THERMOSTABILITY OF PROTEIN IN STARCH DERIVATIVE EMULSION

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ABSTRACT

Heat-induced destabilisation (denaturation) of whey proteins (WP) is one of the major problems limiting their application in food products. Microencapsulation is a relatively new technology that is used for protection and stabilization. The purpose of this research is to determine the protein denaturation kinetic rate order for main fractions of whey protein isolate (WPI) that are β-lactoglobulin (β-Lg) ,α-lactoalbumin (α-Lac) and bovine serum albumin (BSA). The other purpose of this research is to formulate the microencapsulation material. This method is used to minimize the denaturation of the protein. The experiment was conducted start from preparation sample of various composition of whey protein - maltodextrin solution for heating process (water bath and oil bath). Next step is the preparation of sample for further analysis in UPLC. During kinetic denaturation analysis, the parameter was use are temperature, time and via microencapsulation technique. From the research, the best ratio for WPI and maltodexrtrin is 9:1. The percentage denaturation for 9:1 of WPI to maltodextrin ratio is lower compared to the ratio 1:1. Results from analysis of composition of the residual native protein suggested that thermal sensitivity of a-lac is lower than B-lg. The percentage of denaturation of B-lg is faster than a-lac for each temperature with increasing time. High temperature will cause high percentage of denaturation. The temperature 60^0C shows the lowest percentage of denaturation as showed in Figure 4.13 and 4.14. In conclusion, result from this research may be useful to gain understanding of relationship between heat treatment and its effect on functional properties of whey protein.

KESTABILAN TERMA PROTEIN DALAM EMULSI TERBITAN KANJI

ABSTRAK

Ketidakstabilan yang disebabkan oleh haba (penyahaslian) terhadap protein whey adalah salah satu masalah utama yang menghadkan aplikasi mereka dalam industri makanan. Pemikrokapsulan adalah satu teknologi yang agak baru yang digunakan untuk perlindungan dan penstabilan. Tujuan kajian ini adalah untuk menentukan kadar susunan kinetik peyahaslian protein untuk setiap pecahan utama protein whey iaitu β-lactoglobulin (β-Lg) ,α-lactoalbumin (α-Lac) and Bovine Serum Albumin (BSA). Tujuan lain kajian ini juga adalah untuk merumuskan bahan pemikrokapsulan. Kaedah ini digunakan untuk meminimumkan penyahaslian protein. Eksperimen telah dijalankan bermula dari penyediaan sampel yang pelbagai komposisi larutan protein whey-maltodexrtin untuk proses pemanasan di dalam 'shaking water bath' dan 'oil bath'. Langkah seterusnya ialah penyediaan sampel untuk analisis selanjutnya dalam UPLC. Semasa analisis penyahaslian kinetik, parameter yang di gunakan adalah suhu, masa dan melalui teknik pemikrokapsulan. Daripada kajian, nisbah yang terbaik untuk WPI dan maldodextrin ialah 9:1. Peratusan penyahaslian untuk 9:1, nisbah WPI : maltodextrin rendah berbanding dengan nisbah 1:1. Hasil daripada analisis komposisi sisa protein asli menunjukkan sensitiviti haba a-lac lebih rendah berbanding B-lg. Peratusan penyahaslian B-lg lebih laju berbanding a-lac untuk setiap suhu dengan peningkatan masa. Suhu yang tinggi akan menyebabkan peratusan penyahaslian semakin tinggi. Pada suhu 60^0C menunjukkan peratusan peyahaslian yang paling rendah seperti yang dipaparkan di gambarajah 4.13 dan 4.14. Kesimpulannya, hasil daripada kajian ini, mungkin berguna untuk mendapatkan pemahaman tentang hubungan antara rawatan haba dan kesan ke atas sifat-sifat fungsi protein whey.

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LIST OF ABBREVIATIONS

WP	-	Whey Protein
WPI	-	Whey Protein
a-lac	-	a-lac
B-lg	-	B-lactoglobin
BSA	-	Bovine Serum Albumin
UPLC	-	Ultra Performance Liquid Chromatography

LIST OF SYMBOLS

^0C	-	Degree Celcius
%	-	Percent
G	-	Gram
L	-	Liter
Rpm	-	rotation per minutes
M	-	Molarity
Ml	-	milliliter
I	-	Length
C	-	Concentration
Mg	-	Miligram

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production (Hudson et al., 2000). Some preclinical studies in rodents have suggested that whey protein may possess anti-inflammatory or anti-cancer properties; however, human data are lacking. As part of aware about growing health and wellness, it is important to improve the protein content of products. Many food and beverage manufacturers use whey proteins as the protein source of choice for product innovation. Expanded utilization of these ingredients holds great potential for creating even more new formulation possibilities through improvements in whey quality and performance. This report summarizes recent research on improving the heat stability of whey protein ingredients, thus helping product developers utilize them in more applications. Whey proteins represent about 20 percent of the milk proteins, which remain in the serum phase

during the process of making cheese and are later processed into many different ingredients (Dissanayake et al., 2009). The primary whey proteins are beta-lactoglobulin (β-lg), alpha-lactalbumin (α-lac), bovine serum albumin,immunoglobulins and proteose peptones.

In the food industry, encapsulation process can be applied for a variety of reasons. Encapsulation is a useful tool to improve delivery of bioactive molecules (e.g. antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells (e.g. probiotics) into foods (Nedovic et al.,2011). The microencapsulation technique is very important in order to improve the quality of whey protein. For encapsulation technique, spray drying is the most extensively applied (Nedovic et al., 2011). This technique is used in the food industry because it is flexible, continuous, but more important an economical operation. There are some advantages of microencapsulation. For example, encapsulation can be used to protect sensitive materials such as flavouring agents, aroma, vitamins, etc. from oxygen in air, and for controlled release of encapsulated substance (Faldt et al., 1996). The objective of encapsulation of active ingredients is to provide improved stability in final products and during processing. The study of this work is the evaluation of the effects of WPI conditions on the microencapsulating formulation (different ratio of matodextrin) by using the different parameters (temperature and time). The study focused on the reaction kinetic of WPI denaturation upon heating (60^0C, 70^0C, 80^0C, 90^0C, 100^0C and 120^0C). The native structure of proteins is the thermodynamically most stable conformation which is formed under precise physiological conditions and presents a net product of different intra- and inter-molecular attractive and repulsive forces (Damodaran et al., 2008; Shirley, 1995). However, different environmental changes such as heat can affect the protein native structure. The major changes in the

secondary, tertiary or quaternary protein structures without breaking the backbone peptide bonds are known as protein denaturation. In the case of globular proteins, the denaturation leads to consequent protein aggregation affecting their solubility as well as all other functional properties (Damodaran et al., 2008; Anema and McKenna, 1996).

Milk, in comparison with many other foods, is remarkably heat stable, which allows for the manufacture of a range of heat-sterilized products. Numerous reactions that influence the nutritional and functional properties of the milk and the subsequent products can occur during the heating process (Walstra et al., 1984). These reactions can occur because it is dependent on heating conditions like temperature and duration of heat treatment. It is important to have an understanding of these reactions so that heating conditions can be applied to achieve the desired functional properties in milk products and to keep undesirable reactions to a minimum. The whey proteins retain their native conformation only within relatively limited temperature ranges. Exposing these proteins to extremes of temperature results in denaturation of the proteins, defined as a considerable change in the native conformation in which the three-dimensional (tertiary) structure of the polypeptide chain is converted to a lower state of order.

1.2 Problem Statement

Numerous reactions that influence the nutritional and functional properties of the milk and the subsequent products can occur during the heating process (Walstra et al., 1984). This is reactions happen dependent on the heating condition

(temperature and duration of heat treatment). It is important to study of the rate order of the kinetic denaturation in each main fraction of whey protein so that to have an understanding of these reactions so that heating conditions can be applied to achieve the desired functional properties in milk products and to keep undesirable reactions to a minimum. Protein will be denatured. The method of using an encapsulation of active ingredients is to provide improved stability in final products and during processing (Nedovica et al., 2011). This method is used to minimize the denaturation of the protein. Many researchers like Kehoe et al., 2011 and Anema et al., 2006 have study about WPI denaturation only, but without addition of maltodextrin. This work focused on WPI denaturation with different ratio of WPI to maltodextrin with different temperature and time.

1.3 Research Objective

The objective of this experiment are to determine the protein denaturation kinetic rate order for main fractions of whey protein isolate (WPI) that are β-lactoglobulin (β-Lg) ,α-lactoalbumin (α-La) and bovine serum albumin (BSA) and to formulate the new microencapsulation material.

1.4 Scope of Research

- To study the rate order of kinetic denaturation in different parameters.

- To minimize the denaturation of process by changing the parameters such as temperature and via microencapsulation.
- To establish relationship between parameters condition and protein denaturation by using Ultra Performance liquid chromatography (UPLC).

1.5 Rationale and Significance

This study focused on the kinetic and thermodynamic parameters for the individual whey protein denaturation in whey. It will lead to a better understanding of the relationship between heat treatment and its effect on the functional properties of milk products by study of the kinetics of thermal denaturation of the individual whey proteins. The important of experimental kinetic studies is the development of mathematical models to describe the rate of particular reactions as a function of various experimental variables the objective of this study also to gain an understanding of the thermodynamic parameters (temperature and time) important in the reaction process.

1.6 Thesis outline

In this research, it will divide into five chapters. Firstly, **Chapter 1** is an overview about this research. It consists of the introduction on whey protein, microencapsulation and study of kinetic denaturation which gives a brief idea on what microencapsulation process and the effect to the protein. The problem statement, objective and the scope of the study also are included in this chapter.

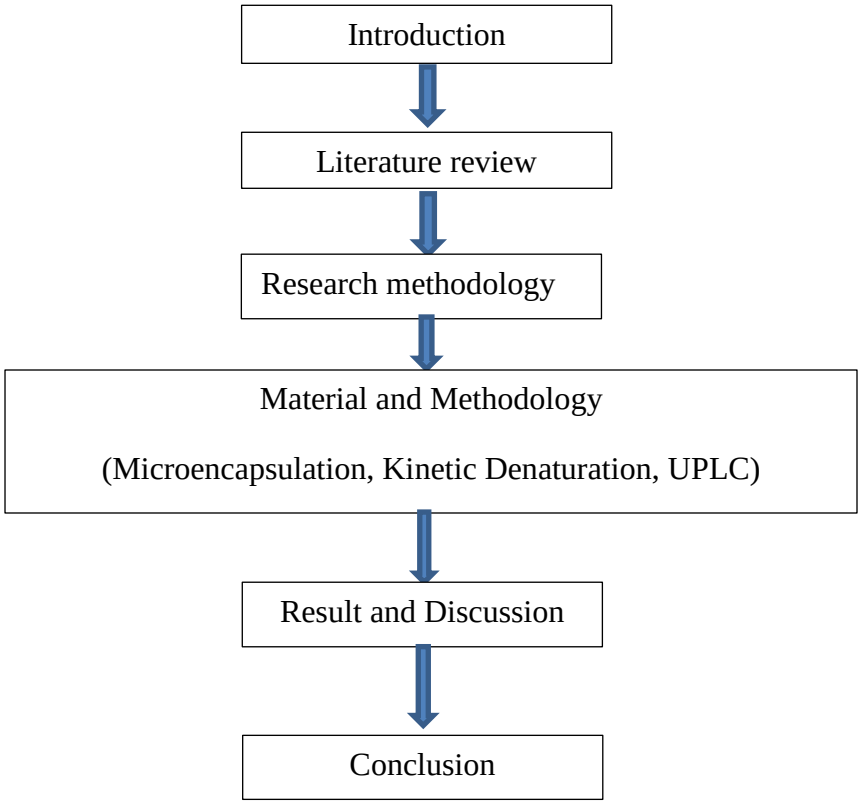

Figure 1.0 The road map for thesis

Chapter 2 is about literature review on protein denaturation process of whey protein and about microencapsulation. This chapter includes all parameters taken to minimize protein denaturation. In this chapter, all the relevant journal, technical paper and books taken from those researches will be studied and discussed.

Chapter 3 will be covered the parts of experimental set up and will be explained more details on equipment and material used, methodology and operating procedures. The techniques and the algorithms that will be used in performing this study will be applied. The method and techniques used for this system is described in detail.

Chapter 4 will be covered on the results and discussion of the research during the operation process. All the experimental result and data will be discussed in details which are including the effects of microencapsulation, rate order of kinetic denaturation in different parameters. The detailed report on the product quality analysis was evaluated. Implementation of process that is involved during development of this analysis is explained in detail in this chapter.

Lastly, **Chapter 5** will be discussed on the conclusion can be made for the study and some recommendations can be taken.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The native structure of proteins is the thermodynamically most stable conformation which is formed under precise physiological conditions and presents a net product of different intra- and inter-molecular attractive and repulsive forces (Damodaran et al., 2008; Shirley, 1995). However, different environmental changes such as heat, pressure, chemicals etc. can affect the protein native structure. The major changes in the secondary, tertiary or quaternary protein structures without breaking the backbone peptide bonds are known as protein denaturation. Protein denaturation minimize by using microencapsulation process. Microencapsulation may be defined as the process of enveloping or surrounding one substance within another substance on a very small scale. Microcapsules may be with a continuous wall surrounding the core, spherically shaped, while others are asymmetrically and

variably shaped, with a quantity of smaller droplets of core material embedded throughout the microcapsule (Umer et al., 2011).

2.2 Whey Protein

Milk contains two primary sources of protein, the caseins and whey (Marshall et al., 2004). After processing occurs, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. There are some components in the whey include beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals. Whey derived from buttermilk versus cheese also contains the lipid sphingomyelin.

Table 2.1 Whey protein composition (source: Kimberlee et al., 2006)

Whey Protein	WPC %	WPI %
α-lactalbumin	12 to 16	14 to 15
β-lactoglobulin	50 to 60	44 to 69
Glycomacropeptide (GMP)	15 to 21	2 to 20
Serum albumin	3 to 5	1 to 3
Immunoglobulins	5 to 8	2 to 3
Lactoferrin	<1	Not reported

Whey, a protein complex derived from milk, is being touted as a functional food with a number of health benefits. For example, whey has the ability to act as an

antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent (Marshall et al., 2004). The primary mechanism by which whey is thought to exert its effects is by intracellular conversion of the amino acid cysteine to glutathione, a potent intracellular antioxidant. A number of clinical trials have successfully been performed using whey in the treatment of cancer, HIV, hepatitis B, cardiovascular disease, osteoporosis, and as an antimicrobial agent. Whey protein has also exhibited benefit in the arena of exercise performance and enhancement.

Whey proteins are widely used because of their high nutritional value and technological characteristics as gelling agents, emulsifiers, texture modifiers, thickening, and foaming agents (Klein et al., 2010). The main fractions of whey proteins are β-lactoglobulin (β-Lg), α-lactoalbumin (α-La), and bovine serum albumin (BSA).

Table 2.2 Components Found in Whey Protein (source: Klein et al., 2010)

Whey Components	% of Whey Protein	Benefits
Beta-Lactoglobulin	50-55%	• Source of essential and branched chain amino acids
Alpha-Lactalbumin	20-25%	• Primary protein found in human breast milk • Source of essential and branched chain amino acids
Bovine Serum Albumin	5-10%	• Source of essential amino acids • Large protein

2.2.1 Beta-Lactoglobulin (β-Lg)

β-Lg is quantitatively the dominant whey protein (58% (w/w), and was first discovered in 1934. When in isolated form, it exhibits a low solubility (despite its globular nature) and a low ionic strength. Synthesized in the mammary gland of

ruminants (and other species) and designed to be included in milk, this protein has several genetic variants – of which β-Lg A is the most common. It is composed mainly of b-sheet motifs, and consists of 162 amino acid residues – which lead to a molecular weight of ca. 18,277 kDa (Eigel et al., 1984). Its quaternary structure depends on the medium pH: it occurs mainly as a stable dimmer, with a molecular weight of 36,700 kDa, at pH values between 7 and 5.2; as an octamer, with a molecular weight of ca. 140,000 kDa, at pH values between 5.2 and 3.5; and as a monomer, with two-cysteine residues per monomer, at pH 3.0 and above 8.0 (de Wit, 1989). β-Lg plays a role in transfer of passive immunity to the newborn, and in regulation of phosphorus metabolism at the mammary gland (Farrell et al., 1987).

Figure 2.0 Structure of Beta-Lactoglobulin (β-Lg) (source: Google, 2013)

2.2.2 Alpha-Lactalbumin (α-La)

Alpha-Lactalbumin (α-La) is the second major globular protein in the whey of milks from various mammalian species. It is a low molecular weight (14.2 kDa) compact metalloprotein accounting for 20% of the proteins in bovine whey. α-La is a modifier protein of the lactose synthase complex in the mammary cell with eight

cysteine residues which exist as four intramolecular disulfides. It contains 123 amino acid residues (its sequence is quite homologous to that of lysozyme), which lead to a molecular weight of 14,175 kDa (Brew et al., 1970). Three genetic variants have already been identified – A, B and C (Fox, 1989). Its globular structure is stabilized by four disulphide bonds, at pH values in the range 5.4–9.0 (Evans, 1982). α-La contributes to reduce the risk of incidence of some cancers – as it constrains cell division, when incubated in distinct mammalian intestinal cell lines (Ganjam et al., 1997). This protein also was demonstrated to possess antiproliferative effects in colon adenocarcinoma cell lines (Caco-2 or HT-29 monolayers), delaying initiation of cell aptoesis, after 4 days of growth with low concentrations (10–25 lg/ml) of such protein (Sternhagen et al., 2001).

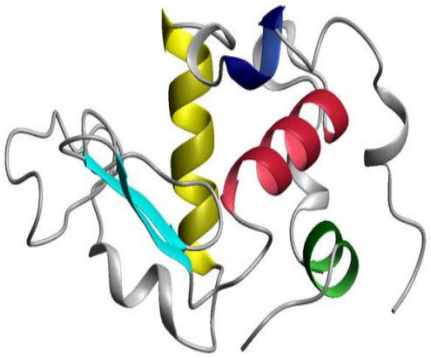

Figure 2.1 Structure of Alpha-Lactalbumin (α-La) (source: Google, 2013)

2.2.3 Bovine Serum Albumin (BSA)

BSA is not synthesized in the mammary gland, but appears instead in milk following passive leakage from the blood stream. It contains 582 amino acid residues, which lead to a molecular weight of 66,267 kDa; it also possesses 17

intermolecular disulphide bridges and one thiol group at residue 34 (Fox, 1989). Because of its size and higher levels of structure, BSA can bind to free fatty acids and other lipids, as well as flavour compounds (Kinsella et al.,1989) – a feature that is severely hampered upon denaturation. Its heat-induced gelation at pH 6.5 is initiated by an intermolecular thiol– disulphide interchange, similar to what happens with β-Lg (de Wit, 1989). One important property that has been associated to BSA is the ability to inhibit tumour growth; in vitro incubation with human breast cancer cell line MCF-7 has provided adequate evidence thereof, which lies on modulation of activities of the autocrine growth regulatory factors (Laursen et al., 1990). The aforementioned binding properties of BSA depend on the fatty acid (or other small molecules) in stake (Brown et al., 1982). Binding to fatty acids, that are stored in the human body as fat, allow it to participate in synthesis of lipids – which are a part of all outer and inner cell membranes, and which provide energy; this issue has been reviewed at some length (Choi et al, 2002). The antioxidant activities of this protein have been tackled (Tong et al., 2000). BSA has been shown to in vitro protect lipids against phenolicinduced oxidation (Smit et al., 1992).

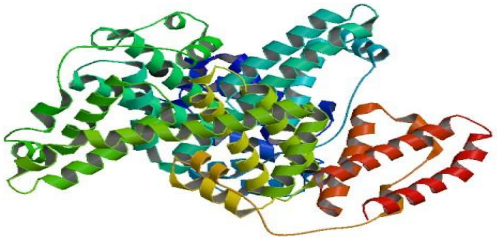

Figure 2.2 Structure of Bovine Serum Albumin (BSA) (source: Google, 2013)