsion had most of the fat present in particles with diameters less than 0.4 μm while the particles at the top of the samples had most of the particles with diameters ranging from 2 to 20 μm. It is evident that this emulsion experienced not only creaming but some aggregation or possible coalescence since there was a large increase in the size of fat droplets at the top of the container. Polysorbates are nonionic emulsifiers and can displace proteins from the interfaces promoting aggregation with a mechanism similar to that of monoglycerides. However, polysorbates have a greater HLB value (10) compared to monoglycerides (3.7) and nonionic water soluble emulsifiers are generally more effective at displacing milk proteins from the oil water interface than are nonionic oil soluble emulsifiers (Courthaudon and others 1991, Dickinson and Hong 1994). This can

explain the greater instability of emulsions with polysorbates than emulsions with monoglycerides. Emulsions with polysorbates formed aggregates during the heat treatment. Emulsions that contained the emulsifiers were very unstable and reliable measures of the fat layer were not obtained.

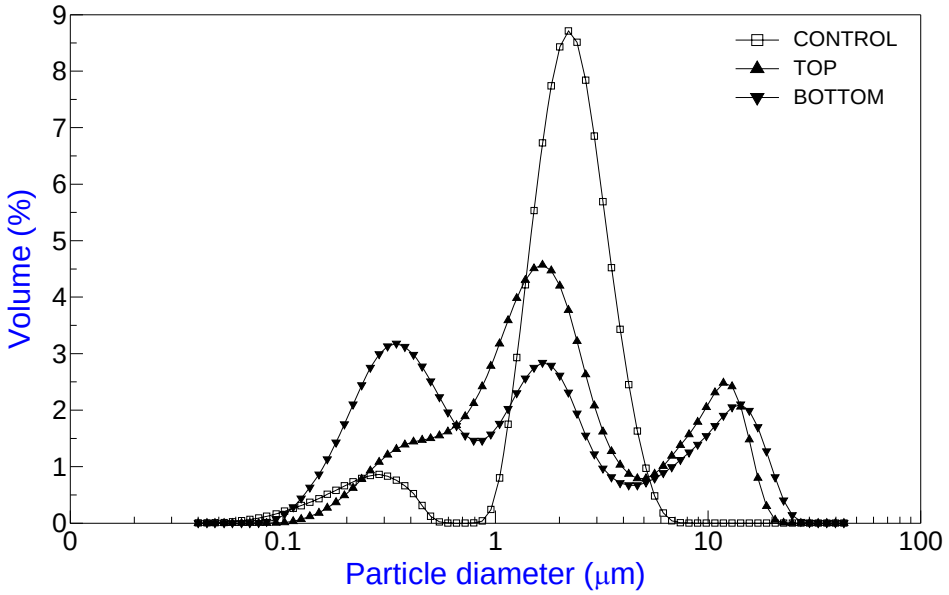


Fig. 28. Particle size distribution of emulsions containing SSL with 5% protein and homogenized at 20 MPa.

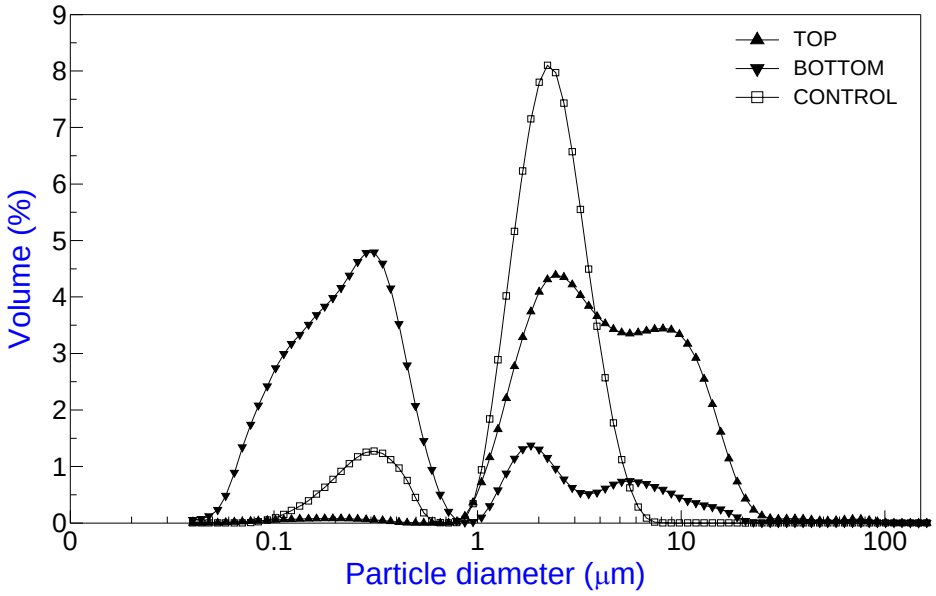


Fig. 29. Particle size distribution of emulsions containing polysorbates with 3% protein and homogenized at 20 MPa.

5.3.4 Effect of hydrocolloids

Dvs and particle size distribution

Neither protein concentration nor the interaction between protein concentration and hydrocolloids affected the particle size. However, the presence of hydrocolloids in the emulsions had a significant effect on particle size (Table 26, Fig. 30). Emulsions that contained LBG had a smaller mean particle size compared to all other emulsions except the control (Table 27). It was thought that some impurities in the sample of LBG could have created artificially reduced particles so an additional experiment with a different brand of LBG was conducted. Results from the second experiment showed that the particle size of emulsions that contained LBG was not different from those emulsions that contained the other hydrocolloids. Particles size distributions of emulsions containing hydrocolloids with 3% and 5% protein are presented in Fig 31 and 32. Emulsions containing LBG had an increase in the size of the peak representing particles with small diameters.

Viscosity

The use of hydrocolloids and protein concentration significantly ($p=0.000$) affected the viscosity of the emulsions (Table 26). Emulsions containing 5% protein had higher viscosity than emulsions that contained 3% protein. Emulsions that contained a hydrocolloid had a higher viscosity than the control (1.5 cP) (Table 27, Fig. 33). Xanthan gum and ι-CGN produced the highest viscosities with overall means of 4.83 and 3.01 cP, respectively. No differences in the viscosity of emulsions were found between κ-CGN and LBG where these emulsions had mean viscosities of 2.04 and 2.16 cP, respectively. Differences in viscosity are expected since the gums are structurally different. κ-CGN and ι-CGN viscosities are affected by the cations present in the system. κ-CGN selects for potassium ions to stabilize junction zones whereas iota selects for calcium (Imeson 2000). No minerals were added in the system. However, the WPI powder used contained 0.50% calcium and 0.42% potassium.

Table 26. Probability values for the effect of protein concentration and hydrocolloids determined by ANOVA.

Model	Dvs (μm)	Surface Area (cm^2/ml)	Fat layer (mm)	Viscosity (cP)
Protein	.368	.368	.002	.000
Hydrocolloids	.003	.001	.000	.000
Prot x Hydro	.975	.872	.448	.000
R^2	.459	.522	.613	.992

Table 27. Effect of protein concentration and hydrocolloids type on physical properties of whey protein stabilized emulsions.

	Dvs[1] (μm)	Surface Area[1] (cm^2/ml)	Fat layer[1] (mm)	Viscosity[1] (cP)
Protein (%)				
3 %	0.956a	63554a	1.95a	2.52a
5 %	0.928a	65421a	1.61b	2.92b
Hydrocolloids				
Control	0.949ab	63387a	1.75ab	1.53a
ι-CGN	0.981b	61458a	1.27b	3.01b
κ-CGN	1.003b	60181a	1.62ab	2.04c
LBG	0.811a	74510b	1.81a	2.16c
XG	0.966b	62901a	2.46c	4.83d

[1]Means within columns for each variable followed by the same letter are not significantly different (α=0.05).

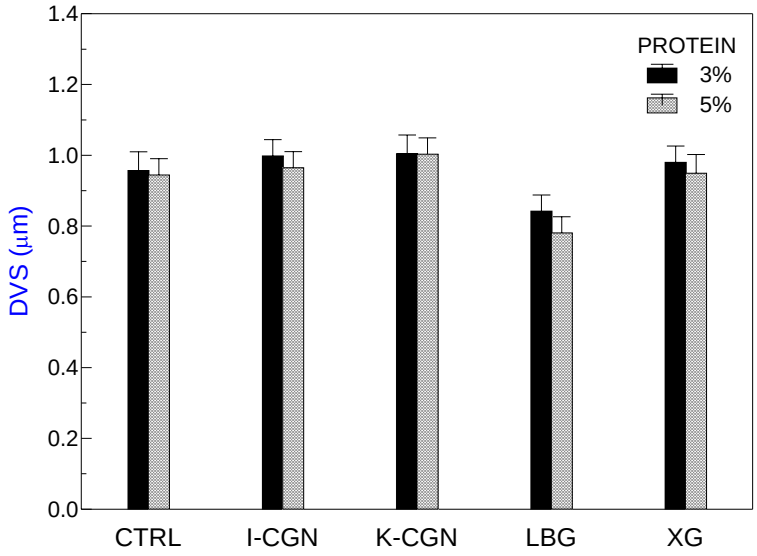


Fig. 30. Effect of different hydrocolloids and protein content on dvs.

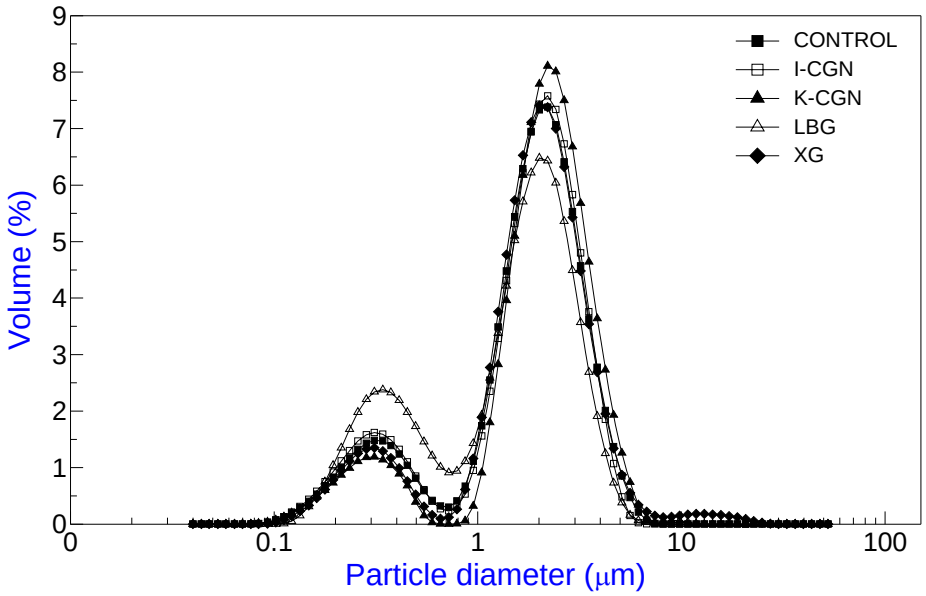


Fig. 31. Particle size distribution of emulsions containing hydrocolloids and 3% protein.

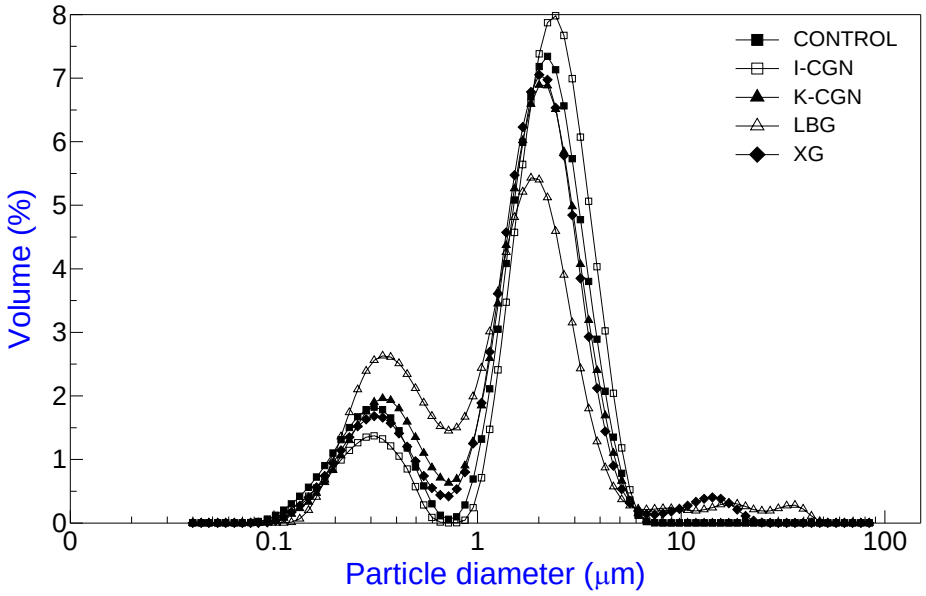


Fig. 32. Particle size distribution of emulsions containing hydrocolloids and 5% protein.

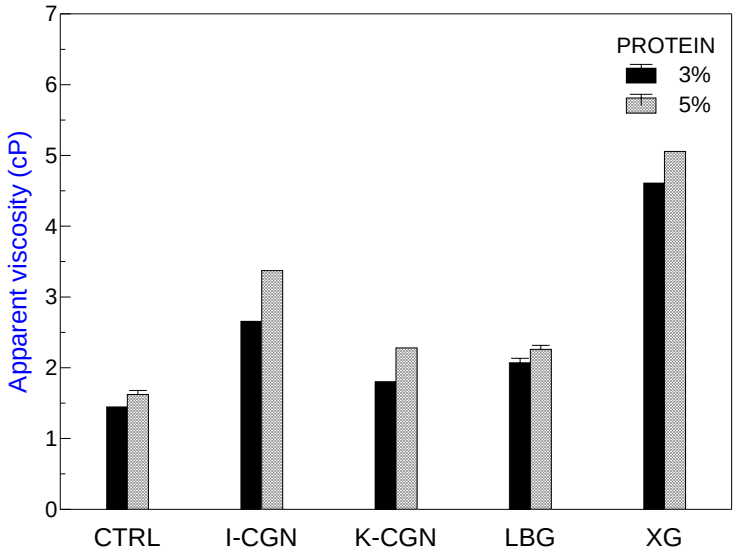


Fig. 33. Effect of hydrocolloids and protein on viscosity.

Creaming stability

The presence of hydrocolloids in the emulsions caused the viscosity in the emulsions to increase so an increase in emulsion stability was expected. However, the emulsion that contained xanthan gum and had the highest viscosity had the lowest emulsion stability (Fig. 34 and Table 27). Xantham gum might have caused flocculation through the depletion flocculation mechanism and clusters of fat globules can promote creaming. Xanthan gum has been reported to cause thermodynamic incompatibility in whey protein solutions (Bryant and McClements 2000) and depletion flocculation in whey protein emulsions (Euston and others 2002). Xanthan molecules have a large hydrodynamic radius and they tend to be excluded from the gap between two approaching droplets. The region between the droplets becomes depleted of xanthan and rich in solvent compared to the bulk phase resulting in an osmotic flow of water from the region between two droplets into the bulk phase which promotes flocculation of the droplets (Euston and others 2002).

The highest creaming stability was found with emulsions containing ι-CGN. Excluding XG, ι-CGN had the highest viscosity, which might explain why it created a more stable emulsion compared to hydrocolloids.

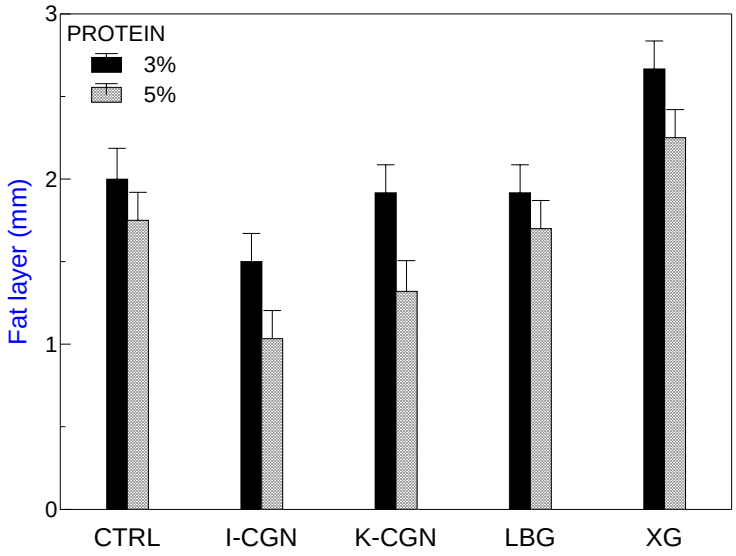


Fig. 34. Effect of hydrocolloids and protein on the fat layer.

Effect of heat treatment

The emulsions that contained hydrocolloids and 3 or 5 % protein formed gels after the heat treatment (table 28). The control made with a protein concentration of 3% protein had some aggregation but did not form a gel. It is evident that the presence of hydrocolloids in the emulsions caused this emulsions to be less heat stable than the control. Heat stability was measured by visual observation of aggregates and gel formation so mechanisms that might have caused the instability cannot be inferred. Euston and others (2002) studied the kinetics of droplet aggregation in heated whey protein-stabilized emulsions which contained polysaccharides (20% soya oil and 1% protein). Xanthan and carrageenan gums increased the heat aggregation rate constant in the heated systems. The mechanism suggested for this observation was depletion flocculation in emulsions containing xanthan gum. It was reasoned that if droplets are closer together at the time of heating, the likelihood of aggregation would be increased.

Bryant and McClements 2000 reported a thermodynamic incompatibility between xanthan gum and WPI with the incompatibility worse if the whey proteins were denatured. They suggested that heat denatured WPI forms aggregates with a molar mass that is much greater than that of the individual whey protein molecules, consequently, the free energy term associated with the entropy of mixing that favors the random distribution of molecules through the sytem is reduced in the heat denatured system. This causes thermodynamic incompatibility to be more pronounced in a solution containing aggregated proteins than individual proteins.

Zhang and Foegeding (2003) using turbidity measurements studied the behavior of ß-lg with different polysaccharides upon heating at 80°C. λ-CGN increased stability while κ-CGN decrease stability and ι-CGN at low concentrations, < 0.075% in 3% protein solutions, increased stability. When ß-lg concentration was 6 and 9%, adding ι-CGN increased the aggregation of ß-lg. Capron and others 1999 and Croguennoc and others 2001 reported that the presence of κ-CGN in solutions of heat denatured ß-lg does not influence the rate of protein consumption to form heat aggregates nor the structure of the aggregates. However, it increases the aggregation rate and at higher κ-CGN concentrations it induced phase separation and lead to the formation of protein-rich spherical microdomains.

No references were found regarding LBG and whey protein but the observations of this experiment made it clear that heat aggregation was more pronounced in this sample than in the control. LBG is a non ionic hydrocolloid and creaming stability data does not suggest that it promoted depletion flocculation. It seems that thermodynamic instability increased locally the concentration of protein in the system and promoted heat aggregation of the proteins during the heat process. However, hydrocolloids can also protect whey proteins from heat denaturation. Zhang and others (2004) and Zhang and Foegeding (2003) reported that dextran sulfate and λ-CGN can inhibit heat denaturation and aggregation of ß-lg. The mechanism by which dextran sulfate and λ-carrageenan enhance the thermal stability of ß-lg seems to be associated with changes in the tertiary structure. Changes in the secondary structure appear to be unaffected. They studied the

heat denaturation of ß-lg using DSC and near-UV circular dichroism. These studies showed that the denaturation temperature was about 4.6 °C higher in the presence of dextran sulfate, as compared with ß-lg alone, whereas in the presence of λ-CGN the difference in denaturation temperature was about 1.2 °C. Euston and others 2002 showed that propylene glycol alginate which may form an electrostatic complex with whey proteins did not affect the heat stability. These experiments were conducted at temperatures lower than sterilization temperatures. The ability of those hydrocolloids to perform well in sterilized whey protein beverages needs to be evaluated.

Table 28. Heat stability of whey protein emulsions with hydrocolloids.

	3 % Protein	5% Protein
Control	X	G
ι-CGN	G	G
κ-CGN	G	G
LBG	G	G
XG	G	G

X some aggregates, G gel formation

5.4 Part 4: Storage stability

Storage time affected the physical properties of the retort processed products (Table 29). Apparent viscosity (AV), Creaming index (CI), Particle size index (PI), mean particle size in the top (MPS-T) and bottom (MPS-B) of the emulsions changed during storage. pH was the only parameter that did not change during storage (Table 29). The effect of storage time on the mean of the physical properties parameters is presented in table 30. The effect the interactions: storage time and homogenization pressure, storage time and concentration of lecithin and storage time and concentration of phosphate on the different physical properties of the emulsions are shown in Fig. 35, 36 and 37, respectively.

Apparent viscosity

Apparent viscosity decreased during storage (*p*-value = .000, Table 29). Mean apparent viscosity decreased from 5.5 cP at day 1 to 3.6 cP at day 14, but there was no difference between the viscosity of the samples after 14 and 28 days of storage (Table 30). This decreased viscosity of approximately 2.5 cP after 28 days of storage might not be of practical importance even though it was statistically significant. The decreased viscosity indicated changes to the structure of the emulsions during storage, which might have been caused by the formation of reversible interactions during the heat processing. The mean viscosity of the unheated emulsions was 4.14 cP but after retort processing the emulsions had a mean viscosity increased of 5.5 cP. Heat treatment can cause proteins to form intermolecular interactions such as hydrophobic and sulfhydryl reactions (Sawyer 2003) that would increase the viscosity of the system. However, these intermolecular interactions might have been reversible during storage to cause the viscosity to decrease to 3.4 cP after 28 days of storage. Unfolded whey proteins might have changed to a conformation that provided less resistance to shearing. Some conformational changes could be attributed to renaturation of α-lactalbumin. α-lactalbumin has been reported to renature following heat denaturation (Fox 2003). If this reaction is slow more renatured α-lactalbumin might be present after 14 days of storage compared to day 1. The structure of whey proteins adsorbed at the interface of fat

droplets has been reported to change with time. Whey proteins form secondary layers at the surface of the oil droplets due to slow polymerization of the protein in the adsorbed layer via the interchange between sulfhydryl and disulphide groups (Monahan and others 1993, Dickinson and Matsumura 1991, Dickinson 1997). However, these findings were reported in emulsions that were not subjected to heat treatment, but formation of secondary layers can be expected in heat treated emulsions.

The interactions between main factors and storage time on apparent viscosity were not significant (Table 29 and Fig 35a, 36a and 37a), which indicated that the change in viscosity of the retorted products during storage was independent of the pressure used to homogenize the emulsions or the concentrations of phosphates and lecithin.

pH

The pH of the samples did not change during storage (*p*–value =.702, Table 29). It was possible that the pH could have changed during storage if the polyphosphates were subjected to hydrolysis; however, no changes in pH were found which indicated that degradation of the phosphorous compounds did not happen during the storage period.

Table 29 Probability values for the effect of homogenization pressure, lecithin, phosphate and storage time on physical properties of retorted emulsions.

	pH	AV[1]	CI[2]	PI[3]	MPS-T[4]	MPS-B[5]
Intercept	.000	.000	.000	.000	.000	.000
HP	.017	.163	.003	.038	.000	.000
Lecithin	.586	.263	.835	.699	.661	.492
Phosphates	.002	.002	.209	.007	.806	.018
Day	.702	.000	.000	.000	.000	.000
Day*HP	.495	.111	.003	.302	.230	.002
Day*Lec	.706	.092	.889	.272	.524	.596
Day*Phosp	.006	.719	.425	.021	.479	.114

[1] Apparent viscosity (cP)
[2] Creaming index
[3] Particle size index
[4] Mean particle size of top layer
[5] Mean particle size of bottom layer

Table 30. Effect of storage time on physical properties of retorted emulsions.

Storage time	AV[1]	pH	CI[2]	PI[3]	MPS-T[4]	MPS-B[5]
Day 1	5.52a	6.984a	1.046a	1.060a	0.412a	0.383a
Day 14	3.61b	6.973a	1.540b	1.828b	0.523b	0.287b
Day 28	3.47b	6.986a	2.245c	2.941c	0.623c	0.225c

[1] Apparent viscosity (cP)
[2] Creaming index
[3] Particle size index
[4] Mean particle size of top layer
[5] Mean particle size of bottom layer

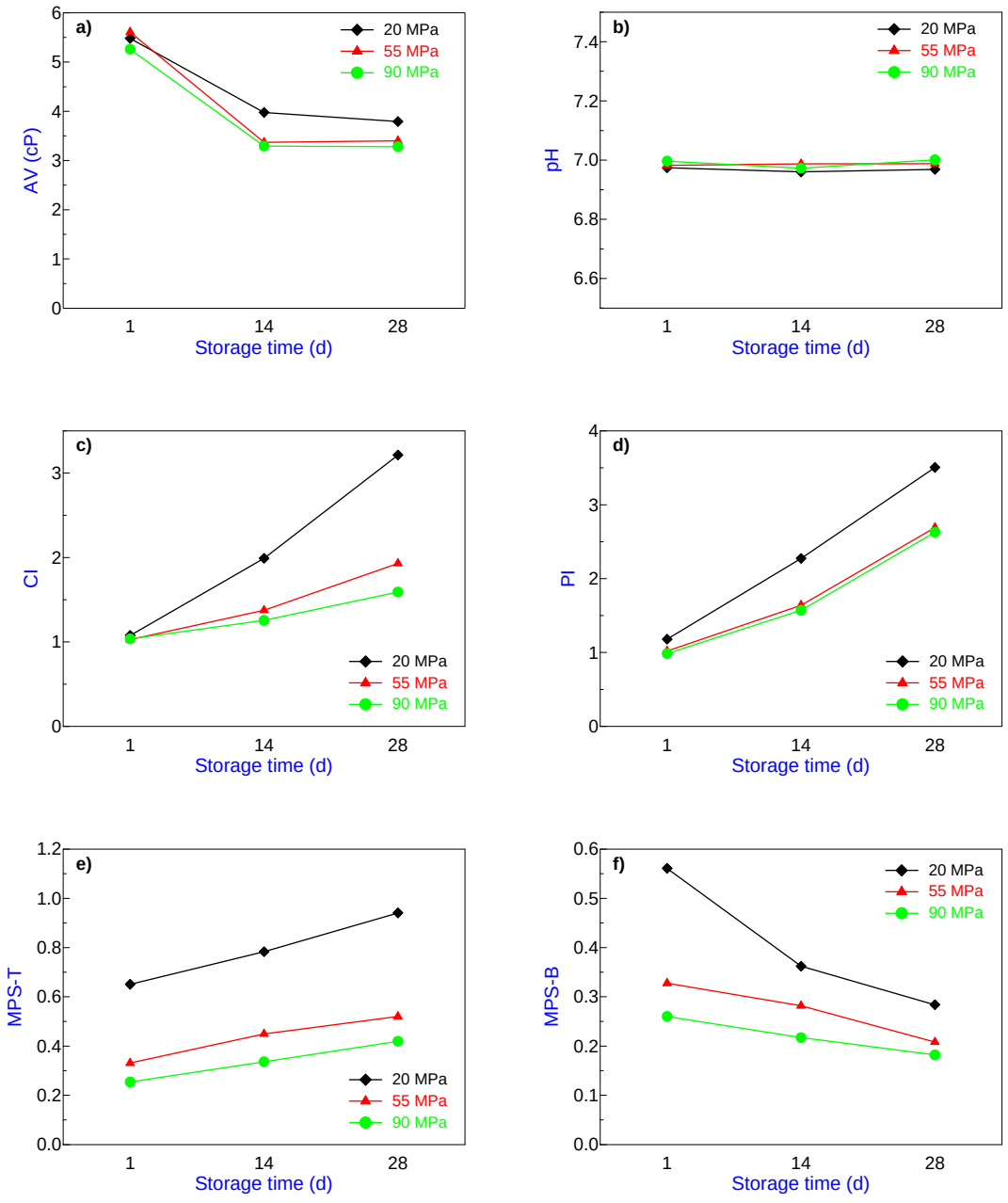


Fig. 35. Effect of storage time and homogenization pressure on a) AV, b) pH, c) CI, d)PI, e) MPS-T and f) MPS-B.

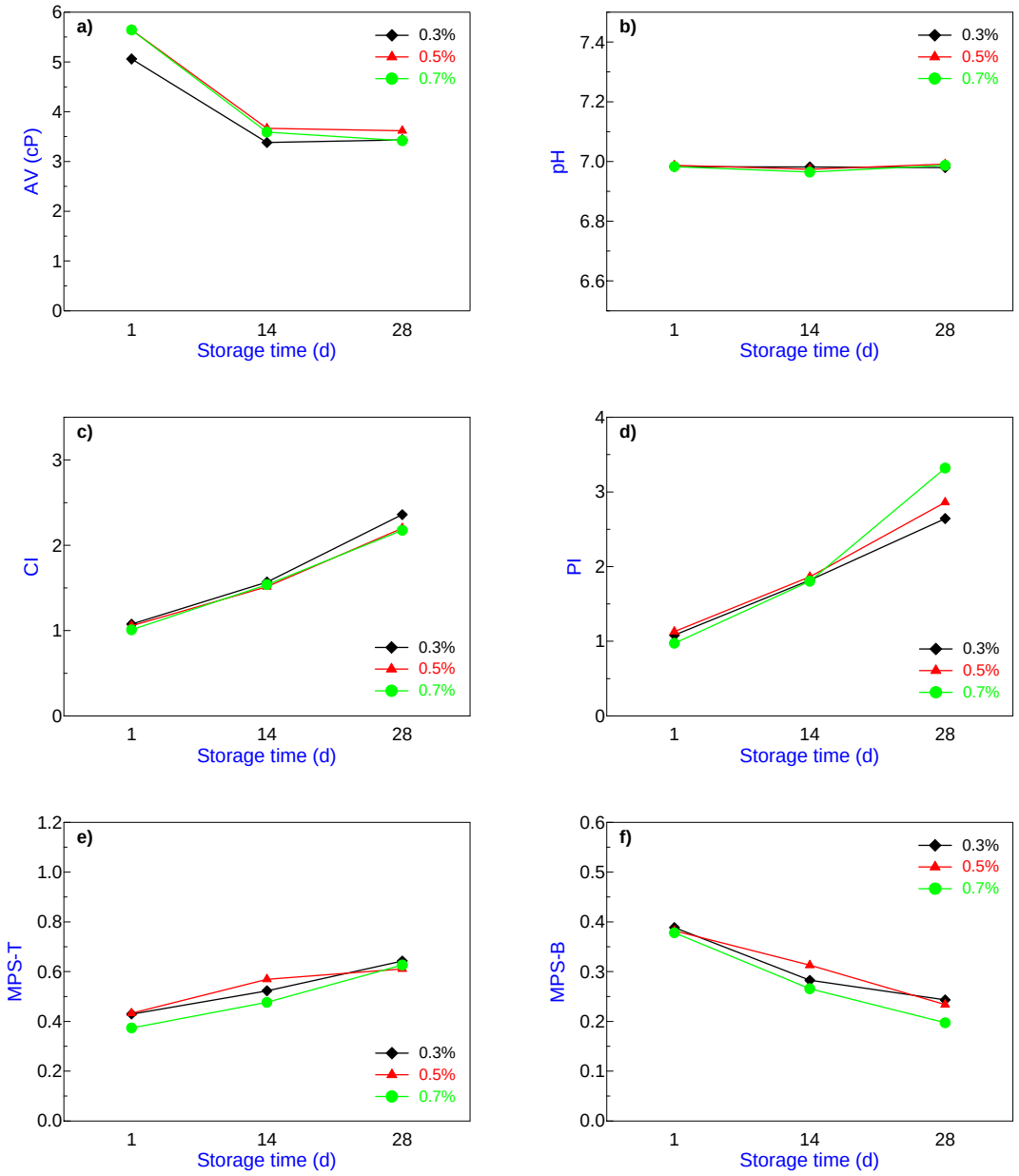


Fig. 36. Effect of storage time and concentration of lecithin on a) AV, b) pH, c) CI, d)PI, e) MPS-T and f) MPS-B.

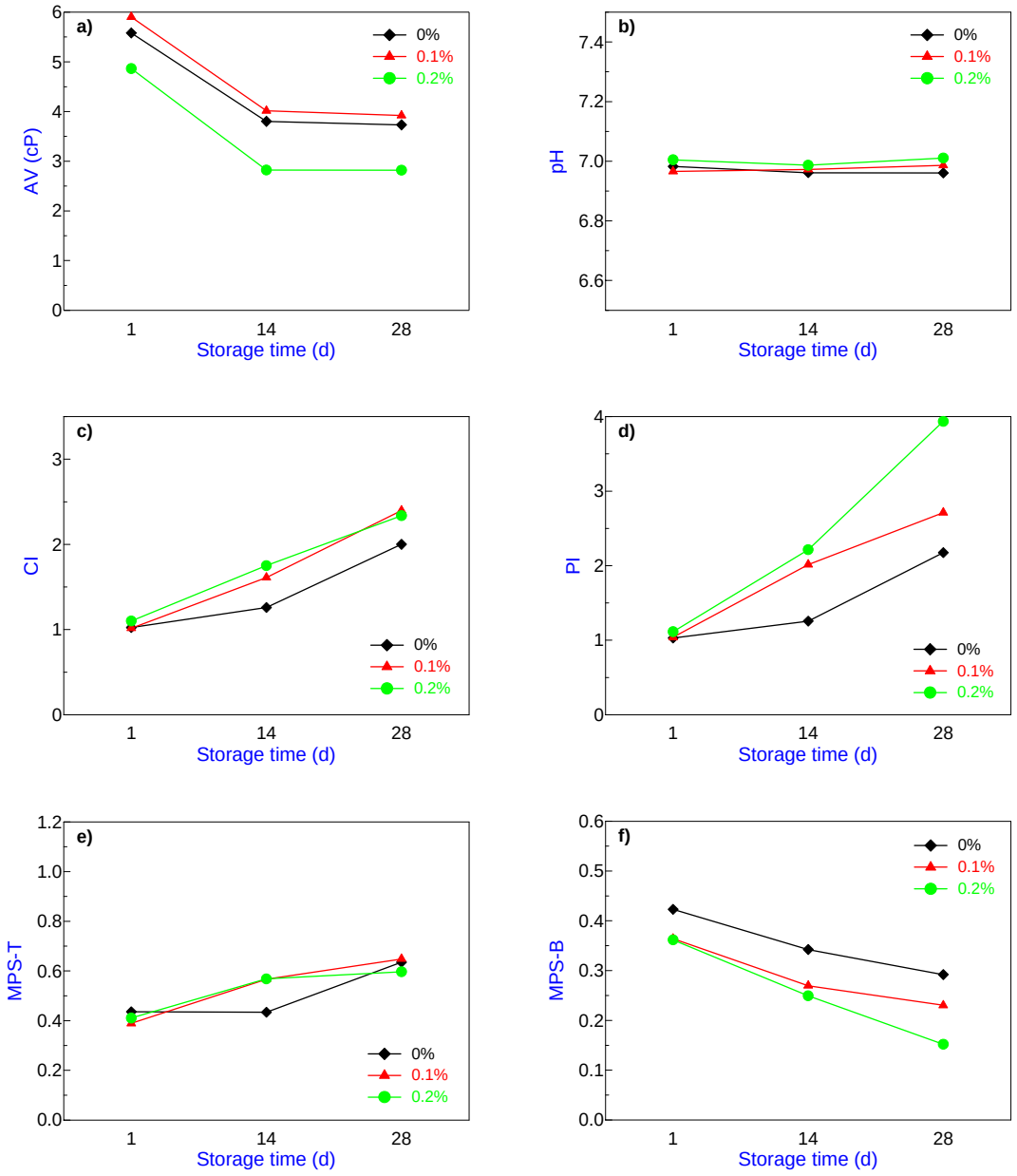


Fig. 37. Effect of storage time and concentration of phosphates on a) AV, b) pH, c) CI, d)PI, e) MPS-T and f) MPS-B.

Creaming index

Creaming index changed during storage (*p*-value=.000, Table 29). The creaming index which is a measure of emulsion stability increased over time from an average of 1.04 at day 1 to 2.24 at day 28 (Table 30). Values greater than 1 indicate creaming of the emulsion so optimum values for the creaming index are values close to 1. An increase in the value of the creaming index is likely to occur during storage since the fat droplets which are the least dense particles on the emulsions will move towards the top of the container according to Stoke's law (eq. 3).

Interactions between the storage time and homogenization pressure, storage time and lecithin, and storage time and phosphate are plotted in Fig 35c, 36c and 37c, respectively. The interaction between homogenization pressure and storage time was significant (p-value=0.003, Table 29) and it is evident from Fig 35c that the change of creaming index with time strongly depends on the pressure of homogenization. The change in the creaming index during storage in emulsions homogenized at 90 and 55 MPa was not as pronounced as in emulsions homogenized at 20 MPa. The creaming index of emulsions homogenized at 90 MPa after 28 days of storage is approximately 1.5 whereas the creaming index for emulsions homogenized at 20 MPa is 3.2. The decreased in the creaming index caused by the use of higher homogenization pressure is explained by the smaller sizes of fat droplets in emulsions homogenized at higher pressures (Fig 35e and 35f). Small fat droplets will move to the top of the container more slowly therefore the concentration of fat on the top of the container is lower in emulsions homogenized at high homogenization pressures. Also small fat globules are denser than big fat globules since the ratio of protein adsorbed at the interface of fat droplets to fat volume is higher in small droplets compared to big droplets, making small fat droplets denser than big fat globules. Denser fat droplets will move at a lower speed to the top of the container producing emulsions with low creaming index values.

The interaction between storage and phosphates was insignificant, but the trend of the data shows that samples with phosphates were more susceptible to creaming (Fig. 37c).

Particle size index

The particle size index changed during storage time (*p*-value=.000, Table 29). Mean particle size index values for the emulsions increased from 1.06 at day 1 to 2.94 at day 28 (Table 30), which indicated that these emulsions had poor stability against separation after 28 days of storage. The significant interaction between storage time and phosphates (p-value=0.021, Table 29 and Fig. 37d) indicated that change in the particle size index over time is dependent on the concentration of phosphates in the emulsions. Emulsions without phosphates had a smaller change in the particle size index during storage compared to emulsions containing phosphates (Fig. 37d). Data shows that the increase in particle size index in emulsions containing phosphates is due to an increase in smaller fat droplets at the bottom of the container compared to emulsions without phosphates (Fig. 37f). Mean particle size of particles from the top layer of the can was not affected by the use of phosphates (Fig. 37e). Mean particle size of particles at the bottom decreased over time and this change seems to be more pronounced in emulsions containing 0.2% phosphates. Phosphates might have caused changes in the continuous phase of the emulsions permitting fat droplets to move upwards faster. The decrease in the viscosity of emulsions containing phosphates (Fig. 37a) confirmed the effect of phosphates in the continuous phase. Phosphates prevented heat aggregation as reported in part 3. Phosphates have been reported to prevent heat denaturation of β-lactoglobulin by altering the structure of water (Kella and Kinsella 1988a). Particles suspended in emulsions containing phosphates are expected to segregate at different rates compared to emulsions without phosphates, causing the change of PI values during storage to be different between emulsions with and without phosphates.

Emulsions homogenized either at 55 or 90 MPa had a smaller increase in the PI during storage than emulsions homogenized at 20 MPa (Fig. 35d), however the interaction between homogenization pressure and storage was not significant (p-value=.302, Table 29). The average PI after 28 days of storage for emulsions homogenized at 20 MPa was 3.50 whereas for emulsions homogenized at 55 and 90 MPa the PI was 2.69 and 2.62, respectively. Particle size index is less likely to increase during storage in emulsions homogenized at 90 MPa because the fat droplets contained in emulsions homogenized at the higher homogenization pressure are smaller and denser and will move upward more slowly compared to bigger fat droplets contained in the emulsion homogenized at 20 MPa.

In conclusion, the physical properties of retorted emulsions changed during storage. Changes in the creaming index and the particle size index during storage strongly depended on the pressure applied during homogenization and phosphate concentration, but not on the concentration of lecithin studied. The use of high homogenization pressure produced emulsions with the best storage stability indicated by the smaller change of the particle size index and creaming index during storage compared to emulsions homogenized at lower homogenization pressures. Emulsions with phosphates produced emulsions with poor storage stability indicated by the large change in the particle size index and creaming index during storage.

5.5 Part 5: Optimization of parameters

Response surface analysis was conducted on the physical parameters of the emulsions after 28 days of storage. The primary objective of the surface response analysis was to determine the conditions that provide values of the particle size index and the creaming index close to one, which relates to emulsions with the best stability. PI values > 1 might be associated with creaming whereas values < 1 can be associated with sedimentation. Regression formulas for the physical properties of the emulsions (Table 31) and prediction profilers (Fig. 38) were obtained using JMP software.

Particle size index

The regression parameters obtained (Table 31) and prediction profilers (Fig. 38), provided clear evidence that when the homogenization pressure was increased the particle sized index decreased. In contrast an increased concentration of lecithin and phosphate caused the particle size index to increase. Particle size index strongly depended on the concentration of phosphates (Table 31).

Particle size index surface plots as a function phosphate and lecithin at HP of 20, 55 and 90 MPa are shown in Fig. 39. The particle size index under the experimental conditions was always greater than 1 and indicated that the size of the particles in the top layer of the emulsions was always larger than the size of the fat droplets in the bottom of the container. Emulsions homogenized at 20 MPa had PI values higher than 3 at all concentrations of phosphate and lecithin studied (Fig. 39a) and exhibited poor stability during storage. An increase in phosphate concentration from 0 to 0.2% caused the PI values to increase to 4. Changes in the concentration of lecithin did not have an effect on the particle size index. Emulsions homogenized at 55 MPa had PI values of approximately 1.8 when the concentration of phosphates and lecithin were at their minimum concentrations. The PI values increased when the concentration of phosphates and lecithin was increased.

The effect of the concentration of phosphate was more important than the effect of lecithin concentration on changes of the PI. The lowest PI values in emulsions homogenized at 90 MPa were about 1.4 when concentration of lecithin was less than 0.5% and concentration of phosphates was less than 0.05%. Increase in the PI values caused by an increase in the concentration of phosphates is attributed to the decrease in the MPS-B. Phosphates caused a slight but insignificant decrease in the diameter of the fat droplets in the top (*p*-value 0.685, table 31and Fig. 38) and significant decreases in the particle size in the bottom of the container (*p*-value 0.001, table 31 and Fig 38) which causes the PI ratio to be increased. This decrease in the MPS-B might be explained by the decrease in viscosity associated with the use of phosphates (*p*-value 0.042 Table 31, Fig. 38 and 41). Fat droplets moving upwards are opposed by the viscosity of the continuous phase and emulsions containing phosphates had lower viscosity than emulsions without phosphates allowing droplets to move faster to the top of the container. Emulsions with the best values of particle size index were obtained when emulsions were homogenized at 90 MPa and had the lowest concentrations of phosphates and lecithin.

Table 31. Regression formulas for particle size index, creaming index, apparent viscosity, pH, mean particle size top and bottom.

term	PI	*p*-value	CI	*p*-value
Intercept	1.343	0.241	3.374	0.003
HP	-0.013	0.187	-0.023	0.007
LEC	1.694	0.291	-0.459	0.638
PHOS	8.800	0.028	1.679	0.402
HP*HP	0.000	0.414	0.000	0.140
HP*LEC	0.023	0.714	0.045	0.275
LEC*LEC	3.031	0.786	1.666	0.815
HP*PHOS	0.064	0.603	-0.016	0.837
LEC*PHOS	5.250	0.806	3.537	0.796
PHOSP*PHOS	34.375	0.453	-22.896	0.435

term	AV	*p*-value	pH	*p*-value
Intercept	4.835	0.000	6.933	0.000
HP	-0.008	0.001	0.000	0.001
LEC	0.356	0.355	0.019	0.147
PHOS	-5.062	0.000	0.250	0.000
HP*HP	0.000	0.243	0.000	0.407
HP*LEC	-0.001	0.898	-0.001	0.060
LEC*LEC	-7.109	0.018	-0.198	0.057
HP*PHOS	0.057	0.073	0.000	1.000
LEC*PHOS	-9.562	0.087	0.000	1.000
PHOSP*PHOS	-69.437	0.000	-0.042	0.902

term	MPS-T	*p*-value	MPS-B	*p*-value
Intercept	0.975	0.002	0.430	0.000
HP	-0.007	0.002	-0.001	0.003
LEC	-0.040	0.865	-0.115	0.056
PHOS	-0.193	0.685	-0.699	0.001
HP*HP	0.000	0.059	0.000	0.130
HP*LEC	0.012	0.248	0.002	0.368
LEC*LEC	0.601	0.730	-0.336	0.370
HP*PHOS	0.006	0.756	0.002	0.558
LEC*PHOS	-1.688	0.617	0.000	1.000
PHOSP*PHOS	-3.246	0.643	-0.821	0.575

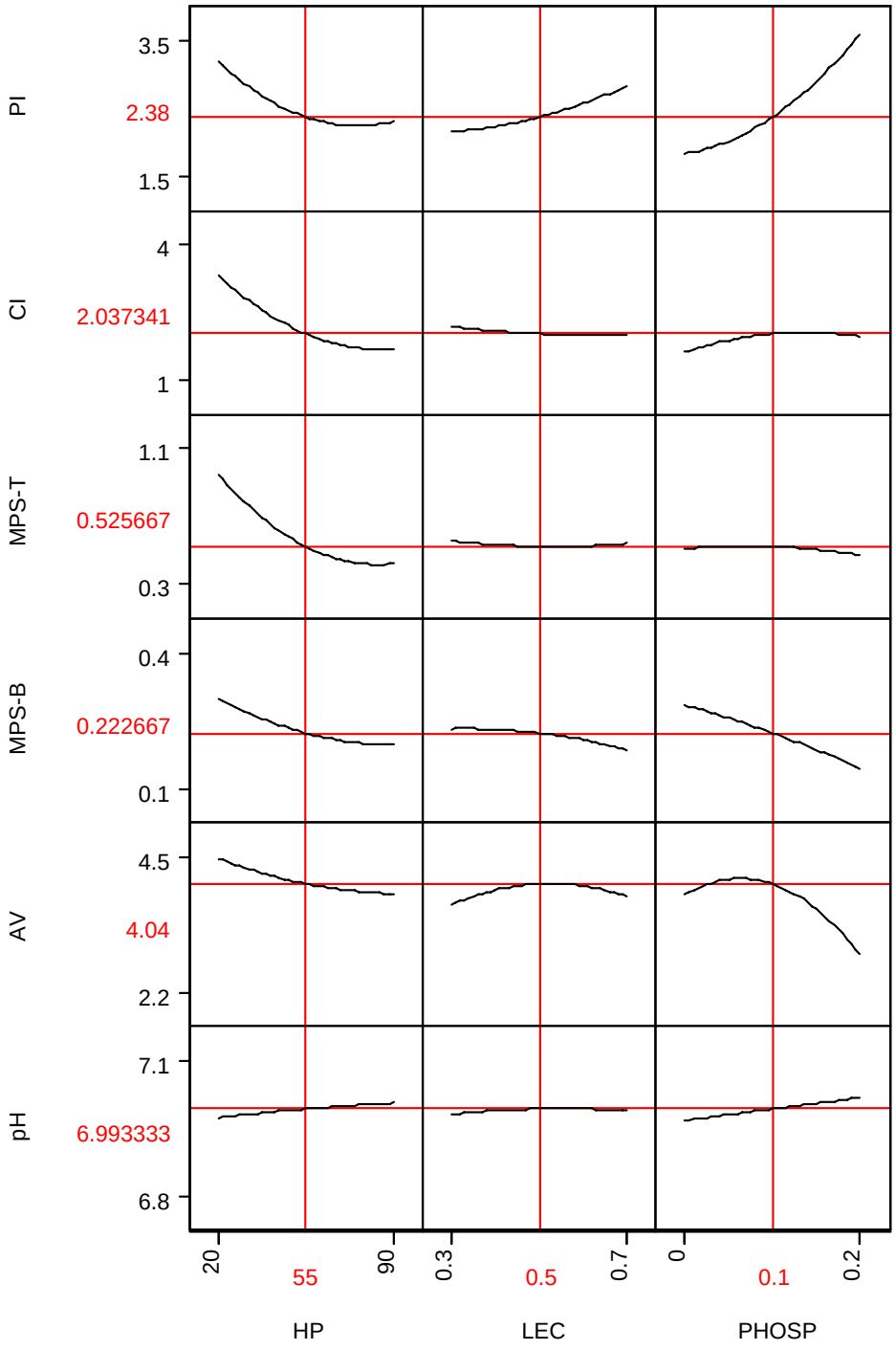


Fig. 38. Prediction profilers of physical properties of emulsions.

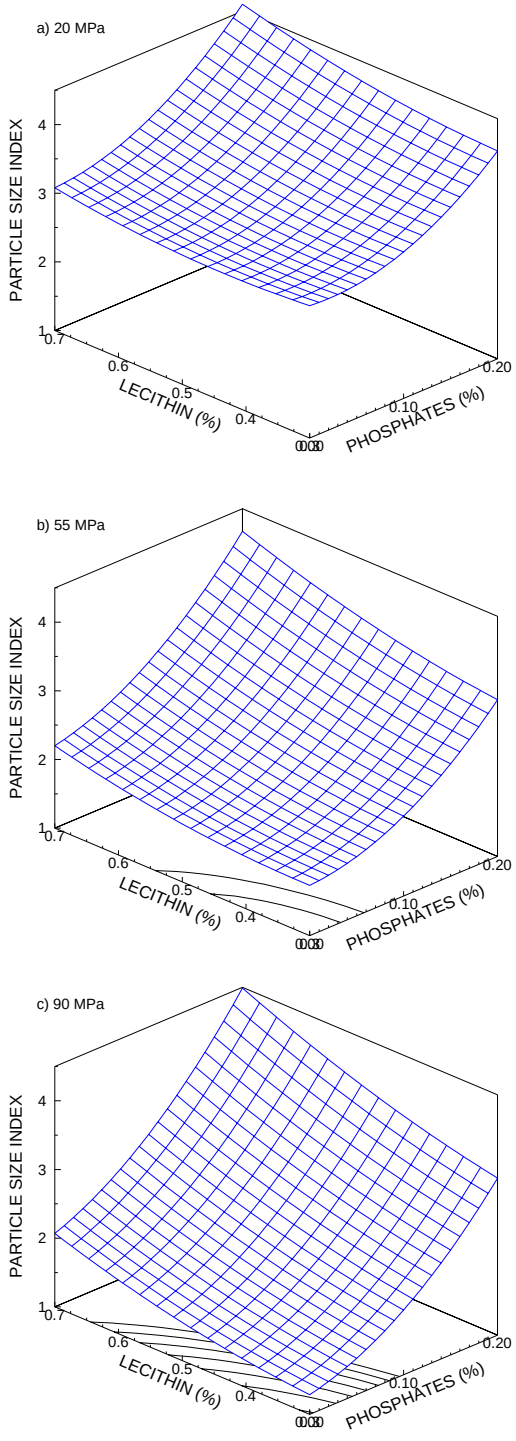


Fig. 39. Effect lecithin and phosphates concentration on PI at a) 20 b) 55 and c) 90MPa.

Creaming index

Regression parameters (Table 31) and prediction profilers (Fig. 38) illustrate that creaming index values were decreased by an increase in the homogenization pressure (*p*-value 0.007) and the values were slightly affected by the concentration of lecithin and phosphates however not significantly (*p*-values 0.638 and 0.402, respectively).

Creaming index surface plots as a function of phosphate and lecithin concentration at homogenization pressures of 20, 55 and 90 MPa are shown in Fig. 40. Optimal creaming values of 1 were not achieved under the experimental conditions used. Creaming index values were always > 1 which indicated creaming had occurred in the emulsions. However CI values close to 1 were obtained when the homogenization pressure was increased to 90 MPa (Fig. 40c) at the low concentration of phosphates and lecithin. The increase in creaming stability in emulsions homogenized at high homogenization pressure is explained by the smaller particles and more dense fat droplets in these emulsions. Creaming can be predicted by Stoke's law (Eq. 3) which explains why smaller and more dense particles cream at a slower rate. There was a trend that showed that increasing the concentration of phosphates caused a slight increase in the creaming index while increasing the concentration of lecithin decreased the creaming index. However the effect of lecithin and phosphates was not significant.

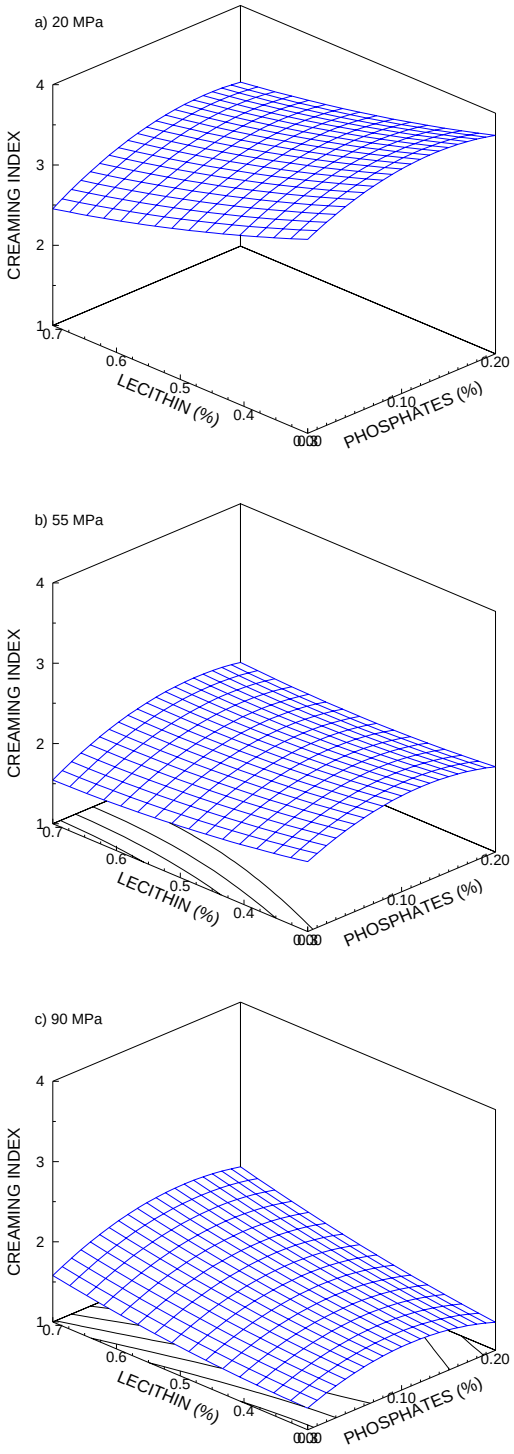


Fig. 40. Effect lecithin and phosphates concentration on CI at a) 20 b) 55 and c) 90MPa.

Apparent viscosity

Regression parameters (Table 31) and prediction profilers (Fig 38) illustrate that apparent viscosity was affected by the pressure of homogenization (*p*-value=0.000) and concentration of phosphates (*p*-value=0.0001). However, changes in the concentration of lecithin did not affect the viscosity (*p*-value= 0.355).

Surface plots show that viscosity decreased as the pressure of homogenization was increased from 20 to 90 MPa (Fig.41). Viscosity decreased from 4.4 cP in emulsions homogenized at 20 MPa, to 3.87 cP in emulsions homogenized at 90 MPa when the concentration of lecithin and phosphates was maintained at 0.5% and 0.10%, respectively. An increase in viscosity was expected in emulsions homogenized at the higher homogenization pressure since the viscosity of diluted emulsions (μ) follows Einstein equation (Berg 2004, Becher 2001): $\mu = \mu_0(1 + k\phi)$, where μ_o is the viscosity of the medium, φ is the volume fraction of the particles , and k is a constant equal to 2.5. Higher homogenization pressures might cause a slight increase in the volume fraction since more protein is adsorbed at the interfaces increasing the effective volume fraction. However, the viscosity decreased with the increase in homogenization pressure. The viscosity of the aqueous phase might have been decreased by increased homogenization pressure and this can be explained by the higher depletion of protein from the aqueous phase in the emulsions homogenized at 90 MPa compared to emulsions homogenized at 20 MPa. Fewer protein interactions in the aqueous phase during the heat treatment are expected with lower concentrations of protein in the aqueous phase and subsequently a decrease in the apparent viscosity. The same effect of homogenization pressure on viscosity was confirmed in emulsions after 1 and 14 days of storage. However viscosity was not affected by the homogenization pressure in emulsions before the heat treatment which supports the hypothesis that changes in the viscosity are due to interactions of proteins during the heat treatment and emulsions homogenized at higher homogenization pressures had fewer interactions which produced emulsions with lower viscosity.

Phosphates decreased the apparent viscosity of the emulsions (*p*-value=0.0001). Emulsions without phosphates but with 0.5% lecithin and homogenized at 20 MPa had an apparent viscosity of 4.48 cP. An increase in the concentration of phosphates to 0.1% did not change the viscosity. However, increasing the concentration of phosphates to 0.2% decreased the apparent viscosity to 3.0 cP (Fig 41a). Emulsions homogenized at 55 and 90 MPa (Fig 41b and c) exhibited similar behaviour. The decreased in viscosity caused by the change in the concentration of phosphates might be attributed to the protective effect of polyphosphates on the denaturation of whey proteins. Whey proteins exposed to heat treatment undergo inter- and intramolecular interactions; especially, hydrophobic and disulphide interactions which can cause an increase in the viscosity of solutions. However, when phosphates are present in the formulations, whey proteins might be protected against heat denaturation. Kosmotropic salts including phosphates and citrates have been reported to increase the stability of ß-lactoglobulin against heat by delaying the dissociation of ß-lactoglobulin dimmers which is the initial step in the heat denaturation of ß-lactoglobulin, This protective effect on the protein is probably caused by changes in the structure of water (Kella and Kinsella 1988a).

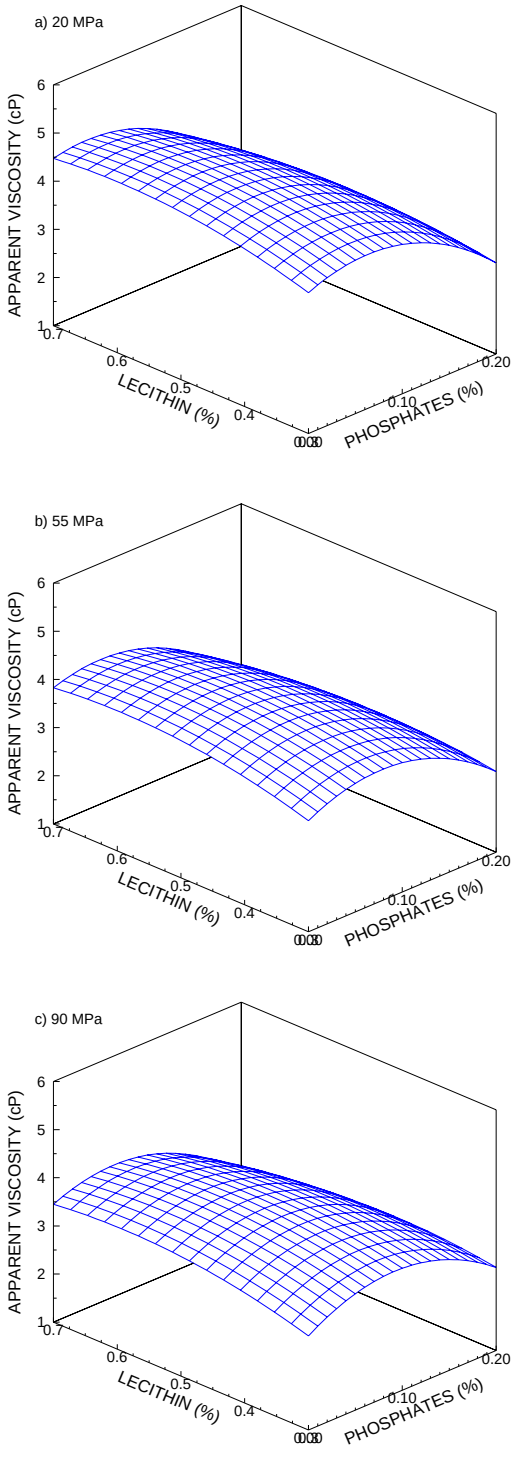


Fig. 41.Effect lecithin and phosphates concentration on AV at a) 20 b) 55 and c) 90MPa.

The optimal homogenization pressure, concentration of lecithin and phosphates that provide simultaneously the best particle size index and creaming index (values of 1), were determined by analyzing the maximum desirability functions of the prediction profilers using JMP software. The results are shown in Fig. 42. Emulsions, formulated with 0.3% lecithin and 0% phosphates and homogenized at 90 MPa were the best with a creaming index of 1.26 and particle size index of 1.28. This emulsion had an apparent viscosity of 2.95.

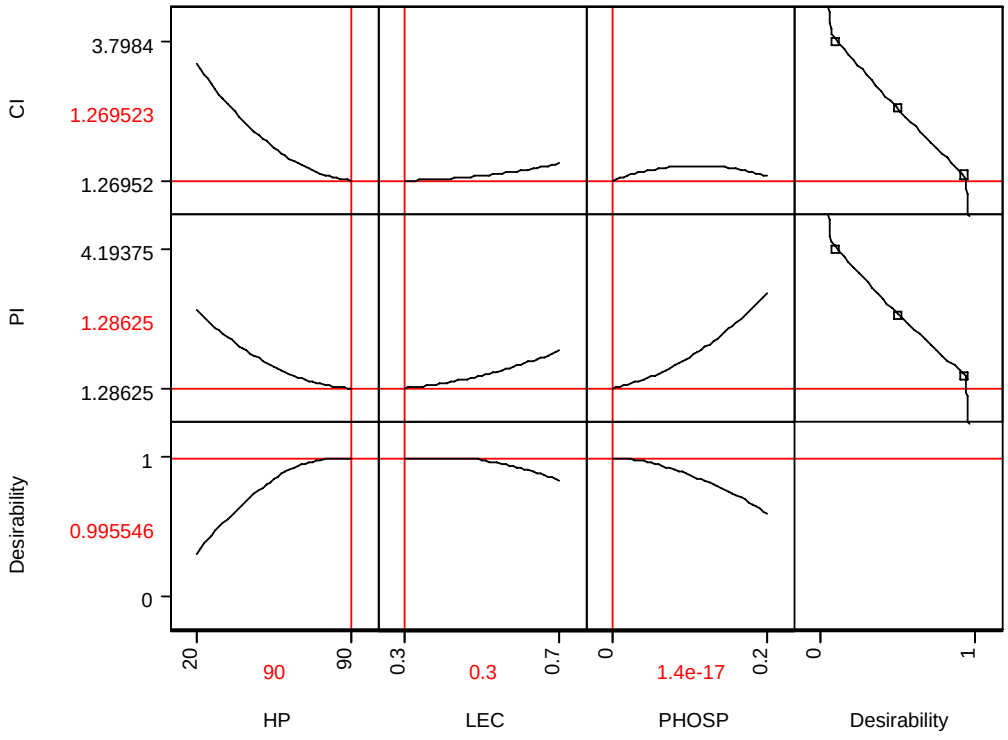


Fig. 42. Optimization of emulsions as a function of homogenization pressure and concentration of lecithin and phosphate.

CHAPTER VI
CONCLUSIONS

The composition of the emulsions and pressure applied during homogenization modified the dvs and the surface area of the fat droplets in experiments in part I. An increase in homogenization pressure from 20 to 90 MPa caused a 57% decrease in the dvs with an increase of surface area of 135%. The dvs decreased 14% when the protein concentration was increased from 1 to 9% and the surface area increased 27%. The concentration of fat did not affect those parameters. It is evident that homogenization pressure had about 5x impact on dvs and surface area compared to protein concentration. Whey protein beverages containing high protein concentration were unstable when heated. Emulsions with >1% protein formed aggregates during the heat treatment. Homogenization pressure did not affect the heat stability. Different additives were used to improve the heat stability of sterilized whey protein beverages. Phospholipids and polyphosphates were effective for improving the heat stability in whey protein solutions and emulsions. Regular, hydrolyzed and acetylated lecithin improved the heat stability of emulsions containing up to 5% protein. Milkfat influenced the mechanism by which lecithins improve the heat stability of emulsions because the protective effect of lecithins against heat aggregation was not evident when beverages did not contain fat. Modified lecithins, which had a higher HLB value compared to regular lecithin, provided more protection against heat denaturation. The mechanism by which lecithin improved the heat stability is associated with protein-lecithin interactions mainly at the interface of the fat droplets. Emulsions containing lecithin were more stable against creaming than emulsion without lecithin and acetylated lecithin created the most stable emulsion. Improvement in the emulsion stability seems to be associated with a more negative charge at the interface of the fat droplets in emulsions containing lecithin increasing electrostatic repulsions.

Polyphosphates improved the heat stability of whey protein beverages. However, polyphosphates with a degree of polymerization of approximately 4 units were the only

effective polyphosphates for an increased heat stability of beverages with 5% protein. With the use of polyphosphates it is possible to create retorted clear protein beverages containing up to 5% protein. Polyphosphates did not affect the emulsions stability or particle size distribution compared to emulsions without phosphates. The mechanism by which polyphosphates improved the heat stability might be associated with changes in the structure of water that prevented aggregation of whey proteins.

Hydrocolloids usually required in formulations to improve the long term stability of emulsions and provide desired texture and viscosity were found to have a detrimental effect on the heat stability of whey protein emulsions, most probably through thermodynamic incompatibility that locally increased the concentration of proteins and promoted heat aggregation.

Homogenization pressure and concentration of acetylated lecithin and polyphosphates were chosen to study the storage stability of whey protein beverages containing 5% protein and 3% fat. The creaming index and particle size index which are indicators of the stability of the emulsions changed over 28 d of storage and indicated creaming of the emulsions. The use of homogenization pressures of 55 and 90 MPa compared to 20 MPa reduced the magnitude of the change of the particle size index and creaming index during storage. Inclusion of polyphosphates in the formulations enhanced the change in particle size index and creaming index and reduced the stability of the emulsions.

Optimization of parameters showed that emulsions formulated with 0.3% lecithin without polyphosphates and homogenized at 90 MPa had the best stability after 28 d of storage. However, the creaming index and particle size index were > 1 which indicated creaming of the emulsions. The use of additives that can increase viscosity without causing detrimental effects on the heat stability of the emulsions is needed to improve creaming stability of whey protein retorted beverages. Another approach is the combination of proteins such as caseinate and whey proteins, caseins have disordered molecular structures and can protrude longer distances from the fat droplet interface increasing steric repulsion and improving emulsion stability.

REFERENCES

Al-Mashikh SA, Nakai S.1987. Reduction of ß-lactoglobulin content of cheese whey by polyphosphate precipitation. Journal of Food Science 52(5):1237-40.

Angelo AJ.1989. A brief introduction to food emulsions and emulsifiers. In: Charalambous G, Doxastakis G, editors. Food emulsifiers chemistry, technology, functional properties and applications.1st ed. New York: Elsevier. p 1-7.

A.O.A.C . 1984. Official methods of analysis. 14th ed. Washington D.C.:Association of Official Analytical Chemists.

Becher P. 2001. Emulsions: theory and practice. Washington D.C.: American Chemical Society Oxford University Press. 513 p.

Berg JC. 2004. An introduction to surfaces, colloids and nanoscience. Notes on Surface and Colloid Science short course; 2004 July 12-16; Department of Chemical Engineering. Seattle W.A.: University of Washington.

Bergenståhl B.1997. Physicochemical aspects of an emulsifier functionality. In: Hasenhuettl GL, Hartel RW, editors. Food emulsifiers and their applications. New York: Chapman Hall. p.147-172.

Bhatia S. 2001. Effect of protein source and high homogenization pressure on the storage stability of retort processed dilute whey protein emulsions. MS thesis, Texas A&M University, College Station.

Brown EM, Caroll RJ, Pfeffer PE, Sampugna J. 1983. Complex formation in sonicated mixtures of ß-lactoglobulin and phosphatidylcholine. Lipids 18:111-118.

Bryant CM, McClements DJ. 2000. Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. Food Hydrocolloids 14:383-390.

Cano-Ruiz, M. E. 1996. Effect of high pressure homogenization on the milk fat globule membrane and its relation with the emulsion stability of milk. MS thesis, Texas A&M University, College Station.

Capron I, Nicolai T, Durand D. 1999. Heat induced aggregation and gelation of ß-lactoglobulin in the presence of κ-carrageenan. Food Hydrocolloids 13:1-5.

Claesson PM, Blomberg E, Poptoshev E. 2004. Surface forces and emulsion stability. In: Friberg SE, Larson K, Sjöblom J. Food emulsions. 4th ed. New York: Marcel Dekker. p. 267-97.

Courthaudon JL, Dickinson E, Christie WW. 1991. Competitive adsorption of lecithin and ß-casein in oil-in-water emulsions. Journal of Agricultural and Food Chemistry 39:1365-68.

Croguennoc P, Nicolai T, Durand D. 2001. Phase separation and association of globular protein aggregates in the presence of polysaccharides: 2. Heated mixtures of native ß-lactoglobulin and κ-carrageenan. Langmuir 17:4380-85.

Dannenberg F, Kessler H. 1988. Reaction kinetics of the denaturation of whey protein in milk. Journal of Food Science 53:258-63.

Danner T, Schubert H. 2001. Coalescence processes in emulsions. In: Dickinson E, Miller R, editors. Food colloids fundamentals of formulation. Cambridge, UK: Royal Society of Chemistry. p.116-24.

Demetriades K, Coupland J, McClements D. 1997. Physical properties of whey protein stabilized emulsions as related to pH and NaCl. J Food Science 62:342-347.

Dickinson E. 1997. Properties of emulsions stabilized with milk proteins: overview of some recent developments. J. Dairy Sci. 80:2607-19.

Dickinson E, Euston SR.1991. Stability of food emulsions containing both protein and polysaccharide. In: Dickinson E, editor. Food polymers, gels, and colloids, Special publication no. 82. London: Royal Society of Chemisty. pp. 132-46.

Dickinson E, Hong S. 1994. Surface coverage of β-Lactoglobulin at the oil-water interface: influence of protein heat treatment and various emulsifiers. Journal of Agricultural and Food Chemistry 42:1602-06.

Dickinson E, Matsumura Y. 1991. Time-dependent polymerization of β-Lactoglobulin through disulphide bonds at the oil-water interface in emulsions. International Journal of Biology and Macromolecules 13:26-30.

Dickinson E and Matsumura Y. 1994. Proteins at liquid interfaces: role of the molten globule state. Colloids Surfaces B. 3:1-17.

Doig SD, Diks RM. 2003. Toolbox for modification of the lecithin headgroup. European Journal of Lipid Science and Technology 105(7):368-76.

Dybing ST, Parsons JG, Martin JH, Spurgeon KR. 1982. Effect of sodium hexametaphosphate on cottage cheese yields. Journal of Dairy Science. 65: 544-51.

Euston S, Finnigan S, Hirst R. 2000. Aggregation kinetics of heated whey protein-stabilized emulsions. Food Hydrocolloids 14:155-161.

Euston S, Finnigan S, Hirst R. 2001. Aggregation kinetics of heated whey protein-stabilized emulsions: effect of low-molecular weight emulsifiers. Food Hydrocolloids 15:253-262.

Euston S, Finnigan S and Hirst R. 2002. Kinetics of droplet aggregation in heated whey protein- stabilized emulsions: effect of polysaccharides. Food hydrocolloids 16:499-505.

Fox P. 2003. Milk proteins: general and historical aspect. In: Fox P, McSweeney P, editors. Advanced dairy chemistry volume 1: proteins, 3rd ed. New York: Kluwer Academic/Plenum publishers. p 319-86.

Friberg SE. 1997. Emulsion stability. In: Friberg SE, Larsson K, editors. Food emulsions. 3rd ed. Ed. New York: Marcel Dekker, Inc. p 1-55.

Garti N. 2002. Food emulsifiers: structure-reactivity relationships, design and applications. In: Marangoni AG, Narine SS, editors. Physical properties of lipids.1st ed. New York: Marcel Dekker. p.265-386.

Giroux HJ, Britten M. 2004. Heat treatment of whey proteins in the presence of anionic surfactants. Food Hydrocolloids 18:685-92.

Glandfor K, Thomasow J. 1977. Behavior of polyphosphates during production and storage of long-life milk products. Z. Lebensm. Unters. Forsch. 25:709-13

Hardy E, Sweetsur A, West I and Muir D. 1985. Heat stability of concentrated milk: enhancement of initial heat stability by incorporation of food grade lecithin. Journal of Food Technology 20:97-105

Harwalkar VR, Vreeman HJ.1978. Effect of added phosphate and storage on changes in ultra-high temperature short-time sterilized concentrated skim-milk. Netherlands Milk Dairy Journal. 32: 204-10

Havea P, Singh H, Creamer L. 2001. Characterization of heat-induced aggregates of β-lactoglobulin, α-lactalbumin and bovine serum albumin in a whey protein concentrate environment. Journal of Dairy Research 68:483-497.

Hossen M. 2005. Enzyme catalyzed synthesis of structured phospholipids with conjugated linoleic acid and plant sterol. Ph. D. Dissertation, Texas A&M University, College Station, TX.

Huffman L, Harper J. 1999. Symposium: Marketing dairy value through technology. Maximizing the value of milk through separation technologies. Journal of Dairy Science 82:2238-44.

Hunt J, Dalgleish D. 1994. Adsorption behavior of whey protein isolate and caseinate in soya oil-in-water emulsions. Food Hydrocolloids 8(2):175-187.

Hunt J, Dalgleish D. 1995. Heat stability of oil-in-water emulsions containing milk proteins: effect of ionic strength and pH. Journal of Food Science 60:1120-23, 1131.

Imeson AP. 2000. Carrageenan. In Phillips GO, Williams PA, editors. Handbook of hydrocolloids. Cambridge, UK: Woodhead Publishing Limited. p 87-102.

Israelachvili J. 2003. Intermolecular and surface forces. 2nd ed. San Diego, California: Academic press. 450 p.

Jones MN. 1975. A theoretical approach to the binding of amphipathic molecules to globular proteins. Biochemical Journal, Cellular Aspects 151:109-14.

Ju Z and Kilara A 1998. Textural properties of cold-set gels induced from heat-denatured whey protein isolates. Journal of Food Science 63: 288-92.

Kella NK and Kinsella JE. 1988a. Structural stability of ß-lactoglobulin in the presence of kosmotropic salts. A kinetic and thermodynamic study. International Journal of Peptide and Protein Research 32:396-405.

Kella NK and Kinsella JE. 1988b. Enhanced thermodynamic stability of ß-lactoglobulin at low pH. A possible mechanism. Biochemistry Journal 255:113-118.

Kepacka M, Pijanowski E. 1970. Effect of polyphosphates on the colloidal stability of evaporated batch-sterilized milk. Milchwissenschaft 25:709-12

Khan S, Ghatak PK, Bandyopadhyay AK. 1992. Heat stability of buffalo milk as affected by phosphate salts addition. Indian Journal of Dairy Science 45(9):461-464.

Kocak HR, Zadow JG. 1986. Storage problems in UHT milk: age gelation. Food Technol Aust. 38(1):148-52

Kontopidis G, Holt C, Sawyer L. 2004. Invited review: β-Lactoglobulin: binding properties, structure, and function. Journal of Dairy Science 87:785-796.

Kristensen A, Nylander T, Paulsson M, Carlsson A. 1997. Calorimetric studies of interactions between ß-lactoglobulin and phospholipids in solutions. International Dairy Journal 7:87-92.

Krog NJ, Sparsø FV. 2004. Food emulsifiers: their chemical and physical properties. In: Friberg SE, Larson K, Sjöblom J. Food emulsions. 4th ed. New York: Marcel Dekker. p 45-91.

Kulaev IS, Vagabov VM, Kulakovskaya TV. 2004 The Biochemistry of inorganic polyphosphates. 2nd edition. Hoboken, New Jersey: John Wiley and Sons. 294 p.

Lin Chu-An 2002 Effects of homogenization pressure and selected additives on the physico-chemical properties of retort sterilized dairy beverages. Ph. D. Dissertation. Texas A&M University, College Station.

McCrae CH. 1999. Heat stability of milk emulsions: phospholipid-protein interactions. International Dairy Journal 9:227-231.

Melachouris N. 1972. Interaction of ß-lactoglobulin with polyphosphates. Journal of Agricultural and Food Chemistry. 20: 798- 802

Melachouris N, inventor; Stauffer Chemical Company, assignee.1977 Aug 23. Process for the recovery of whey protein having improved solution clarity using polyphosphates. U.S. patent 4,043,990.

Molins RA.1991. Phosphates in food. 1st ed. Boca Raton, Florida: CRC Press. 261 p.

Monahan F, McClements D, German J. 1996. Disulphide-mediated polymerization reactions and physical properties of heated WPI-stabilized emulsions. Journal of Food Science 61:504-509.

.

Monahan F, McClements D, Kinsella J. 1993. Polymerization of whey proteins in whey protein-stabilized emulsions. Journal of Agricultural and Food Chemistry 41:1826-29.

Montilla A and Calvo MM. 1997. Goat's milk stability during heat treatment: effect of pH and phosphates. Journal of Agricultural and Food Chemistry. 45(3):931-94.

Perez-Hernandez G. 2001. Effect of milk fat globule membranes on emulsion stability of recombined sterilized milk. MS thesis. Texas A&M University, College Station.

Perez-Hernandez G, Hossen M, Richter R, Hernandez E. 2004. Effect of different phospholipids on emulsion stability. In: 95th AOCS Annual meeting book of abstracts; 2004 May 9-12; Cincinnati, Ohio.

Perez-Hernandez G, Suhareli R, Richter R. 2002. Effect of phosphates and protein concentration on physical properties of sterilized whey protein emulsions: In IFT Annual meeting book of abstracts; 2002 June 22-26; Anaheim, California. Chicago: Institute of Food Technologists.

Sarker DK, Wilde PJ and Clark DC. 1995. Competitive adsorption of L-α-lysophosphatidylcholine/ß-lactoglobulin mixtures at the interfaces of foams and foam lamellae. Colloids and Surfaces B: Biointerfaces 3:349-56.

Sawyer L 2003. β-lactoglobulin. In: Fox PF, McSweeney PL, editors. Advanced dairy chemistry volume 1: Proteins, 3rd ed. New York: Kluwer Academic/Plenum Publishers. P 319-86.

Sher A, Mallangi CR, Jacobson MR, Vadehra DV, Wedral RE, inventors; 2002. Shelf-stable calcium fortified milk and dairy products. U.S. patent 6,455,082B1.

Singh H, Havea P. 2003. Thermal denaturation, aggregation and gelation of whey proteins. In: Fox PF, McSweeney PL, editors. Advanced dairy chemistry volume 1: Proteins, 3rd ed. New York: Kluwer Academic/Plenum Publishers. p 1261-82.

Singh H, Sharma R, Tokley R. 1992. Influence of incorporation of soya lecithin into skim milk powder on the heat stability of recombined evaporated milk. The Australian Journal of Dairy Technology 47:33-7.

Smoluchowski M. 1917. Verusch einer Mathematischen Theorie der Koagulationskinetic Kolloider Lösungen, Z. Phys. Chem. 92, 129-168

Sünder A, Scherze I, Muschiolik G. 2001. Physico-chemical characteristics of oil-in-water emulsions based on whey protein-phospholipid mixtures. Colloids and Surfaces B: Biointerfaces 21:75-85.

Suhareli R. 2003. Effect of homogenization pressure and whey protein source on rheological properties of heat-set whey protein emulsion gels. MS thesis. Texas A&M University, College Station.

Sworn G. 2000. Xanthan gum. In: Phillips GO, Williams PA, editors. Handbook of hydrocolloids. Cambridge, UK: Woodhead Publishing Limited. p 103-115.

Syrbe A, Bauer WJ and Klostermeyer H. 1998. Polymer science concepts in dairy systems – an overview of milk protein and food hydrocolloid interaction. International Dairy Journal 8:179-193.

Szuhaj B and Sipos E. 1989. Food emulsifiers from the soybean. In: Charalambous G, Doxastakis G, editors. Food emulsifiers chemistry, technology, functional properties and applications. New York: Elsevier. p 113-86.

Van Aken GA. 2004. Coalescence mechanisms in protein-stabilized emulsions. In Friberg SE, Larson K, Sjöblom J, editors. Food emulsions. 4th ed. New York: Marcel Dekker. p. 299-325

Vanapalli SA, Coupland JN. 2004. Orthokinetic stability of food emulsions. In Friberg SE, Larson K, Sjöblom J, editors. Food emulsions. 4th ed. New York: Marcel Dekker. p. 327-351

Wabel CT. 1998. Influence of lecithin on structure and stability of parenteral fat emulsions. Ph.D. Dissertation. Friedrich-Alexander University of Erlangen-Nuremberg**.** Erlangen, Germany. Available from: dissertationen@ccc.chemie.uni-erlangen.de.

Walstra P, Geurts TJ, Noomen A, Jellema A, van Boekel MAJS. 1999. Dairy technology priniciples of milk properties and processes. New York: Marcel Dekker. 727p.

Wielinga WC. 2000. Galactomannans. In: Phillips GO and Williams PA, editors. Handbook of hydrocolloids. Cambridge, UK: Woodhead Publishing Limited. p 137-54.

Wingard WH. 1972. Protein separation from whey using a solubilized phosphate, U.S. Patent 3,637,643. 1972 Jan 25.

Wit J. 1998. Nutritional and functional characteristics of whey proteins in food products. Journal of Dairy Science 81:597-608.

Wüstneck R, Moser B, Karageorgiewa G, Mushchiolik G. 2000. Proceedings of Eighth Food Colloids Meeting; 2000 Apr 2-5; Potsdam, Germany. pp. T40.

Yamauchi K, Shimizu M, Kamiya T. 1980. Emulsifying properties of whey protein. Journal of food science 45:1237-1242.

Zernel M. 2003. Mechanisms of dairy modulation of adiposity. Journal of Nutrition. 133:252S-256S.

Zhang G, Foegeding EA. 2003. Heat-induced phase behavior of ß-lactoglobulin/polysaccharide mixtures. Food Hydrocolloids 17:785-792.

Zhang G, Foegeding EA, Hardin CC. 2004. Effect of sulfated polysaccharides on heat-induced structural changes in ß-lactoglobulin. Journal of Agricultural and Food Chemistry 52:3975-3981.

VITA

Name: Gabriela Perez-Hernandez

Permanent address: Herradero 117
Salamanca, Gto. 36710
Mexico

Parents: Manuel Perez Corral and Gloria Hernandez de Perez

Date of Birth: September 25, 1973

Place of Birth: Salamanca, Guanajuato, Mexico

Marital Status: Single

Educational Background:

Bachelor of Science, University of Guanajuato, Mexico
August 1996
Master of Science, Texas A&M University, College Station, Texas, US
August 1998

Major Field: Food Science and Technology