

Enhancing the Functionality of Delactosed Whey by Enzymatic Hydrolysis Using Response Surface Methodology Approach



December 2012

By: Baraem (Pam) Ismail University of Minnesota

Edem Folly, graduate student

Funded in part by:

Midwest Dairy Association

Abstract

The use of Delactosed whey (DLW) in various food applications is limited. Amplifying the functionality of the protein fraction (26-33%) of DLW can lead to expanded use of such product. Given that limited enzymatic hydrolysis can improve the functional properties of whey proteins in general, it is hypothesized that subjecting DLW to enzymatic proteolysis will lead to enhanced functionality. In order to achieve the desired functionality enhancement, hydrolysis parameters and conditions need to be optimized. Response surface methodology approach will be pursued to identify the best conditions that will lead to a directed enhancement in several functional properties. Functionality enhancement that will be suitable for a processed meat application will be targeted.

Background Information

Significance of Whey Powders/Ingredients

World-wide an increasing amount of whey is available due to increasing cheese production. For decades scientists have been working on identifying beneficial components in whey that contribute to functional and nutritional enhancement in various food products, with an ultimate intention of benefiting the dairy industry/farmers through increasing the value of the whey. Whey is now recognized as a "value-added" ingredient because of its highly functional and nutritional properties. Technological advances have enabled researchers to enhance the functionality and utilization of whey by producing more concentrated and specialized forms. With several different whey ingredients available to product developers, new applications continue to evolve.

The most valuable component of whey is its protein, which delivers both enhanced functionality and nutrition to many formulations. Technological advances including efficient and cost-effective unit processes for concentration, fractionation, dehydration as well as structure-function modifications (Smithers, 2008) resulted in the development of several whey protein ingredients including whey protein concentrates (WPC, 35-80% protein), whey protein isolates (WPI, 90-96% protein) and whey protein hydrolysates (WPH). Other whey ingredients available in the market include whey powder, demineralized whey permeate powder and delactosed whey.

Delactosed whey (DLW) is produced from sweet whey by concentrating, crystallizing and separating of lactose, followed by spray drying. The functional and nutritive properties of the protein fractions in the DLW are mostly retained. However, given its relatively low protein content (26-33%), the use of DLW in various food applications is limited. The main use of DLW is animal feed. *However, amplifying the functionality of the protein fraction of DLW can lead to expanded food applications, and thus increased market value.*

Significance of Whey Protein

Whey protein is considered a high quality protein since it is rich in all essential amino acids, and has a biological value that is 15 % greater than the former benchmark egg protein. Additionally, whey protein has unique physicochemical characteristics, which allow it to have versatile functionality, such as foaming, gelation and emulsification (Foegeding et al., 2002). As compared to other food proteins, whey protein has great solubility under acidic conditions (Pelegrine & Gasparetto, 2005), which makes it the protein-of-choice for protein-fortified acidic beverages. Because of their nutritional and functional properties, researchers have intensified efforts to expand the utilization of whey proteins as food ingredients. Today, whey protein ingredients are used to replace other proteins or to improve the functional properties of many food products such as baby food, luncheon meat, ice cream, baked goods, and beverages (Spellman et al., 2005).

Technology and cost reduction incentives have resulted in significant increase in whey protein utilization in various food applications. However, a considerable percentage of whey protein products remain unused (Balagtas et al., 2003). Additionally, processing imparts some challenges pertaining mainly to whey protein thermal stability (Zhang et al., 2004), solubility (Sava et al., 2005), aggregation during storage (Zhou et al., 2008 a & b), gelation (Foegeding et al., 2002), foaming and emulsion thermal stability (Ye & Sing, 2006), sensory quality (Beecher et al., 2008), and delivery of optimal nutritional and biological quality (Maier et al., 2006). Therefore, research remains in progress to address some of the mentioned challenges and devise new ingredient opportunities via enhanced concentration and processing technologies and various protein modification approaches.

Approaches to modify protein functional and biological properties are numerous encompassing chemical, physical and enzymatic treatments that cause changes in protein conformation and physicochemical characteristics. The most recently researched protein modification include Maillard conjugation (Jiménez-Castaño et al., 2007; Guzey & McClements, 2007; Zhu et al., 2008); physical modification utilizing high pressure treatment (Bouaouina et al. 2006; Belloque et al., 2007; Chicón et al., 2009), ultrasound treatment (Jambark et al., 2008), and supercritical carbon dioxide treatment (Zhong & Jin, 2007); and enzymatic modification, which is extensively researched (Cheison et al., 2007; Creusot & Gruppen 2008; Pouliot et al., 2009; Spellman et al., 2009). <u>These researchers and many others have shown that limited enzymatic hydrolysis of whey proteins can result in markedly improved functionality and biological activities.</u>

Whey Protein Hydrolysate

Enzymatic production and use of functional and bioactive hydrolysates have gained prominence in the food industry due to the escalating consumer awareness and demand of healthy foods. Healthy foods are commonly referred to as functional foods that provide physiological benefits beyond their nutritional value. The global market size of functional foods is witnessing 8% increase per year and is estimated to reach the value of \$100 billion by 2012 (Smithers, 2008). Therefore, the food industry is facing the challenge of choosing healthier ingredients that are economical and can sustain or even improve texture and overall quality of the final product. Whey protein hydrolysate serves as an excellent choice for many food applications due to its enhanced functional and biological properties as compared to its native counterpart. Whey protein hydrolysates currently are produced using enzymatic hydrolysis rather than chemical hydrolysis. Chemical hydrolysis causes loss in some essential amino acids, such as tryptophan, and can result in a product with high amount of free amino acids, which can encumber the body's osmotic balance (Mahmoud, 1994). Enzymatic hydrolysis, on the other hand, is preferred due to the mild hydrolysis conditions employed that result in low free amino acid content, improved digestibility, and enhanced bioactivity; and also due to the availability of a wide range of enzymes that are considered safe (GRAS) and natural.

Upon hydrolysis of whey proteins, a number of biologically active peptides can be released (Meisel, 2005). Biologically active peptides gained prevalence due to promising positive effects on human health and prevention of chronic diseases. The biological peptides contribute to several health benefits, including but not limited to antihypertensive, antioxidant, anticancerous, opioid, antibacterial, and mineral binding activity (Ariyoshi, 1993; Schlimme & Meisel, 1995; Clare & Swaisgood, 2000). Additionally, whey proteins, having a globular structure, can resist enzymatic

digestion, therefore, hydrolysates provide an excellent protein source for infants with underdeveloped digestive tract (Zeiger et al., 1986) and for patients with digestive tract disorders, such as impaired luminal hydrolysis, reduced absorptive capacity and or specific gastric or heptic failure (Potter & Tomé, 2008). Moreover, WPH is a nutritious protein source for individuals with whey protein allergy (Guadix et al., 2006). Also, WPH is added to weight reduction/management drinks (Haque et al., 2010).

Several enzymes are currently used in industry and others are being researched for the production of WPH with tailored functionality and biological activity. The functional and biological properties of the WPH depend to a great extent on the type of enzyme used (specificity and selectivity), hydrolysis conditions employed (enzyme-to-substrate ratio, incubation temperature, pH and time), and the source of the protein. <u>Optimization of these conditions can lead to a directed functional enhancement.</u>

Whey Protein Hydrolysate Functionality

Whey proteins, in general, have excellent functional properties; however, in order to expand the whey protein market and compete with meat proteins that have excellent gelation property, and egg proteins that have excellent foaming and emulsification properties, as well as other functional protein ingredients, whey protein must be tailor-processed to have superior functionality. Processing and economic challenges and the growing demand for versatile food products that meet the requirement and expectations of the consumer, have engaged producers and academic researchers in a long-term commitment to enhance whey protein functionality.

Functionality of proteins can be improved via limited hydrolysis that impart changes in the molecular size, conformation, and strength of inter- and intramolecular interactions (Guan et al., 2007). Limited hydrolysis is generally defined by the degree of hydrolysis that ranges between 2-8%. With increased hydrolysis (>10% DH), flavor challenges can be encountered due to the production of bitter peptides. In many cases, limited hydrolysis leads to increased solubility due to the reduced molecular weight and the increased hydrophilicity resulting from the increase in free carboxyl and amine groups. Proteolysis of whey protein with various enzymes and under specific hydrolysis conditions has led to enhanced solubility over a wide range of pH (Jost & Monti, 1977; O'Keefe & Kelly, 1981; Perea et al., 1993; Mutilangi et al., 1996). Heat stability also was improved upon partial hydrolysis due to the loss of secondary structure, thus contributing to reduced structural changes upon heating (Foegeding et al., 2002). However, hydrolysis of whey protein in some cases caused aggregation and gelation, mostly due to the exposure of buried hydrophobic residues and/or release of specific peptides that promote peptide-peptide and peptide-protein aggregation (Creusot & Gurppen, 2007). Limited enzymatic hydrolysis also was found to be successful in improving interfacial properties of whey protein, both foaming and emulsification properties (Foegeding et al., 2002). Enzymatic modifications widened the use of whey proteins as functional food ingredients. However, the choice of enzyme and hydrolysis conditions is what determines the best application of the WPH produced (i.e. either for enhanced solubility, gelation or emulsification purposes).

A wide range of enzymes and enzymatic conditions have been tested for the production of WPH with enhanced functional and biological properties. However, industrial feasibility for many of the successful bench scale production of functionally enhanced WPH remains to be seen. This is mainly due to the great variability in the results obtained, which originates from the many variables that can influence the characteristics of the WPH produced, including specificity of the enzyme, source of the protein and enzymatic reaction parameters. *Fundamental understanding of the effect of these*

Amplifying the Functionality of the Protein Fraction of DLW

Given that limited hydrolysis can improve the functional properties of whey proteins in general, it is hypothesized that subjecting DLW to enzymatic proteolysis will lead to enhanced functionality. In order to achieve the desired functionality enhancement, hydrolysis parameters and conditions need to be optimized. Response surface methodology approach will be pursued to identify the best conditions that will lead to a directed enhancement in several functional properties, including water holding capacity, gelation, viscosity, emulsification and solubility. Functionality enhancement that will be suitable for a processed meat application will be targeted.

One of the main applications of whey protein ingredients is processed meat. Quality improvement, nutritional optimization and cost-effectiveness are key drivers in using whey protein ingredients in processed meats. The use of whey protein ingredients, mainly WPC and WPI, in processed meats (ground meats, emulsion products, coarse ground products, and whole muscle products) improve flavor, texture, emulsification, water binding, cook yield, and finished product functional performance. *Therefore, our overall objective is to enhance the functionality of DLW by enzymatic hydrolysis using response surface methodology (RSM) approach with a focus on processed meat application.*

Objectives

1) Optimize the functionality of DLW using enzymatic hydrolysis by either alcalase or papain following RSM approach.

2) Determine the functional properties and quality of beef patties fortified with selected DLW hydrolysates in comparison to DLW, WPI, and WPC 34.

Materials and Methods

Materials and Methods for Objective One:

Materials

Samples of DLW, WPC34 and WPI were kindly provided by Valley Queen (Milbank, SD, USA), PGP International (Woodland, CA, USA) and Main Street Ingredients (La Crosse, WI, USA), respectively. Bicinchoninic acid (BCA) protein assay kit (23235) was purchased from Pierce (Rockford, IL, USA). Food grade Alcalase 2.4 L and papain (65,600 USP/mg) were provided by Novozymes (Bagsvaerd, Denmark) and American Laboratories Incorporated (Omaha, NE, USA), respectively. Pre-stained broad-range molecular weight (MW) standard (161-0318), Laemmli sample buffer (161-0737), 10X Tris/glycine/SDS running buffer (161-0732), ammonium persulfate (161-0700), 40% Acrylamide/Bis solution, 37.5:1 (2.6% C) (161-0148), and N,N,N',N'-tetra-methyl-ethylenedimine (TEMED) (161-0800) were purchased from BioRad (Hercules, CA, USA). Sudan Red 7B and soybean oil were purchased from Sigma Inc. Other analytical reagent grade chemicals were purchased from Fisher Scientific (Pittsburg, PA, USA) and Sigma-Aldrich (St Louis, MO, USA).

Experimental Design

To order to determine the optimal hydrolysis conditions, a Box-Behnken design with 4 independent variables at 3 levels (Table 1) was generated and implemented. The variables chosen, temperature (x_1) , enzyme-to-substrate

(e/s) ratio (x₂), time (x₃) and pH (x₄), are known to have the most significant influence on the DH and protein functionality. The design was generated using SAS and had 27 experimental units that included 3 center points to assess the pure error (Tables 2 and 3). The three levels for each independent variable were coded as -1, 0, 1. Each experimental unit was run in triplicate and the means of DH and measured functional properties were recorded as the y responses. The behavior of each response was explained by a quadratic equation (Eq. 1). To evaluate the effect of the independent variables on the response and generate tridimensional response plots, R 2.14.1 was used. The conditions at which the responses were maximal/optimal were then determined. To validate the model, experimental data was obtained using the predicted optimized levels.

$$y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} x_i x_j$$
 Eq.1

where y is the dependent variable (DH, EA, EC or ES); β_0 , β_i , β_{ii} , β_{ii} , and β_{ij} are the linear, quadratic and interactions coefficients estimated by the model; x_i and x_j are the levels of the independent variables.

Protein Analysis

Protein analysis of DLW, WPC 34, WPI and hydrolyzed DLW samples was performed using a Nitrogen analyzer (LECO[®] TruSpec TM, St. Joseph, MI, USA) according to the AOAC Dumas method (AOAC method 990.03).

Table 1. Natural and coded variable levels used for the hydrolysis of DLW using Alcalase and papain following the RSM approach.

	Cod	مطلم	vola	Natu	ral Lev				
	Cou	eu Lev	veis	Alcal	ase		Ра	pain	
Temperature (°C), X1	-1	0	1	45	55	65	60	65	70
Enzyme-to-substrate ratio (%, v/w), X2	-1	0	1	0.5	1	1.5	0.5	1	1.5
Time (min), X3	-1	0	1	15	45	75	15	45	75
рН, Х4	-1	0	1	6.5	7.5	8.5	6.5	7.5	8.5

Hydrolysis of DLW and Determination of DH Using the pH-STAT Method

Hydrolysis of DLW was done following the pH-STAT method (Adler-Nissens, 1986) using a Mettler Toledo titrator (DL 22 food and beverage analyzer, Mettler Toledo LLC, Columbus, OH, US). Briefly, 25 mL protein solutions (5% protein w/v) of DLW were prepared in phosphate buffer (pH 7, 0.01 M) and placed in a water bath set at the desired temperature with constant stirring. The pH was adjusted using 2 M HCl or 0.5 M NaOH to the final desired pH, and the enzyme was added to the solution at a specific e/s ratio (as per the experimental runs presented in Tables 2 and 3). The pH drop was monitored and readjusted back to the original value using 0.5 M NaOH. The total volume of NaOH used to keep the pH constant was then recorded and DH was calculated according to Eq. 2.

Where B is the volume of NaOH (mL); N_b is the normality of the base; MP is the mass of the protein; h_{tot} is the total number of peptide bonds of the protein; α is the degree of dissociation of the α -NH2 groups as determined according the method described by Silva et al. (2010). Please see Appendix B for example calculations.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein distribution of DLW, WPC 34 and WPI, and hydrolysis pattern of the hydrolyzed DLW samples were monitored following SDS-PAGE under non-reducing conditions as outlined by Laemmli (1970). Each sample was dissolved in DDW (~50 mg protein/mL), and an aliquot (100 μ L) was mixed 1:2 (v/v) with Laemmli buffer and boiled for 5 min. Pre-stained broad range MW standard, protein standards, controls and hydrolyzed samples (8 μ L; containing ~ 133 μ g protein) were loaded onto 18-well hand-cast 20% acrylamide and 4% stacking gels. The gels were electrophoresed at a constant voltage of 200 V for approximately 1 h, then were stained using Coomassie Brilliant Blue (G-250) for another hour followed by de-staining. Molecular Imager Gel DoxTM XR system (Bio-Rad Laboratories, Hercules, CA) was used to scan the gels.

Dure	Coded Lev	vels			Natural Levels					
Run	X ₁	X ₂	X ₃	X ₄	X ₁	X ₂	X ₃	X ₄		
1	-1	0	-1	0	45	1	15	7.5		
2	0	0	0	0	55	1	45	7.5		
3	-1	1	0	0	45	1.5	45	7.5		
4	1	0	0	1	65	1	45	8.5		
5	1	0	-1	0	65	1	15	7.5		
6	0	1	0	1	55	1.5	45	8.5		
7	0	0	1	1	55	1	75	8.5		
8	-1	0	0	-1	45	1	45	6.5		
9	1	-1	0	0	65	0.5	45	7.5		
10	0	-1	0	1	55	0.5	45	8.5		
11	1	0	0	-1	65	1	45	6.5		
12	0	1	-1	0	55	1.5	15	7.5		
13	-1	0	0	1	45	1	45	8.5		
14	0	-1	0	-1	55	0.5	45	6.5		
15	0	-1	1	0	55	0.5	75	7.5		
16	0	0	0	0	55	1	45	7.5		
17	0	0	-1	1	55	1	15	8.5		
18	0	1	1	0	55	1.5	75	7.5		
19	-1	0	1	0	45	1	75	7.5		
20	0	-1	-1	0	55	0.5	15	7.5		
21	1	1	0	0	65	1.5	45	7.5		
22	0	1	0	-1	55	1.5	45	6.5		
23	1	0	1	0	65	1	75	7.5		
24	0	0	0	0	55	1	45	7.5		
25	0	0	1	-1	55	1	75	6.5		
26	-1	-1	0	0	45	0.5	45	7.5		
27	0	0	-1	-1	55	1	15	6.5		

Table 2. The 27 experimental units (runs) performed for DLW hydrolysis using Alcalase following the Box-behnken design.

Bup	Coded Le	evels			Natural Levels					
Kull	X ₁	X ₂	X ₃	X ₄	X ₁	X ₂	X ₃	X ₄		
1	-1	0	-1	0	60	1	15	7.5		
2	0	0	0	0	65	1	45	7.5		
3	-1	1	0	0	60	1.5	45	7.5		
4	1	0	0	1	70	1	45	8.5		
5	1	0	-1	0	70	1	15	7.5		
6	0	1	0	1	65	1.5	45	8.5		
7	0	0	1	1	65	1	75	8.5		
8	-1	0	0	-1	60	1	45	6.5		
9	1	-1	0	0	70	0.5	45	7.5		
10	0	-1	0	1	65	0.5	45	8.5		
11	1	0	0	-1	70	1	45	6.5		
12	0	1	-1	0	65	1.5	15	7.5		
13	-1	0	0	1	60	1	45	8.5		
14	0	-1	0	-1	65	0.5	45	6.5		
15	0	-1	1	0	65	0.5	75	7.5		
16	0	0	0	0	65	1	45	7.5		
17	0	0	-1	1	65	1	15	8.5		
18	0	1	1	0	65	1.5	75	7.5		
19	-1	0	1	0	60	1	75	7.5		
20	0	-1	-1	0	65	0.5	15	7.5		
21	1	1	0	0	70	1.5	45	7.5		
22	0	1	0	-1	65	1.5	45	6.5		
23	1	0	1	0	70	1	75	7.5		
24	0	0	0	0	65	1	45	7.5		
25	0	0	1	-1	65	1	75	6.5		
26	-1	-1	0	0	60	0.5	45	7.5		
27	0	0	-1	-1	65	1	15	6.5		

Table 3. The 27 experimental units (runs) performed for DLW hydrolysis using papain following the Box-behnken design.

Emulsification Capacity (EC)

Emulsification capacity was measured following the method outlined by Rickert et al. (2004) with some modifications. Briefly, sample dispersions (10 mL of a 1% protein solution, w/v) of DLW, WPC 34, WPI and hydrolyzed DLW were prepared in DDW and adjusted to pH 7.0 with 2N HCl or NaOH. Soybean oil dyed with 4 µg/mL of Sudan Red 7B was continuously blended into the dispersion at 37 mL/min flow rate using a homogenizer (IKA® RW 20 Digital, IKA Works, Inc., NC, US) with the stirrer (R 1342 propeller stirrer, 4-bladed) rotating at 830 rpm. This was done until phase inversion was observed. Emulsification capacity was expressed as g oil/g protein.

Emulsification Activity (EA) and Stability (ES)

The ES index and EA were measured according to the methods outlined by Tang et al. (2003) and Chove et al. (2001), with modifications. An aliquot (6 mL) of protein solutions (0.1% protein w/v), prepared in DDW, was added to 2 mL of soybean oil and homogenized using a homogenizer (Power Gen 700, Fisher Scientific, Pittsburg, PA, US) set at the least rotational speed (~ 10,000 rpm) of the instrument for 2 min. Right after the homogenization, 50 μ L of the emulsion (pipetted from the bottom of the beaker) was diluted in 5 mL of 0.1% SDS, vortexed for 5 sec, and absorbance measured at 500 nm using a spectophotometer (Beckman DU 640 B, Beckman Coulter, Inc., CA, US). The addition of 0.1% SDS was to avoid creaming from occurring. At t=0, the absorbance value was recorded as the EA. Another measurement was taken at t=10 min after which the ES was determined using Eq. 3.

 $ES = (A_0/A_0-A_{10}) \times 10.$

Eq. 3

Where A_0 is the absorbance at t=0, and A_{10} the absorbance at t=10min.

Eq. 4

Heat-induced Gelation

Heat-induced gelation was monitored following the method outlined by Kong et al. (2008), with some modification. Protein solutions (10% protein, w/v) were prepared in DDW and transferred to a mold (1.2 cm x 3 cm). The mold was filled to three quarters, heated at 95 °C for 30 min in a water bath, and cooled to room temperature (~ 23 °C) on an ice bath. The gel formed was compressed using a Texture Analyzer (TA-TX Plus, Stable Micro Systems LTD, Surrey, UK) until ruptured at room temperature, and the force was recorded. The probe used was 10 mm in diameter and the speed was set at 1 mm/s.

Water holding capacity (WHC)

Measurement of WHC was conducted as outlined by Sternberg et al. (1976), with some modifications. Protein solutions (10% protein, w/v) were prepared in DDW and adjusted to pH 7 using 2 M HCl or NaOH. One mL of each prepared solution was pipetted into a microcentrifuge tube and the weight was recorded (T_1). The samples were then heated at 100 °C for 10 min. After heating the tubes were weighed (T_2), inverted for 10 min, and weighed again (T3). The WHC was determined using Eq. 4.

WHC = $100 \times [(T_3 - T_1)/(T_2 - T_1)]$

Statistical analysis

Several response surface models, first order, first order with two-way interactions, and second order, were compared to determine the best fit. Polynomial regression equations were generated and used to construct

RSM plots. The point optimization method was used to optimize each variable level for maximum response. Experimental data obtained using the predicted optimized levels were analyzed using analysis of variance (ANOVA) utilizing XLSTAT for Windows (2010). Significant ($P \le 0.05$) differences between the means were determined using Tukey-Kramer multiple means comparison test.

Materials and Methods for Objective Two:

Materials

Ground meat was purchased from Andrew-Boss Laboratory-Meat Science (University of Minnesota, St Paul, 55108). Samples of DLW (24 % protein), WPC34 (34% protein) and WPI (90 % protein) were kindly provided by Valley Queen (Milbank, SD, USA), Main Street Ingredients (La Crosse, WI, USA), PGP International (Woodland, CA, USA), respectively. Food grade Alcalase 2.4 L (2.4 AU-A/g) and papain (65,600 USP/mg) were provided by Novozymes (Bagsvaerd, Denmark) and American Laboratories Incorporated (Omaha, NE, USA), respectively. Food grade hydrochloride acid (A481-212) and sodium hydroxide (1310-73-2) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Dextrose was purchased from Science Company (Denver, CO, USA). Other formulation ingredients, including the spices, were purchased from local stores.

Preparation of DLW Hydrolysates

Delactosed whey was hydrolyzed using food grade Alcalase at pH 8.5, 45 °C, enzyme to substrate (E/S) ratio of 1.5 (%, v/w), for 15 min. Delactosed whey was also hydrolyzed using food grade papain at pH 8.5, 70 °C, enzyme to substrate (E/S) ratio of 1.5 (%, v/w), for 75 min. The hydrolysis conditions were optimized in our previous study to produce DLW hydrolyzed with enhanced emulsification properties. Hydrolysis of DLW with Alcalase and papain under these conditions resulted in enhanced emulsification activity (EA) and emulsification capacity (EC), respectively. The hydrolysates were produced in a food grade lab. For each enzyme treatment, one batch of 2000 mL DLW solution (5% protein, w/v) was prepared by diluting 416.7 g of DLW in water (assuming a protein content of 24% as stated on the specification sheet of DLW). The obtained solution was then heated to the required temperature using a water bath (Isotemp, Immersion Circulator, Model 730, Fisher Scientific, Pittsburg, PA, USA). After the temperature was reached, the pH of the solution was adjusted to the desired value, using 2M food grade NaOH or HCl. Then, the necessary amount of enzyme was added, and the solution was continuously stirred until the end of the hydrolysis time. The pH was maintained constant throughout the hydrolysis time by using 2M food grade NaOH or HCl. At the end of the hydrolysis time, samples were boiled for 5 min to inactivate the enzyme, and were lyophilized using a food grade lyophilizer, then were stored at -20 °C for later use. The protein content of both Alcalase and papain DLW hydrolysates was ~ 23.5% as determined by Dumas, AOAC 990.03, using a Nitrogen Analyzer (LECO[®] TruSpec TM, St. Joseph, MI, USA).

Beef Formulation

In triplicate, ground beef patties were formulated using the DLW hydrolysates, non-hydrolyzed DLW, WPC34 or WPI (Table 4). A control sample was formulated without the addition of any whey protein ingredient. The amount added of each whey ingredient was standardized based on a delivery of 15 g of whey proteins. Compared to the control more water (100 g vs. 30 g) was added to the formulations containing whey protein, to compensate for the reduced amount of beef, which has ~ 70% moisture, and to account for the increased water holding capacity upon addition of whey protein.

Fat and Moisture Analysis

Fat and moisture contents of all the formulated beef patties were determined using the Sohxlet method (AOAC 960.39) and the vacuum oven method (AOAC 950.46), respectively.

	Treatment										
Ingredient	Control		DLW		DLWH ^a	DLWH ^a			WPI		
	g	%	g	%	g	%	G	%	g	%	
Lean beef	1000	94.7	960	84.5	960	84.5	960	85.0	960	87.1	
DLW	-	-	50	4.4	-	-	-	-	-	-	
aDLMH	-	-	-	-	50	4.4	-	-	-	-	
WPC 34	-	-	-	-	-	-	45	3.98	-	-	
WPI	-	-	-	-	-	-	-	-	17	1.54	
Salt	12.5	1.18	12.5	1.1	12.5	1.1	12.5	1.1	12.5	1.13	
Dextrose	10	0.95	10	0.88	10	0.88	10	0.88	10	0.91	
Garlic powder	1.3	0.12	1.3	0.11	1.3	0.11	1.3	0.12	1.3	0.12	
^b Na Glut	1.3	0.12	1.3	0.11	1.3	0.11	1.3	0.12	1.3	0.12	
Water	30	2.84	100	8.81	100	8.81	100	8.84	100	9.07	
Total	1056	100	1136	100	1136	100	1131	100	1103	100	

Table 4. Formulation of beef patties using different whey protein ingredients.

^aDLWH= DLW-hydrolysate, either the Alcalase or the papain hydrolysate; ^bNa Glut.= Monosodium glutamate.

Cook Loss Determination

Prior to cooking, the ground beef was thoroughly mixed with the ingredients (Table 14) using a Kitchen Aid[®] Pro 600 Mixer equipped with a KitchenAid Flat Beater. The ground beef was then mixed manually for a few minutes and refrigerated for one hour. Cook loss was determined according to the method outlined by Barbut (2010), with some modifications. Ground beef (35 g) was weighed, in triplicate, in 50- mL centrifuge tubes. The tubes were heated to 72 °C in a water bath for 75 min. The tubes were then cooled down to room temperature (23 °C) under running cold water for 15 min. The liquid released was decanted into 15 mL tubes and placed at 5-7 °C overnight. After refrigeration, separation into water, fat, and solid components was achieved. Volume of water and fat were recorded and converted to mass using their respective densities. Water and fat losses were determined as the percentage of released water and fat to the total moisture and fat content of the initial beef mix, respectively.

Cooking of the Beef Patties

Formulated beef samples were molded using a patty former into patties with \sim 4 in in diameter and ½ in in thickness. The patties were cooked to a core temperature of 160 °F, which took about 4 to 6 min, using a preheated George Foreman grill. The purpose of using a George Foreman grill was to ensure uniform cooking on each side of patties and, thus, reduce cooking-induced differences in texture and appearance.

Texture Analysis

Texture analysis was performed based on the method outlined by Barbut (2010), with some modifications. Prior to texture analysis, six cores (12-mm diameter, 10-mm height) were isolated from the center of each cooked patty (three patties cooked per treatment). The cores were then submitted to compression tests using a TA-XT2 analyzer (Texture Technologies Corp., Scarsdale, NY, USA). A moving flat plate descending at 1mm/s was employed to compress the cores. The other test parameters were set as follows: pre-test speed, 1.0 mm/s; test speed, 1.0 mm/s; post-test speed, 10.0 mm/s; trigger force, 12 g; penetration distance, 8 mm. The maximum force necessary to compress the samples to the set penetration distance was then recorded.

Sensory analysis

Subjects

Ten subjects (9 females, 1 male, ages 23-55 years) were recruited from the trained panel maintained by the Sensory Center at the University of Minnesota. All subjects were tasters of 6-n proporthiouracil (PROP) and free from food allergies or sensitivities. Subjects were paid for participating in a two-day study.

Test Samples

Formulated beef patties (Table 14) were cooked as described above. Cooking was conducted in a different room to avoid extraneous odors in the testing area. Cooked patties were cut crosswise into 4 equal pieces, and served hot to panelists in plates coded with random 3-digit numbers. The presentation order of the test samples was based on a Latin Squares design balanced to prevent bias and carryover effects.

Training session

The subjects were given a training session before they participated in the test session. During the training, the subjects were introduced and familiarized with different sensory attributes of interest, saltiness, bitterness off-flavor, juiciness, firmness and toughness of the cooked beef patties. Using an intensity rating scale from 0 = imperceptible, to 20 = extremely intense (Lawless, 2010), the subjects practiced evaluating the mentioned sensory attributes of cooked beef patties formulated with and without whey protein. The judges were not informed about the composition of the patties.

Test Sessions

Two test sessions were held to duplicate the sensory evaluation of the beef patties by the trained panel. During each session, all 6 samples, the control, and the beef patties formulated with Alcalase hydrolysate, papain hydrolysate, DLW, WPC 34 and WPI (Table 17) were evaluated for juiciness, firmness, toughness, saltiness, bitterness and off-flavor using the intensity rating scale from 0 to 20 (see sensory evaluation form in Appendix E). Panelists were instructed to rinse their mouth with water between samples.

Statistical Analysis

In order to compare the functional and sensory attributes of the different beef patties, analysis of variance (ANOVA) was carried out utilizing XLSTAT for Windows (2012). The independent variable was the type of whey protein added to the beef mix. For the sensory data, judges were treated as a random factor. When the

independent variable effect was found significant ($P \le 0.05$), significant differences among the means were determined using Tukey-Kramer multiple means comparison test or Fisher's LSD test.

Results and Discussion

Results and Discussion for Objective One:

Protein Content and Profile

The protein content of DLW and hydrolysates ranged between ~ 20-23 %, and was, as anticipated, less than that of WPC 34 and WPI (Table 5). Protein profiling revealed the presence of the two major whey proteins, β lactoglobulin and α -lactalbumin (β -lg and α -la) among other whey proteins and glycomacropeptides in DLW, WPI and WPC. After hydrolysis of DLW using either Alcalase or papain under the various hydrolysis conditions, major protein bands faded or disappeared, and new peptide bands with molecular weight less than 6 kDa were visualized. Some qualitative differences in the intensity of the intact protein and the released peptides can be visualized among the different DLW hydrolysates. This observation is in line with the observed differences in DH, as will be discussed in the following sections.

Sample	Protein content (%)
DLW	22.49
Alcalase-hydrolyzed DLW (runs 1 to 27)	20.99 – 23.68
Papain-hydrolyzed DLW (runs 1 to 27)	20.65 – 23.26
WPI	90.02
WPC34	34.00*

Table 5. Protein content of DLW, hydrolyzed DLW, WPI and WPC34.

* As provided on the specification sheet of the product.

Response Surface Modeling

Several response surface models were compared to determine the best fit for the RSM data collected (Tables 6 and 7). First order, first order with two-way interactions, and second order were the models tested (Tables 8 and 9). When the most complex model didn't fit significantly better at $P \le 0.05$, the next simplest one was chosen for further analysis. The point within the range of experimentation with the highest fitted value was determined. Using the model with the best fit (high R² and $P \le 0.05$) for each response, prediction equations were generated (Table 10). For EC, Log (ES) and EA of Alcalase hydrolysates, the first order with two-way interactions models were selected, while for DH second order model was selected (Tables 8 and 10). For EC, Log (ES), EA and DH of papain hydrolysates, second order, first order, first order with two-way interactions, and second order models were selected, respectively (Tables 9 and 10). The predicted response obtained when using the generated equations, were close to the actual responses, more so for the DH and EA (Tables 6 and 7).

Run	Code	ed Vai	riables	\$	EC (g oil / g	protein)	ES (mi	n)	EA		DH (%)	
	X 1	X ₂	X ₃	X 4	Α	Р	Α	Р	Α	Р	Α	Р
1	-1	0	-1	0	1586	1451	28.9	28.8	0.58	0.60	3.02	2.02
2	0	0	0	0	2006	1731	53.9	41.7	0.49	0.49	8.02	7.94
3	-1	1	0	0	1994	1847	37.7	34.7	0.70	0.66	6.57	6.57
4	1	0	0	1	1587	1428	79.3	95.5	0.32	0.29	9.18	9.06
5	1	0	-1	0	2027	1989	39.8	33.1	0.54	0.53	5.88	5.45
6	0	1	0	1	1425	1637	143	118	0.26	0.32	11.7	11.09
7	0	0	1	1	1848	1690	63.3	91.2	0.35	0.30	10.6	9.80
8	-1	0	0	-1	1309	1579	21.8	20.4	0.51	0.58	6.75	7.38
9	1	-1	0	0	1612	1883	35.2	36.3	0.45	0.50	7.32	7.22
10	0	-1	0	1	1872	1798	36.9	33.1	0.56	0.60	6.66	6.48
11	1	0	0	-1	1897	1911	33.7	36.3	0.41	0.45	14	13.99
12	0	1	-1	0	1874	1927	28.7	37.2	0.55	0.60	6.39	6.30
13	-1	0	0	1	1911	2007	38.4	40.7	0.64	0.64	6.4	6.91
14	0	-1	0	-1	2110	1824	22	26.3	0.48	0.46	9.92	10.06
15	0	-1	1	0	1998	2109	39.4	34.7	0.53	0.53	7.51	8.12
16	0	0	0	0	1767	1731	47	41.7	0.49	0.49	7.68	7.94
17	0	0	-1	1	1678	1745	39.6	42.7	0.7	0.63	5.53	6.68
18	0	1	1	0	1290	1376	84.8	87.1	0.28	0.29	11.2	12.08
19	-1	0	1	0	2079	2135	30	28.8	0.59	0.62	6.63	6.63
20	0	-1	-1	0	1435	1513	23	25.7	0.49	0.53	3.11	2.78
21	1	1	0	0	1444	1456	96.7	95.5	0.22	0.24	11.2	11.29
22	0	1	0	-1	1667	1666	26.1	28.2	0.57	0.57	13.2	12.91
23	1	0	1	0	1197	1350	134	105	0.20	0.21	11.4	11.97
24	0	0	0	0	2000	1731	42.8	41.7	0.54	0.49	8.14	7.94
25	0	0	1	-1	1912	1795	27.6	33.1	0.59	0.53	16.2	14.94
26	-1	-1	0	0	1626	1739	24.5	24.0	0.57	0.56	3.33	3.18
27	0	0	-1	-1	1588	1695	24.8	22.4	0.57	0.50	6.23	6.94

Table 6. Actual (A) and predicted (P) responses for the 27 DLW hydrolysates obtained using Alcalase.

Refer to Table 3 for natural variable levels.

Run	Code	ed Var	riables	*	EC (g oil / g	protein)	ES (mi	n)	EA		DH (%)	
	X 1	X ₂	X ₃	X 4	Α	Р	Α	Р	Α	Р	Α	Р
1	-1	0	-1	0	1850	1832	31.9	38.0	0.51	0.54	4.39	3.41
2	0	0	0	0	1941	2002	44	49.0	0.41	0.39	6.88	5.72
3	-1	1	0	0	1861	1940	81.6	67.6	0.34	0.34	6.88	6.86
4	1	0	0	1	2000	2017	51.3	47.9	0.47	0.48	8.81	7.01
5	1	0	-1	0	1953	1928	42.7	51.3	0.44	0.42	4.96	4.95
6	0	1	0	1	1918	1958	43.9	56.2	0.47	0.50	7.06	7.31
7	0	0	1	1	1956	2062	38.5	45.7	0.63	0.57	6.73	6.53
8	-1	0	0	-1	1979	1909	29.3	33.9	0.49	0.46	10.2	11.47
9	1	-1	0	0	1861	1933	43	39.8	0.41	0.40	4.18	4.18
10	0	-1	0	1	1823	1647	37.7	42.7	0.55	0.58	3.03	4.03
11	1	0	0	-1	1981	1875	58.5	67.6	0.25	0.26	12.4	12.39
12	0	1	-1	0	1908	1796	52.4	53.7	0.49	0.45	6.29	6.29
13	-1	0	0	1	1826	1879	56	51.3	0.59	0.55	4.63	4.15
14	0	-1	0	-1	1979	1842	26.2	24.5	0.57	0.55	8.85	9.10
15	0	-1	1	0	1696	1754	29.1	28.8	0.52	0.55	4.96	4.48
16	0	0	0	0	2062	2002	60.6	49.0	0.38	0.39	4.88	5.72
17	0	0	-1	1	1821	1780	59.6	53.7	0.58	0.58	3.92	5.15
18	0	1	1	0	1954	1749	101	102.3	0.23	0.25	9.64	9.57
19	-1	0	1	0	1965	1893	50.2	46.8	0.41	0.45	5.30	5.81
20	0	-1	-1	0	1519	1672	35.9	36.3	0.59	0.56	2.67	2.25
21	1	1	0	0	1539	1659	99.1	81.3	0.23	0.24	8.15	8.49
22	0	1	0	-1	1571	1650	91.4	95.5	0.25	0.23	15.4	14.94
23	1	0	1	0	1980	1901	66.0	63.1	0.32	0.30	6.57	8.05
24	0	0	0	0	2003	2002	62.4	49.0	0.39	0.39	5.4	5.72
25	0	0	1	-1	1550	1741	56.7	64.6	0.33	0.32	15.5	14.24
26	-1	-1	0	0	1518	1548	27.7	26.3	0.59	0.58	2.39	2.03
27	0	0	-1	-1	1945	1988	41	36.3	0.47	0.53	9.95	10.13

Table 7. Act	ual (A)	and	predicted	(P) res	ponses	for the	- 27	DLW h	vdrol	vsates	obtained	using	papain.
TUDIC 7. ACC	uui	ana	predicted	<u>ب</u> ا	1103	ponses	TOT UN	/		yurur	ysuics	obtanica	using	pupum.

Refer to Table 3 for natural variable levels.

Effect of the Hydrolysis Parameters on the DH of DLW

Using Alcalase and papain, the experimental DH values ranged from 3.02% to 16.2% and 2.39% to 15.4%, respectively (Tables 6 and 7). When using Alcalase, temperature and time appeared to be the major driving factors (Figure 1, see DH quadratic equation when using Alcalase Table 10). As incubation temperature and time increased, DH increased. Maximum DH was obtained at the highest temperature and longest incubation time. The optimum temperature for Alcalase, per the specification sheet and reported literature (Babji, 2011), is between 55-70 °C. Thus, it is anticipated that higher DH values can be reached beyond the range of experimentation. Based on the results obtained within the range of experimentation, Alcalase had the highest activity at 65 °C. At this temperature there was a constant increase in DH as time increased. When hydrolyzing a protein, the number of cleaved peptide bonds increases as a function of time.

Response	Model	DF ^d	SS ^e	MS ^f	F	p-value	Lack of Fit F	Lack of Fit p-value	R ²
	FO ^a	4	126169	31542	0.79	0.55	4.39	0.20	0.07
	FO + 2WI ^b	6	1052276	175379	4.39	0.01	2.25	0.35	0.65
EC	SO ^c	4	147521	36880	0.92	0.48	2.36	0.33	0.73
	Residuals	12	478908	39909					
	Lack of fit	10	441558	44156					
	Pure error	2	37351	18675					
	FO	4	1.10	0.28	36.86	0.00	5.52	0.16	0.79
	FO + 2WI	6	0.17	0.03	3.82	0.02	3.10	0.27	0.92
Log (ES)	SO	4	0.03	0.01	0.88	0.50	3.31	0.25	0.94
	Residuals	12	0.09	0.01					
	Lack of fit	10	0.08	0.01					
	Pure error	2	0.01	0.00					
	FO	4	0.27	0.07	24.45	0.00	12.31	0.08	0.56
	FO + 2WI	6	0.17	0.03	10.37	0.00	3.47	0.25	0.91
EA	SO	4	0.01	0.00	0.97	0.46	3.61	0.24	0.93
	Residuals	12	0.03	0.00					
	Lack of fit	10	0.03	0.00					
	Pure error	2	0.00	0.00					
	FO	4	214.19	53.55	81.03	0.00	58.93	0.02	0.76
	FO + 2WI	6	12.80	2.13	3.23	0.04	68.34	0.01	0.80
DH	SO	4	47.38	11.85	17.92	0.00	13.55	0.07	0.97
	Residuals	12	7.93	0.66					
	Lack of fit	10	7.82	0.78					
	Pure error	2	0.12	0.06					

Table 8. ANOVA for the various responses using Alcalase RSM data.

^a FO: first order; ^b FO + 2WI: first order plus two-way interaction; ^c SO: second order; ^d DF: degrees of freedom; ^eSS: sum of squares; ^fMS: mean sum of squares.

Response	Model	\mathbf{DF}^{d}	SS ^e	MS ^f	F	p-value	Lack of Fit F	Lack of Fit p-value	R ²
	FO ^a	4	29193	7298	0.34	0.84	9.52	0.10	0.04
	FO + 2WI ^b	6	258088	43015	2.02	0.14	8.60	0.11	0.39
EC	SO ^c	4	196281	49070	2.30	0.12	6.73	0.14	0.65
	Residuals	12	256026	21335					
	Lack of fit	10	248639	24864					
	Pure error	2	7387	3694					
	FO	4	0.46	0.11	18.09	0.00	1.62	0.45	0.65
	FO + 2WI	6	0.15	0.03	3.97	0.02	0.80	0.68	0.87
Log (ES)	SO	4	0.02	0.00	0.69	0.61	0.87	0.64	0.89
	Residuals	12	0.08	0.01					
	Lack of fit	10	0.06	0.01					
	Pure error	2	0.01	0.01					
	FO	4	0.28	0.07	45.27	0.00	18.63	0.05	0.75
	FO + 2WI	6	0.04	0.01	4.16	0.02	15.47	0.06	0.85
EA	SO	4	0.04	0.01	5.74	0.01	7.30	0.13	0.95
	Residuals	12	0.02	0.00					
	Lack of fit	10	0.02	0.00					
	Pure error	2	0.00	0.00					
	FO	4	216.73	54.18	40.85	0.00	4.29	0.21	0.70
	FO + 2WI	6	4.90	0.82	0.62	0.71	5.80	0.16	0.71
DH	SO	4	73.07	18.27	13.77	0.00	1.29	0.51	0.95
	Residuals	12	15.91	1.33					
	Lack of fit	10	13.78	1.38					
	Pure error	2	2.14	1.07					

^a FO: first order; ^b FO + 2WI: first order plus two-way interaction; ^c SO: second order; ^d DF: degrees of freedom; ^e SS: sum of squares; ^f MS: mean sum of squares.

Enzyme	Response	Quadratic Polynomial Equations	R ²	P-value
	EC (FO ^a + 2WI ^b)	$Y_1 = 1731.17 - 61.79X_1 - 79.91X_2 + 11.23X_3 - 13.60X_4 - 133.62X_1X_2 - 330.89X_1X_3 - 228.03X_1X_4 - 286.69X_2X_3 - 1.05X_2X_4 - 38.51X_3X_4$	0.65	0.01
Alcalase	Log (ES) (FO + 2WI)	$Y_2 = 1.62 + 0.15X_1 + 0.14X_2 + 0.12X_3 + 0.18X_4 + 0.06X_1X_2 + 0.13X_1X_3 + 0.03X_1X_4 + 0.06X_2X_3 + 0.13X_2X_4 + 0.04X_3X_4$	0.92	0.02
	EA (FO + 2WI)	$Y_3 = 0.49 - 0.12X_1 - 0.04X_2 - 0.07X_3 - 0.02X_4 - 0.09X_1X_2 - 0.09X_1X_3 - 0.06X_1X_4 - 0.08X_2X_3 - 0.10X_2X_4 - 0.09X_3X_4$	0.91	0.00
	DH (SO ^c)	$Y_{4}=7.94 + 2.19X_{1} + 1.87X_{2} + 2.78X_{3} - 1.35X_{4} + 0.17X_{1}X_{2} - 0.48X_{1}X_{3} - 1.12X_{1}X_{4} - 0.11X_{2}X_{3} + 0.44X_{2}X_{4} - 1.22X_{3}X_{4} - 0.84X_{1}^{2} - 0.04X_{1}^{2} - 0.59X_{1}^{2} + 2.23X_{1}^{2}$	0.97	0.00
	EC	$Y_{c}=2001.93 + 25.97X_{1} + 29.69X_{2} + 8.70X_{2} + 28.30X_{4} -$	0.65	0.12
	(SO)	$166.7X_{1}X_{2} - 22.08X_{1}X_{3} + 43.06X_{1}X_{4} - 32.53X_{2}X_{3} + 125.7X_{2}X_{4} + 132.4X_{3}X_{4} - 43.22X_{1}^{2} - 188.66X_{2}^{2} - 70,37X_{3}^{2} - 38.75X_{4}^{2}$		
	Log (ES) (FO + 2WI)	$Y_6 = 1.69 + 0.07X_1 + 0.18X_2 + 0.04X_3 + 0.005X_4 - 0.03X_1X_2 - 0.002X_1X_3 - 0.08X_1X_4 + 0.09X_2X_3 - 0.12X_2X_4 - 0.08X_3X_4$	0.87	0.02
Papain	EA (SO)	$Y_{7}=0.39 - 0.07X_{1} - 0.10X_{2} - 0.05X_{3} + 0.08X_{4} + 0.02X_{1}X_{2} - 0.005X_{1}X_{3} + 0.03X_{1}X_{4} - 0.05X_{2}X_{3} + 0.06X_{2}X_{4} + 0.05X_{3}X_{4} - 0.01X_{1}^{2} - 0.01X_{2}^{2} + 0.05X_{3}^{2} + 0.06X_{4}^{2}$	0.95	0.01
	DH (SO)	$Y_{8}=5.72 + 0.94X_{1} + 2.28X_{2} + 1.37X_{3} - 3.17X_{4} - 0.13X_{1}X_{2} + 0.17X_{1}X_{3} + 0.48X_{1}X_{4} + 0.26X_{2}X_{3} - 0.64X_{2}X_{4} - 0.68X_{3}X_{4} - 0.21X_{1}^{2} - 0.12X_{1}^{2} + 0.05X_{1}^{2} + 3.25X_{1}^{2}$	0.95	0.00

Table 10. Equations derived using RSM models with the best fit for the prediction of the dependent variables (Y_{1} - Y_{8}), with temperature as X_{1} , enzyme-to-substrate (e/s) ratio as X_{2} , time as X_{3} and pH as X_{4} .

^a FO: first order; ^b FO + 2WI: first order plus two-way interaction; ^c SO: second order



Figure 1. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on DH when Alcalase was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.

Within the parameters of the hydrolysis conditions tested using Alcalase, as e/s ratio increased DH increased (Figure 1). The substrate concentration used was kept at levels exceeding the K_m of the enzyme, maintaining a zero order reaction with respect to the substrate. Thus, with an increase in e/s ratio, higher enzyme activity was achieved, as demonstrated by the observed increase in DH. The DH response to pH change, on the other hand,

was mostly a saddle response, with an overall negative correlation, as shown in the quadratic equation (Table 11). Under most of the conditions tested, minimum DH was observed when the pH approached 7.5.

When using papain, pH and e/s ratio were the most driving factors (Figure 2, see also DH quadratic equation when using papain in Table 10). The quadratic equation showed an overall negative correlation between the DH and pH (Table 10). The highest DH was obtained at the lowest pH level tested. The optimal pH range of papain is from 5.0 to 7.0 (Whitehurst and Oort, 2009). Thus, a higher DH values can be reached at pH lower than 6.5, which is beyond the range of experimentation. Although when using papain, e/s had a higher impact than when using Alcalase, the noted trend in the DH response was similar.

The effect of temperature on DH when using papain was not as significant as when using Alcalase. This is mostly attributed to the fact that the range of temperature tested falls within the actual optimum temperature for papain (Babji, 2011). Finally, and as expected, DH increased as incubation time increased, within the range tested.

Effect of the Hydrolysis Parameters on the Emulsification Properties of DLW

Effect on Emulsification Capacity

Of the four hydrolysis parameters, when Alcalase was used, temperature and e/s ratio had the greatest effect on EC (refer to the quadratic equation for EC using Alcalase in Table 10). And the most pronounced interaction was between temperature and time. At a constant pH (8.5) and e/s ratio (0.5), The highest incubation temperature and longest time resulted in minimum EC (Figure 3). Also, at the highest pH (8.5) and the longest time (75 min), the EC decreased as e/s ratio and temperature increased. On the other hand, EC increased as hydrolysis temperature decreased, reaching maximum levels at the lowest temperature, lowest e/s ratio, longest time and highest pH.

When papain was used, none of the individual variables had a significant effect on EC, however the interaction effect of temperature and e/s, and that of time and pH were significant. The second order model, which was significant only when $P \le 0.12$, was selected to generate the quadratic equation since it had the highest R and lowest *P*-value among the tested models. As incubation pH increased EC increased, reaching a high values at pH 8.5 when the temperature was in the low 60s °C, e/s between 1-1.5%, and when time was at the highest level (Figure 4).

The conditions that elicited high EC, when using either Alcalase or papain, corresponded to a moderate DH, 5-10%, while the conditions that elicited low EC, corresponded to a relatively high DH, 10-17 %. For example, at pH 8.5, 60-65 °C, 70-75 min and e/s ratio of 1-1.5 %, when papain was used, the DH was 5-10% (Figure 2), while EC was at maximum (Figure 4). While, at pH 6.5, at the highest temperature, and at the highest level for time and e/s ratio, DH was greater than 15% (Figure 4) and EC was at a minimum (Figure 4). Similarly, when Alcalase enzyme was used, at the highest temperature and time levels and lowest pH and e/s ratio, DH was > 15% (Figure 1) and EC was in the mid range (~ 1,700 g oil per/1 g protein) (Figure 3). The EC was ~ 2,200 g oil per/1 g protein and DH was less than 10% at the lowest temperature and highest e/s ratio time and pH levels (Figures 1 & 3). Thus, higher DH is detrimental to the EC.

A positive correlation exists between emulsification properties and peptide chain length. It was suggested that a peptide with a minimum length of 20 residues possesses good emulsifying and interfacial properties (Gauthier et al, 1993). Higher DH indicates an elevated levels of short-chain peptides, and hence a reduced EC. In general, desired functionalities are obtained by controlling the DH. A moderate DH imparts increased molecular flexibility to the globular whey protein, reduced average molecular weight, increased number of charged groups and increased surface hydrophobicity (Kitts and Weiler, 2003), allowing faster movement and rearrangements at the interface.



Figure 2. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on DH when papain was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.



Figure 3. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the emulsification capacity when Alcalase was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.



Slice at temperature=62, e.s=1.3

Slice at Temperature =62, pH = 8.5

Figure 4. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the emulsification capacity when papain was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.

Effect on Emulsification Stability and Activity

When Alcalase was used all of the tested variables and interactions were positively correlated with the ES response (after converting the data to log values). However, when papain was used some of the interactions were negatively correlated with the ES response (refer to the quadratic equations for ES in Table 10). Within the experimental range tested, a maximum value can not be predicted since the ES seemed to continue increasing with the increase in the levels of the independent variables (Figures 5 and 6). Therefore, the true optimal point was probably not within the range tested since none of the plots showed an inflection or a saddle point.

The effect of DH on the ES response at the various conditions tested is not as apparent as that on EC. A Pearson correlation test was performed, using both Alcalase and papain data (a total of 54 observations), to determine the significance of the association. A positive correlation (R= 0.368) was found between the DH and ES, indicating that the ES response tends to increase as DH increases. Although the R value is relatively low, it is statistically significant (P = 0.006).

The DLW hydrolysates that had really high ES had very low EA (Tables 6 and 7). At low EA, coalescence occured almost immediately after homogeneization, leading to abnormaly high ES values. For most of the highly hydrolyzed samples, coalescence caused the denominator $(A_0 - A_{10})$ in Eq.4 to have very low values as a result of A_{10} being too close to A_0 , thus giving an erraneous high ES value. Hence, more focus was given to the EA response.

When Alcalase was used, all of the tested variables and interactions were negatively correlated with the EA response. However, when papain was used, the pH and some of the interactions were positively correlated with the EA response (refer to the quadratic equations for EA in Table 10). The effect of pH on EA was not statistically significant when Alcalase was used. The effect of hydrolysis time on EA, however, was dependent on the pH and temperature. Emulsification activity approached maximum at lower temperature and shorter time (Figure 7). When papain was used, the EA seemed to increase as pH moved away from 7.5 (Figure 8). Similar to when Alcalase was used, EA approached maximum at lower temperature and time. However, at higher pH (8.5), as time increased EA increased when temperature was held at 60 °C.

Similar to what was observed of EC, EA was improved upon limited hydrolysis by both enzymes. When Alcalase was used, the EA of the hydrolysates with relatively high DH was lower than that of the non-hydrolyzed DLW. Non-hydrolyzed delactosed whey had an EA value of 0.365, however the hydrolysates of runs 6 and 18 had EA values of 0.260 and 0.280, and a DH of 11.7% and 11.2%, respectively (Table 6). At the highest pH 8.5, e/s ratio temperature and time, the DH was between 10-15% (Figure 1), which corresponded to very low EA (Figure 7). Maximal EA responses where observed at the lowest temperature and time combinations (Figure 7), which corresponded to low to moderate DH values of 2-8%, (Table 6 and Figure 1). Similarly, when papain enzyme was used, conditions that elicited a relatively high DH % resulted in reduced EA, and visa versa (Table 7, Figures 2 and 8). When running a Pearson correlation test, a negative correlation (R= - 0.481) was found to be significant at P < 0.001. While at moderate DH the hydrophobic/hydrophylic balance was most likely improved, at high DH it was distrupted. At lower/moderate DH, moderate size peptides are generated that are more likely to contain both hydrophobic and hydrophilic moieties. These peptides can rearrange at the interface more easily than the globular protein (Gauthier et al., 1993). Characterization and profiling of the peptides produced using either Alcalase or papain, would further explain the observed effect of DH on the EA of hydrolyzed DLW.



Figure 5. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the Log (ES, emulsification stability) when Alcalase was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.



Figure 6. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the Log (ES, emulsification stability) when papain was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.



Figure 7. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the emulsification activity when Alcalase was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.



Figure 8. RSM plots for the effect of temperature, enzyme-to-substrate ratio (e.s), time and pH on the emulsification activity when papain was used. Each plot shows the effect of two variables while keeping the other two constant at a level that elicits the maximum response.

Effect of Hydrolysis on the Heat-Induced Gelation and WHC of DLW

The heat-induced gel formed using non-hydrolyzed DLW at 10% (w/v) protein concentration had a rupture force of 12.3 \pm 1.2 N and WHC of 74.1 \pm 1.25%. On the other hand, none of the DLW hydrolysates formed a heat-induced gel at 10%, 15% or even 20% protein concentration. As a result, the water holding capacity could not be determined. Hydrolysis under the conditions tested had a detrimental impact on the gelling property of DLW. Ju et al. (1995) found that whey protein hydrolysates produced using trypsin did not gel at pH 7.0 and 3.0, yet formed a gel when the pH was close to the iso-electric point (pI) of whey protein (pH 5.2). For future studies, it is recommended to monitor heat-induced gelation of DLW hydrolysates at different pH and ionic strengths.

Whey protein hydrolysates (DH 2.8-4.9%) produced using BLP enzyme (one of the enzymes found in Alcalase), however, formed strong gels at pH 7.0 (Ju et al., 1995). Contrary to their findings, our hydrolyzed DLW to a similar DH, using Alcalase, did not form a gel at pH 7. This can be partially attributed to the high lactose content of DLW that could have interfered in the formation of gels using hydrolyzed DLW. Lactose was reported to have a negative impact on gelation (Wong et al., 1996). Since the objective of the study was to find low cost and simple ways to amplify the functionalities of DLW, we did not attempt to eliminate the lactose to evaluate its impact. However, it would be interesting in future studies to investigate its effects on heat-induced gelation.

Additionally, whey protein gelation property can be affected by the source of whey. Pallavicini et al. (1988) showed that the coagulation properties of acid whey can be improved upon Alcalase hydrolysis. Sweet whey, however, did not coagulate under the same conditions, unless CaCl₂ was added. The DLW used in this study was produced from sweet whey, a fact that can partially explain our observation. Further studies investigating the effect of limited hydrolysis on DLW gelling property using different environmental parameters are a natural follow up to this work.

Emulsification Properties of Hydrolyzed DLW under the Optimized Conditions predicted by the RSM models

The predicted and actual responses of the 27 hydrolysates (Tables 6 and 7) were relatively close, indicating that the chosen models had a relatively good fit. Yet, when hydrolyzing samples under the predicted optimal conditions, actual responses drifted away from predicted responses in some cases (Table 11). Based on the RSM analysis, hydrolysis conditions that resulted in maximal response were designated as the optimal hydrolysis conditions.

Under the optimal Alcalase and papain hydrolysis conditions determined following the first order + two-way interaction model and the second order model (Table 10), respectively, the experimental (actual) values for EC were not close to the predicted values (Table 11). The R^2 of both models (0.65 for both) was relatively low, hence the observed difference between the predicted and the actual response.

Table 11. Actual (A) and predicted (P) emulsification capacity and activity (EC and EA) responses for Alcalase and papain DLW hydrolysates produced under optimized conditions as determined by RSM analysis.

Enzyme	Parameter	рН	Temp (°C)	Time (min)	E/S	Ρ	A
Alcalase	EC (g oil/g protein)	8.5	45	75	0.5	2544	1571
	EA	8.5	45	15	1.5	0.75	0.77
Papain	EC (g oil/g protein)	8.5	62	75	1.3	2084	1678
	EA	6.5	60	15	0.5	0.75	0.41

Under the optimal Alcalase hydrolysis conditions determined following the first order + two-way interaction model (Table 10), the experimental (actual) value for EA was very close to the predicted value (Table 11). The R^2 of the model was relatively high (0.91) with a *P*-value of < 0.001. Surprisingly, however, the actual value for EA was far from the predicted values under the optimal papain hydrolysis conditions, although the R^2 for the second order model was relatively high with *P*-value of 0.01.

A possible reason for the variation observed between predicted and actual responses could be attributed to the viscosity of the emulsion (Pearce and Kinsella, 1978). When very viscous emulsions are formed, the mixing of oil may become inefficient or incomplete and thus lead to erroneous EC and EA values. In the present study, viscous emulsions were often obtained with hydrolysates that had low DH, while those that had high DH formed emulsions that were much less viscous. The DH of the hydrolysate produced under the optimal papain hydrolysis conditions was only 2.54%, which was relatively low.

The optimal conditions derived from the RSM models might not be the true optimal conditions due the fact that the range of levels used for each variable were deemed narrow. In several cases no inflexion or saddle point was obvious. Therefore, the optimal conditions for the highest responses might be outside of the range tested. For future studies, it is recommended to widen the range of the levels used for each variable.

Emulsification Properties of DLW hydrolyzed under Optimized Conditions as Compared to DLW, WPI and WPC 34

The DLW hydrolysates, produced using Alcalase under optimal conditions for maximal EC and EA, had significantly better emulsification properties than the non-hydrolyzed DLW (Table 12). While the EC and ES values of these hydrolysates were comparable to those of WPC 34 and WPI, their EA values were significantly higher (P < 0.05). Both hydrolysates had moderate DH, less than 6%, further confirming that moderate hydrolysis result in enhanced functionality.

On the other hand, the DLW hydrolysates, produced using papain under optimal conditions for maximal EA, did not show any improvement in the emulsification properties of DLW. This observation could be partially attributed to the low DH obtained (2.54%). This observation highlights the importance of considering the extent of hydrolysis when aiming at optimizing hydrolysis conditions.

The DLW hydrolysate, produced using papain under optimal conditions for maximal EC, had significantly higher EC than DLW, WPC 34 and WPI, yet low EA. Within the range tested for papain, the derived optimal conditions did not conclusively result in enhanced emulsification properties.

Part to Carach	DU (0()	EC		ES (min)	
Protein Sample	DH (%)	(g oil/g protein)	EA		
WPI	-	1738 ^ª	0.662 ^b	31.93 ^{bc}	
DLW	-	1387 ^{cd}	0.365 ^d	19.63 ^c	
WPC 34	-	1454 ^{bcd}	0.485 ^c	21.37 ^c	
EA optimized with alcalase hydrolysis (45 °C, e/s = 1.5, 15 min, pH 8.5)	5.92 ^{a*}	1643 ^{ab}	0.772 ^ª	57.26 ^b	
EC optimized with alcalase hydrolysis (45 °C, e/s = 0.5, 75 min, pH 8.5)	5.73ª	1571 ^{abc}	0.742 ^ª	49.08 ^{bc}	
EA optimized with papain hydrolysis 60 °C, e/s = 0.5, 15 min, pH 6.5)	2.54 ^b	1316 ^d	0.409 ^d	21.78 ^c	
EC optimized with papain hydrolysis (62 °C, e/s = 1.3, 75 min, pH 8.5)	5.83ª	1732 ^a	0.239 ^e	77.69 ^ª	

Table 12. Mean values (n = 3) of DH and emulsification parameters of hydrolyzed DLW produced under selected optimized conditions, in comparison to WPI, WPC 34, and non-hydrolyzed DLW.

* Means in each column followed by the same lower case letter are not significantly different according to either Duncan or Tukey-Kramer multiple means comparison test ($P \le 0.05$); n=3.

Results and Discussion for Objective Two:

Cook Loss

Although the same beef sample was used in all formulation and water content was adjusted for consistency, slight differences in the initial moisture and fat contents were observed among the different beef formulations (Table 13). Theses variations could be attributed to some losses during handling of the beef patties and distribution to various containers. It seems that some formulations were more prone to lose moisture and fat during handling than others.

No significant difference in water loss (15-20%) upon cooking was observed among the control and beef patties formulated with DLW, WPI and WPC34 (Table 14). However, in beef patties formulated with hydrolyzed DLW, both the Alcalase and the papain hydrolysate, lost about half as much moisture (Table 13). Similarly, the fat loss

in the beef patties formulated with hydrolyzed DLW was about 5 times less than that of the control, and about two times less than that of the beef patty formulated with WPC34. The percent loss in fat upon cooking of beef patties formulated with hydrolyzed DLW was not significantly different from that of the beef patties formulated with WPI. Considering both the fat and the moisture losses, the total cook loss of the control beef patty was ~ 18% while that of the beef patties formulated with hydrolyzed DLW was only ~7%. The total cook loss of the beef patty formulated with WPI was ~14%.

These observations confirmed the enhancement in the emulsification properties of hydrolyzed DLW. The enhanced emulsification properties of hydrolyzed DLW led to improved retention in both moisture and fat, similar if not greater than that of WPI. Similar findings were reported by Barbut (2007, 2010), confirming the positive impact of hydrolysates with superior emulsification properties, on cook loss of meat products.

Along with the formation of an interfacial protein film, gelation is important for fat stabilization in meat products (Zorba, Kurt and Gençcelep, 2005; Youssef and Barbut, 2010). The hydrolyzed DLW did not form a thermally induced gel (Chapter two), however, it might have formed intermolecular interactions with meat proteins and thus enhanced the formation of a gel upon cooking and better entrapment of water and fat. This assumption needs further confirmation in a model system containing both meat proteins and DLW.

Non-hydrolyzed DLW had a lower cook loss (~ 10%) than WPI and WPC34. This finding was initially thought to be the result of the high levels of lactose in DLW. However, previous work indicated that lactose had no impact on the cook loss of meat products. Barbut (2010) monitored the cook loss of batters fortified with lactose and no significant difference was found between the control and products fortified with lactose. Thus, the high lactose content of DLW was not likely the cause of the improved cook loss observed in patties fortified with DLW. The difference observed between DLW and the other non-hydrolyzed whey protein ingredients might actually be due to differences in pH, ionic strength and initial processing of the whey ingredients. The pH was not monitored, but the samples fortified with DLW contain more salt because DLW is slightly higher in mineral (5%) than WPI (2-3%). Additionally, the matching of the protein content of DLW to that of WPI in the formulations must have resulted in amplified amount of minerals in DLW patties. The higher mineral content might have facilitated improvement in the gelation and emulsification properties of the proteins in the system, thus leading to better water and fat retention.

Beef Patties Fortified with	Initial Moisture	Initial Fat	Water loss	Fat Loss
	Content (%)	Content (%)	(%)	(%)
Alcalase Hydrolyzed DLW	68.30	14.89	8.37 ^b	13.0 ^c
Papain Hydrolyzed DLW	64.49	14.12	8.12 ^b	17.9 ^{bc}
DLW	58.97	16.41	15.3 ^ª	9.60 ^c
WPI	58.40	17.51	19.6 ^ª	17.7 ^{bc}
WPC34	57.21	16.55	15.8 ^ª	24.8 ^b
Control	65.37	12.02	16.0 ^ª	62.6 ^ª

Table 13. Initial moisture and fat content of the different beef patties and % loss in moisture and fat upon cooking.

^{*} Means in each column followed by the same lower case letter are not significantly different according to either Duncan or Tukey-Kramer multiple means comparison test ($P \le 0.05$); n = 3.

Texture Analysis

The beef patty formulated with Alcalase hydrolyzed DLW had the highest compression force, which was not significantly different from that of the beef patty formulated with WPI (Figure 9). The compression force of the beef patty formulation with papain hydrolyzed DLW was not significantly different from those of the control and the beef patties formulated with WPC34 and non-hydrolyzed DLW.



Figure 9. Compression forces of the control and of beef patties formulated with WPI, WPC34, non-hydrolyzed DLW, Alcalase hydrolyzed DLW, and papain hydrolyzed DLW. Different lower case letters above the bars indicate significant differences according to Tukey-Kramer multiple means comparison test ($P \le 0.05$); n = 18.

A higher compression force indicates better elasticity and reduced potential to crumble upon processing (Barbut, 2010). The observed increase in compression force in beef patties formulated with Alcalase hydrolyzed DLW could be the result of either the co-gelling of whey proteins with meat proteins, or an increase in elasticity due to a greater fat retention. Previous studies suggested that, reducing the fat content significantly decreased the hardness of meat batters. Because water provides less resistance to compression (Youssef and Barbut, 2010), increased proportions of water lead to decreased hardness as well. Good emulsifiers such as caseinates were shown to increase the hardness of beef frankfurters (Barbut, 2010).

Although fat and moisture losses of beef patties formulated with Alcalase hydrolyzed DLW and those of papain hydrolyzed DLW were not significantly different (Table 13), compression forces were significantly (P < 0.05) different (Figure 9). This might be due to the differences in the strength of the matrix formed, possibly a result of the differences in DH between the two hydrolysates. The DH (5.92%) of Alcalase hydrolyzed DLW was significantly lower than that (7.76%) of papain hydrolyzed DLW (Chapter two). The higher DH coupled with the nature of the peptides released could have potentially caused the formation of a weaker matrix.

Similarly, the enhanced retention of water and fat of the beef patties formulated with non-hydrolyzed DLW did not contribute to enhanced texture, as compared to that of the control and those formulated with WPI and WPC34. This observation could be attributed to several reasons including but not limited to the salt and lactose content and their interaction effects on the protein matrix formed (Wong et al. 1996).

Sensory Analysis

No significant differences (P < 0.05) were observed in any of the evaluated sensory attributes among the different beef patties (Table 14). Overall, the panelists did not discriminate between the different beef samples. This observation potentially indicated that the formulation with hydrolyzed DLW did not affect the sensory quality of the beef patties, although instrumental measurements indicated functional differences.

Most importantly, no bitterness or off-flavor was detected in the samples formulated with hydrolyzed DLW. Both hydrolysates had a DH lower than 8% (Objective One results). At a DH of less than 8%, no perceived bitterness due to released peptides is expected (Rios et al. 2004).

The tested DLW hydrolysates can thus be incorporated into meat products to amplify the functional properties without jeopardizing the overall sensory quality. During reformulation, food developers aim at making favorable changes for functional and economic benefits, without incurring any perceivable changes in flavor (Lawless, 2010).

Table 14. Mean rating (\pm SE) of the different sensory attributes (n = 20) of the control and the beef patties formulated with Alcalase hydrolyzed DLW, papain hydrolyzed DLW, non-hydrolyzed DLW, WPI, and WPC34.

Beef Patty	Sensory Attributes [*]										
,	Off-flavor	Bitterness		Saltiness		Juiciness		Firmness		Toughness	
Alcalase Hydrolyzed DLW	0.45 ± 0.18	0.42 0.15	±	8.13 0.57	±	9.62 0.65	±	7.16 ± 05	55	7.32 0.77	±
Papain Hydrolyzed DLW	0.28 ± 0.16	0.12 0.07	±	8.64 0.72	±	9.54 0.59	±	7.70 0.60	±	8.33 0.74	±
DLW	0.46 ± 0.31	0.08 0.05	±	8.68 0.74	±	8.39 0.63	±	7.91 0.72	±	8.27 0.79	±
WPI	0.55 ± 0.22	0.16 0.11	±	8.29 0.75	±	9.21 0.58	±	7.26 0.45	±	7.94 0.72	±
WPC 34	0.06 ± 0.05	0.21 0.11	±	7.96 0.60	±	8.61 0.61	±	7.69 0.52	±	8.14 0.70	±
Control	1.05 ± 0.29	0.32 0.14	±	8.12 0.65	±	8.31 0.60	±	7.75 0.58	±	8.76 0.72	±

* No significant difference (P < 0.05) was observed in any of the evaluated sensory attributes among the different beef patties; n = 20 (ANOVA Table 26 in Appendix G).

Conclusions

The RSM approach to enhance the functionality of DLW demonstrated the effect of each hydrolysis parameter on the DH and on the assessed functional properties. It also provided the optimized enzymatic hydrolysis conditions that elicited desired functional enhancement. Moderate and controlled hydrolysis of DLW using Alcalase or papain significantly amplified its functional properties. Specifically, the emulsification capacity and activity of the DLW hydrolysates produced under optimized conditions were comparable to and sometimes greater than that of WPI, which is known for its exceptional functionality. The extent of hydrolysis had a pronounced effect on the final functionality. Moderate DH levels resulted in enhanced emulsification properties, while high DH was detrimental. Additionally, within the ranges tested, samples' ability to gel was impaired by hydrolysis even at low DH.

Nevertheless, results of this work showed, for the first time, that the functionality of the protein component of DLW can be amplified upon limited and controlled hydrolysis. The functional performance of DLW hydrolysates in formulated beef patties was comparable to that of WPI. Both Alcalase and papain hydrolyzed DLW, with enhanced emulsification properties, resulted in reduced loss in moisture and fat of cooked beef patties, and thus reduced cook loss. Of the two hydrolysates, DLW hydrolyzed with Alcalase under optimized conditions enhanced the texture of beef patties as compared to a control. Additionally, the hydrolysates did not jeopardize the sensory quality of the beef patties.

Overall, results indicated that DLW subjected to controlled and limited/moderate hydrolysis can easily replace WPI in processed meat applications. The use of hydrolyzed DLW in meat products will result in a dual benefit to both the dairy industry through adding value to a dairy byproduct, and to the meat industry by reducing cost.

Further hydrolysis optimization using RSM with wider ranges is recommended to confirm the true optimal hydrolysis conditions for a directed improvement in functionality. Even though the results of the present study show that limited hydrolysis can significantly improve emulsification properties, there is still room for improvement of the response variables and determination of their true optimized conditions. Some of the tridimensional plots did not show a saddle point, indicating that the response variables would potentially increase more outside the limit of the ranges tested. Therefore extension of the tested ranges is needed to determine true optimized response variables with possibly higher values. Wider ranges may lead to identifying conditions that can actually improve the gelling properties. Future work should also address the impact of lactose and salt contents on the gelation properties of DLW.

With more confirmatory work a functionally enhanced DLW may be produced that would have a great potential to reduce processing cost by replacing WPI in various food applications, while maintaining acceptable quality.

References

- Adler-Nissens, J. (1986). Enzymic hydrolysis of food proteins. (1st ed.). New York: Elsevier applied science publishers.
- Agboola, S.O., Singh, H., Munro, P., Dalgleish, D.G., & Singh, A.M. (1998). Destabilization of oil-in-water emulsions formed using highly hydrolysed whey proteins. *Journal of Agricultural and Food Chemistry*, 46, 84–90.
- Ahmedna, M., Prinyawiwatkul, W., & Rao, R. (1999). Solubilized wheat protein isolate: functional properties and potential food applications. *Journal of Agricultural and Food Chemistry*, 47, 1340-1345.
- Ariyoshi, Y. (1993). Angiotensin converting enzyme inhibitors derived from food proteins. *Trends Food Science and Technology*, 4, 139-144.
- Aziznia, S., Khosrowshahi, A., Madadlou, A., & Rahimi, J. (2008). Whey protein concentrate and gum tragacanth as fat replacers in nonfat yogurt: chemical, physical, and microstructural properties. *Journal of Dairy Science*, 91, 2545-2552.
- Balagtas, J.V., F.M.Hutchinson, J.M.Krochta, and D.A. Sumner. (2003). Anticipating market effects of new uses for whey and evaluating returns to research and development. *Journal of Dairy Science*, 86, 1662-1672.
- Barbut, S. (2007). Effect of hydrolyzed and regular dairy proteins on the texture, colour and microstructure of poultry meat emulsions. *British Poultry Science*, 48, 655-660.
- Barbut, S. (2010). Effects of milk powder and its components on texture, yield, and color of a lean poultry meat model system. *Poultry Science*, 89, 1320–1324.
- Beecher, J. W., Drake, M. A., Luck, P. J., & Foegeding, E. A. (2008). Factors regulating astringency of whey protein beverages. *Journal of Dairy Science*, 91, 2553-2560.
- Belloque, J., Chicón, R., & López-Fandiño, R. (2007). Unfolding and refolding of β-lactoglobulin subjected to high hydrostatic pressure at different pH values and temperatures and its influence on proteolysis. *Journal of Agricultural and Food Chemistry*, 55, 5282-5288.
- Blaschek, K. M., Wendorf, W. L. & Rankin S. A. (2007). Survey of salty and sweet whey composition from various cheese plants in Wisconsin. *Journal of Dairy Science*, 90, 2029-2034.
- Bouaouina, H., Desrumaux, A., Loisel, C., & Legrand, J. (2006). Functional properties of whey proteins as affected by dynamic high-pressure treatment. *International Dairy Journal*, 16, 275-284.
- Bonnaillie, L. M., & Tomasula, P. M. (2008). Whey protein fractionation. In C.I. Onwulata, & P.J. Huth (Eds.), Whey processing, functionality and health benefits (pp 15–38). Massachusetts: Wiley-Blackwell and Institute of Food Technologists Press.
- Bouaouin, H., Desrumaux, A., Loisel, C., & Legrand J. (2006). Functional properties of whey proteins as affected by dynamic high-pressure treatment. *International Dairy Journal*, 16, 275-284.
- Britten, M., Giroux, H.J., Jean, Y., & Rodrigue, N. (1994). Composite blends from heat denatured and undenatured whey protein: emulsifying properties. *International Dairy Journal*, 4, 25-26.

- Caessens, P.W.J.R., Daamen, W.F., Gruppen, H., Visser, S., & Voragen, A.G.J. (1999). *θ*-lactoglobulin hydrolysis 2 Peptide identification, SH/SS exchange, and functional properties of hydrolysate fractions formed by the action of plasmin. *Journal of Agricultural and Food Chemistry*, 47, 2980–2999.
- Chaplin, L. C., & Andrew, A. T. (1989). Functional properties of peptides derived from casein proteolysis. *Journal of Dairy Research*, 56, 544-548.
- Cheison, S. C., Wang, Z., & Xu, S. Y. (2007). Multivariate strategy in screening of enzymes to be used for whey protein hydrolysis in an enzymatic membrane reactor. *International Dairy Journal*, 17, 393-402.
- Chicón, H., Belloque, J. Alonso, E., & López-Fandiño, R. (2009). Antibody binding and functional properties of whey protein hydrolysates obtained under high pressure. *Food Hydrocolloids*, 23, 593-599.
- Chove, B. E., Grandison, A. S., & Lewis, M. J. (2001). Emulsifying properties of soy protein isolate fractions obtained by iso-electric precipitation. *Journal of the Science of Food and Agriculture*, 81, 759-763.
- Clare, D. A., & Swaisgood, H. E. (2000). Bioactive milk peptides: a prospectus. *Journal of Dairy Science*, 86, 1187-1195.
- Creusot, N., & Gruppen, H. (2007). Enzyme-induced aggregation and gelation of proteins. *Biotechnology Advances*, 25, 597-601.
- Creusot, N., & Gruppen, H. (2008). Hydrolysis of whey protein isolate with *Bacillus licheniformis* protease: aggregating capacities of peptide fractions. *Journal of Agricultural and Food Chemistry*, 56, 10332-10339.
- Cruz, A. G., Faria, J. A. F., Walter, E. H. M., Andrade, R. R., Cavalcanti, R. N., Oliveira, C. A. F., Granato, D. (2010). Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology. *Journal of Dairy Science*, 93, 5059-5068.
- De Wit, J.N., & van Kessel, T. (1996). Effects of ionic strength on the solubility of whey protein products: A colloid chemical approach. *Food Hydrocolloids*, 10, 143-149.
- Dalgleish, D. G., & Singh, A. M. (1998). The emulsifying properties of hydrolysates of whey protein. *Journal of Dairy Science*, 81, 918–924.

Damodaran, S., Parkin, K.L., Fennema, O.R. (2008). Fennema's Food Chemistry. (4th ed.). Boca Raton: CRC Press.

- Davis, J. P., Doucet, D., & Foegeding, E. A. (2005). Foaming and interfacial properties of β-lactoglobulin. *Journal* of colloid and Interface Science, 288, 412-422.
- Deeslie, W. D., & Cheryan, M. (1988). Functional properties of soy protein hydrolysates from a continuous ultrafiltration reactor. *Journal of Agricultural and Food Chemistry*, 36, 26-31.
- Doucet, D., Gauthier, S. F., Otter, D. E., & Foegeding, E. A. (2003). Enzyme-induced gelation of extensively hydrolyzed whey proteins by alcalase: Comparison with the plastein reaction and characterization of interactions. *Journal of Agricultural and Food Chemistry*, 51, 6036–6042.
- Euston, S. R., Finnigan, S. R., & Hirst, R. L. (2001). Aggregation kinetics of heated whey protein-stabilised emulsions: effect of low-molecular weight emulsifiers. *Food Hydrocolloids*, 15, 253-262.
- Etzel, M.R. (2004). Manufacture and use of dairy protein fractions. *Journal of Nutrition*, 134, 996-1002.

- Fagan, C. C., Castillo, M., Payne, F. A., O'Donnell, C.P., & O'Callaghan, D. J. (2007). Effect of cutting time, temperature, and calcium on curd moisture, whey fat losses, and curd yield by response surface methodology. *Journal of Dairy Science*, 90, 4499-4512.
- Farrell Jr., H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K., Hicks, C. L., Hollar, C. M., Ng-Kwai-Hang, K. F., Swaisgood, H. E. (2004). Nomenclature of the proteins of cows' milk sixth revision. *Journal of Dairy Science*, 87, 1641-1674.
- Fennema, O.R. (1996). Food chemistry. (3rd ed.). New York: Marcel Dekker.
- Ferreira, I., Pinho, O., Mota, M.V., Tavares, P., Pereira, A., & Goncalves, M. P. (2007). Preparation of ingredients containing an ACE inhibitory peptide by tryptic hydrolysis of whey protein concentrates. *International Dairy Journal*, 17, 481-487.
- Foegeding, E. A., Davis J. P., Doucet D., & McGuffey M. K. (2002). Advances in modifying and understanding whey protein functionally. *Trends in Food Science and Technology*, 13, 151-159.
- Foegeding, E.A. & Luck, P.J. (2003). Whey Protein Products. In H. Roginski, J. W. Fuquay, P. F. Fox (Eds.), Encyclopedia of Dairy Sciences (pp. 1957-1960). New York: Academic Press.
- Food and Drug Administration (FDA). (1999). Food labeling: Health claims, soy protein and coronary heart disease. *Fed. Regist*, 57, 699-733.
- Gauthier, S. F., Paquin, P., Pouliot, Y., & Turgeon, S. (1993). Surface activity and related functional properties of peptides obtained from whey proteins. *Journal of Dairy Science*, 76, 321-328.
- Gauthier, S. F., Pouliot, Y., & Saint-Sauveur, D. (2006). Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins (Review). *International Dairy Journal*, 16, 1315-1323.
- Gobbetti, M., Minerini F., & Rizzello, C. G. (2004). Angiotensin I-converting-enzyme-inhibitory and antimicrobial bioactive peptides. *International Journal of Dairy Technology*, 57, 173–188.
- Goodall, S., Grandison, A. S., Jauregi, P. J. & Price J. (2009). Selective separation of the major whey proteins using ion exchange membranes. *Journal of Dairy Science*, 91, 1-10.
- Groleau, P.E., Morin, P., Gauthier, S. F., & Pouliot, Y. (2003). Effect of physicochemical conditions on peptidepeptide interactions in a tryptic hydrolysate of *θ*-lactoglobulin and identification of aggregating peptides. *Journal of Agricultural and Food Chemistry*, 51, 4370-4375.
- Guadix, A., Camacho, F. & Guadix, E. M. (2006). Production of whey protein hydrolysates with reduced allergenicity in a stable membrane reactor. *Journal of Food Engineering*, 72, 398-405.
- Guan, X., Yao, H. Y., Chen, Z. X., Shan, L., & Zhang, M. D. (2007). Some functional properties of oat bran protein concentrate modified by trypsin. *Food Chemistry*, 101, 163-170.
- Guzey, D., & McClements, D. J. (2007). Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by beta-lactoglobulin-pectin complexes. *Journal of Agricultural & Food Chemistry*. 55, (2): 475-485.
- Haque ZU; Bohoua GL; Williams J; Mikel WB. (2010). Influence of whey peptides on the surface activity of k-casein and ß-lactoglobulin A. *Society of Dairy Technology*, 63, 190-196.
- Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, 53, 588-593.

- Hongsprabhas, P., & Barbut, S. (1999). Use of cold-set whey protein gelation to improve poultry meat batters. *Poultry Science*, 78, 1074-1078.
- Huang, X. L., Catignani, G. L., Swaisgood, H. E., (1999). Modification of rheological properties of whey protein isolates by limited proteolysis. *Food / Nahrung*, 43, 79–85.
- Hughes, E., Mullen, A. M., & Troy, D. J. (1997). Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. *Journal of Meat Science*, 48, 169-180.
- Hustad, O., & Richardson, T. (1972). Rigid Polyurethane Foams Extended with Partially Deladosed Whey Powder. *Journal of Dairy Science*, 55, 1388-1395.
- Inoue, K., Ochi, H., Taketsuka, M., Saito, H., Sakurai, K., Ichihashi, N., Iwatsuki, K., & Kokubo, S. (2008). Modeling of the effect of freezer conditions on the principal constituent parameters of ice cream by using response surface methodology. *Journal of Dairy Science*, 91, 1722-1732.
- Ipsen, R., Otte, J., Sharma, R., Nielsen, A., Gram, H. L., & Bruun, Q. K. (2001). Effect of limited hydrolysis on the interfacial rheology and foaming properties of β-lactoglobulin A. *Colloids and Surfaces B: Biointerfaces*, 21, 173–178.
- Jambark, A. E., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, 86, 281-287.
- Jiménez-Castaño, L., Villamiel, M., & López-Fandiño, R. (2007). Glycosylation of individual whey proteins by Maillard reaction using dextran of different molecular mass. *Food Hydrocolloids*, 21, 433-443.
- Jost, R., & Monti, J. C. (1977). Partial enzymatic hydrolysis of whey protein by trypsin. *Journal of Dairy Science*, 60, 1387-1393.
- Ju, Z. Y., Hettiarachchy, N., & Kilara A. (1999). Thermal properties of whey protein aggregates. *Journal of Dairy Science*, 82, 1882-1889.
- Ju, Z. Y., Otte, J., Madsen, J. S., & Qvist K. B. (1995). Effects of limited proteolysis on gelation and gel properties of whey protein isolate. *Journal of Dairy Science*, 78, 2119-2128.
- Kaneko, T., Wu, B. T. & Nakai, S. (1985). Selective concentration of bovine immunoglobulins and α-lactalbumin from acid whey using FeCl₃. *Journal of Food Science*, 50, 1531-1537.
- Kerker, M. (1969). The scattering of light and other electromagnetic radiation. (1st ed.). London: Academic Press.
- Kilara, A., & Panyam, D. (2003). Peptides from milk proteins and their properties. *Critical Reviews in Food Science and Nutrition*, 43, 607-633.
- Kim, S.B., Seo I.S., Khan, M.A., Ki, K.S., Nam, M.S., Kim, H.S. (2007). Separation of iron-binding protein from whey through enzymatic hydrolysis. *International Dairy Journal*, 17, 625–631.
- Kitts, D.D., & Weiler, K. (2003). Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design*, 9, 1309-1323.

Klink, I.M., Phillips, & R.J., Dungan, S.R. (2010). Effect of emulsion drop-size distribution upon coalescence in simple shear flow: a population balance study. *Journal of colloid and interface science*, 353, 467-475.

- Kneifel, W., Paquin, P., Abert T., & Richard, J.-P. (1991). Water holding capacity of proteins with special regard to milk proteins and methododical aspects. *Journal of Dairy Science*, 74, 2027-2041.
- Kong, X., Li, X., Wang, H., Hua, Y., & Huang, Y. (2008). Effect of lipoxgenase activity in defatted soybean flour on the gelling properties of soybean protein isolate. *Food Chemistry*, 106, 1093-1099.
- Konrad, G., Kleinschmidt, T., Rohenkohl, H., Reimerides, & E. H. (2005). Peptic partial hydrolysis of whey protein concentrate for modifying the surface properties of whey protein. II. Effects on the emulsifying and foaming properties. *Milchwissenschaft*, 60, 195-198.
- Kontopidis, G., Holt, C. & Sawyer, L., 2004. Invited review: β-lactoglobulin: binding properties structure and function. *Journal of Dairy Science*, 87, 785 796.
- Korhonen, H., Pihlanto-Leppälä, A., Rantamäki, P., & Tupasela, T. (1998). Impact of processing on bioactive proteins and peptides. *Trends in Food Science and Technology*, 9, 307–319.
- Kuwata, T., Pham, A. M., Ma, C., Nakai, Y. (1985). Elimination of β-lactoglobulin from whey to simulate human milk protein. *Journal of Food Science*, 50, 605–609.
- Laca, A., Paredes, B., Diaz, M. (2011). Thermal behavior of lyophilized egg yolk fractions. *Journal of Food Engineering*, 102, 77-86.
- Lawless, H. T., & Heymann, H. (2010). Sensory evaluation of food. (2nd ed.). New York: Springer-Verlag.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.
- Lenth, R. V. (2009). Response surface methods in R, using rsm. Journal of Statistical Software, 32, 1-17.
- Liu, D., Zhou, P., Liu, X., & Labuza, T. P. (2008). Moisture-induced aggregation of whey proteins in a protein/buffer model system. *Journal of Agricultural and Food Chemistry*, 56, 4534-4540.
- Lupano, C. E. (1994). Effect of heat treatments in very acidic conditions on whey protein isolate properties. *Journal of Dairy Science*, 77, 2191-2198.
- Madsen, J.S., Ahmt, T.Ø., Otte, J., Halkier, T., & Qvist, K.B. (1997). Hydrolysis of β-lactoglobulin by four different proteinases monitored by capillary electrophoresis and high performance liquid chromatography. *International Dairy Journal*, 7, 399–409.
- Maduko, C.O., Akoh, C.C., Park, Y.W. (2007). Enzymatic Production of Infant Milk Fat Analogs Containing Palmitic Acid: Optimization of Reactions by Response Surface Methodology. *Journal of Dairy Science*, 90, 2147-2154.
- Madureira, A.R., Pereira, C.I., Gomes, A.M.P., Pintado, M.E., & Malcata, F.X. (2007). Bovine whey proteins overview on their main biological properties. *Food Research International*, 40, 1197–1211.
- Mahmoud, M. I. (1994). Physiological and functional properties of protein hydrolysates in nutritional products. *Food Technology*, 48, 89-95.

- Maier, I., Okun, V. M., Pittner, F., & Lindner, W. (2006). Changes in peptic digestibility of bovine β-lactoglobulin as a result of food processing studied by capillary electrophoresis and immunochemical methods. *Journal of Chromatography B*, 841, 160-167.
- Mate, J. I., & Krochta, J. M. (1994). ß-lactoglobulin separation from whey protein isolate on a large scale. *Journal* of Food Science, 59, 1111-1114.
- Matoba, T., & Hata, T. (1972). Relationship between bitterness of peptides and their chemical structures. *Agriculture and Biological Chemistry, 36*, 1423-1431.
- Meisel, H. (2005). Biochemical properties of peptides encrypted in bovine milk proteins. *Current Medicinal Chemistry*, 12, 1905-1919.
- Meisel, H., & FitzGerald, R.J. (2000). Opioid peptides encrypted in intact milk protein sequences. *The British Journal of Nu*trition, 84, 27-31.
- Mine, Y. and Shahidi, F. 2006. Nutraceutical proteins and peptides in health and disease: an overview. Boca Raton: CRC Press Taylor & Francis Group.
- Mishra, S., Mann, B., & Joshi, V.K. (2001). Functional improvement of whey protein concentrates on interaction with pectin. *Food Hydrocolloids*, 15, pp. 9–15.
- Modler, H. W. & Emmons. D. B. (1977). Properties of Whey Protein Concentrate Prepared by Heating Under Acidic Conditions. *Journal of Dairy Science*, 60, 177-184.
- Mutilangi, W. A. W., Panyam, D., & Kilara A. (1996). Functional properties of hydrolysates from proteolysis of heat denatured whey protein hydrolysates. *Journal of Food Science*, 61, 270-275.
- Myers, R. H., Montgomery, D.C., & Anderson-Cook, C. M. (2009). Response surface methodology: product and process optimization using designed experiments. (3rd ed.). New York : John Wiley & Sons, (Chapter 1).
- Nakamura, T., Sado H., Syukunobe Y., & Hirata T. (1993). Antigenicity of whey protein hydrolysates prepared by combination of two proteinases. *Milchwissenschaft*, 48, 667-670.
- Nielsen PM, Petersen D., & Dambmann C. (2001). Improved method for determining food protein degree oh hydrolysis. *J Food Sci.*, 66, 642-646.
- Nielsen, M. A., Coulter, S. T., Morr, C. V., & Roseneau, J. R. (1973). Four factor response surface experimental design for evaluating the role of processing variables upon protein denaturation in heated whey systems. *Journal of Dairy Science*, 56, 76-83.
- O'Keefe, A. M. Kelly, J. (1981). Solubilization of denatured whey protein. *Netherlands Milk and Dairy Journal*, 35, 392.
- Onwulata, C. I., & Huth, P. J. (2009). Whey processing, functionality and health Benefits. (1st ed.). New Jersey:Wiley-Blackwell (Chapter 5).
- Otani, H. (1987). Antigenically reactive regions of bovine milk proteins, Japan. *Agriculture Research Quarterly*, 21, 135–142.
- Otte, J., Ju, Z. Y., Faergemand, M., Lomholt, S. B., & Qvist K. B. (1996). Protease-induced aggregation and gelation of whey proteins. *Journal of Food Science*, 61, 911, 923–915, 923.
- Pallavicini, C., Pagliarini, E., & Per, C. (1988). Effect of a proteolytic enzyme preparation (Alcalase) on whey proteins. *Journal of Dairy Science*, 71, 887-892.

- Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation a of turbimetric technique. *Journal of Agricultural and Food Chemistry*, 26, 716-723.
- Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. Lebensmittel-Wissenschaft und- Technologie, 38, 77-80.
- Pelegrine, D.H.G., & Gasparetto C.A. (2006). A comparative study between ovalbumin and lactoglobulin fouling in a tube hot surface. *Journal of Food Engineering*, 73, 394-401.
- Pena-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science*, 64, 259-263.
- Perea, A., Ugalde, U., Rodriguez, I. & Serra, J.L. (1993). Preparation and characterization of whey protein hydrolysates: applications in industrial whey bioconversion processes. *Enzyme and Microbial Technology*, 15, 418-423.
- Potter M and Tome D (2008) Comparison of digestibility and quality of intact proteins with their respective hydrolysates. *Journal of AOAC International* 91 1002–1005.
- Pouliot, Y., Guy, M. M., Tremblay, M., Gaonac'h, A. C., Ting, B. P. C. P, Gauthier, S. F., & Voyer, N. (2009). Isolation and characterization of an aggregating peptide from a tryptic hydrolysate of whey proteins. *Journal of Agricultural and Food Chemistry*, 57, 3760-3764.
- R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rabiey, L., & Britten, M. (2009a). Effect of protein composition on the rheological properties acid-induced whey protein gels. *Food Hydrocolloids*, 23, 973-979.
- Rabiey, L., & Britten, M. (2009b). Effect of whey protein enzymatic hydrolysis on the rheological properties of acid-induced gels. *Food Hydrocolloids*, 23, 2302-2308.
- Rahali, V., Chobert, J.-M., Haertlé, T., & Guéguen, J. (2000). Emulsification of chemical and enzymatic hydrolysates of β-lactoglobulin. Characterization of the peptides adsorbed at the interface. Nahrung, 44, 89–95.
- Rickert, D.A., Johnson, L. A. & Murphy, P. A. (2004). Functional properties of improved glycinin and β-conglycinin fractions. *Journal of Food Science*, 69, 303-311.
- Ryan, K. N. B., Vardhanabhuti, B., Jaramillo, D.P., van Zanten, J.H., Coupland, J.N., & Foegeding, E.A. (2011). Stability and mechanism of whey protein soluble aggregates thermally treated with salts. *Food Hydrocolloids*, 27, 411-420.
- Sava, N., van der Plancken, I., Claeys, W., & Hendrickx, M. (2005). The kinetics of heat-induced structural changes of beta-lactoglobulin. *Journal of Dairy Science*, 88, 1646-1653.
- See, S. F., Hoo, L. L. & Babji A. S. (2011). Optimization of enzymatic hydrolysis of Salmon (Salmo salar) skin by Alcalase. *International Food Research Journal*, 18, 1359-1365.
- Schingoethe, D. J. (1976). Whey utilization in animal feeding: a summary and evaluation. *Journal of dairy science*, 59, 556-570.
- Schlimme, E., & Meisel, H. (1995). Bioactive peptides derived from milk proteins. Structural, physiological and analytical aspects. *Die Nahrung*, 39, 1-20.

- Schmidt, R. H., Packard, V. S. & Morris H. A. (1984). Effect of processing on whey protein functionality. *Journal of Dairy Science*, 67, 2723-2733.
- Serdaroglu, M. (2006). Improving low fat meatball characteristics by adding whey powder. *Meat Science*, 72, 155–163.
- Silva, V.M., Park, K.J., Hunbinger , A D. (2010). Optimization of the enzymatic hydrolysis of mussel meat. *Journal* of Food Science, 75, 36-42.
- Sinha, R., Radha, C., Prakash, J., & Kaul, P. (2007). Whey protein hydrolysate: functional properties, nutritional quality and utilization in beverage formulation. *Food Chemistry*, 101, 1484-1491.
- Smilowitz, J. T., Dillard, C. J., & German, J. B. (2005). Milk beyond essential nutrients: The metabolic food. *Australian Journal of Dairy Technology*, 60, 77-83.
- Smithers, G.W. (2008). Whey and whey proteins from 'gutter to gold'. International Dairy Journal, 18, 695-704.
- Spellman, D., Kenny, P., O'Cuinn, G., & FitzGerald, R. J. (2005). Aggregation properties of whey protein hydrolysates generated with Bacillus licheniformis proteinase activities. *Journal of Agricultural and Food Chemistry*, 5, 1258-1265.
- Spellman, D., O'Cuinn, G., & FitzGerald, R.J. (2009). Bitterness in *Bacillus* proteinase hydrolysates of whey proteins. *Food Chemistry*, 114, 440-446.
- Sternberg, M., Chiang, J.-P., & Eberts, N. J. (1975). Cheese whey proteins isolated with polyacrylic acid. *Journal of Dairy Science*, 59, 1042-1050.
- Sun, C., Gunasekaran, S., & Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids*, 21, 555-564.
- Tamime, A. Y. (2009). Dairy powders and concentrated products. (1st ed.). New Jersey: John Wiley & Sons.
- Tang, C.H.; Choi, S.M.; & Ma, C.Y. (2007). Study of thermal properties and heat-induced denaturation and aggregation of soy proteins by modulated differential scanning calorimetry. *Int. J. Biol. Macromol.*, 40, 96-104.
- Tang, S., Hettiarachchy, N. S., Horax R., & Eswaranandam, S. (2003). Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. *Journal of Food Science*, 68, 152-157.
- Tong, P.S., Jordan, W.K., & Houghton, G. (2009). Modeling of the effect of freezer conditions on the hardness of ice cream using response surface methodology. *Journal of Dairy Science*, 92, 5834-5842.
- Turgeon, S.L., Gauthier, S.F., Molle, D., & Leonil, J. (1992). Interfacial properties of tryptic peptides of βlactoglobulin. *Journal of Agricultural and Food Chemistry*, 40, 669–675.
- van Der Ven, C., Gruppen, H., de Bont, D.B.A., & Voragen, A.G.J. (2002). Correlations between biochemical characteristics and foam-forming and stabilizing ability of whey and casein hydrolysates. *Journal of agricultural and Food Chemistry*. 50, 2938-2946.
- Vasbinder, A.J. & de Kruif C.G. (2003). Casein-whey protein interactions in heated milk: the influence of pH. *International Dairy Journal*, 13, 603.
- Vyas, H.K., Izco, J.M., & Jiménez-Flores R. (2002). Scale-up of native β-lactoglobulin affinity separation process. *Journal of Dairy Science*, 85, 1639-1645.

- Webb, N. B., Ivey, F. J., Jones, V. A., & Monroe, J. R. (1970). The measurement of emulsifying capacity by electrical resistance. *Journal of Food Science*, 35, 501-504.
- Whitaker, J. R., Shahidi, F., Munguia, A. L., Yada, R. Y., & Fuller, G. (1997). Functional properties of proteins and lipids. (5th ed.). Washington, DC : American Chemical *Society*.
- Whitehurst, R.J., & Van Oort M. (2009). Enzymes in Food Technology. (1st ed.). Massachusetts: Wiley-Blackwell and Institute of Food Technologists Press.
- Wong, D.W.S. (1986). Mechanism and Theory in Food Chemistry. (1st ed.). New York: Van Nostrand Reinhold International Company Limited.
- Wong, P.Y.Y., & Kitts D. D. (2003). A comparison of the buttermilk solids functional properties to nonfat dried milk, soy protein isolate, dried egg white, and egg yolk powders. *Journal of Dairy Science*, 86, 746-754.
- Ye, A., & Singh, H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: influence of polysaccharides. *Food Hydrocolloides*, 20, 269-276.
- Youssef, M.K., & S. Barbut (2010). Fat reduction in comminuted meat products-effects of beef fat, regular and pre-emulsified canola oil. *Meat Science*, 87, 356-360.
- Zeiger, R.S., Heller, S., Mellon, M., O'Conner, R., & Hamburger, R.N. (1986). Effectiveness of dietary manipulation in the prevention of food allergy in infants. *Journal of Allergy and Clinical Immunology*, 78, 224-238.
- Zhang, G., Foegeding, E. A., & Hardin, C. C. (2004). Effect of sulfated polysaccharides on heat-induced structural changes in β-lactoglobulin. *Journal of Agricultural and Food Chemistry*, 52, 3975-3981.
- Zhong, Q. & Jin, M. (2008). Enhanced functionalities of whey proteins treated with supercritical carbon dioxide. *Journal of Dairy Science*, 91, 490-499.
- Zhou, P., Lui, X., & Labuza, T. P. (2008a). Moisture-induced aggregation of whey proteins in a protein/buffer model system. *Journal of Agriculture and Food Chemistry*, 56, 2048-2054.
- Zhou, P., Lui, X., & Labuza, T. P. (2008b). Effects of moisture-induced whey protein aggregation on protein conformation, the state of water molecules, and the microstructure and texture of high-protein-containing matrix. *Journal of Agriculture and Food Chemistry*, 56, 4534-4540.
- Zhu, D., Damodaran, S., & Lucey, J.A., 2008. The formation of WPI-dextran conjugates in aqueous solutions. *Journal of Agricultural and Food Chemistry*, 56, 7113-7118.
- Zorba, O., Kurt, S., & Genccelep, H. (2005). The effects of different levels of skim milk powder and whey powder on apparent yield stress and density of different meat emulsions. *Food Hydrocolloids*, 19, 149-155.