1-1-2011

Thermostability Of Sweet Whey As Influenced By Thermization And Addition Of Enzymatic Hydrolyzate Of Caseinate

Jingming Tao

THERMOSTABILITY OF SWEET WHEY AS INFLUENCED BY THERMIZATION AND ADDITION OF ENZYMATIC HYDROLYZATE OF CASEINATE

By

Jingming Tao

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Food Science, Nutrition, and Health Promotion
in the Department of Food Science, Nutrition and Health Promotion

Mississippi State, Mississippi

December 2011

THERMOSTABILITY OF SWEET WHEY AS INFLUENCED BY THERMIZATION AND ADDITION OF ENZYMATIC HYDROLYZATE OF CASEINATE

By

Jingming Tao

Approved:

Z. Zee Haque Professor of Food Science, Nutrition and Health Promotion (Major Professor)	J. Byron Williams Assistant Professor of Food Science, Nutrition and Health Promotion (Committee Member)
Ramakrishna Nannapaneni Assistant Research Professor of Food Science, Nutrition and Health Promotion (Committee Member)	Diane K. Tidwell Associate Professor of Food Science, Nutrition and Health Promotion (Committee Member)
Juan L. Silva Professor and Interim Department Head of Food Science, Nutrition and Health Promotion (Graduate Coordinator)	George M. Hopper Dean of College of Agriculture and Life Science

Name: Jingming Tao

Date of Degree: December 9, 2011

Institution: Mississippi State University

Major Field: Food Science, Nutrition, and Health Promotion

Major Professor: Z. Zee Haque

Title of Study: THERMOSTABILITY OF SWEET WHEY AS INFLUENCED BY THERMIZATION AND ADDITION OF ENZYMATIC HYDROLYZATE OF CASEINATE

Pages in Study: 56

Candidate for Degree of Master of Science

Sweet whey is the liquid that separates from the cheese curd during manufacture of cheeses like Edam and Cheddar. Though highly nutritious, problems associated with whey utilizations include variability of desired functional attributes and lack of thermostability (TS), an attribute that is imperative in retort or pasteurization stable high protein drinks. The objective of this study was to determine the influence of pre-heat treatment (thermization) of fresh sweet whey and/or addition of casein hydrolyzate on the subsequent TS of whey protein concentrates (WPC). Fresh sweet wheys were obtained from the Mississippi State University Dairy Plant, separated, thermized for different time periods (5-30 min) at 70°C, vacuum evaporated, and spray dried to obtain WPC. Thermization of Edam and Cheddar whey for 5 and 10 min significantly enhanced TS across all pH (3-7.5) levels studied. Addition of the hydrolyzate to thermized and not thermized Edam whey significantly enhanced the TS.

DEDICATION

I would like to dedicate this research paper to my parents Liyan Tao and Zhongning Cui.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my major professor Dr. Zahur Zee Haque for his guidance and encouragement in this research process.

I also would like to express my gratitude to my committee members: Dr. Diane Tidwell, Dr. J. Byron Williams, and Dr. Ramakrishna Nannapaneni. Special thanks are extended to Mr. David Hall, Jimmy Fox, Mark, TJ, James, and Anthony in the MSU dairy plant for their help with this project. I would like to thank Sashie, who always helps and supports me.

Finally, I would like to express my deepest appreciation to my parents, who gave me life, raised me up, and always support me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sweet whey originates from rennet-coagulated cheese production such as Cheddar or Edam cheese (Tunick 2008). It is commonly used in food systems for its thickening and emulsifying properties. Thermostability (TS) is an important functionality that impacts potential utilization of whey solids in retort/pasteurization stable protein rich food systems such as health drinks. This is particularly meaningful if the TS is high at low pH environments. Protein structure, which plays a critical role in the functionality of proteins, is changed by thermal treatment (Ji and Haque 2003). According to Oldfield et al.(2005) irreversible denaturation of major and minor whey proteins occurred mainly during preheating, Olson and Haque (1998) observed during a survey of five whey plants from different regions of the US, that processes like vacuum evaporation (VE) and lactose crystallization adversely affected emulsifying properties. Previous studies indicated that whey proteins are denatured on heating above 70°C (Jelen and Rattray 1995; Boye and Alli 2000).

However, the effect of different pre-heating or thermization and temp on the functionality of whey proteins has not been thoroughly studied. Haque et. al (Haque 1993; Bohoua and Haque 2006) have reported significant changes in surface activity of milk proteins as reflected by contact angle changes. In a review article, Haque reported alterations of milk protein functionality as a result of the addition of milk protein hydrolyzate (Haque 1993).

Our present research investigates the effect of various levels of pre-heat treatment, or thermization, at 70°C of fresh sweet whey with and without added casein hydrolyzate on its TS.

CHAPTER II

LITERATURE REVIEW

2.1 Whey

Whey is the liquid part of milk remaining after separation of the curd in cheese making. About 8-9 liters of whey is produced for every kilogram of cheese manufactured (Jelen 2003). Cheese making presumably originated in the Fertile Crescent at approximately 6000 B.C. after it was noticed that an acid-coagulated milk gel separated into curds and whey (Onwulata and Huth 2008). Since early centuries, our ancestors made a valiant effort to utilize whey in different forms. For example, in the middle ages whey was applied as a pharmaceutical drug, a component of soothing salves for burns, a skin balm, a potion to inspire vitality and to restore hair, fed to pigs or other livestock, spread on soil as fertilizer, but rarely was it used as a food for humans (Kosikowski 1982; Meat and Livestock Commisssion 2003).

Cheese production has more than doubled in the past 30 years in the United States (Davis, Blayney et al. 2010), as has the production of fluid whey. Most factories simply discharged the waste whey into nearby streams or rivers before the environmental regulations took hold (Cryan, 2001). However, raw whey is a potent pollutant with a biological oxygen demand in 5 days (BOD_5) of 30,000-50,000 ppm (Marwaha and Kennedy 1988) and 68,000 ppm of chemical oxygen demand (COD) (Webb and Whittier 1970). A study conducted by Watson et al. (1977) in the early 1970s showed that spraying whey on the ground to a depth of 25 mm improved the yield of corn and hay

without increasing groundwater pollution. In the 1980s, sewage authorities regulated whey disposal, and eliminated dumping of raw whey into most streams or sewage systems (Kosikowski 1982).

Whey used to be considered a byproduct and of low commercial value. However, with the discoveries of the high nutritional value and other properties as food additives, whey has become an attractive dairy product. Approximately 85% of the total milk used for manufacturing cheese is discarded as whey, which may contain as much as 55% of total milk nutrients (Panesar 2007). The most abundant of these nutrients are lactose, soluble proteins, lipids, and mineral salts (Tango and Ghaly 1999; Onwulata and Tomasula 2004). Although mainly used as a food component, whey waste is the source of great economical losses for the dairy industry, since almost 50% of the total worldwide production is disposed of in waste water treatment plants or on farm fields (Rech and Ayub 2007).

The world production of liquid whey was 85 million metric tons per year in 1983 (Zall 1984), from which the United States produced14.2 million metric tons. According to the data from the Food and Agricultural Organization of the United Nations, over 18 million metric tons of cheese was produced worldwide in 2004. From the cheese data, we could project approximately 150 million metric tons of liquid whey was produced in the same year. Today, the United States still manufactures more than one quarter of the world's dry whey and lactose, 935,000 metric tons, manufactured at over 200 dairy plants (U.S. Dairy Export Council 2011).

Depending on the type of cheese being manufactured, there are two types of liquid whey produced: sweet and acid. Acid whey comes mainly from cottage and ricotta cheeses and has a lower pH value, less than 5.1. Acid whey typically has higher ash and

lower protein content than sweet whey. Because of its acidic flavor and high saline content, its use in alimentation has been limited (Weetall, Havewala et al. 1974; Kosikowski 1982; Mawson 1994). Sweet whey comes from manufacturing Edam, Cheddar, Swiss and similar types of cheese and has a pH value of at least 5.6. Sweet whey contains less acid and calcium, and more lactose than acid whey. Most U.S. whey products are derived from sweet whey.

2.2 Whey Processing

Today, because of the lactose, minerals and protein content, whey is becoming a a highly sought-after product. Thus, a wide range of whey products have been developed by different whey processing technologies. Whey can be processed by various methods such as separation, concentration, fractionation of solids, pasteurization, vacuum evaporation (VE), ultrafiltration, reverse osmosis, ion exchange, gel filtration, electro-dialysis, crystallization and spray-drying (Kosaric and Asher 1985; Spreer and Mixa 1998; Bylund 2003).

Whey must be processed as soon as possible after collection, as its temperature and composition promote the growth of bacteria. Consequently, the whey should be quickly cooled to about 5°C, to temporarily stop bacterial growth (Bylund 2003).

The first stage is filtering of the curd particles left in the whey, followed by separation of fat and casein fines, partly to increase the economic yield and partly because these constituents interfere with subsequent treatment. Casein fines have an adverse effect on fat separation, and therefore, should be removed first (Bylund 2003).

Pasteurization is a critical step in the preservation of whey for further processing. Whey is heated to 62.8°F for 30 minutes or to higher temperatures for shorter times, to

retard the growth of the lactic-acid-producing bacteria, which have been active during the cheesemaking process and to destroy the pathogenic organisms that might be present if milk was not pasteurized before making the cheese (Webb and Whittier 1948).

The initial trial for concentrating and drying of whey was invented in the 1920s, which involved four methods: conventional hot roller milk dryers; heating until a concentrated liquid was obtained, cooling to solidification, and then extruding in a tunnel; two-stage steam heating and a combination of spray drying and rotary drum drying (Gillies 1974). In 1933, the long-tube multiple-effect evaporator was applied to whey processing. Evaporation in the first effect takes place around 77°C and in the second at around 45°C (Kosikowski and Mistry 1997), finally leaving concentrated whey with 45% of total solids (Kosikowski 1982). The spray dryer started being used in whey processing since 1937, and it is still the most widely used method for whey drying. The concentrated whey is dispersed by a rotary wheel or nozzle atomizer into a drying chamber through a stream of hot air, producing a powder with 10-14% moisture (Deis 1997) . Drying and concentrating whey reduces water for transport, makes for easier handling of the product, and increases keeping quality. Theoretically, conversion of the entire world's fluid or liquid whey annually into powder would result in about 5 billion kilograms.

However, using dried whey in a food system is limited due to the various functional properties of different components. Fractionation techniques can be used to remove some of the undesirable components or to recover the most valuable whey components (Jelen 1979). Lactose crystallization could be used to recover the lactose from whey. There are two methods depending on the raw material: untreated but concentrated whey and. from which the protein has been removed by other methods (Bylund 2003). Membrane filtration, a relative new technique to the whey industry, was

introduced in the early 1970s. It allowed for the separation and fractionation of whey proteins while retaining their solubility (Wingerd 1971; Onwulata and Huth 2008). The principle of membrane filtration is the solids components in whey consists of different particle sizes. By selecting different pore sizes for filters or membranes and applying pressure on them, it is possible to divide whey into different fractions. In the whey industry, there are five types of membrane filtration: ultrafiltration (UF), microfiltration (MF), nanofiltration (NF), electrodialysis (ED), and reverse osmosis (RO), that may sometimes be used in combinations. Membrane filtration is followed by spray drying to obtain dry products of different protein contents. The dried product is called whey protein concentrate (WPC).

The ion-exchange is the process which eliminates insoluble solids from solutions on a constant electro-chemical basis. An ion exchange process employs resin beads to adsorb minerals from solution in exchange for other ionic species. The resins have a limited capacity for this so that when they are completely saturated, the adsorbed minerals must be removed and the resins regenerated before reuse (Bylund 2003). An ion-exchange tower is often used in combination with membrane filtration to make a high protein content product: whey protein isolate (WPI), which contains more than 90% of protein (Huffman and Harper 1999).

The data from USDA showed that in 2008, the United States produced 502 million kilograms of total dried whey, 192 million kilograms of WPC, and 19.6 million kilograms of whey protein isolate. In general, production of whey powder, demineralised whey powder, lactose and delactosed whey powder preponderate the processed whey market. However, there is a gradual shift towards new and functional products that will

change the image of whey from an unwanted byproduct to an important raw material to the food system.

2.3 Whey Composition

The composition of whey and whey products depend on milk composition, cheese manufacturing, pasteurization temperature, methods of production, purification, and concentration (Mavropoulou and Kosikowski 1973; Josephson, Rizvi et al. 1975; Onwulata and Huth 2008). Cheese whey is a watery solution containing about 7% solids. The solids contain about 10-12% protein, the rest being mainly lactose (74%), mineral salts such as sodium chloride, potassium chloride, and calcium salt (8%), milk fat (3%), lactic and citric acid (Kosikowski and Wierzbicki 1973; Coton 1976; Marwaha and Kennedy 1988; Morr 1989; Gradinaru, Biliaderis et al. 2003). In addition, water soluble vitamins such as thiamine, riboflavin, ascorbic acid, and lecithin are present in whey (Mavropoulou and Kosikowski 1973).

A typical composition of liquid sweet whey, in one liter, has 6-10 g of protein, 46-52 g of lactose and 2.5 to 4.7 g of minerals. For acid whey, there is 6-8g, 44-46g and 4.3-7.2 g of protein, lactose and minerals, respectively, in one liter of fluid (Jelen 2003). Mavropoulou and Kosikowski (1973) reported that the acid whey and sweet whey dried powders have compositional differences in protein, lactic acid, mineral elements and lecithin content. Dried sweet whey has a higher protein and lactose content, while dried acid whey has a higher amino acid, mineral, lactic acid and lecithin content (Mavropoulou and Kosikowski 1973; Kosikowski 1982).

Whey protein concentrate may contain 20-89% protein, 4-50% lactose, and 3-7% minerals (Jelen 2003). A combination of UF and diafiltration removes minerals and

lactose, which makes a WPC product with more than 50% protein content (Kelly 2002). Whey protein isolate has at least 90% protein, 2-3% minerals and virtually no lactose. A typical chemical composition of commercial whey protein concentrate (WPC) and whey protein isolate were shown in Table 2.1.

Whey protein concentrate and WPI are high protein products. The major whey proteins are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and the heavy and light-chain immunoglobulins (Igs). Lactoferrin (LF) and lactoperoxidase are also present in whey proteins but in minor quantities. Glycomacropeptides (GMPs) and low-molecular-weight products can be formed by enzyme degradation of caseins during the cheese making process (De Wit 1989). The chemical and physicochemical properties of the major whey proteins were listed in Table 2.2.

Table 2.1 Chemical composition of commercial whey protein concentrate (WPC) and whey protein isolate (WPI) (%) (Morr and Foegeding 1990)

	WPC		WPI	
	Range	Mean SD	Range	Mean SD
Moisture	4.14-6.01	5.31±0.66	2.4-5.57	3.75±1.34
Protein	72.0-76.6	73.8±1.64	88.6-92.7	91.0±1.73
Nonprotein N Compounds	0.93-4.56	3.09±1.33	0.29-0.34	0.32±0.02
Lactose	2.13-5.75	3.92±1.20	0.42-0.46	0.44±0.02
Total lipids	3.3-7.38	5.00±1.27	0.39-0.67	0.57±0.13
Phospholipids	0.8-1.54	1.28±0.23	0.11-0.31	0.21±0.08
Ash	2.52-6.04	4.28±1.29	1.37-2.15	1.82±0.33
Sodium	0.15-1.71	1.04±0.65	0.36-0.42	0.39±0.03
Potassium	0.07-0.46	0.25±0.17	0.10-0.16	0.13±0.04
Calcium	0.23-1.05	0.46±0.27	0.20-0.24	0.22±0.03
Magnesium	0.02-0.40	0.09±0.12	0.02-0.03	0.02±0.02
Phosphorus	0.02-1.30	0.44±0.35	0.05	0

Composing approximately 50% of all whey proteins, β-LG are important because they have many sites for binding minerals, lipids, and fat-soluble vitamins (Severin and Wenshui 2005; Yalcin 2006). One-fourth of the whey proteins is α-LA, a calcium binding protein, which heightens the absorption of calcium. Known as transport protein for insoluble fatty acids in the blood circulatory system, BSAs are responsible for binding fatty acids and other small molecules (Spector 1975). Immunoglobulins include IgG, IgA, and IgM, with IgG making up 80% of the immunoglobulins. The IgG has four polypeptide chains, namely two light and two heavy chains joined by disulfide bridges (De Wit and Klarenbeek 1984), and may prevent and fight against the effects of bacteria (de Wit, Klarenbeek et al. 1978). For minor whey protein, LF helps in immune system functions and can kill pathogenic microorganisms (Leon-Sicairos, Lopez-Soto et al. 2006; Van Der Kraan, Nazmi et al. 2006), while lactoperoxidase has antibacterial properties (Floris, Recio et al. 2003; Severin and Wenshui 2005; Yalcin 2006). Glycomacropeptide is a bioactive peptide. With its unique composition and characteristics, GMP offers health promoting effects with multiple applications.

In a recent study, it was found that the whey protein in commercial Cheddar cheese samples had 53.8-64.8% β-LG, 22.4-26.4% α-LA, 2.9-7.1% Igs, 2.1-4.0% BSA, 0.3 to 2.4% LF, and 3.6-14.8% other protein (Blaschek, Wendorff et al. 2007).

Free amino acid level in whey powder is between 1.0 to 11 g/kg (Mavropoulou and Kosikowski 1973). Glutamic acid, proline, lysine, tyrosine, aspartic acid, and arginine were the major free amino acids in whey powder. The combined free amino acids and soluble peptides are almost six times of the amount of free amino acids. Glutamic acid, proline, threonine, aspartic acid, lysine, and serine are the predominating amino acids (Mavropoulou and Kosikowski 1973).

Table 2.2 Chemical and Physicochemical Properties of Whey Proteins (Eigel, Butler et al. 1984).

	β-LG	α-LA	BSA	Igs
Isoelectric point	5.2	4.2-4.5	4.7-4.9	5.5-8.3
Concentration in whey, g/l	2-4	0.6-1.7	0.4	0.4-1.0
Concentration in whey, % w/w	56-60	18-24	6-12	6-12
Molecular weight, daltons	18,000	14,000	66,000	>146,000
Average hydrophobicity, kcal/residue	1075	1020	995	NA
Total amino acid residues/mol	162	123	582	NA
Apolar residues/mol	54	44	163	NA
Cysteine residues/mol	5	8	35	NA
Disulfide residues/mol	2	4	17	NA
Sulfhydryl residues/mol	1	0	1	NA
Lysine residues/mol	15	12	59	NA
Glutamic acid residues/mol	16	8	59	NA
Aspartic acid residues/mol	10	9	39	NA

2.4 Whey Utilization

In ancient Greece, whey was known as “healing water” by Greek physicians. In 460 B.C., Hippocrates, the father of medicine, prescribed whey for an assortment of human ailments (Vasey 2006). During the middle ages, many doctors recommended whey as pharmaceutical drugs. Until the 1940s, dyspepsia, uremia, arthritis, gout, liver diseases, anemia, and even tuberculosis were treated with the ingestion of up to 1500 g of whey per day in Western Europe (Holsinger, Posati et al. 1974).

Due to the poor facilities and equipment, the use of whey as feeding material for hogs can be traced back to ancient Rome (Schingoethe 1976). There are several methods by which whey can be utilized by animals, such as feeding liquid or condensed whey directly to animals and addition of whey or whey product to ensiled forage to feeding of dried whey products. Schingoethe (1976) concluded that ruminants can consume up to 30% of their dry-matter intake as liquid whey without impaired performance while swine

may experience diarrhea when more than 20% of their dry matter intake is liquid whey. Liquid whey protein after fermentation, ammoniation and condensation becomes an acceptable supplement for ruminants. Because of the effects of lactose in preventing coccidiosis and the effect of riboflavin on the growth of chicks, hatchability effects and prevention of curled toe paralysis, dried whey has been used in feeding chickens (Webb and Whittier 1948).

Whey was not used in food systems until the 1930s. Peter and Bell (1930) studied the foaming properties of whey protein and compared it with that of egg albumin. The foam made from concentrated whey was increased by neutralization or by addition of small quantities of tannic acid, saponin, or bisulfites. The preparation of a heat-coagulable foaming agent by the neutralization and filtration of the mother liquid from lactose manufacture has also been studied (Beeching and Severn 1943). However, when coagulated by heat, a whey protein whip will not be able to support the other ingredients to replace egg white in certain cakes and custards. Some studies showed the possibility of partial replacement of egg white protein with WPC or WPI in angel food cake formulation, due to the high calcium and lipid content of WPC from pretreatment and microfiltration and the foaming and heat induced gelation properties of WPI (Jelen 1973; Morr, Swenson et al. 1973; Haggett 1976; Khan, Rooney et al. 1979; Morr and Foegeding 1990; Phillips, Schulman et al. 1990; Morr 1992; Morr and Ha 1993; Lawson 1994; Zhu and Damodaran 1994; Arozarena, Bertholo et al. 2001). In Ramsdell & Webb's study (1938), sweetened condensed whey was whipped in four minutes to foam, which had 15 hours stability and 200% overrun. The whipping foam was used as topping, icings, fruit whips and other similar products.

With the growth of the cheese industry, many efforts have been made to develop a suitable product for food use from the large volumes of whey (Djurić, Carić et al. 2004). Since the preparation of the whey-based beverage is the cheapest and most efficient product that is produced by draining the whey from the cheese vat, pasteurization, deodorization, flavoring, and packaged for consumption, many studies have been done. In 1898, Graeff (1898) invented a simple way of whey processing: heating, deaerating and charging with carbon dioxide and formaldehyde under pressure. Whey flavor, especially the acid whey flavor, is most compatible with citrus flavors and high acceptability was reported from cheddar and cottage wheys (Demott 1972; Holsinger, Posati et al. 1974; Haque and Ji 2003). Though using cheese whey to make a beverage is one of the technologically simplest approaches, it is commercially the most difficult to utilize in the human food chain (Jelen, Currie et al. 1987). Rivella, a milk whey drink produced first in Switzerland in 1952, was considered to be the most commercially successful whey beverage sold in most of the Western European countries. It was thought impossible in the United States to manufacture a whey beverage which could be sold at a price competitive with a snack beverage (Holsinger 1973). Rivella attempted to expand to the United States in the 2000s, but failed. New attempts to develop whey-based beverages for consumers have never stopped. County Life Vitamins (Hauppauge, NY) introduced as Biochem 100% Whey Ready to Drink in berry, tropical and orange cream flavors in late 2009 (Nutraceuticals World 2009). Whey can also be blended with dairy ingredients and used in preparation of soup, baking foods, candies, soup and sauce, cheese and cheese foods, spirit vinegar, and food acidulant (Webb and Whittier 1948).

2.5 Whey as Functional Food

In the 1980s, the term "functional food" was first used in Japan. It was defined as food product fortified with special components which would have beneficial physiological effects in addition to its nutritive value (Goldberg 1994; Hardy 2000; Kwak and Jukes 2001). Though this term is still under use today, Japanese authorities highlighted three conditions that functional food must fulfill; the food should have naturally occurring ingredients, not capsules, tablets or powders; it can be consumed as part of daily diet; and it can be used to prevent or control a disease or regulate a biological process or mechanism. However, there is no universally accepted definition of functional food. Several terms, such as nutraceuticals, medical food, vitafood, dietary supplements, and fortified foods, have been used world-wide. The global market for functional food and beverages is highly dynamic. According to the French Technology Press Office, the market is over $128 billion with the largest in Europe ($34 billion). The United States and Japan also play important roles with $26 and $22 billion, respectively. The growth of the industry is expected to rise 16 percent annually (Trueman 2009).

Whey has been used to prevent and treat diseases for thousands of years in various cultures and societies. Whey contains a number of proteins, which not only play important roles in nutrition, but also have specific physiological roles in vivo. The primary proteins in whey, β-LG, α-LA, and lactoferrin, have positive biological effects including: anti-carcinogenic, immunostimulatory, digestive function, anti-microbial, etc., in human and animal studies (Bounous, Batist et al. 1991; Perez, Sanchez et al. 1992; Meisel and Schlimme 1996).

2.6 Functional properties of whey

There are several proposed definitions for the functionality of food proteins. Pour-El (1981) suggested functional property as, "Any property of a food or food ingredient except its nutritional ones, which affects its use." In Kinsella's (1976) definition, functional properties are, "Those physical and chemical properties which affect the behavior of proteins in food system during processing, storage, preparation and consumption." The functional properties of a food protein are related to its structure; however, currently, the relationship is not understood in adequate features to allow designing protein structure to achieve a specific functionality. Nevertheless, some general features of the relationship between structure and functionality can be outlined. Protein functional properties are related to several general molecular characteristics including: hydrophobicity, hydration, surface activity, and the type of protein-protein interactions favored by partially unfolded structures. The functional characteristics associated with these molecular properties are listed in Table 2.3.

Table 2.3 Relationship between Protein Molecular Properties and Functionality (Fox 1982).

Molecular property	Associated functional properties
Hydration	Solubility, dispersibility, swelling, viscosity, gelation, water absorption
Surface activity	Emulsification, fat adsorption, foaming, whipping
Protein-protein interactive potential	Aggregation, cohesion, texturization, gelation, elasticity, extrudability
Molecular structure or architecture yielding organoleptic properties	Color, flavor, odor

Functional properties of whey proteins include physiochemical characteristics of protein which affect the properties of food products. Functionalities of whey protein depend on the source of cheese, processing methods, and composition of the products

(De Wit 1990; Huffman and Harper 1999). Many factors associated with whey protein production have an effect on functionality of whey protein, such as farm practices, cheese production methods, acid or rennet coagulation, and spray drying method (Onwulata, Konstance et al. 2004). The functionality of whey protein can be studied in model systems, model food systems, and in real food. Nevertheless, testing functionality in real food is more difficult than in a model system due to the complexity of food systems.

2.7 Whey Solubility

Since WPC has been considered functional food and an important ingredient in food systems, it has received attention from nutritionists and food scientists. Because it influence on other functional properties significantly, solubility is one of the most important functional properties. The high solubility of protein will offer good emulsion, form, gelation, flavor binding, and whipping properties (Nakai and Li-Chan 1985; De Wit 1989). Solubility of protein relates to surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interactions with water. Thermodynamically, the protein solubility refers to the protein concentration in the single or two-phase system (protein solution in liquid-liquid phases or liquid-solid phases) at the equilibrium state (Hall 1996). Mathematically, the solubility of protein is defined as the amount of protein present in the liquid phase in relation to the total amount of protein in the liquid and solid phases at equilibrium. The solubility of WPC is influenced by several factors, such as native or denatured state, pH, temperature, pressure, nature and concentration of salts, and concentration of protein (Hall 1996; Pelegrine and Gasparetto 2005). Proteins are least soluble in the pH range close to their isoelectric point (pI). Because at lower or higher pH, there are more same sign ionic charges, which will produce repulsion among

the molecules and increase the solubility (De Wit 1989; Hall 1996; Mann and Malik 1996; Kontopidis, Holt et al. 2004). On the other hand, whey proteins have a pI of 4.5 and are soluble at this range. Since whey proteins are soluble at a wide range of pH, it is possible to utilize whey in various food products. Morr (1975) and Mulvihill and Donovan (1987) have studied the mechanisms of thermal denaturation and insolubilization of whey. Heat treatment and acidification of whey have been developed to recover the protein in insoluble form (Hidalgo and Gamper 1977).

Solubility of whey powders in water ranges from 91.4 to 99.7% and in 5% sodium chlorine solutions from 72.8 to 98.2%. Acid whey powders generally are more stable to storage at room temperature than sweet whey powders (Mavropoulou and Kosikowski 1973). Heating whey protein concentrated solutions at neutral pH causes up to 70% loss in solubility (Hidalgo and Gamper 1977; Shon and Haque 2007).

Though it is important to study the whey protein solubility and stability close to neutral pH, limited quantitative data are available on the thermal sensitivity of WPC.

2.8 Whey stability, thermostability, denaturation and thermodenaturation

In a food system, stability is one of the most important factors to the functionality of the food. If the protein's conformation is changed, its functionality would be altered as well. Denaturation is closely related to the structural stability of the native protein. It is critical to preparation, processing, nutritional value, quality and safety of the food proteins (Brandts 1964; Tanford, C.B. Anfinsen et al. 1970; Mulvihill and Donovan 1987). Beta- lactoglobulin and α-LA are the major proteins in whey, thus properties of these proteins influence the overall functionality of whey proteins.

Thermostability of whey proteins is referred to as the property that makes whey proteins resistant to irreversible changes in physical structure at high temperatures. The heat treatments used during processing and preservation of whey protein products can seriously affect the native state and thermostability of whey proteins. Consequently, a better understanding of the behavior of whey proteins during heat exposure is essential for the control of their functional properties during recovery and application of whey protein products.

Protein denaturation can be induced by heat, organic solvent, detergents, surface spreading, and extremes in pH. Since heating is most always involved in the processing and preparation of food, it has been considered to be the most important form of chemical and physical denaturation methods.

Thermodenaturation occurs when hydrogen hydrophobic and other non-covalent bonds are broken by heat (Mulvihill and Donovan 1987). The protein denaturation happens in two steps. First, the protein is altered non-covalently, which is also called the denaturation stage; then the irreversible aggregation happens, and precipitation may follow. The high thermal energy addition might induce the covalent bonds to be broken and lead to thermal degradation (McKenzie 1970; Mulvihill and Donovan 1987). Whey proteins are generally considered denatured around 72°C. Denaturation of serum proteins in whey has been used in recovering whey proteins since the 1970s. Heat treatment at or above the pI, followed by gravitational settling, filtering, or centrifugation are usually used in the industry (Modler and Emmons 1977).

2.9 Whey pretreatment methods

To increase cheese yield, several pretreatment methods have been studied and some have already been used commercially. These methods include high-heat treatment, ultrafiltration, and microfiltration. Different pretreatments were used at different processing stages, thus affecting different property changes. High-heating pretreatment is mostly used in the production of fresh and semi-soft cheese to develop desired texture (Banks, Law et al. 1993; Picone, Takeuchi et al. 2011). The whey protein is then denatured and transferred to the cheese matrix. The critical temperature for whey protein's physical properties is around 70°C, at which protein solubility and emulsifying activity begin to decline, while the water binding and viscosity increases (Kester and Richardson 1984). The study of Wang et al.(2006) showed protein aggregation occurred through both hydrophobic interaction and formation of intermolecular disulfide bonds when whey protein exposed to temperature above 70°C. More severe thermization causes protein denaturation and loss of aqueous solubility and decreased overall functional behavior (Kester and Richardson 1984).

The ultrasonic process is relatively new pretreatment method. Gulseren et al. (2007) suggested that sonication can change the functional properties of bovine serum albumin by forming an ultrasonically induced state, which is different from a thermal, mechanically, or solvent induced state. Ashokkumar et al. (2009) use short-time ultrasound treatment after the heat pretreatment. The sonication process broke the aggregation of the proteins in pretreating step and preventedthe reformation on subsequent heating. Due to the reduction in the size of insoluble aggreagetion, sonication at 20 kHz increase the clarity of WPC soluction. However, the WPI soluction did not

observed significant changes might due to the absence of large aggregated in the initial solution or the difference in composition (Zisu, Lee et al. 2011).

2.10 Protein Hydrolyzate

Protein hydrolyzates are produced from purified protein sources by heating with acid or addition of proteolytic enzymes (Bucci and Unlu 2000). As a result of cleavage of peptide bonds, hydrolyzation of protein is broken down proteins into peptides of different sizes and free amino acids. Enzymatic hydrolysis of protein by using selective proteases provides more moderate conditions of the process, such as pH (6–8) and temperature (40–60 °C), and few or no undesirable side reactions or products. Whereas acid and alkaline hydrolysis tend to be a difficult process to control and yield products with reduced nutritional qualities(Sinha, Radha et al. 2007). Chemical hydrolysis can form toxic substances, such as lysino-alanine (Lahl and Grindstaff 1989). The peptides produced by proteolysis have smaller molecular sizes and less secondary structure than native proteins and may be expected to have increased solubility near the isoelectric point, decreased viscosity and significant changes in the foaming, gelling and emulsifying properties from those of original proteins (Chobert, Bertrand-Harb et al. 1988; Nollet 2004).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Citric acid and disodium phosphate were purchased from Sigma-Aldrich. Milk used in cheese making was obtained from mixed cattle herd (16.7% Jersey and 83.3% Holstein) from Mississippi State University Dairy Farm, Starkville, MS, USA. Enzymatic hydrolyzate of caseinate was made by the previous study (Haque and Mozaffar 1992).

3.2 Methods

3.2.1 Whey processing

Fresh whey from the manufacture of Edam and Cheddar cheese was collected from Mississippi State University Dairy Plant, and skimmed through a de-creaming separator (Mueller, Springfield, MO). Aliquotes were flash frozen in liquid nitrogen and freeze dried using a laboratory-scale freeze-dryer (Labconco, Kansas City, MO), and stored at -10°C until needed as the cold control. The reminder of the whey was thermized using a pasteurizer (Mueller, Springfield, MO) at 70°C for 5, 10, 15, 20 and 30 min (5T, 10T, 15T, 20T, and 30T, respectively) to obtain treatments. Samples were cooled to 25°C using a plate heat exchange. The liquid whey was processed immediately after thermization (Figure 3.1). Concentrate whey was made by VE using a falling film vacuum-evaporator (Model 26061, Year 1990, APV, Sorborg, Denmark) using a chamber temperature range of 65-70°C. The vacuum pressure was held at 0.3 kPa/cm^3 until the

solids content reached 24-29% (w/v) as opposed to an initial solids content of 5.8-6.8%, measured using a hand-held refractometer (0° -32° Brix range) (Fisher Scientific, Pittsburgh, PA).The concentrated whey product was then spray-dried using a spray-dryer (APV, Sorborg, Denmark) at an inlet air temperature of 220-260°C atomizing fluid whey to whey powder. The temperature at the sample collecting outlet of the spray dryer was between 50-70°C.

3.2.2 Isoionic buffer

McIlvaine's isoionic buffer was made using 0.1M citric acid and 0.2 M Na_2HPO_3 $2H_2O$ as described by McIlvaine (1921). The pH levels used were 3, 4.5, 6, and 7.5 as shown in Table 3.1.

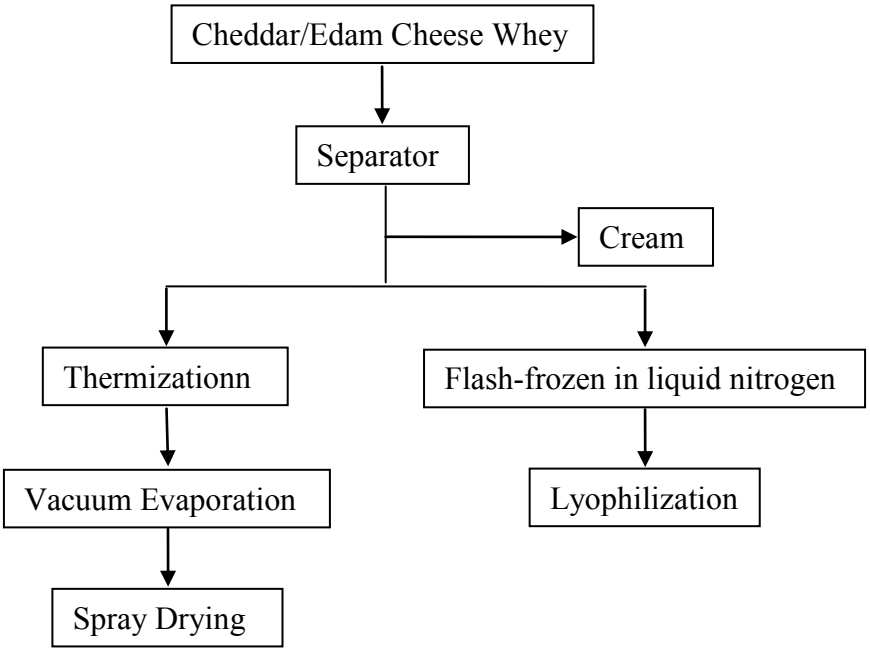

Figure 3.1 Flow chart for manufacture of whey protein concentrates.

Table 3.1 The volume of two stock solutions to make standard McIlvaine's buffer.

pH	Volume of citric acid (mL)	Volume of Na_2HPO_3 $2H_2O$ (mL)
3	79.45	20.55
4.5	54.58	45.42
6	36.85	63.15
7.5	7.75	92.25

3.2.3 Dissolved Solid content

The concentrates of dissolved solids in the aqueous solutions were tested by using a Fisher-Scientific brand hand-held Brix refractormeter (0-32°) (Pittsburgh, PA). Measurements were taken before thermization, after thermization, and after VE process. One drop of sample was applied on a prism and the total solid content was read through the ocular window.

3.2.4 The pH value of whey fluid

The pH of the whey was measured using a mini lab ISFET portable pH meter model IQ125 (Carlsbad, California).

3.2.5 Thermostability (TS)

The TS value of 1% (w/v) Edam or Cheddar whey was determined in isoionic McIlvaine's buffer (pH 3.0, 4.5, 6.0, and 7.5). The TS of 0.01 % (w/v) casein hydrolysate with 1% (w/v) of sweet whey was also tested. Four ml of sample dispersions were put in test tubes, heated at 82°C for 0, 1, 5, 10, and 20 min, quickly cooled to 22°C using an ice bath, and the transmittance values at 600 nm (T_{600}) was measured (Thermo Scientific Biomate 3 UV- Vis Spectrophotometer , Madison, WI). The ratio of T_{600} of heated to that of unheated provide a reflection of the TS. For example, low transmittance reflected low dispersibility of the sample. The sample showed similar transmittance after the heat

treatment compared to the unheated sample was considered thermostable. Three-dimensional plots of thermostability were generated using the correlation of thermostability as a function of heating time and pH by Axum software (Cambridge, MA). The x, y, and z axes and ranges being the same, the surface plots which are spline fits of mean data points (n=5), reflect the TS changes as a result of initial thermization and pH of whey samples. The connecting line was forced to go through every one of the original data points.

3.3 Statistical design

The experiment was conducted using a completely randomized design with three replications. When significant differences occurred among treatments, Fisher's least significant test (LSD) (Fisher 1949) was used to separate treatment means ($P<0.05$). SAS software version 9.2 phase 2 (Cary, NC) was used to perform the statistical analysis.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Physical properties and pH

The fluid Edam and Cheddar whey are light yellowish in color. After whey samples concentrated by VE, the color of the samples become darker. Generally, Edam whey has a slightly lighter color than Cheddar whey. The spray dried whey powders of both types have a cream and whitish yellow color. They were fine grained similar to milk powder and had a milky smell. Though Edam and Cheddar whey are both sweet whey, they have some different physical properties. The data in Table 4.1 showed the pH of the Edam whey fluid sample before and after heat treatment, and before the evaporation process. The pH of the Cheddar whey fluid samples are shown in Table 4.2. The pH of Edam whey fluid was between 6.3-6.6 before heat treatment; while Cheddar whey had a lower pH, ranging from 5.9-6.4. The pH of the whey samples decreased after VE processing. The concentrations of dissolved solids of whey samples are shown in Tables 4.3 and 4.4. Edam whey has a lower solid content (ranging from 6.2 to 6.8%) compared to cheddar whey (6.4-6.8%).

4.2 Thermostability

Thermization significantly enhanced TS of Edam whey at acidic pH 3 (Figures 4.1-4.7 and Table 4.5-4.8). In Edam treatment, the TS of the cold control showed the lowest TS in almost all pH levels in all treatments. The vacuum evaporation process help protein clustering and non-clustering in globular proteins which helped enhanced the TS.

At pH 4.5, the TS was minimized for all treatments except 5T (see Figure 4.3 and Table 4.6) and 15T (see Figure 4.5 and Table 4.6). As pH 4.5 is near the isoelectric point of the major whey proteins, β-lactoglobulin and α-lactalbumin (Kella and Kinsella 1988), the protein-protein interactions were the highest at this pH, because the electrostatic forces are lowest and less water interacted with proteins. It was also observed that at pH 3 (see Table 4.7), the TS had the highest value for all control and treated treatments. The TS of the cold control was the least compared to all treatments including the un-thermized controls. Treatments 5 (see Figure 4.3) and 15 (see Figure 4.7) showed marked enhancement of TS throughout the pH range studied, and thermal exposure with the minimally thermized 5T was significantly ($p<0.05$) the best. Interestingly, TS of Edam whey at pH 4.5 (see Table 4.6) was significantly improved ($p<0.05$) compared to both controls and all other treatments.

Control and treated Cheddar whey (see Table 4.9-4.12 and Figure 4.) had the least TS at pH 4.5, with one exception that was observed for the 10 min treatment (see Figure 4.10). The highest TSs were found at pH 3.0 (see Table 4.8) in all treatments. From the three dimensional plot for Cheddar whey, the control TS (see Figure 4.8) was significantly and dramatically lower at all pHs particularly as heat-exposure time increased. Treatment improved ($p<0.05$) this desirable attribute with 10T being the best at all pHs, and heat exposures.

The difference between the two sweet wheys is conceivably due to differences in the types and amount of peptides that are generated during the manufacturing process. The TS of non-thermized Edam and Cheddar whey were compared (see Tables 4.17-4.20). The TS of Edam whey had significantly higher values in all pH and heating time durations. . Edam is a type of cheese that originally was produced in Netherland, which

was produced by heating up to 15 min at 35°C. Cheddar cheese was involved with 37.8-39.4°C heating for 45 min (Kosikowski 1982). Cheddaring involves lengthy warm cutting and folding steps that facilitate starter culture protease activity (Kosikowski 1982). Thus, Cheddar whey contains relatively higher amount of peptides than Edam whey.

The mere addition of a trace amount of enzymatic hydrolyzate of casein (CH) (0.01%, w/v) treatments, TS of Edam whey control (see Figure 4.14), 10T+CH (see Figure 4.16) and 15T+CH (see Table 4.16) significantly increased at pH 4.5(Tables 4.14). Interestingly, TS of Edam whey+CH at pH 4.5, which is close to the pI of the major whey protein, β-lactoglobulin, was improved ($p<0.05$) compared to both controls and all other treatments. Addition of CH enhanced TS by up to 30%, compared to control, when the thermization was for 10 min. On the other hand, 5T+CH (see Figure 4.15), 20T+CH (see Figure 4.17) and 30T+CH (see Figure 4.18) did not cause significant changes compared to 5T, 20T and 30T, respectively. Treatment significantly improved this disable attribute with 10T (see Tables 4.13-16) being the best at all pH and heat exposures.

Adding enzymatic hydrolyzate of casinate to Cheddar whey did not help improve the TS significantly (see Tables 4.21-24), actually decreased due to Cheddar whey having a higher peptide content than Edam whey (Kucukoner and Haque 1998).

Thermostability is a desirable functionality for food proteins, especially in high protein content beverages which required pasteurization. Heat treatment usually develops protein precipitation. However, after short-time thermization pretreatment, the TS significantly increased ($p<0.05$). The increased TS may be an indication of protein-protein association and protein-peptide association increased. Milk peptides can

profoundly impact association tendency of proteins (Haque 1993). Proteins contain hydrophilic and hydrophobic groups. With the increase of the temperature, the protein molecules need to cleavage the hydrophobic residues for associate with other ligands to decrease hydrocarbon-aqueous interface (Haque and Mozaffar 1992).

Table 4.1 The pH values of fluid Edam whey.

Treatments	Before thermization (%)	After thermization (%)	After evaporation (%)
Control	6.4	6.4	5.8
5T*	6.4	6.4	6.2
10T*	6.3	6.3	5.9
15T*	6.6	6.6	6
20T*	6.6	6.6	6.1
30T*	6.4	6.4	6

* 5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.2 The pH values of fluid Cheddar whey.

Treatments	Before thermization (%)	After thermization (%)	After evaporation (%)
Control	5.9	5.9	5.3
5T*	5.9	5.9	5.3
10T*	6.4	6.4	5.7
15T*	6	5.9	5.3
30T*	6.2	6.2	5.8

* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.3 Solids content of Edam whey.

Treatments	Before thermization (%)	After thermization (%)	Before spray drying (%)
Control	6.4	6.4	26.4
5T*	6.2	6.4	28
10T*	6.4	6.4	27.4
15T*	6.2	6.2	26
20T*	6.8	6.8	24.6
30T*	6.6	6.6	26

* 5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.4 Solids content of Cheddar whey.

Treatments	Before thermization (%)	After thermization (%)	Before spray drying (%)
Control	6.7	6.7	24
5T*	6.8	6.8	25
10T*	6.8	6.8	27.6
15T*	6.4	6.4	22.8
30T*	6.8	6.8	24

* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

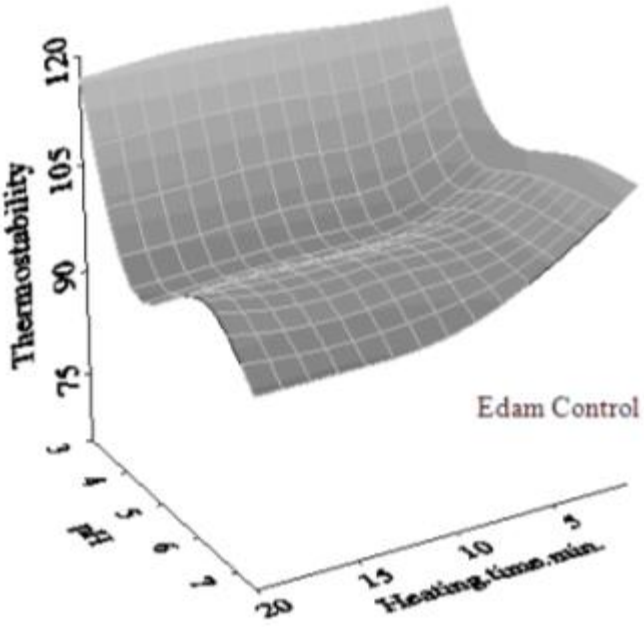

Figure 4.1 Thermostability of untreated (not thermized) spray dried Edam whey as affected by duration of heating at 82°C and pH3-7.5.

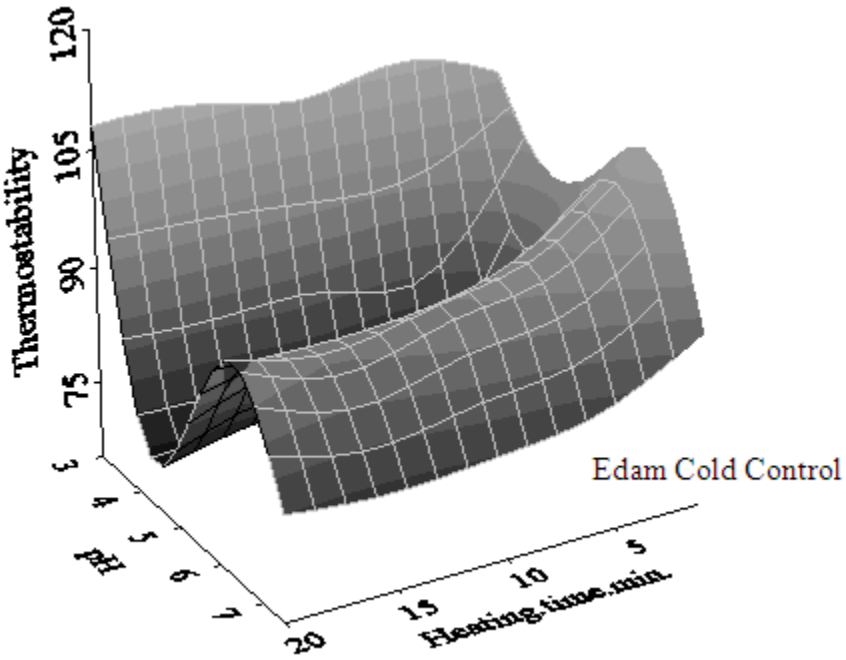

Figure 4.2 Thermostability of untreated (not thermized) freeze dried Edam whey at 70°C as affected by duration of heating at 82°C and pH3-7.5.

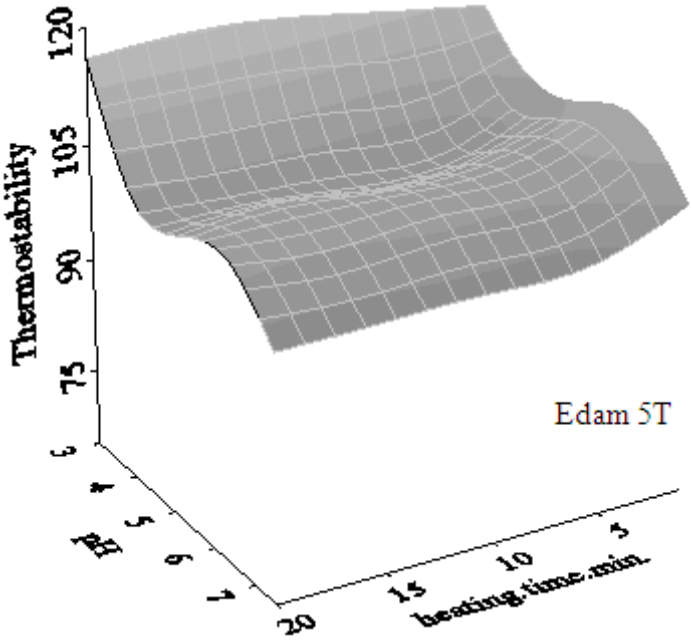

Figure 4.3 Thermostability of spray dried Edam whey thermized (pre-heated) for 5 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

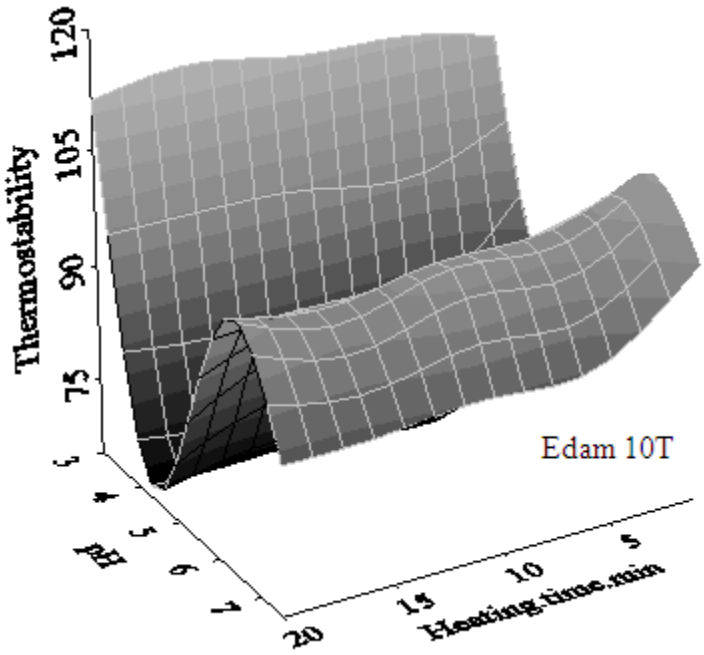

Figure 4.4 Thermostability of spray dried Edam whey thermized (pre-heated) for 10 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

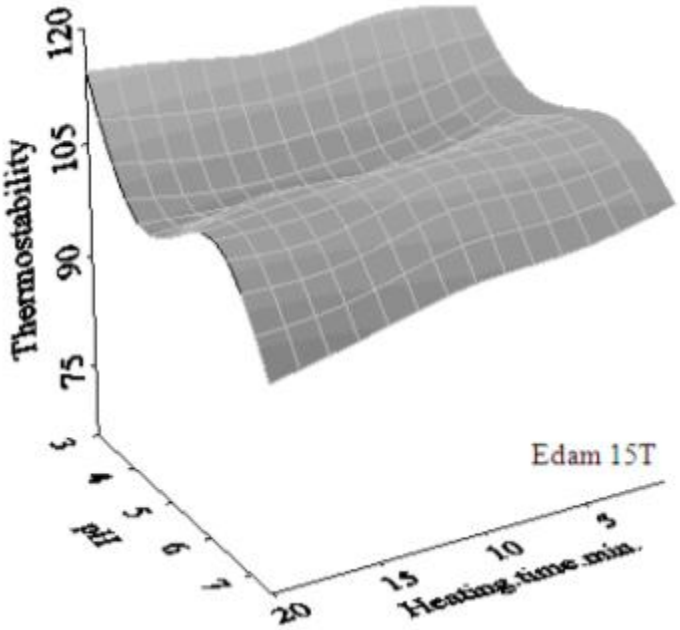

Figure 4.5 Thermostability of spray dried Edam whey thermized (pre-heated) for 15 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

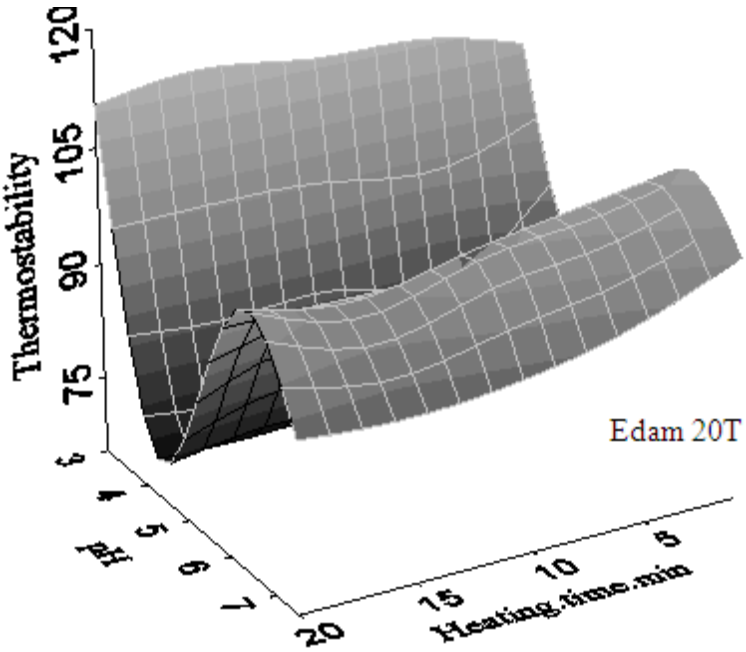

Figure 4.6 Thermostability of spray dried Edam whey thermized (pre-heated) for 20 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

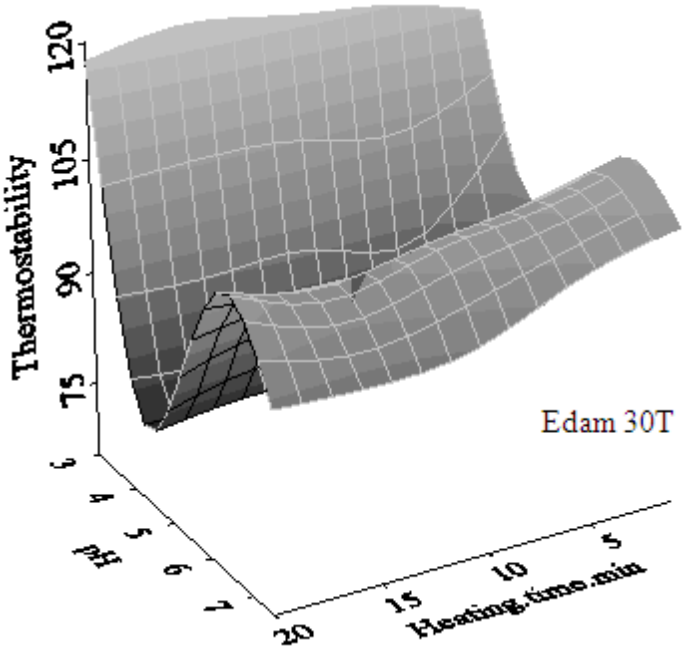

Figure 4.7 Thermostability of spray dried Edam whey thermized (pre-heated) for 30 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

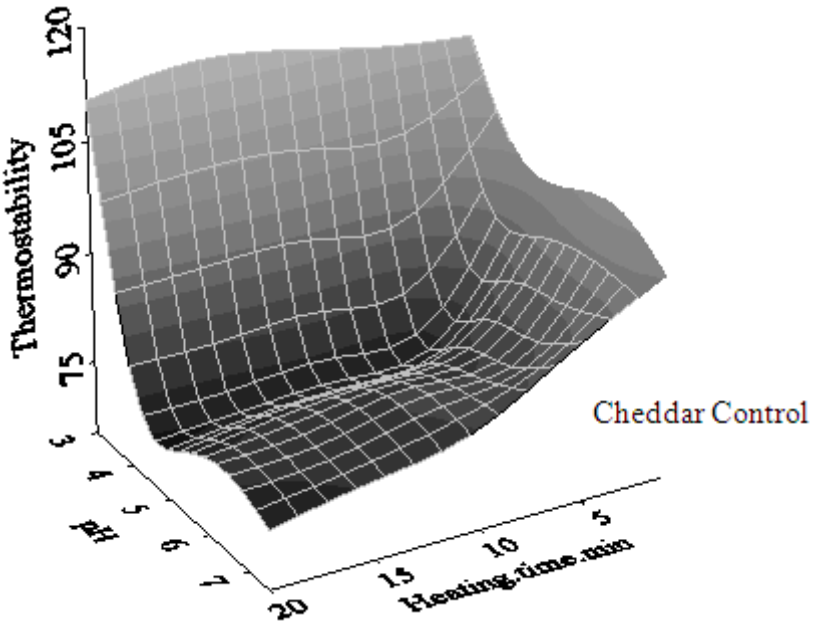

Figure 4.8 Thermostability of untreated (not thermized) spray dried Cheddar whey as affected by duration of heating at 82°C and pH3-7.5.

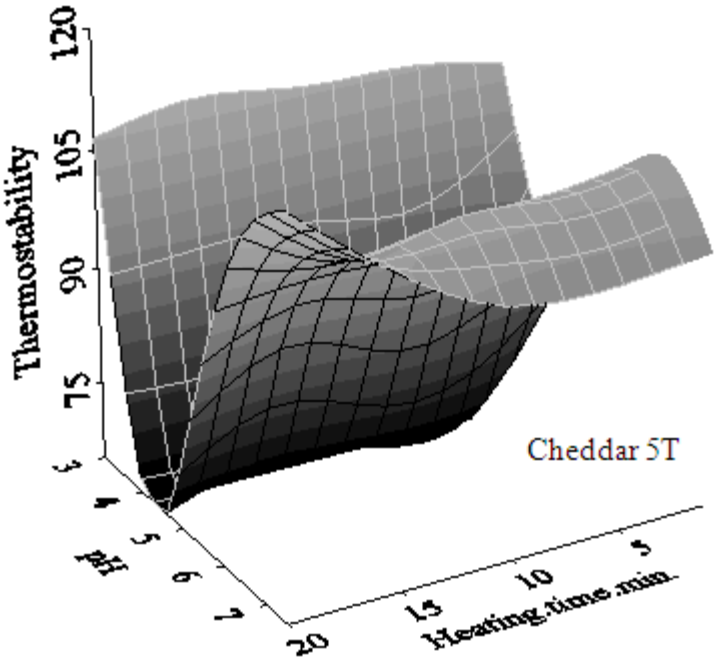

Figure 4.9 Thermostability of spray dried Cheddar whey thermized (pre-heated) for 5 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

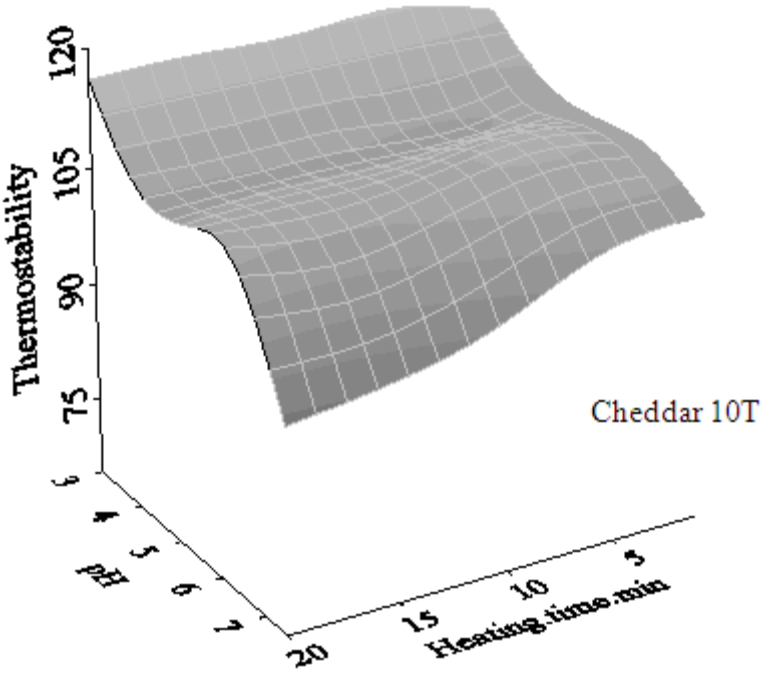

Figure 4.10 Thermostability of spray dried Cheddar whey thermized (pre-heated) for 10 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH pH3-7.5.

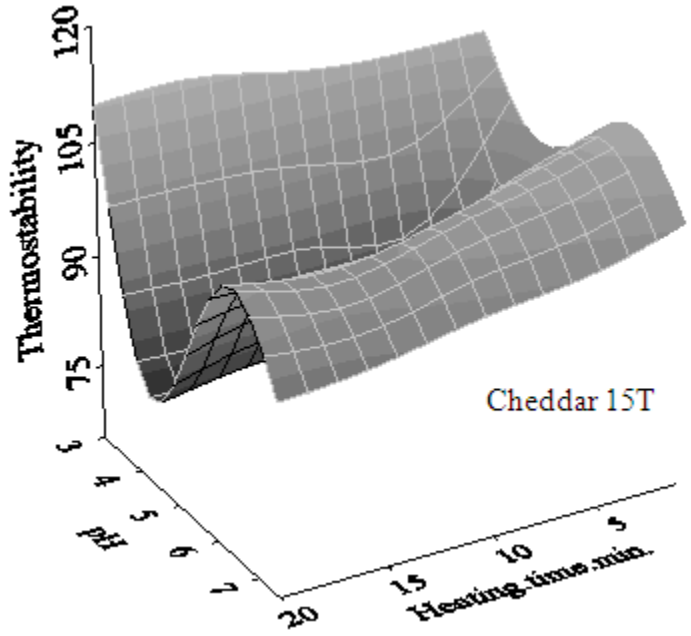

Figure 4.11 Thermostability of spray dried Cheddar whey thermized (pre-heated) for 15 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

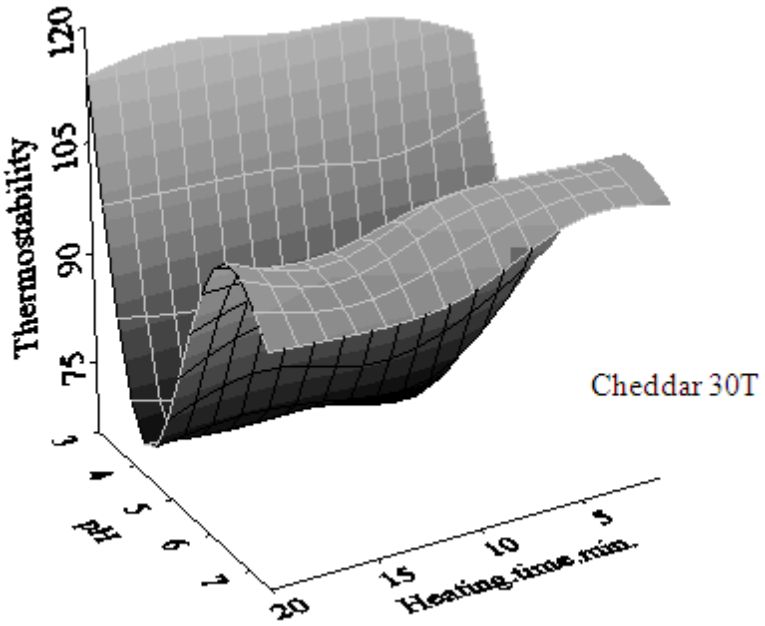

Figure 4.12 Thermostability of spray dried Cheddar whey thermized (pre-heated) for 30 minutes at 70°C as affected by duration of subsequent heating at 82°C and pH3-7.5.

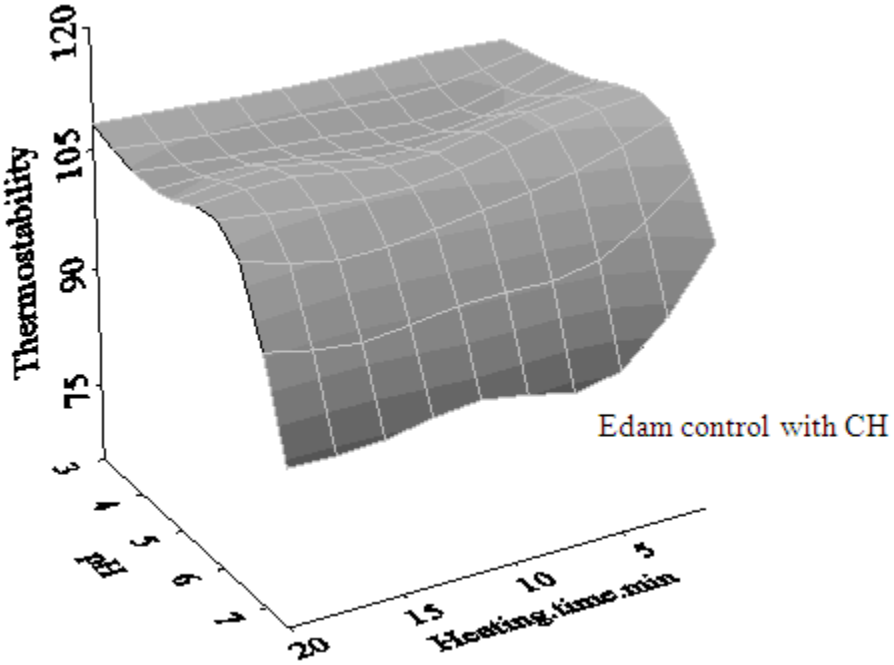

Figure 4.13 Thermostability of untreated (not thermized) spray dried Edam whey with addition of enzymatic hydrolyzate of caseinate as affected by duration of heating at 82°C and pH3-7.5.

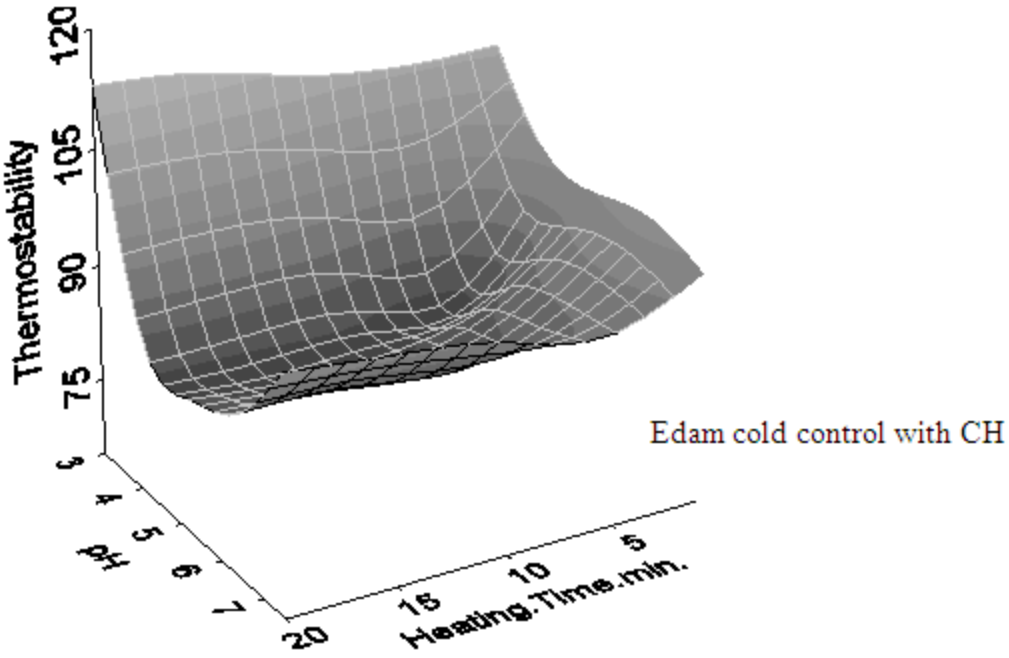

Figure 4.14 Thermostability of untreated (not thermized) freeze dried Edam whey with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of heating at 82°C and pH3-7.5.

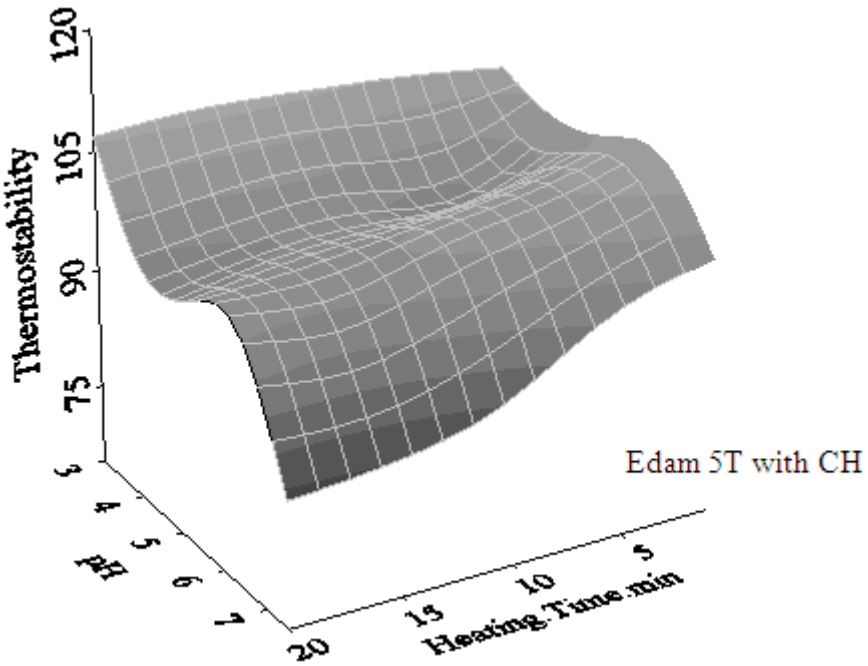

Figure 4.15 Thermostability of spray dried Edam whey thermized (pre-heated) for 5 minutes at 70°C with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of subsequent heating at 82°C and pH3-7.5.

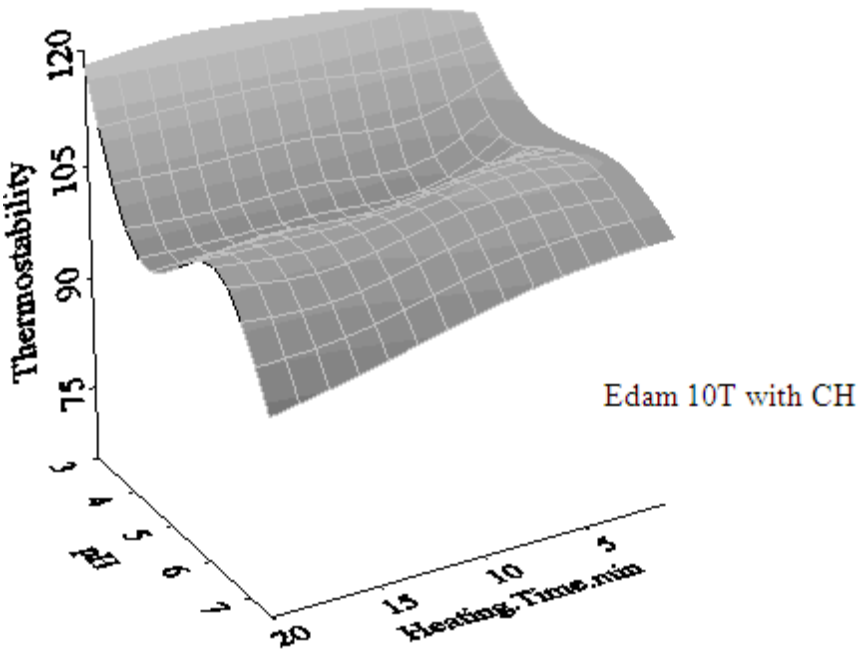

Figure 4.16 Thermostability of spray dried Edam whey thermized (pre-heated) for 10 minutes at 70°C with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of subsequent heating at 82°C and pH3-7.5.

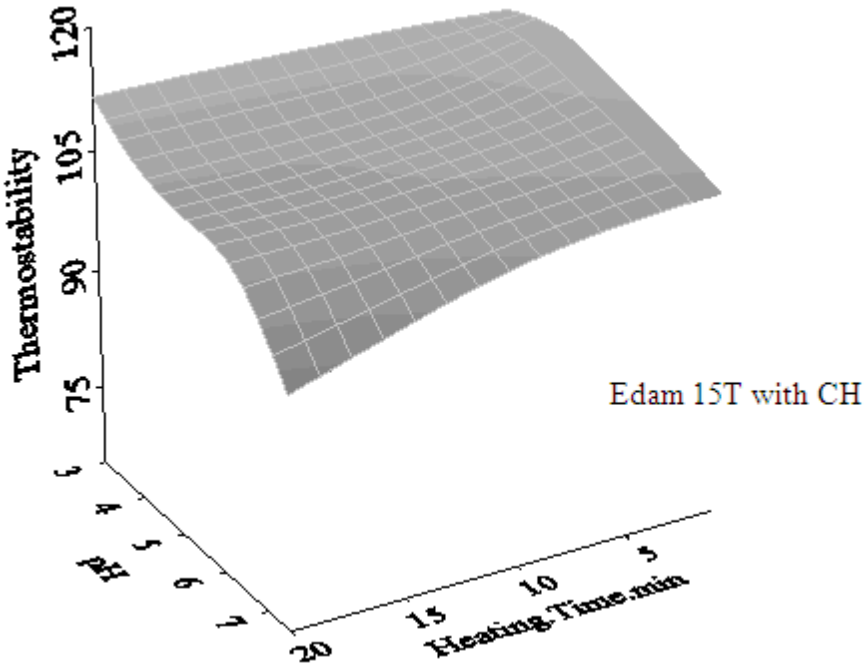

Figure 4.17 Thermostability of spray dried Edam whey thermized (pre-heated) for 15 minutes at 70°C with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of subsequent heating at 82°C and pH3-7.5.

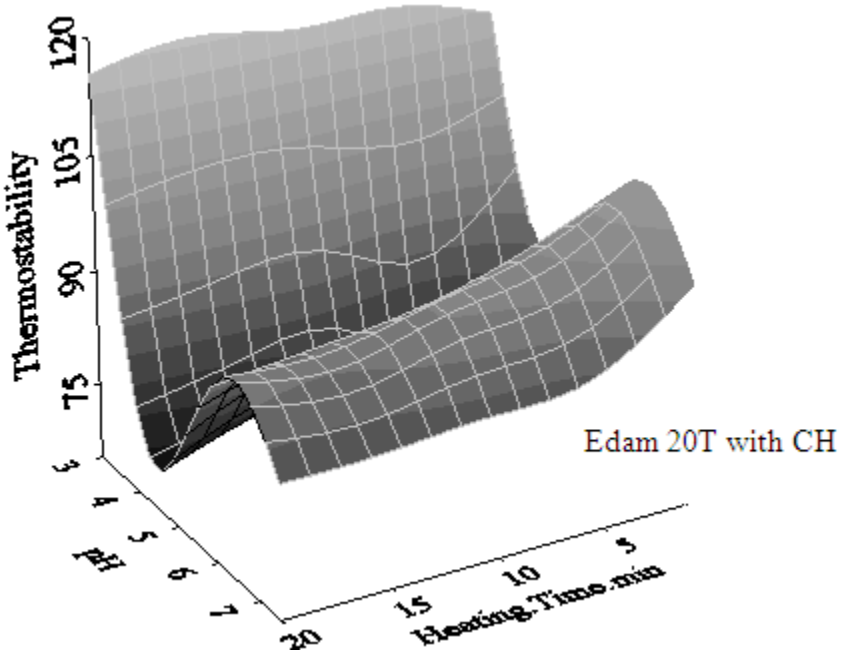

Figure 4.18 Thermostability of spray dried Edam whey thermized (pre-heated) for 20 minutes at 70°C with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of subsequent heating at 82°C and pH3-7.5

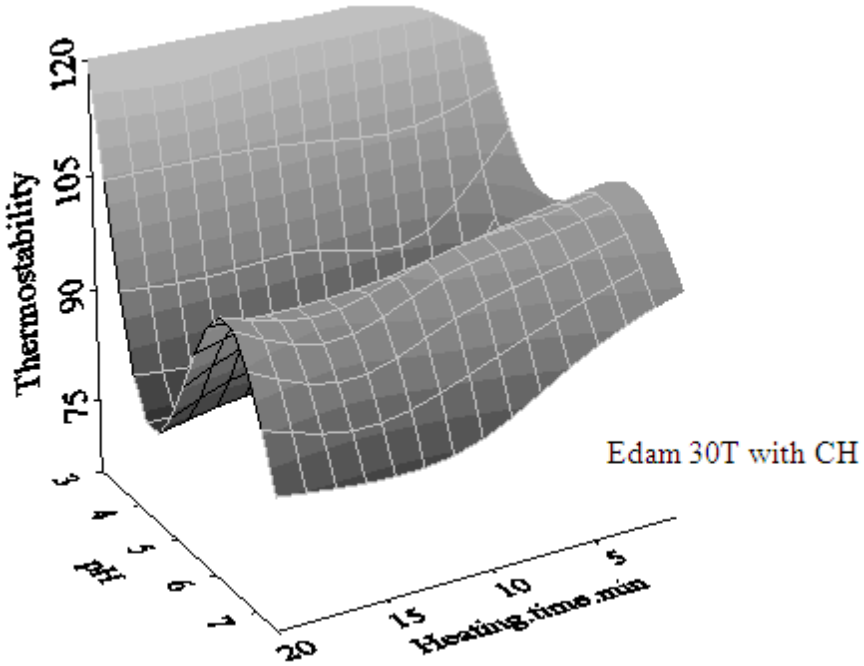

Figure 4.19 Thermostability of spray dried Edam whey thermized (pre-heated) for30 minutes at 70°C with addition of enzymatic hydrolyzate of caseinate (CH) as affected by duration of subsequent heating at 82°C and pH3-7.5.

Table 4.5 Thermostability of Edam whey at pH=3

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	116.43[a]	116.13[a]	117.07[a]	116.89[a]
Cold control	103.57[d]	107.12[b]	104.71[c]	107.99[b]
5T*	112.16[b]	113.63[a]	115.47[a]	116.09[a]
10T*	108.28[c]	109.28[b]	109.71[b]	111.06[b]
15T*	112.15[b]	113.52[a]	111.78[b]	114.41[a]
20T*	107.21[c]	108.98[b]	109.43[b]	110.42[b]
30T*	113.57[b]	115.50[a]	116.75[a]	117.65[a]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.6 Thermostability of Edam whey at pH4.5

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	101.38[a]	95.88[b]	93.04[b]	90.57[b]
Cold control	92.98[b]	74.04[c]	70.96[d]	67.66[d]
5T*	105.03[a]	100.49[a]	99.12[a]	99.75[a]
10T*	77.79[v]	67.56[e]	65.34[f]	62.95[e]
15T*	104.37[a]	101.08[a]	98.34[a]	97.89[a]
20T*	80.68[c]	70.53[d]	68.61[e]	66.67[d]
30T*	90.34[b]	76.76[c]	74.05[c]	71.73[d]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.7 Thermostability of Edam whey at pH6

	Time			
Treatment	1 min	5 min	10 min	20 min
Control	106.63^{b}	100.67^{cd}	99.83^{c}	100.33^{bc}
Cold control	106.57^{b}	97.57^{d}	93.79^{d}	93.16^{d}
5T*	110.32^{a}	105.85^{ab}	105.29^{ab}	105.36^{a}
10T*	102.53^{c}	98.87^{d}	98.36^{c}	97.70^{c}
15T*	109.09^{a}	108.85^{a}	108.74^{a}	106.25^{a}
20T*	102.98^{c}	101.21^{cd}	99.24^{c}	98.61^{bc}
30T*	105.96^{b}	103.00^{bc}	101.66^{bc}	101.73^{b}

$^{a-e}$ Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.8 Thermostability of Edam whey at pH7.5

	Time			
Treatment	1 min	5 min	10 min	20 min
Control	107.43^{a}	99.83^{a}	93.45^{b}	91.72^{b}
Cold control	87.27^{d}	82.46^{d}	79.28^{d}	78.60^{e}
5T*	102.7b^{a}	98.80^{a}	97.86^{a}	97.17^{a}
10T*	95.76^{c}	87.47^{c}	86.09^{c}	84.11^{d}
15T*	102.47^{b}	100.13^{a}	98.85^{a}	92.37^{b}
20T*	96.47^{c}	92.03^{b}	88.34^{c}	87.07^{c}
30T*	101.51^{b}	99.05^{a}	93.29^{b}	91.48^{b}

$^{a-e}$ Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.9 Thermostability of Cheddar whey at pH3

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	108.07[b]	108.92[b]	110.67[b]	110.44[b]
5T*	104.71[c]	105.72[b]	105.72[c]	106.44[c]
10T*	113.92[a]	116.39[a]	115.99[a]	116.06[a]
15T*	108.62[b]	107.79[c]	107.80[c]	109.89[b]
30T*	108.39[b]	111.37[d]	112.25[b]	113.65[a]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.10 Thermostability of Cheddar whey at pH4.5

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	93.74[c]	78.06[c]	72.99[c]	69.90[c]
5T*	82.41[d]	65.99[d]	63.43[e]	60.48[e]
10T*	107.51[a]	105.47[b]	105.28[a]	104.68[a]
15T*	97.21[b]	81.13[b]	77.18[b]	73.78[b]
30T*	80.44[d]	69.28[d]	67.01[d]	66.03[d]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.11 Thermostability of Cheddar whey at pH6

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	98.29[c]	82.95[c]	80.69[d]	76.94[c]
5T*	104.96[b]	102.90[b]	102.69[bc]	102.00[b]
10T*	108.79[a]	110.73[a]	109.58[a]	108.95[a]
15T*	108.40[a]	105.09[b]	101.40[c]	100.27[b]
30T*	103.05[b]	102.81[b]	103.47[b]	101.54[b]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.12 Thermostability of Cheddar whey at pH7.5

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	92.67[d]	87.01[c]	78.68[c]	72.97[d]
5T*	97.59[c]	97.02[b]	97.77[a]	115.15[a]
10T*	104.04[a]	102.93[a]	97.33[a]	91.26[c]
15T*	99.91[bc]	97.19[b]	94.93[b]	90.23[c]
30T*	102.31[ab]	103.28[a]	97.74[a]	96.05[b]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)

* 5T, 10T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively.

Table 4.13 Thermostability of Edam whey with addition of enzymatic hydrolyzate of caseinate at pH3

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	107.6[c]	108.0[d]	107.5[c]	108.1[e]
Cold control	107.1[c]	106.9[d]	108.0[c]	112.5[d]
5T*	104.2[d]	105.7[d]	106.5[c]	106.8[e]
10T*	114.3[a]	116.8[ab]	118.3[a]	118.0[b]
15T*	111.5[b]	112.1[c]	112.0[b]	111.6[d]
20T*	112.5[ab]	114.4[bc]	114.4[b]	115.4[c]
30T*	112.3[ab]	118.1[a]	118.6[a]	120.7[a]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)

*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively

Table 4.14 Thermostability of Edam whey with addition of enzymatic hydrolyzate of caseinate at pH4.5

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	108.2[b]	105.0[b]	104.0[ab]	105.0[a]
Cold control	95.1[e]	83.1[e]	82.0[c]	80.6[d]
5T*	101.3[d]	96.0[d]	95.3[b]	92.3[c]
10T*	104.5[c]	98.8[c]	97.1[b]	95.8[b]
15T*	112.6[a]	109.3[a]	107.2[a]	105.7[a]
20T*	82.5[f]	72.7[f]	81.6[c]	67.2[f]
30T*	94.7[e]	79.1[e]	76.0[c]	73.7[e]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively

Table 4.15 Thermostability of Edam whey with addition of enzymatic hydrolyzate of caseinate at pH6

Treatment	Time			
	1 min	5 min	10 min	20 min
Control	112.2[a]	111.1[a]	107.8[a]	109.4[a]
Cold control	98.0[e]	89.8[e]	84.1[d]	85.0[e]
5T*	106.4[bc]	101.2[c]	100.1[b]	98.8[c]
10T*	106.6[bc]	107.9[b]	106.3[a]	105.9[b]
15T*	108.9[b]	107.2[b]	106.3[a]	105.1[b]
20T*	103.1[d]	97.8[d]	94.4[c]	91.3[d]
30T*	105.3[cd]	103.8[c]	102.6[b]	101.4[c]

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively

Table 4.16 Thermostability of Edam whey with whey protein enzymatic hydrolyzate at pH7.5

	Time			
Treatment	1 min	5 min	10 min	20 min
Control	98.7^{cd}	87.2^{d}	87.3^{c}	84.7^{c}
Cold control	94.8^{e}	94.3^{cd}	92.4^{c}	95.7^{a}
5T*	97.1^{cd}	94.3^{c}	86.6^{d}	81.0^{d}
10T*	101.0^{b}	100.1^{b}	97.7^{b}	91.0^{b}
15T*	105.2^{a}	104.5^{a}	102.2^{a}	93.8^{ab}
20T*	94.1^{e}	86.8^{d}	84.4^{d}	82.6^{cd}
30T*	95.5^{de}	91.4^{c}	83.8^{d}	82.5^{cd}

[a-e] Mean values within the same column with different letters are significantly different. ($p<0.05$)
*5T, 10T, 20T, and 30T refer to 5, 10, 15, 20, and 30 min of thermization, respectively

Table 4.17 Comparison the thermostability of Edam whey control with Cheddar whey control at pH3

	Time			
Treatment	1 min	5 min	10 min	20 min
Edam whey control	116.43^{a}	116.13^{a}	117.077^{a}	116.886^{a}
Cheddar whey control	108.07^{b}	108.92^{b}	110.67^{b}	110.44^{b}

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.18 Comparison the thermostability of Edam whey control with Cheddar whey control at pH4.5

	Time			
Treatment	1 min	5 min	10 min	20 min
Edam whey control	101.38^{a}	95.88^{a}	93.04^{a}	90.57^{a}
Cheddar whey control	93.74^{b}	78.06^{b}	72.99^{b}	69.9^{b}

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.19 Comparison the thermostability of Edam whey control with Cheddar whey control at pH6

	Time			
Treatment	1 min	5 min	10 min	20 min
Edam whey control	106.63[a]	100.67[a]	99.83[a]	100.33[a]
Cheddar whey control	98.28[b]	82.95[b]	80.40[b]	78.94[b]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.20 Comparison the thermostability of Edam whey control with Cheddar whey control at pH7.5

	Time			
Treatment	1 min	5 min	10 min	20 min
Edam whey control	107.42[a]	99.83[a]	93.45[a]	91.72[a]
Cheddar whey control	92.67[b]	87.01[b]	78.68[b]	72.93[b]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.21 Comparison of TS of Cheddar whey control and control with addition of hydrolyzate of casienate at pH3

	Time			
Treatment	1 min	5 min	10 min	20 min
control	108.07[a]	108.92[b]	110.67[a]	110.44[a]
control with hydrolyzate of casienate	101.39[b]	101.71[b]	101.96[b]	101.99[b]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.22 Comparison of TS of Cheddar whey control and control with addition of hydrolyzate of casienate at pH4.5

	Time			
Treatment	1 min	5 min	10 min	20 min
control	93.74[b]	78.06[a]	72.99[a]	69.90[a]
control with hydrolyzate of casienate	96.73[a]	77.48[a]	71.69[a]	69[a]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.23 Comparison of TS of Cheddar whey control and control with addition of hydrolyzate of casienate at pH6

	Time			
Treatment	1 min	5 min	10 min	20 min
control	98.28[a]	82.95[a]	80.69[a]	76.94[a]
control with hydrolyzate of casienate	99.47[a]	81.92[a]	77.08[b]	73.7[b]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

Table 4.24 Comparison of TS of Cheddar whey control and control with addition of hydrolyzate of casienate at pH7.5

	Time			
Treatment	1 min	5 min	10 min	20 min
control	92.67[b]	87[b]	78.68[b]	72.94[b]
control with hydrolyzate of casienate	95.14[a]	90.3[a]	87.72[a]	84.54[a]

[a-b] Mean values within the same column with different letters are significantly different. ($p<0.05$)

CHAPTER V

CONCLUSION

Whey is a byproduct generated during cheese manufacture. Sweet whey, also referred to as cheese whey, is the liquid that separates from the cheese curd when starter cultures and rennet are applied to milk during the manufacture of cheeses like Edam and Cheddar. Though highly nutritious, problems associated with whey utilizations include variability of desired functional attributes and lack of thermostability (TS), an attribute that is imperative in "retort-stable" food application and foods that require pasteurization, e.g., health and diet drinks. This is particularly meaningful if the TS is high at low pH environments. Protein structure plays a critical role in the functionality of proteins in food can be changed by thermal treatment.

The pretreated sweet whey with thermization for a short period of time (5-10 min at 70°C) significantly improved the TS. Added whey protein enzymatic hydrolyzate of caseinate, a significant improvement in the TS of Edam whey was observed when thermization for a short period of time (10-15 min at 70°C) occurred. This research showed the possibility of using thermization as a pretreatment method to improve the thermostability of sweet whey. The TS improved significantly at pH 3 and pH 6 as compared with other pHs. The almost neutral pH could be used in several of areas in food systems, such as fruit drinks. As pretreatment becomes a common practice to improve the functionality of the whey proteins, there are many margin areas that have not been studied yet as related to this practice.

REFERENCES

Arozarena, I., H. Bertholo, et al. (2001). "Study of the total replacement of egg by white lupine protein, emulsifiers and xanthan gum in yellow cakes." European Food Research and Technology **213**(4): 312-316.

Ashokkumar, M., J. Lee, et al. (2009). "Hot topic: Sonication increases the heat stability of whey proteins." Journal of Dairy Science **92**(11): 5353-5356.

Banks, J., A. Law, et al. (1993). The inclusion of whey proteins in cheese. An overview. Cheese yield and factors affecting its control, Brussels, Belgium, International Dairy Federation.

Beeching, E. and G. Severn (1943). British Patent 560,840, July.

Blaschek, K., W. Wendorff, et al. (2007). "Survey of salty and sweet whey composition from various cheese plants in Wisconsin." Journal of Dairy Science **90**(4): 2029-2034.

Bohoua, G. L. and Z. U. Haque (2006). "Effect of whey peptides on surface activities of milk protein." Sciences des Aliments **26**(6): 525-532.

Bounous, G., G. Batist, et al. (1991). "Whey proteins in cancer prevention." Cancer letters **57**(2): 91-94.

Boye, J. I. and I. Alli (2000). "Thermal denaturation of mixtures of alpha-lactalbumin and beta-lactoglobulin: a differential scanning calorimetric study." Food Research International **33**(8): 673-682.

Brandts, J. F. (1964). "The thermodynamics of protein denaturation. II. A model of reversible denaturation and interpretations regarding the stability of chymotrypsinogen." Journal of the American Chemical Society **86**(20): 4302-4314.

Bucci, L. and L. Unlu (2000). Proteins and amino acid supplements in exercise and sport. Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition. J. A. Driskell and I. Wolinsky, CRC Press**:** 191-212.

Bylund, G. (2003). Dairy processing handbook, Tetra Pak Processing Systems AB.

Chobert, J. M., C. Bertrand-Harb, et al. (1988). "Solubility and emulsifying properties of caseins and whey proteins modified enzymically by trypsin." Journal of Agricultural and Food Chemistry **36**(5): 883-892.

Coton, S. (1976). Recovery of dairy waste. Foods from Waste, London, Applied Science Publishers.

Davis, C. G., D. P. Blayney, et al. (2010) Long-term growth in U.S. cheese consumption may slow. 19

De Wit, J. (1990). "Thermal stability and functionality of whey proteins." Journal of Dairy Science **73**(12): 3602-3612.

De Wit, J. N. (1989). Functional properties of whey proteins. Developments in dairy chemistry. P. F. Fox. London, Elsevier Applied Science. **4:** 285-321.

De Wit, J. N. and G. Klarenbeek (1984). "Effects of various heat treatments on structure and solubility of whey proteins." Journal of Dairy Science **67**(11): 2701-2710.

de Wit, J. N., G. Klarenbeek, et al. (1978). A simple method for the clarification of whey. 20th International Dairy Congress, Paris.

Deis, R. (1997). "Spray drying-innovative use of an old process." Food Product Design **7**(2): 97–113.

Demott, B. J. (1972). "Cottage cheese whey used as a beverage." Sunbelt Dairyman **10**(10): 14.

Djurić, M., M. Carić, et al. (2004). "Development of whey-based beverages." European Food Research and Technology **219**(4): 321-328.

Eigel, W., J. Butler, et al. (1984). "Nomenclature of Proteins of Cow's Milk: Fifth Revision1." Journal of Dairy Science **67**(8): 1599-1631.

Fisher, R. A. (1949). The design of experiments. Edinburgh, Oliver and Boyd.

Floris, R., I. Recio, et al. (2003). "Antibacterial and antiviral effects of milk proteins and derivatives thereof." Current Pharmaceutical Design **9**(16): 1257-1275.

Fox, P. (1982). Developments in dairy chemistry. I. Proteins. London, Applied Science Publishers.

Gillies, M. T. (1974). Whey processing and utilization: economic and technical aspects. Park Ridge, N.J., Noyes Data Corp.

Goldberg, I. (1994). Functional foods: designer foods, pharmafoods, nutraceuticals. London, Springer.

Gradinaru, G., C. G. Biliaderis, et al. (2003). "Thermal stability of Hibiscus sabdariffa L. anthocyanins in solution and in solid state: effects of copigmentation and glass transition." Food Chemistry **83**(3): 423-436.

Graeff, F. W. H. (1898). Effervescent milk beverage and method of making same. U. S.

Gulseren, I., D. Guzey, et al. (2007). "Structural and functional changes in ultrasonicated bovine serum albumin solutions." Ultrasonics Sonochemistry **14**(2): 173-183.

Haggett, T. O. R. (1976). "The whipping, foaming and gelling properties of whey protein concentrates." New Zealand Journal of Dairy Science and Technology **11**: 244-250.

Hall, G. M. (1996). Methods of testing protein functionality. London, Blackie Academic & Professional.

Haque, Z. and T. Ji (2003). "Cheddar whey processing and source: II. Effect on non fat ice cream and yoghurt1." International Journal of Food Science & Technology **38**(4): 463-473.

Haque, Z. U. (1993). "Influence of milk peptides in determining the functionality of milk proteins: a review." Journal of dairy science **76**(1): 311-20.

Haque, Z. U. (1993). "Influence of Milk Peptides in Determining the Functionality of Milk Proteins: A Review1." Journal of Dairy Science **76**(1): 311-320.

Haque, Z. U. and Z. Mozaffar (1992). "Casein hydrolysate. II. Functional properties of peptides." Food hydrocolloids **5**(6): 559-571.

Hardy, G. (2000). "Nutraceuticals and functional foods: introduction and meaning." Nutrition **16**(7-8): 688-689.

Hidalgo, J. and E. Gamper (1977). "Solubility and heat stability of whey protein concentrates." Journal of Dairy Science **60**(10): 1515-1518.

Holsinger, V. (1973). The use of cheese whey in beverages. Proceedings of the Whey Products Conference., Chicago.

Holsinger, V., L. Posati, et al. (1974). "Whey beverages: A review." Journal of Dairy Science **57**(8): 849-859.

Huffman, L. M. and W. J. Harper (1999). "Maximizing the value of milk through separation technologies." Journal of Dairy Science **82**(10): 2238-2244.

Jelen, P. (1973). "Whipping Studies with Partially Deloctosed Cheese Whey." Journal of Dairy Science **56**(12): 1505-1511.

Jelen, P. (1979). "Industrial whey processing technology: An overview." Journal of Agricultural and Food Chemistry **27**(4): 658-661.

Jelen, P. (2003). Whey processing: Utilization and Products. Encyclopedia of Dairy Sciences. H. Roginski, J. W. Fuquay and P. F. Fox. New York, Academic Press**:** 2739-2745.

Jelen, P., R. Currie, et al. (1987). "Compositional Analysis of Commercial Whey Drinks." Journal of Dairy Science **70**(4): 892-895.

Jelen, P. and W. Rattray (1995). "Thermal Denaturation of Whey Proteins. Brussels." International Dairy Federation (Special Issue)(9501): 66-85.

Ji, T. and Z. U. Haque (2003). "Cheddar whey processing and source: I. Effect on composition and functional properties of whey protein concentrates." International Journal of Food Science and Technology **38**(4): 453-461.

Josephson, R. V., S. S. H. Rizvi, et al. (1975). "Compositional differences in whey systems." Journal of Food Science **40**(3): 479-483.

Kella, N. and J. E. Kinsella (1988). "Enhanced thermodynamic stability of beta-lactoglobulin at low pH. A possible mechanism." Biochemical Journal **255**(1): 113.

Kelly, P. M. (2002). Membrane Separation. Encyclopedia of Dairy Sciences. H. Roginski, J. W. Fuquay and P. F. Fox. New York, Academic Press**:** 1777-1786.

Kester, J. and T. Richardson (1984). "Modification of whey proteins to improve functionality." Journal of Dairy Science **67**(11): 2757-2774.

Khan, M. N., L. Rooney, et al. (1979). "Baking properties of plasma protein isolate." Journal of Food Science **44**(1): 274-276.

Kinsella, J. E. (1976). "Functional properties of proteins in foods: a survey." Critical Reviews in Food Science and Nutrition **7**(3): 219-280.

Kontopidis, G., C. Holt, et al. (2004). "Invited Review:[beta]-Lactoglobulin: Binding Properties, Structure, and Function." Journal of Dairy Science **87**(4): 785-796.

Kosaric, N. and Y. Asher (1985). The utilization of cheese whey and its components. Agricultural Feedstock and Waste Treatment and Engineering, Springer Berlin / Heidelberg. **32:** 25-60.

Kosikowski, F. and V. Mistry (1997). Cheese and fermented milk foods. Ashfield, MA, New England Cheesemaking Supply Co,.

Kosikowski, F. V. (1982). Cheese and Fermented Milk Foods, Brooklondale, New York, NY.

Kosikowski, F. V. and L. E. Wierzbicki (1973). "Lactose hydrolysis of raw and pasteurized milks by Saccharomyces lactis lactase." Journal of Dairy Science **56**(1): 146-148.

Kucukoner, E. and Z. U. Haque (1998). "Peptide profile of low-fat Edam cheese." Turkish Journal of Veterinary and Animal Sciences **22**: 449-452.

Kwak, N. S. and D. J. Jukes (2001). "Functional foods. Part 1: the development of a regulatory concept." Food Control **12**(2): 99-107.

Lahl, J. W. and D. A. Grindstaff (1989). Spices and seasonings: hydrolysed proteins. . The 6th SIFST symposium on food ingredients--Applications, Status and Safety, Singapore Singapore Institute of Food Science and Technology.

Lawson, M. (1994). "Milk proteins as food ingredients." Food Technology **48**(10): 101.

Leon-Sicairos, N., F. Lopez-Soto, et al. (2006). "Amoebicidal Activity of Milk, Apo-lactoferrin, sIgA and Lysozyme." Clinical Medicine & Research **4**(2): 106-113.

Mann, B. and R. Malik (1996). "Studies on some functional characteristics of whey protein-polysaccharide complex." Journal of food science and technology **33**(3): 202-206.

Marwaha, S. S. and J. F. Kennedy (1988). "Whey—pollution problem and potential utilization." International Journal of Food Science & Technology **23**(4): 323-336.

Mavropoulou, I. and F. Kosikowski (1973). "Composition, solubility, and stability of whey powders." Journal of Dairy Science **56**(9): 1128-1134.

Mavropoulou, I. and F. Kosikowski (1973). "Free amino acids and soluble peptides of whey powders." Journal of Dairy Science **56**(9): 1135-1138.

Mawson, A. J. (1994). "Bioconversions for whey utilization and waste abatement." Bioresource Technology **47**(3): 195-203.

McIlvaine, T. (1921). "A buffer solution for colorimetric comparison." Journal of Biological Chemistry **49**(1): 183.

McKenzie, H. A. (1970). β-Lactoglobulin. New York, Academic Press.

Meat and Livestock Commisssion (2003). "General Guidelines on Liquid Feeding for Pigs." Retrieved February 12, 2008, from www.bpex.org/technical/general/pdf/liquidfeeding.pdf.

Meisel, H. and E. Schlimme (1996). "Bioactive peptides derived from milk proteins: Ingredients for functional foods?" Kieler Milchwirtschaftliche Forschungsberichte **48**(4): 343-357.

Modler, H. and D. Emmons (1977). "Properties of whey protein concentrate prepared by heating under acidic conditions." Journal of Dairy Science **60**(2): 177-184.

Morr, C. (1992). "Improving the texture and functionality of whey protein concentrate." Food Technology **46**(1): 110-113.

Morr, C. and E. Foegeding (1990). "Composition and functionality of commercial whey and milk protein concentrates and isolates: a status report." Food Technology **44**(4).

Morr, C., P. Swenson, et al. (1973). "Functional characteristics of whey protein concentrates." Journal of Food Science **38**(2): 324-330.

Morr, C. V. (1989). Whey Protein: Manufacture. Developments in Dairy Chemistry-4: Proteins. P. F. Fox. London, Elsevier Science Publishers**:** 245-248.

Morr, C. V. and E. Y. Ha (1993). "Whey protein concentrates and isolates: processing and functional properties." Critical Reviews in Food Science and Nutrition **33**(6): 431-476.

Morr, V. (1975). "Symposium: Milk proteins in dairy and food processing." Journal of Dairy Science **58**: 977.

Mulvihill, D. and M. Donovan (1987). "Whey proteins and their thermal denaturation-a review." Irish Journal of Food Science and Technology **11**(1): 43-75.

Nakai, S. and E. Li-Chan (1985). "Structure modification and functionality of whey proteins: quantitative structure-activity relationship approach." Journal of Dairy Science **68**(10): 2763-2772.

Nollet, L. M. L. (2004). Handbook of Food Analysis: Physical characterization and nutrient analysis, Marcel Dekker.

Nutraceuticals World (2009). "Biochem 100% Whey Ready to Drink." Retrieved May 5th, 2010, from http://www.nutraceuticalsworld.com/issues/2009-12/view_products/biochem-100-whey-ready-to-drink/.

Oldfield, D. J., M. W. Taylor, et al. (2005). "Effect of preheating and other process parameters on whey protein reactions during skim milk powder manufacture." International Dairy Journal **15**(5): 501-511.

Olson, D. W. and Z. U. Haque (1998). Composition and functional properties of whey collected from commercial whey processing plants. . 1998 Annual Meeting of the Institute of Food Technologists, Atlanta, GA.

Onwulata, C. and P. Huth (2008). Whey processing, functionality and health benefits. Ames, Iowa, Wiley-Blackwell.

Onwulata, C. and P. Tomasula (2004). "Whey texturization: A way forward." Food Technology **58**(7): 50-55.

Onwulata, C. I., R. P. Konstance, et al. (2004). "Minimizing Variations in Functionality of Whey Protein Concentrates from Different Sources." Journal of Dairy Science **87**(3): 749-756.

Panesar, P. S., Kennedy, J.F., Gandhi, D.N., Bunko, K. (2007). "Bioutilisation of whey for lactic acid production." Food Chemistry **105**: 1-14.

Pelegrine, D. and C. Gasparetto (2005). "Whey proteins solubility as function of temperature and pH." Lebensmittel-Wissenschaft und-Technologie **38**(1): 77-80.

Perez, M. D., L. Sanchez, et al. (1992). "Effect of [beta]-lactoglobulin on the activity of pregastric lipase. A possible role for this protein in ruminant milk." Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism **1123**(2): 151-155.

Peter, P. N. and R. W. Bell (1930). "Normal and Modified Foaming Properties of Whey-Protein and Egg-Albumin Solutions." Industrial & Engineering Chemistry **22**(10): 1124-1128.

Phillips, L., W. Schulman, et al. (1990). "pH and heat treatment effects on foaming of whey protein isolate." Journal of Food Science **55**(4): 1116-1119.

Picone, C. S. F., K. P. Takeuchi, et al. (2011). "Heat-Induced Whey Protein Gels: Effects of pH and the Addition of Sodium Caseinate." Food Biophysics **6**: 77-83.

Pour-El, A. (1981). Protein Functionality: Classification, Definition, and Methodology. Protein Functionality in Foods, American Chemical Society. **147:** 1-19.

Ramsdell, G. and B. Webb (1938). "Sweetened Condensed Whey: Its Manufacture and Properties." Journal of Dairy Science **21**(6): 305-314.

Rech, R. and M. A. Z. Ayub (2007). "Simplified feeding strategies for fed-batch cultivation of Kluyveromyces marxianus in cheese whey." Process Biochemistry **42**(5): 873-877.

Schingoethe, D. J. (1976). "Whey utilization in animal feeding: a summary and evaluation." Journal of Dairy Science **59**(3): 556-570.

Severin, S. and X. Wenshui (2005). "Milk biologically active components as nutraceuticals: review." Critical Reviews in Food Science and Nutrition **45**(7): 645-656.

Shon, J. and Z. Haque (2007). "Functional attributes of native and thermized sour and sweet whey." International Journal of Dairy Technology **60**: 135-162.

Sinha, R., C. Radha, et al. (2007). "Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation." Food Chemistry **101**(4): 1484-1491.

Spector, A. A. (1975). "Fatty acid binding to plasma albumin." The Journal of Lipid Research **16**(3): 165.

Spreer, E. and A. Mixa (1998). Milk and dairy product technology. New York, NY, Marcel Dekker.

Tanford, C., J. J. T. E. C.B. Anfinsen, et al. (1970). Protein Denaturation: Part C. Theoretical Models for The Mechanism of Denaturation. Advances in Protein Chemistry, Academic Press. **24:** 1-95.

Tango, M. S. A. and A. E. Ghaly (1999). "Effect of temperature on lactic acid production from cheese whey using Lactobacillus helveticus under batch conditions." Biomass and Bioenergy **16**(1): 61-78.

Trueman, S. (2009). Functional Foods, Patents and Health Claims. The IP Strategist. Tolland, Connecticut Nerac Inc. **Spring Issue:** 6-7.

Tunick, M. H. (2008). Whey protein production and utilization: a brief history, Wiley-Blackwell. .

U.S. Dairy Export Council (2011). "2009 Year-end Summary." Retrieved July 10th 2011, from http://www.usdec.org/Why/content.cfm?ItemNumber=82452.

Van Der Kraan, M. I. A., K. Nazmi, et al. (2006). "Distinct bactericidal activities of bovine lactoferrin peptides LFampin 268-284 and LFampin 265-284: Asp-Leu-Ile makes a difference." Biochemistry and Cell Biology **84**(3): 358-362.

Vasey, C. (2006). The whey prescription : the healing miracle in milk. Rochester, Vt., Healing Arts Press.

Wang, Q., A. Tolkach, et al. (2006). "Quantitative Assessment of Thermal Denaturation of Bovine alpha-Lactalbumin via Low-Intensity Ultrasound, HPLC, and DSC." Journal of Agricultural and Food Chemistry **54**(18): 6501-6506.

Watson, K. S., A. E. Peterson, et al. (1977). "Benefits of spreading whey on agricultural land." Journal of Water Pollution Control Federation: 24-34.

Webb, B. H. and E. O. Whittier (1948). "The utilization of whey: a review." Journal of Dairy Science **31**(2): 139-164.

Webb, B. H. and E. O. Whittier (1970). Byproducts from milk. Westport, Conn., Avi Pub. Co.

Weetall, H. H., N. B. Havewala, et al. (1974). "The preparation of immobilized lactase and its use in the enzymatic hydrolysis of acid whey." Biotechnology and Bioengineering **16**(3): 295-313.

Wingerd, W. (1971). "Lactalbumin as a food ingredient." Journal of Dairy Science **54**: 1234.

Yalcin, A. (2006). "Emerging therapeutic potential of whey proteins and peptides." Current Pharmaceutical Design **12**(13): 1637-1643.

Zall, R. R. (1984). "Trends in whey fractionation and utilization, a global perspective." Journal of Dairy Science **67**(11): 2621-2629.

Zhu, H. and S. Damodaran (1994). "Heat-induced conformational changes in whey protein isolate and its relation to foaming properties." Journal of Agricultural and Food Chemistry **42**(4): 846-855.

Zisu, B., J. Lee, et al. (2011). "Effect of ultrasound on the physical and functional properties of reconstituted whey protein powders." Journal of Dairy Research **78**: 226-232.