SEPARATION OF GOAT MILK TO WHEY PROTEIN BY CROSS FLOW FILTRATION USING HOLLOW FIBRE MEMBRANE

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ABSTRACT

Separation of whey protein in goat milk by cross flow filtration using hollow fibre membrane is a good way for protein purification . Cross flow filtration is a good protein purification method because it can operate continuously and does not require frequent maintenance , therefore can be applied for industrial use . Protein separation by microfiltration requires pretreatment which includes centrifugation and filtration using Whatman filter paper to remove fat layer . Casein precipitation is done by reducing pH of goat milk to 4.6 to avoid fouling layer on membrane . Microfiltration is run using whey solution of varying pH and cross flow velocity to study concentration of whey protein in permeate during cross flow filtration . Whey protein concentration in sample collected from permeate is analysed using Reverse Phase Chromatograpy (RPC) , where peak obtained is compared with standard to determine whey concentration . pH values selected is from range 6 to 8 and cross flow velocity range from 0.01cm/s to 0.03cm/s . Concentration of whey protein , BSA and β – lactoglobulin shows highest value compared to other pH values . pH 6.5 was an optimum pH because whey protein gets denatured at higher pH and gets precipitated at lower pH . Concentration of whey protein is highest at cross flow velocity value of 0.03 cm/s . Whey protein concentration increases steadily as cross flow velocity increases . Cross flow filtration is an efficient method for protein purification and can be applied in industry .

ABSTRAK

Pemisahan protein whey dalam susu kambing dengan penapisan cross flow dengan menggunakan membran serat berongga adalah cara yang baik untuk pemurnian protein . Penapisan cross flow adalah kaedah pemurnian protein yang baik kerana boleh beroperasi secara terus menerus dan tidak memerlukan rawatan sering , sehingga dapat diterapkan untuk keperluan industri . Pemisahan protein oleh mikrofiltrasi memerlukan pretreatment yang meliputi sentrifugasi dan penapisan dengan menggunakan kertas Whatman penapis untuk menghilangkan lapisan lemak . Kasein presipitasi dilakukan dengan mengurangkan pH susu kambing hingga 4.6 untuk mengelakkan lapisan fouling pada membran . Mikrofiltrasi dijalankan menggunakan larutan whey pH berubah-ubah dan kelajuan cross flow untuk mempelajari konsentrasi whey protein dalam menyerap selama filtrasi cross flow . Whey protein konsentrasi dalam sampel yang dikumpulkan dari permeat dianalisis menggunakan Reverse Phase Chromatograpy (RPC), di mana puncak yang diperolehi dibandingkan dengan standard untuk menentukan kepekatan whey . Nilai pH yang dipilih dari jarak 6 hingga 8 dan julat kelajuan cross flow dari 0.01cm / s ke 0.03cm / s . Kepekatan protein whey , BSA dan β - lactoglobulin menunjukkan nilai tertinggi berbanding dengan nilai pH yang lain . pH 6,5 merupakan pH optimum kerana mendapat whey protein tertinggi . Protein didenaturasi pada pH lebih tinggi dan akan diendapkan pada pH rendah . Konsentrasi Whey protein tertinggi pada kelajuan aliran nilai melintang 0,03 cm / s . Konsentrasi Whey protein terus meningkat dengan peningkatan kelajuan aliran silang . Cross aliran filtrasi merupakan kaedah efisien untuk pemurnian protein dan boleh diaplikasikan dalam industri .

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LIST OF ABBREVIATIONS

CF	concentration factor
CFF	cross flow filtration
MF	microfiltration
MW	molecular weight (Da)
NCN	non-casein nitrogen
NPN	non-protein nitrogen
TN	total nitrogen
TS	total solids
UF	ultrafiltration
Vcf	cross flow velocity (m s-1)
WP	whey proteins
WPI	whey protein isolate
WPN	whey protein nitrogen
ΔPT	transmembrane pressure (kPa)
ΔPTM	mean transmembrane pressure (kPa)

CHAPTER 1

INTRODUCTION

Milk production from dairy goats constitutes an economic activity of increasing importance . When goat's milk is obtained following proper milking practices , no differences in the bacteriological qualities of cow 's and goat 's milk are found (Calisay *et al.*, 1983) . As for cow's milk , goat's milk must be submitted to thermal treatment before further utilization as fluid milk , manufacturing yogurt and cheese , etc . Although the consumption of liquid goat's milk is small , the potential market is large since goat's milk is a good substitute for cow 's milk when bovine milk proteins bring about allergic responses in consumers (Zadow *et al.*, 1983) . One of the most important factors on the stability of milk is the pH. Zadow *et al.* (1983) showed that extensive sedimentation occurred if the pH of the milk was below 6.6 ; above pH 6.7 very little sedimentation occurred. .

Milk proteins have different functional properties such as solubility , foaming , whipping , emulsification , gelation and flavor entrapment ability . Cross flow filtration ,CFF (ultrafiltration , UF and microfiltration , MF) can be used to fractionate these milk

proteins (Maubois and Brule , 1982) . In cross flow filtration , as liquid products flow parallel to the surface of the membrane , the permeate passes through the membrane . The permeate flux depends on the properties of the membrane , product and the operational conditions (transmembrane pressure , cross flow velocity , concentration factor , running time , and temperature) .

Casein constitutes about 78% of the proteins in milk . By preparing different casein concentrations using CFF , desired casein / total protein ratio is obtained and used as standardized cheese milk for making cheese . Low concentrate caseinate retentate and liquid precheese are also obtained (Maubois and Ollivier , 1992) . By diafiltering the retentate, casein is purified up to 90% protein on a total solid basis . The casein-rich fraction can also be separated into fractions such as α-casein and β- casein (Maubois and Ollivier , 1992;Woychick et al., 1992) .

The whey protein (WP) fraction could be concentrated , separated and purified and used in food industries . Substitutes for traditional whipping agents have been investigated over several years . Many of these studies used milk proteins , especially whey protein . Whey protein are traditionally made from cheese whey; the process involved is very complex (Maubois and Ollivier , 1992) and most of the time the product was subjected to physico-chemical modification and / or made with additives (Morr et al., 1973; Phillips and Beuchat, 1981; Mahran et al., 1987; Needs and Huitson, 1991) .

In most of these studies , milk proteins were used as a mixture rather than single protein . Residual lipids found in whey inhibit the functionality of whey protein , especially its foaming ability (Richert et al., 1974) . These are mainly phospholipids from the fat globule membranes (Phillips et al., 1990) . These lipids should be removed to obtain more uniform functional properties . Phillips et al. (1990) also found that the

cheese whey contained foam depressors ; removal of these depressors without altering the properties of whey protein isolates (WPI) was very difficult , because heat treatments used in dairy processing induces denaturation , aggregation , and precipitation in WP (Patel et al., 1990) .

1.1 Background of study

There are a lot of researches that have been done previously on separation of whey protein in goat milk . It has been in recent years that the food industry has developed an interest in membrane processes like microfiltration , ultrafiltration , nanofiltration and reverse osmosis . For example , the dairy industry uses microfiltration (MF) for concentration and separation of macromolecules from milk and whey . MF with hollow fibre membranes has been used for fat separation , caseins concentration and microorganisms removal (cold pasteurization) , being this latter one milder technique in comparison to thermal processing , such as pasteurization or evaporative concentration , which ones produce protein denaturation .

1.2 Problem Statement

To find the optimum parameter required to increase efficiency of protein separation in milk using hollow fibre membrane . The main limitation of this kind of process is macromolecules accumulation and deposition from the feed stream onto the membrane, i.e. fouling , which can be reversible or irreversible . Fouling control leads to a reversible fouling , as in the case of milk protein and colloids , pore blocking or cake formation and concentration polarization in nanofiltration and reverse osmosis . Fouling can be removed through effective membrane rinsing and cleaning ; however , since in doing so there are costs involved (cost of labor , energy , chemicals and water) a consequent effect on process productivity is expected; therefore, the industry should be concerned about membrane cleaning.

Separation of whey protein from goat milk is called protein purification. Protein purification is a series of processes intended to isolate a single type of protein from a complex mixture. Protein purification is vital for the characterisation of the function, structure and interactions of the protein of interest. The various steps in the purification process may free the protein from a matrix that confines it, separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins. Separation of one protein from all others is typically the most laborious aspect of protein purification.

1.3 Objective

The objective of this study was to find the optimal operating parameters for separation of milk proteins into casein-rich and whey protein-rich fractions with good permeate flux using ceramic membranes.

1.4 Scope of Research

To study optimum operational parameters for efficiency of separation using cross flow filtration system. Cross flow filtration was found to remove solids effectively, mcan be operated continuously and does not require frequency change out. Parameters which will be studies is pH and cross flow velocity.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk

Milk is a translucent white liquid produced by the mammary glands of mammals . It provides the primary source of nutrition for young mammals before they are able to digest other types of food . The exact components of raw milk vary by species , but it contains significant amounts of saturated fat , protein and calcium as well as vitamin C . Cow's milk has a pH ranging from 6.4 to 6.8 , making it slightly acidic . Delicious with a slightly sweet and sometimes salty undertone , goat's milk is the milk of choice in most

of the world . Although not popular in the United States , it can be found in markets and health foods stores throughout the year .

Milk is an emulsion or colloid of butterfat globules within a water-based fluid . Unlike cow's milk there is no need to homogenize goat's milk . While the fat globules in cow's milk tend to separate to the surface , the globules in goat's milk are much smaller and will remain suspended in solution . When individuals have sensitivity to cow's milk, goat's milk can sometimes be used as an alternative . In unhomogenized cow's milk , the fat globules average about four micrometers across . The fat-soluble vitamins A , D , E , and K are found within the milk fat portion of the milk .

2.1.1 Protein in goat milk

Goat milk protein forms a softer curd (the term given to the protein clumps that are formed by the action of your stomach acid on the protein) , which makes the protein more easily and rapidly digestible . Theoretically , this more rapid transit through the stomach could be an advantage to infants and children who regurgitate cow's milk easily . Goat's milk may also have advantages when it comes to allergies . Goat's milk contains only trace amounts of an allergenic casein protein , alpha-S1, found in cow's milk . Goat's milk casein is more similar to human milk , yet cow's milk and goat's milk contain similar levels of the other allergenic protein , beta lactoglobulin .

The largest structures in the fluid portion of the milk are casein protein micelles . Each micelle is roughly spherical and about a tenth of a micrometer across . There are four different types of casein proteins , and collectively they make up around 80 percent of the protein in milk , by weight . Most of the casein proteins are bound into the micelles . Some initial studies suggested that specific proteins known to cause allergic reactions may have been present in cow's milk in significant quantities yet largely absent in goat's milk . The alpha-casein proteins , including alpha s1-casein , and the beta-casein proteins were both considered in this regard .

Whey protein is actually a term used to describe a group of globular proteins that can be separated from whey . When cow's milk is used to manufacture cheese , it also leads to the production of whey , which may or may not be discarded. It is a mixture of lactoglobulin , alpha-lactalbumin and serum albumin . Whey Protein Concentrates (WPC) are nothing but powders made by drying the retentates from ultrafiltration (UF) of whey . They are described in terms of their protein concentration , protein content as percentage of dry matter, which ranges from 25 to 80% .

2.2 Cross Flow Filtration

In chemical engineering , biochemical engineering and protein purification , crossflow filtration (also known as tangential flow filtration) is a type of filtration (a particular unit operation) . Crossflow filtration is different from dead-end filtration in which the feed is passed through a membrane or bed , the solids being trapped in the filter and the filtrate being released at the other end . Cross-flow filtration gets its name because the majority of the feed flow travels tangentially *across* the surface of the filter, rather than into the filter . The principle advantage of this is that the filter cake (which can blind the filter) is substantially washed away during the filtration process , increasing the length of time that a filter unit can be operational . It can be a continuous process , unlike batch-wise dead-end filtration .

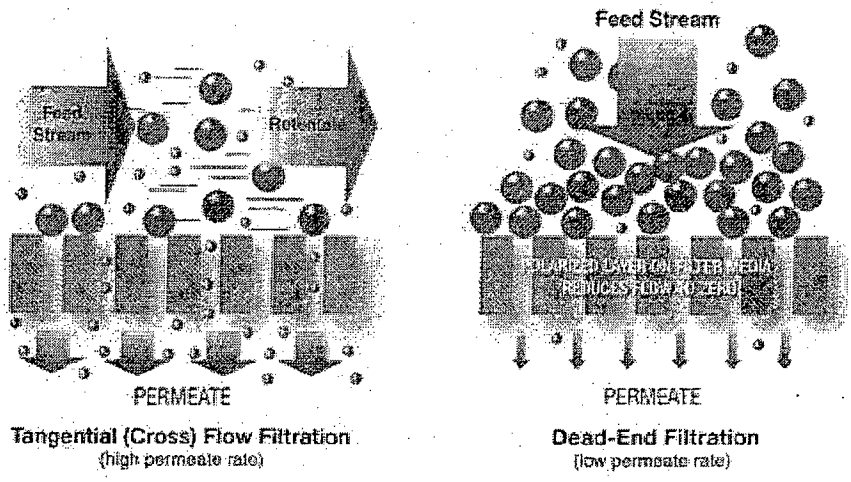

Figure 1: Cross Flow Filtration and Dead-End Filtration

2.2.1 Techniques to improve performance of cross flow filtration

a)Backwashing

In backwashing , the transmembrane pressure is periodically inverted by the use of a secondary pump , so that permeate flows back into the feed , lifting the fouling layer from the surface of the membrane .

b) Process Flow Disruption (PFD)

A technically simpler approach than backwashing is to set the transmembrane pressure to zero by temporarily closing off the permeate outlet , which increases the attrition of the fouling layer without the need for a second pump . PFD is not as effective as backwashing in removing fouling, but can be advantageous .

c) Clean-in-place

Clean-in-place systems are typically used to remove fouling from membranes after extensive use . The CIP process may use detergents , reactive agents such as sodium hypochlorite and acids and alkalis such as citric acid and sodium hydroxide .

d) Diafiltration

In order to effectively remove permeate components from the slurry , fresh solvent may be added to the feed to replace some or all of the permeate volume . This is analogous to the washing of filter cake to remove soluble components .

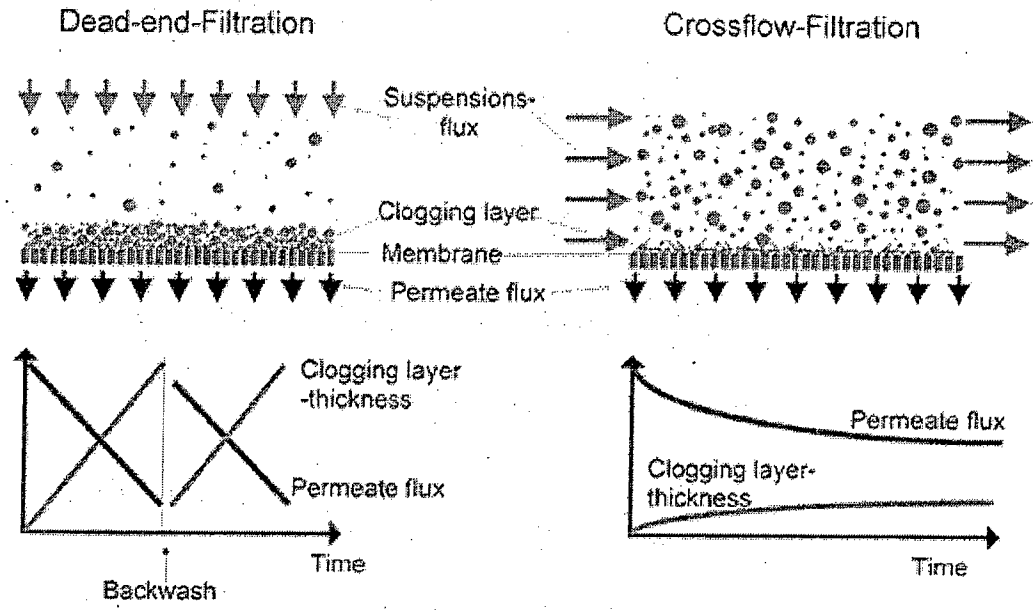

Figure 2: Permeate flux

Cross flow membrane filtration technology has been used widely in industry globally . Filtration membranes can be polymeric or ceramic , depending upon the application . The principles of cross-flow filtration are used in reverse osmosis , nanofiltration , ultrafiltration and microfiltration . When purifying water , it can be very cost effective in comparison to the traditional evaporation methods .

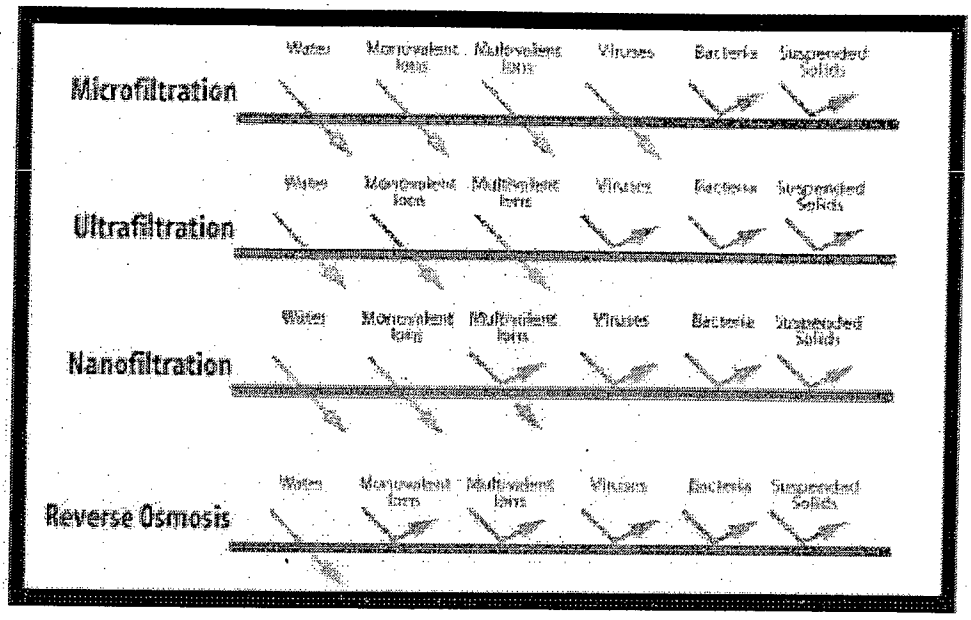

Figure 3: Membrane Process Characteristics

In protein purification, the term Tangential Flow Filtration (TFF) is used to describe cross-flow filtration with membranes . The process can be used at different stages during purification , depending on the type of membrane selected

2.2.2 Application of cross flow filtration in dairy product industry

Cross-flow microfiltration technology is rapidly gaining prominence in the processing of dairy ingredients . Microfiltration, along with other membrane filtration processes like ultrafiltration , reverse osmosis and nanofiltration , is making it possible to produce products with very unique properties and functionalities .

The practical dairy applications of microfiltration are often limited by severe fouling of the membrane , which results in flux decline and changing of the retention characteristics . Microfiltration is a pressure driven process where the Trans Membrane Pressures (TMP) are in the order of 1 to 2 bar which is very critical to the performance of the processes . This makes the plant design and control of operating parameters very important .

In addition , advances in membranes, both organic and inorganic types , have made these processes technically possible and economically viable . While the following are only a few examples of potential separation processes , several new applications are being developed .

Bacteria and Spores Reduction in Fluid Dairy Products

Demand for products with low bacteria and spore counts is increasing. Examples of these are Extended Shelf Life (ESL) milk; pretreatment of cheese milk to prevent gas formation during the cheese aging process, production of skim milk and whole milk powders with very low bacteria and spore counts, and better microbiological quality of

Whey Protein Concentrates. Plants equipped with special ceramic membranes can give at least a 99.99% reduction in bacteria and spores depending on the loading in the feed.

Reduction of Fat in Whey Protein Concentrates (WPC)

The limitation of fat removal from whey by mechanical separation results in high fat content in WPC. This high fat level limits the maximum protein content in the final WPC powder , usually 80-84% depending on the feed quality . Whey Protein Isolates (WPI) require reduction of fat content in the final product to < 0.5 % . This can be accomplished by reducing the fat content further than using mechanical separation only by microfiltration with either ceramic or spiral wound membranes . The choice depends on a variety of parameters such as capital cost , operating cost , and others .

Fractionation of Casein and Whey Proteins in Skim Milk

Separation of casein from whey proteins in skim milk and its concentration gives an opportunity to fortify cheese milk without increasing the whey protein content . This has a positive influence on increasing the capacity of cheese making equipment without affecting the quality of cheese . As an added benefit , whey proteins produced by such a process have some unique functional properties since they have not come in contact with cheese starter , rennet or cheese additives .

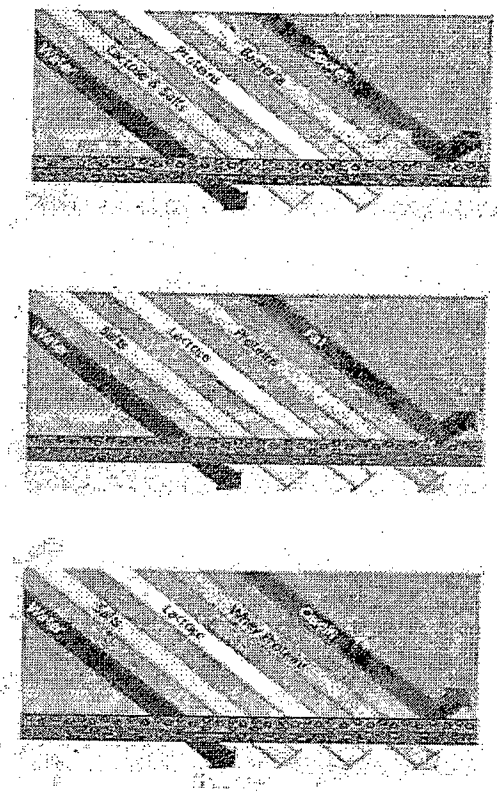

Figure 4 : Component Removal

2.3 Hollow fibre membrane

Multi-layered composite hollow-fiber membrane is a membrane with a completely new structure, and one which has superior permeability to gases . This membrane

features an ultra-thin non-porous membrane sandwiched between two porous membranes . Thanks to its non-multi-porous structure , the membrane prevents permeation of most liquid at the non-pressurized side . As the non-porous membrane is ultra-thin, it has superior performance in letting through gases . As the non-porous membrane is sandwiched between two polyethylene porous membranes , it displays excellent damage resistance . It is possible to remove dissolved oxygen in water to below 1μg/L .

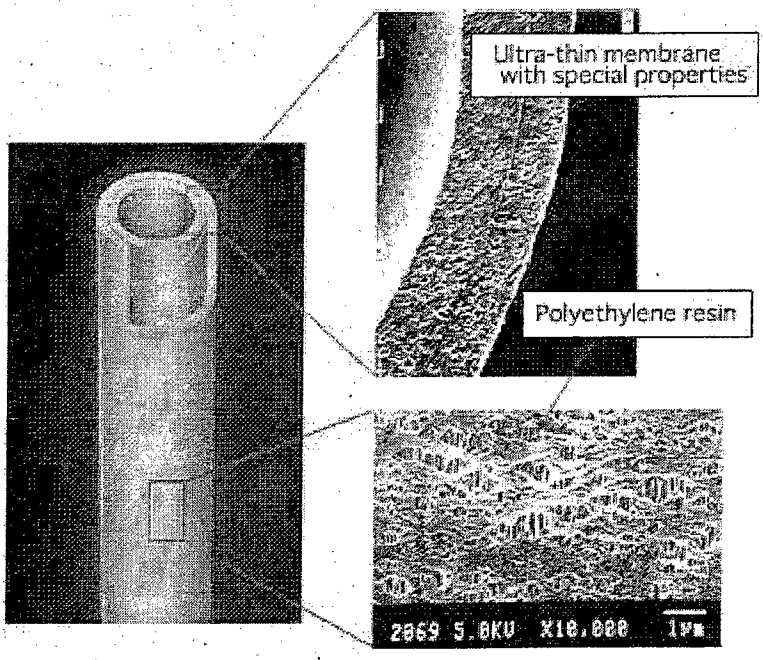

Figure 5 : Hollow fibre membrane structure

The excellent mass-transfer properties conferred by the hollow fiber configuration soon led to numerous commercial applications in various field such as the medical field