WHEY PROTEIN FRACTIONATION USING Q-SEPHAROSE ANION EXCHANGE CHROMATOGRAPHY AND SP-SEPHAROSE CATION EXCHANGE CHROMATOGRAPHY

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ABSTRACT

In dairy industry, whey protein fractionation is an important process that requires an effective method to separate valuable proteins in bovine whey protein. Bovine whey protein can be divided into two components which are major protein and minor proteins. The major protein contains approximately 50% of β-lactoglobulin (β-Lg), 20% of α-lactalbumin (α-Lac), 10% of bovine serum albumin (BSA) and 10% of immunoglobulin (Ig). Cation exchange and anion exchange chromatography is one of advance separation techniques that can fractionate bovine whey protein. All negatively charged proteins in whey were bound simultaneously to an anion exchange column (Q-Sepharose), while all positively charged proteins in whey were bound to a cation exchange column (SP-Sepharose). Whey protein and buffer solution (pH 4 - pH 10) were prepared before run the experiment. The method is based on the use of an ionic column and salt gradient elution buffer (Buffer solution plus 1 M NaCl). The collected fractions were analyzing using SDS-PAGE to determine which fractions that contain protein component. By using UPLC the concentration of protein exists in the fraction had been determined. Protein fractionation at pH 7 gives the best result with highest concentration of protein recover by using Q-Sepharose. While, by using SP-Sepharose show pure β-Lg can be fractionate at pH 5.

Keywords : Whey protein, Anion exchange chromatography, Cation exchange chromatography

PEMERINGKATAN PROTEIN WHEY MENGGUNAKAN PENUKARAN ANION Q-SEPHAROSE KROMATOGRAFÍ DAN PENUKARAN SP-SEPHAROSE KATION KROMATOGRAFI

ABSTRAK

Dalam industri tenusu, pemeringkatan whey protein memerlukan satu kaedah yang berkesan untuk mengasingkan protein yang berharga dalam susu lembu. Whey protein boleh dibahagikan kepada dua komponen iaitu protein utama dan protein kecil. Protein utama yang mengandungi kira-kira 50% β lactoglobulin (β-Lg), 20% α-lactalbumin (α-La), 10% bovine serum albumin (BSA) dan 10% immunoglobulin (Ig). Pertukaran kation dan anion kromatografi pertukaran adalah salah satu teknik pemisahan terbaru yang boleh memeringkatkan whey protein lembu. Semua protein bercas negatif dalam whey terikat serentak kepada ruangan pertukaran anion (Q-Sepharose), manakala semua protein yang bercas positif dalam whey telah terikat kepada lajur pertukaran kation (SP-Sepharose). Whey protein dan penyelesaian penampan (pH 4 - pH 10) telah disediakan sebelum menjalankan eksperimen. Kaedah ini adalah berdasarkan kepada penggunaan ruang ionik dan garam kecerunan elusi penampan (Buffer penyelesaian ditambah 1 M NaCl). Pecahan yang dikumpul telah menganalisis menggunakan SDS-PAGE untuk menentukan pecahan yang mengandungi komponen protein. Dengan menggunakan UPLC kepekatan protein wujud dalam pecahan telah ditentukan. Protein pemeringkatan pada pH 7 memberikan hasil yang terbaik dengan kepekatan tertinggi dengan menggunakan Q-Sepharose. Manakala, dengan menggunakan SP-Sepharose menunjukkan β-Lg yang tulen boleh dipecahkan pada pH 5.

Keywords : Whey protein, Penukaran Anion Q-Sepharose Kromatografí, Penukaran Sp-Sepharose Kation Kromatografi

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α	- Alpha
β	- Beta

CHAPTER 1

INTRODUCTION

1.1 Background Of Study

Whey is a by-product at the end of cheese production. Whey was formed when the layer of cheese curds separated from the milk and the remaining liquid called as whey (Marshall, 2004; Santos et al., 2012). Whey is a dilute solution contains lactose, a variety of proteins, minerals, fat and vitamins (El-Sayed et al., 2011). However, the disposal of whey, having a biological oxygen demand (BOD) value of 35-60 g L-1 and chemical oxygen demand (COD) value of 80-100 g L-1 as sewage that cause severe environmental pollution problems (Bhattacharjee, 2005).

Whey contains all the essential amino acids in the accurate proportions, making it an excellent source of nutrition for both therapeutic and supplementary food (Dairy for Global Nutrition, 2010). On the other hand, the fractionation of bovine whey from cow's milk exhibit beneficial properties for human health than it original milk, including the acquired immune response. Besides that, whey protein fractionation can be emphasize the functional and properties of nutrition of the each of the protein (Bonnaillie and Tomasula, 2012). Figure 1.1 shows the main components of bovine milk where whey proteins correspond to about 18-20% of the total milk

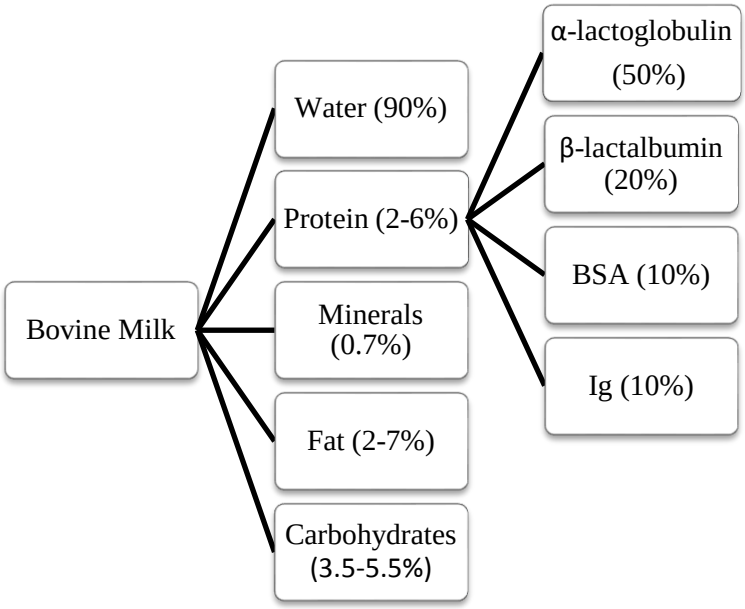

Figure 1.1 Main components in bovine milk (Javonic et al, 2007)

Due to their advantages of individual whey proteins, many efforts had been conducted in dairy industries to develop efficient separation technique to whey protein fractionation. A decade ago, there has been increasing interest in liquid chromatography processes because of the special needs of pharmaceutical and biotechnology (Santos et.

al, 2012). Based on different interaction between chromatography media and component to be separated. They are four mechanism of chromatography, which are:

a) adsorption
b) partitioning
c) size-exclusion
d) ion exchange chromatography.

Adsorption and partitioning chromatography are two oldest methods that utilize a mobile liquid or gaseous phase that is absorbed onto the surface of the stationary solid and based on thin film formed on the surface of solid support by a liquid stationary phase respectively (Gambhir, 2008). Besides that, size exclusion chromatography also known as gel-filtration chromatography is a technique where the proteins pass over a column filled with hydrated porous beads made of a carbohydrate or polyacrylamide polymer. But out of these techniques, ion exchange chromatography has been testified that had very high selectivity, fairly inexpensive and high capacities. This type of chromatography is frequently used in purification step (Lee at. al, 2002).

1.2 Problem Statement

Most of chemical industries produced residue from the upstream processing in the bioreactor. The residue contains many impurities which can produce a new product which give high profit. The dairy industry has conducted many efforts to develop efficient separation technique that can produce new products. Several procedures have been proposed for separation of individual whey proteins including precipitation and membrane separations. However, by using these methods, whey protein is disposed to denaturation and these processes are volume-dependent, which makes the fractionation of whey very expensive. Furthermore, ultrafiltration (UF) membrane also sufficient for complete removal of lactose, nor for the isolation of single pure proteins. Therefore, ion exchange chromatography had been used to recover proteins components as many advantages have been reported for this technology such as no need lengthy column packing procedure, no heat treatment and extreme pH among others (Santos et l., 2012).

1.3 Research Objective

The aim of this study is to separate whey protein components using anion exchange and cation exchange chromatography at different pH.

1.4 RESEARCH SCOPE

The following scope of study was outlined in order to achieve the objective of study:

i. Study the whey protein fractionation using Q-Sepharose anion exchange packed bed chromatography at pH 4 to pH 10.

ii. Study the whey protein fractionation using SP-Sepharose cation exchange packed bed chromatography at pH 4 to pH 10.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter four main topics will be reviewed. The first part is about whey protein. The important and beneficial major individual protein was discussed in second part. In third part, common technologies used in dairy industry were reviewed. The last part was related to the history of protein bioseparation techniques and ion exchange chromatography.

2.2 Whey Protein

Whey protein can be fractionated into whey protein concentrates (WPC) and whey protein isolates (WPI), which typically range in protein from 25 to 90 percent protein (Kimberlee, 2006). Table 2.2 shows the differences between WPC and WPI in term of whey protein composition.

Table 2.1: Whey protein composition (Kimberlee, 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 to5	1 to 3
Immunoglobulins	5 to 8	2 to 3
Lactoferrin	<1	Not reported

Whey protein can be divided into major protein and minor protein. Major protein components have been mention in the introduction. Some example of minor protein such lactoferrin (LF), lactoperoxidase (LP), enzymes, protein components of the MFGM (milk fat globule membrane), proteose-peptone components and glycomacropeptide (Iametti et al.,2002; El-Sayed et al., 2011). Individual proteins have difference properties and functionality. Table 2.2 shows the selected functionality of major whey proteins

(Santos et al., 2011). In Table 2, the physical properties of dairy proteins was summarized (Etzel, 2012).

Table 2.2: Selected function of the major whey proteins.

Protein	Functions
β-Lg	1. Binding and transport of retinol, vitamin D and palmitic acid 2. Enzymic synthesis of prostaglandins. 3. Olfactation, opiodergic, cryptic coloration 4. Anti-hypertensive, anti-cancer,
α-La	1. Binding of calcium absorption 2. Lactose synthesis 3. Tumor cells apoptosis
BSA	1. Transport, metabolism and distribution of ligands 2. Protection from free radicals 3. Contribution to osmotic pressure of blood.
Ig	1. Immunological protection against microbial pathogens and toxins 2. Protect mammary gland against infection

Table 2.3: Physical properties of dairy proteins.

Protein	**Molecular mass (kDa)**	**Concentration (g/L)**	**Isoelectric point (pI)**
α-Lactalbumin	14	3.2	4.7-5.1
β-Lactoglobulin	18	1.2	5.2
Bovine serum albumin	66	0.4	4.9
Immunoglobulin G	150	0.7	5-8
Lactoferrin	77	0.1	7.9
Lactoperoxidease	78	0.03	9.6
κ-Casein	19	3.3	5.8
β-Casein	24	9.3	5.2
αs-Caseins	24	13	4.9/5.3
Glycomocropeptide	8.6	1.5	<3.8

2.3 Important Of Major Individual Protein

2.3.1 α-Lactalbumin

The structure of α-lac is well known and is composed of 123 amino acids and 4 disulfide bridges. The molecular weight is 14.2K Daltons. Bovine α-lac has high homology compare with human α-lac. α-lac is a calcium-binding protein that may have role in calcium transport. Moreover, alpha-lactalbumin is rich in the amino acid cysteine, which is a building block of glutathione, a powerful antioxidant in the body that plays an important role in immunity (Prairie, 2007).

2.3.2 β-Lactoglobulin

β-lg has primary sequence composed of 162 amino acids with 18 K Daltons. It have potential health benefits involving digestive, antimicrobial, immunomodulatory and it is a better foam stabilizer than the other way proteins and can be used in the production of confectionery (Fernandez et al., 2012; Muditha, 2011; Bhattacharjee et al., 2005). Besides that, several researchers stated there have ten genetic variants of bovine β-lg so far; however, the two common variants with equal frequency are β-lg –A and –B

which vary from each other only in two amino acids and the difference given them a bit significant difference in their solubility (Muditha, 2011).

2.3.3 Bovine Serum Albumin (BSA)

Bovine serum albumin (BSA) also one of major protein that can found after purified the bovine whey. BSA is an important source of amino acid including cysteine and methionine from the sulfur. According to Burr (2001), BSA consists of 582 amino acids residue with 17 intramolecular disulfide bonds and single free thiol. Besides the application of BSA in Table 2.1, BSA is very familiar as anticancer properties (Jauregi and Welderufael, 2010).

2.3.4 Immunoglobulin

Immunoglobulin or Ig have five classes which are IgA, IgG, IgD, IgE and IgM Fox and McSweeney (1998). However, only,IgA, and IgM present in milk. For IgG, it can be divided into a subclasses of IgG1 and IgG2. IgG1 found in bovine milk while IgA

in human milk. Morever, Ig is very complex proteins which consists two long and two shorter polypeptide chains linked by disulphides (Saufi, 2010).

2.4 Common application of membrane technology in dairy industry

Dairy industry is one of food processing manufacturer which is known for the high water and energy consumptions that contribute to the major environmental pollution in industrial area (Robert et al., 2000). Thus, in early of 1970s, membrane technology has been using in dairy industry as the new alternative to a few unit operations after considering the separation issues and to improve new product from the dairy production (Pouliot, 2008).

Current technology which are microfiltration (MF), ultrafiltration (UF), nano filtration (NF) and reverse osmosis (RO) are the common pressure-driven membrane unit that has been applied in this industry (Robert et al., 2000). However, those technologies have several weaknesses and create new problem that need high cost to handle it. Table 2.4 shows development of membrane technology since 1960s until 1980s.

Table 2.4: Milestones in the development of membrane technology and its applications in dairy processes since 1960s (Pouliot, 2008)

Year	Advance in membrane technology	Application of membrane in dairy processing
1960s	• Development of reproducible membranes by manufactures	
1970s	• Materials with improved chemical resistance (from cellulose acetate to polysulfone) • First designs of sanitary modules	• Design of whey pre-treatments to prevent membrane fouling • Development of processes for the UF of acid whey • Development of the first UF-based cheese manufacturer processes •
1980s	• Improvement of membrane system hardware (module designs, spacers, anti0telescoping devices) • Development of commercial inorganic (ceramic) membranes	• Using UF or RO membranes to concentrate milk on farm • Defatting of whey (WPI membranes, recovery of minor compounds) • Separations of β-lg & α-lac • Desalting whey with loose-RO (NF) membranes

2.5 Protein bioseparation

Bioseparation which refers to the recovery and purification of protein products from various biological feed streams is an important unit operation in the food, pharmaceutical and biotechnological industry Gopal (2003). Besides that, protein is a biopolymer composed of basic blocks called amino acids and naturally occurring proteins are made up of 20 different amino acids. Protein is by far the most abundant

biopolymers in living cells (constituting about 40 to 70 percent of dry cell weight) and has diverse biological functions as below:

a) Structural components (e.g. collagen, keratin)

b) Catalysts (e.g. enzymes, catalytic antibodies)

c) Transport molecules (e.g. haemoglobin, serum albumin)

d) Regulatory substances (e.g. hormones)

e) Protective molecules (e.g. antibodies)

They are several protein bioseparation techniques to separate the individual protein especially the protein in bovine whey protein. For example, ultrafiltration, supercritical fluid extraction and chromatography that will give different quality of result's request. Many research show this technology has many advantages, such as the faster rate of association between desired protein and functional group; less processing time; ease of scale-up and, best pH or chemical pretreatment that promise protein structures and functionally among the others (Santos et al.,2011).

Principle of chromatography is the difference compound solubility in the mobile respectively with the stationary phase in the column. Once the sample loaded to the system, the proteins were adsorbed to the stationary phase. The gradient salt is to make sure the absorbed proteins desorb. The proteins also will elute at different times, since they were absorbed differently strong Table 2.5 is a summary table of comparison techniques for whey protein fractionation for 10 years ago made by Saufi (2010).

Table 2.5 Comparison techniques for whey protein fractionation (Saufi, 2010)

Author	Protein of interest	Protein source	Material/configuration	Mode in interaction/Ligand
Brochier et al. 2008	β-Lac	Microfiltered whey	HyperCel™column (Pall BioSepra), column volume – 2.5mL, 5 mL, 10mL	Mixed mode – hexyl amine
Etzel et al. 2008	WPI	whey	Mono™ S column (GE Healthcare Technologies), column volume – 2.38 L, 10 cm diameter	Cation exchange – methyl sulphonate
Etzel et al. 2008	WPI	whey	SP Sepharose Bid Bead™ (GE Healthcare Technologies), column volume – 5.34L, 20cm diameter, 17 cm height	Cation exchange –SP
Liang et al. 2006	β-Lac, α-Lac, BSA, IgG	whey	Sephadex™G-200 (GE Healthcare Technologies); 2.6 cm x 70 cm	Gel filtration
Fee and Chand 2006	LF, LP	milk	SP Sepharose Bid Beads, column volume – 5mL	Cation exchange – SP
Schlatterer et al. 2004	β-Lac	whey	Macro-Prep ceramic hydroxyapatite (BioRad), column dimension 12 mm x 88 mm	-
Turhan and Etzel 2004	α-Lac, WPI	Lactic acid whey	SP Sepharose Big Beads, column volume – 80mL	Cation exchange – SP