# FRACTIONATION OF MILK PROTEINS FROM SKIM MILK USING MICROFILTRATION

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY AT MASSEY UNIVERSITY

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# ABSTRACT

The possibility of fractionation of milk proteins from skim milk using microfiltration (MF) was investigated in this project. Pilot scale ultrafiltration/microfiltration equipment (Koch model) was used. Three available MF membranes, 600, 601 and 603, with pore sizes of 1.99μ, 0.85μ and 0.17μ, respectively, were evaluated. The most suitable membrane was found to be MF 603.

By microfiltration to concentration factor ($CF_C$) 7, permeation of 46% non-casein nitrogen (NCN) was achieved in contrast to 1% for casein. Using diafiltration with deionised water to a CF 567, permeation of 80% NCN occurred. Therefore, it is possible to obtain a casein-enriched fraction from the MF retentate and a non-casein nitrogen enriched fraction from the permeate by the MF process using MF membrane 603.

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# CHAPTER 1

# INTRODUCTION

Microfiltration is a pressure-driven membrane separation process. The separation is based on the ability of various polymeric or ceramic membranes to discriminate between molecules with different molecular shape, size and charge. Compared with ultrafiltration, microfiltration involves a more open membrane which has membrane pore sizes in the range of 0.1-10μ.

Caseins in milk are present as spherical biocolloids with salts, called casein micelles, 80% (w/w) of which have diameters in the range 30-600nm (Lin *et al.*, 1971; Beaton, 1979; Donnelly *et al.*, 1984), whereas the whey proteins exist as much smaller, globular and soluble species. The major whey proteins (β-lactoglobulin and α-lactalbumin) have diameters in the range 1-4nm (Beaton, 1979). Therefore, the process for the preparation of micellar whole casein could exploit these differences. According to Möller (1985), molecular weight cut-offs in the membrane of about 30,000 could be used.

Limited trials by Smithers *et al.* (1991) indicated that the microfiltration membrane based physical separation of whey from casein micelles in milk is commercially feasible.

Membranes with pore sizes 100nm and 200nm were used by Woychik *et al.* (1992) to evaluate the potential of microfiltration to produce permeate and retentate fractions with different mean micellar sizes, possibly varying casein composition and altered casein/whey protein ratios. They reported that altered casein/whey protein ratios of 0.75-0.90 in the 100nm and 200nm permeates, respectively, and 5.0-7.7 in the retentates were achieved.

The purpose of this project was to select an appropriate microfiltration membrane to assess the possibility of fractionation of milk proteins from skim milk.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 UF/MF Membrane Technology

### 2.1.1 Definition and Principles

Ultrafiltration can be defined as a pressure-driven membrane process that can be used in the separation and concentration of substances having molecular weight between $10^3$-$10^6$ dalton (molecular size 0.001-0.02μ) (Renner *et al.*, 1991). The feed which has a certain composition is separated into two streams when it flows over the membrane, i.e. a permeate stream and a retentate stream. The permeate stream is the fraction of the feed stream which passes through the membrane whereas the retentate stream is the fraction retained, as permeate (filtrate) is removed.

As the membrane pore size increases, it becomes permeable to relatively low molecular weight solutes as well as solvents, e.g. minerals, lactose and vitamins in milk. Consequently, the higher molecular weight substances in the milk are concentrated.

In the membrane separation process, three constituents i.e. the membrane, the module and the fluid are very important for the final result. The sequence of events when the feed proceeds along the membrane is summarised below (Glover, 1985) :

1) the feed becomes more concentrated and more protein is transported towards the membrane;
2) the concentration gradient between deposit and feed decreases. Thus the back diffusion of material away from the membrane is decreased, causing the transport of solids from the membrane to decrease;
3) the viscosity of the feed increases with increase in concentration, reducing the back diffusion away from the membrane;
4) based on the facts above, the thickness and the resistance of the deposit layer increase which lead to a decline in flux along the membrane.

Finally equilibrium is reached when the transport of solids to the membrane by the ultrafiltration process is balanced by the back diffusion away from the membrane due to the concentration gradient.

The operation of the microfiltration process is essentially the same as ultrafiltration. The main differences between UF and MF were given in Table 1 (Renner *et al.*, 1991).

**Table 1. Differences between microfiltration and ultrafiltration**

| Parameters | Microfiltration | Ultrafiltration |
|---|---|---|
| Size of solutes retained (dalton) | over $10^6$ (0.01-10μ) | $10^3$-$10^6$ (0.001-0.02μ) |
| Operating pressure (bar) | < 2 | 1-15 |
| Mechanism of the membrane retention | molecular screening | molecular screening |
| Typical flux ($L/m^2/h$) | >300 | 30-300 |

Table 1 shows that the mechanisms of the membrane retention for both microfiltration and ultrafiltration processes are the same, which are molecular screening. The only difference between them is the pore sizes of the membranes. The microfiltration involves an even more open membrane than the ultrafiltration. Therefore, the microfiltration process has higher typical flux and lower operating pressure than that using the ultrafiltration process.

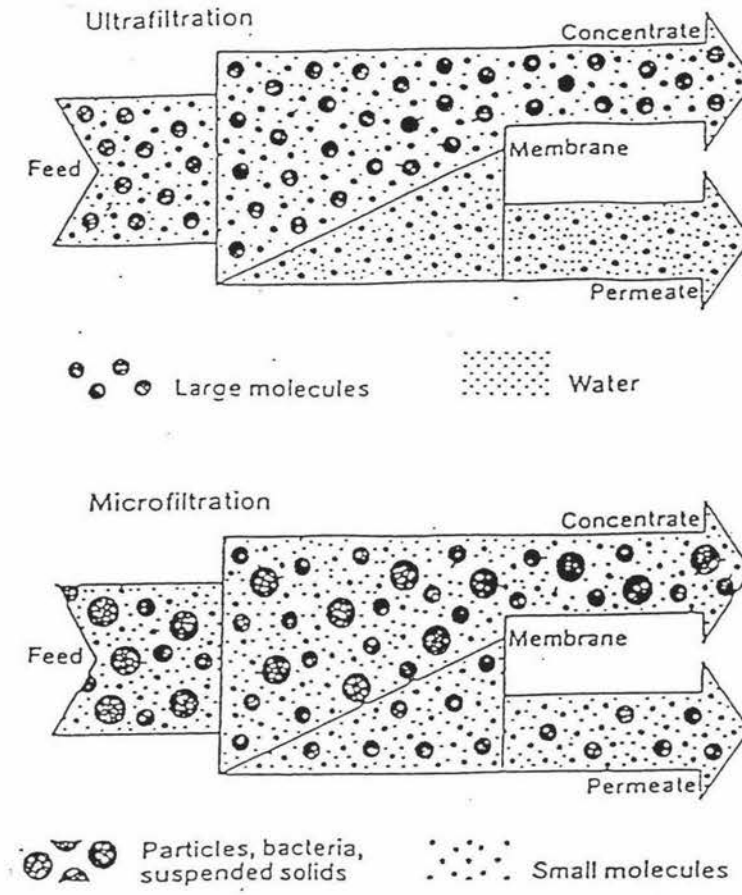


**Fig. 1.** Principles of microfiltration and ultrafiltration (Nielsen, 1990)

Fig. 1 shows that principles of microfiltration and ultrafiltration are basically same, i.e. both of them can be defined as a pressure-driven membrane process which can be used in the separation and concentration of substances. During the ultrafiltration process, some small molecules such as salts, lactose pass through the membrane, whereas molecules such as proteins are rejected by the membrane. However, during the microfiltration process, besides some small molecules, some bigger molecules such as proteins can also pass through the membrane. Only very big particles such as colloids, suspended particles, bacteria and some virus can be rejected by the microfiltration membrane.

## 2.1.2 UF/MF Membranes and Characteristics

### UF Membranes

A variety of UF membranes are available for use in a wide range of applications. Most of the ultrafiltration membranes used commercially these days are prepared from polymeric materials by a phase inversion process (Mulder, 1991). These materials include:

polysulfone/polyether sulfone/sulfonated polysulfone,

polyvinylidene fluoride,

polyacrylonitrile and related block-copolymers,

cellulosics (e.g. cellulose acetate),

polyamide/polyether imide etc.

inorganic (ceramic) materials have been also used.

The first successful membranes on a large scale were made of cellulose acetate. However, cellulose acetate membranes suffer from a number of disadvantages (Glover *et al.*, 1978), particularly for dairy processes, since it is an ester and a polysaccharide which is subject to hydrolysis and confines its use to a pH range of 3-7 and an upper temperature limit of 35°C. These conditions result in restrictions on cleaning and sterilisation. In order to overcome this problem, a second generation of membranes such as polysulfones was developed, which could withstand wider ranges of pH (2-12) and temperature (up to 80°C) than cellulose acetate. In addition, they also have better resistance to compaction under pressure, and to chlorine which is a favourite cleaning and sterilising agent in dairying. The latest inorganic membrane-zirconium oxide is available commercially. Its advantages lie in its great mechanical strength, withstanding pressures up to 20 atmospheres without creep, tolerating the whole pH range and temperatures up to 400°C.

### Membrane characteristics

Membranes are usually characterised in terms of the molecular weights of components they will retain, but this cannot be an exact description of membrane performance because they function as sieves so that molecular size and shape, and to some extent charges affect properties. The Table 2 shows some important properties and measurements of the UF membranes.

**Table 2. Important properties of ultrafiltration membranes** (Mulder, 1991)

| Property | Method of measurement | Significance |
|---|---|---|
| Surface pore size | Electron microscopy | Most critical property of UF membranes |
| Pore size | Electron microscopy | Affects separation |
| Distribution | Solute passage testing | Efficiency |
| Percent porosity | Electron microscopy, thickness and weight measurement | Membrane life and flux |
| Rejection | Solute passage testing | Separation and yields |
| Flux | Water flux under standard conditions(e.g. 1 atm 30°C) | Minimising membrane area |
| Temperature stability | Compatibility testing | Membrane sterilizability life and flux |
| Solvent resistance | Compatibility testing | Membrane life |
| Pressure resistance | Compatibility testing | Membrane life |

The quality of the separation or concentration achieved in UF process will depend upon the characteristics of the selected membrane. According to Lewis (1982), the desired characteristics should be:

1) uniform pore size (sharp molecular weight cut-off value),
2) high permeability,
3) cheapness and reproducibility,
4) resistance to a wide range of chemicals, including solvents, acids, alkalis and sanitising agents.

Furthermore, it should not react with or physically alter the proteins being processed.

### 2.1.3 Membrane Modules and Process Design

It is known that the membrane itself is thin and needs mechanical support against the pressure applied. The support itself should also be porous. The membrane and its support, together, are normally known as the module, in which the membrane area is packed into the smallest unit. The module is considered as the central part of a membrane installation. Several types of module design have been developed for commercial equipment. The most important of these have the membrane in the form of tube, a flat plate or sheet, a spiral or hollow fibres. Fig. 2 shows a schematic drawing of a single module design.

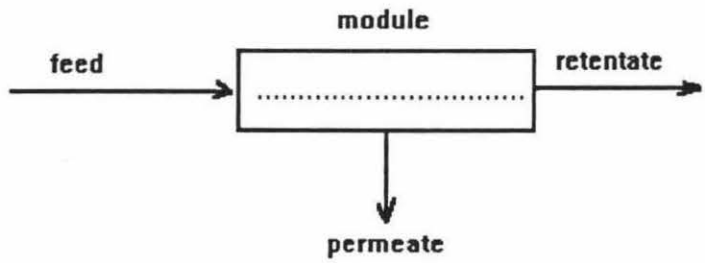


**Fig. 2.** Schematic drawing of a single module design (Mulder, 1991).

There are a number of module designs, but generally based on two types of membrane configurations: i) flat and ii) tubular. Plate-and-frame and spiral-wound modules involve flat membranes whereas tubular and hollow fibre modules are based on tubular membrane configurations. The followings are the schematic drawings of above different modules.

The essential difference between these arrangements is the space between adjacent membranes, termed the flow channel, which ranges from 25mm in the tubes down to about 0.5mm for the flat and spirally wound types. The different modules have different characteristics.

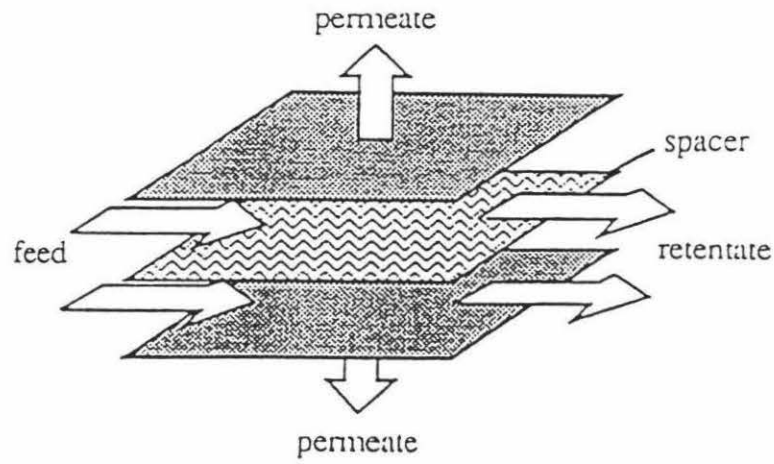


**Fig. 3.** Schematic drawing of a plate-and -frame module (Mulder, 1991).

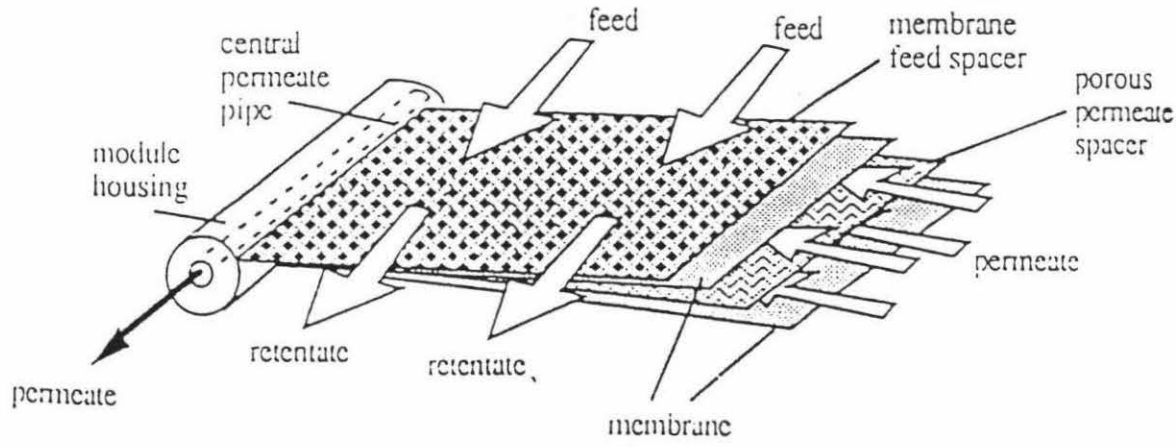


**Fig. 4.** Schematic drawing of a spiral-wound module (Mulder, 1991).

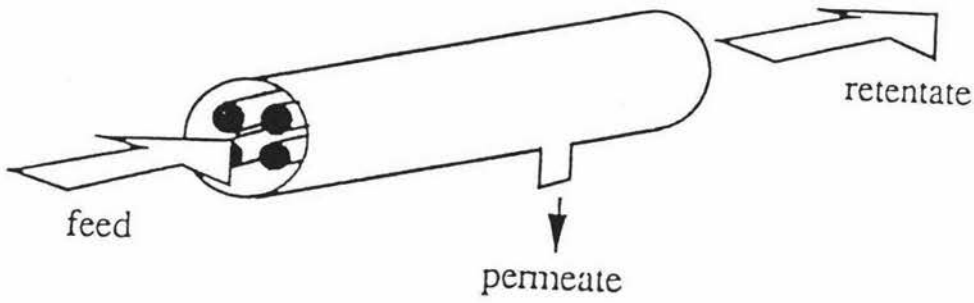


**Fig. 5.** Schematic drawing of a tubular module (Mulder, 1991).

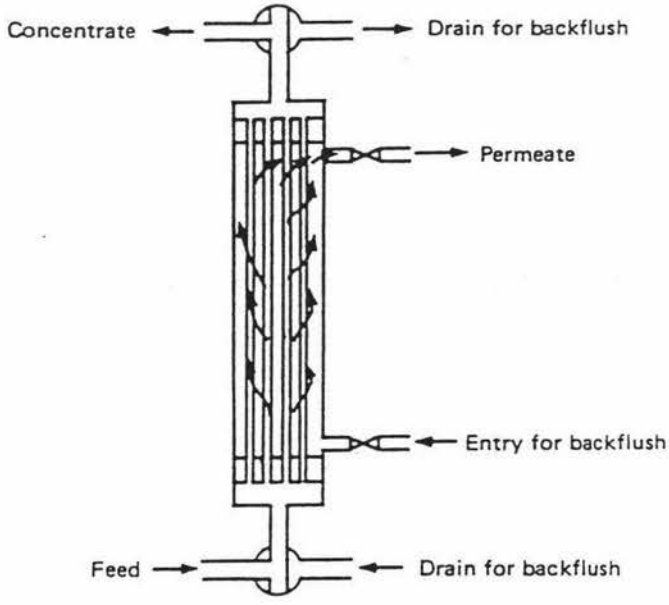


**Fig. 6.** Schematic drawing of a hollow fibre module (Glover, 1985).

The choice of the module is mainly determined by economic considerations. In dairy industry, tubular or plate-and-frame modules are mainly used. Spirals are now becoming dominant. The qualitative comparison of various membrane configuration is showed in Table 3.

**Table 3. Qualitative comparison of various membrane configurations** (Mulder, 1991).

| | Tubular | Plate-and-frame | Spiral-wound | Hollow fibre |
|---|---|---|---|---|
| Packing density | low | ----------> | | very high |
| Investment | high | ----------> | | low |
| Fouling tendency | low | ----------> | | very high |
| Cleaning | good | ----------> | | poor |
| Operation cost | high | ----------> | | low |
| Membrane replacement | yes/no | yes | no | no |

The design of membrane filtration systems can differ significantly because of the large number of applications and module configurations. In principle, two basic methods which are a single-stage or a multi-stage process can be used to achieve different extent of separation. A batch system is usually used for small-scale applications.

## 2.1.4 Polarisation Phenomena and Membrane Fouling

### Concentration polarisation

It is desirable to operate the plant under the optimum conditions, i.e. to maximise the flux whilst at the same time minimising the energy costs. However, the maximum flux is often determined, not by the membrane itself, but by the formation of a layer of rejected material adjacent to the membrane surface. During UF and MF, solute is brought to the membrane surface by convective transport, and a portion of the solvent is removed from fluid. This results in a higher local concentration of the solute at the membrane surface compared to the bulk. This concentrated layer offers an extra resistance to transport of molecules through the membrane and hence reduces the flux. This solute built-up is referred to as concentration polarisation. The phenomenon of concentration polarisation is illustrated in Fig. 7.

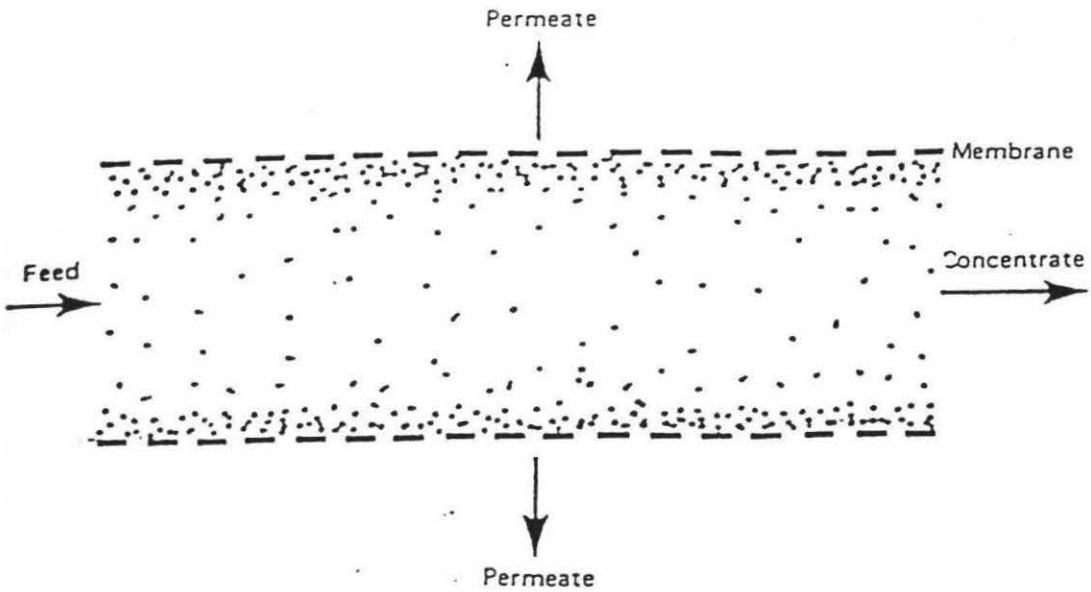


**Fig. 7.** Concentration polarisation-collection of solids near the membrane where permeate is extracted (Glover, 1985)

Glover (1985) stated that within seconds of the start of ultrafiltration, solids begin to collect near the membrane. They are then adsorbed on the membrane surface and invade the pores, causing a rapid decline in permeate flux. Aimar *et al.* (1988) reported that the flux decline occurred in three successive stages. Initially, reversible concentration polarisation builds up within the first minute, leading to a rapid drop in flux. Then, the flux continues to decline, a sharp decrease during the first hour due to either protein deposition or particle deposition, followed by a slow decrease over three hours due to convective deposition of particles.

**Membrane fouling**

Generally, membrane fouling is characterised by an "irreversible" decline in flux during processing. A clear distinction must be made between concentration polarisation and fouling. Concentration polarisation is the development of a concentration gradient of the retained components near the membrane. It is a function of the hydrodynamic conditions in the membrane system and it is not directly influenced by the physical properties of the membrane, i.e. pore size and porosity. However, fouling is the deposition of material on

the membrane surface or in its pores, leading to a change in the membrane behaviour. Fouling is the "coupling" of deposited material to the membrane through the intermediate step of concentration polarisation. Fouling is also different from membrane compaction, which is the compression of the membrane structure under the transmembrane pressure, causing a decrease in membrane permeability (Gekas, 1988).

Fouling results in a lower flux (capacity), a change in retention (separation factor) and worsened cleaning problems. Hallström *et al.* (1989) described the deposition mechanism by combining two of the currently available deposition models; cake layer formation and pore blocking. The model described by Hallström *et al.* (1989) for UF has been divided into three stages:

***First stage***

A deposition occurs on the membrane surface and at the entrances to the pores. The deposit resistance decreases with increasing permeability, i.e. increasing permeability (which is generally considered as an increasing in pore size) will result in a lessened resistance of the pore-associated deposit. Of course, the deposition is also influenced by the solute (protein), other ingredients (salts) and pH.

***Second stage***

When the deposition continuously occurs on the top of the first deposited layer, it restricts the original membrane pores to a much smaller degree. The amount deposited varies for different membrane materials.

***Third stage***

The second stage proceeds continuously into the third, where the solute molecules finally bridge the pore mouths completely, at first the smaller pores, then progressing to the larger pores. Once the membrane is fouled, the deposits change the characteristics of the exposed surface in both hydrophobicity and pore size distribution.

In practice, there are two types of membrane fouling, i.e. surface (temporary) fouling and pore (permanent) fouling, which must be distinguished. Surface (temporary) fouling is

that foulant appears as an evenly deposited layer on the membrane surface. This type of fouling can be easily removed by cleaning solutions and the permeation rate of the fouled membrane can be regenerated by cleaning. This is the most common fouling observed in UF plants (Renner *et al.*, 1991). In ultrafiltration, fouling occurs predominantly on the membrane surface where a protein layer forms a dynamic membrane which controls membrane behaviour. Pore (permanent) fouling involves mineral complexes that diffuse into the membrane blocking the pores of the separation layer of the membrane (Nisbet *et al.*, 1981). The fouling is characterized by an uneven distribution of the foulant and compression of the separation zone. The flux of the membrane fouled with this type of material cannot be regenerated by cleaning. This type of fouling determines the lifetime of the membrane (Renner *et al.*, 1991). In microfiltration, MF membranes do not merely operate on molecular weight rejection as do UF. Severe pore plugging by protein occurs, in spite of the pore being an order of magnitude larger than the protein. Internal fouling appears to dominate with large pores, resulting in a change of apparent pore size, pore size distribution and pore density of the membrane. Merin & Daufin (1990) stated that MF is susceptible to fouling by the following mechanisms:

- adsorption and adhesion of particles and solute to surface;
- concentration polarization and cake formation;
- pore blocking.

Adsorption and adhesion are related to interactions between the membrane and feed components due to variety of forces, which could be eliminated by varying the membrane material.

Concentration polarization and cake formation can be diminished by using proper operating parameters, such as high shear force to enhance the mass transfer coefficient.

Pore blocking is of great importance, especially due to the close size of the rejected and transmitted components. The pores must have minimum attraction to the permeated components to enhance MF performance.

In fact, fouling is a complex phenomenon. The predominant fouling mechanism is a function of the experimental conditions, and is influenced by operating conditions, membrane properties and the properties of the feed material. Retention and selectivity are dependent upon the primary membrane structure, rather than the dynamic membrane (Marshall *et al.*, 1993).

The main cause of membrane fouling is the deposition of submicron particles on the membrane surface and/or the crystallisation and precipitation of smaller solutes on the surface and within the pores of the membrane (Glover, 1985).

Hallström *et al.* (1989) stated that the hydrophobicity of the membrane surface is considered as one of the main characteristics governing the fouling process. The hydrophobicity of the membrane is mainly dependent on the chemical composition of the polymer material rather than the surface roughness or the pore size.

Richert *et al.* (1974) reported that the residual lipids, which are mainly phospholipoproteins coming from the fat globule membranes, may absorb strongly on membrane materials because of their amphoteric and amphiphilic properties and consequently leading to an irreversible fouling. Lee & Merson (1976) found that α-La and β-Lg caused most hindrance to flux. Characterisation of proteinaceous membrane foulants was examined by Tong *et al.* (1988). They found that whey proteins, α-La and β-Lg, accounted for 95% of the proteinaceous membrane foulants and very little casein was identified as membrane foulant. The reason why α-La and β-Lg preferentially adsorb on membranes during milk UF was stated unclear at that stage. This is quite different from the results reported by Patel *et al.* (1985), in which caseins were considered primary foulants.

The study of soluble proteins and Ca and P salts in the fouling deposit on a mineral microfiltration membrane in the processing of milk was carried out by Vetier *et al.* (1988). They found that Ca and P salts increased fouling, which was probably by allowing better adsorption of casein micelles on the alumina and acting as intermicellar matter bonds in the deposit.

In summary, in UF, the amount of protein deposited within the membrane pores is small compared with that on the membrane surface (Marshall *et al.*, 1993). However, in MF, there is a tendency of large particles or colloidal aggregates to be trapped in the pores, followed by cake formation on the surface of the membrane, and the creation of a new membrane layer. This layer starts to govern the overall filtration characteristics and is independent in its rejection properties of the initial pore size of the membrane (Merin *et al.*, 1990).

**Methods used to reduce fouling**

Limitations of a wider application of UF/MF in the dairy industry is mainly because of membrane fouling during the process. Therefore, reduction of fouling to a minimum extent becomes important. To reduce concentration polarisation and fouling as far as possible, the membrane process is generally operated in a cross-flow mode by which the feed flows parallel to the membrane surface with inlet feed stream entering the membrane module at a certain composition. However, methods used to reduce fouling need to be varied due to the complexity of the fouling phenomenon. This means that each separation problem requires its own specific treatment, although several approaches can be distinguished. The methods suggested were briefly described as following by Mulder (1991).

**Pretreatment of the feed solution**

Pretreatment methods employed include: heat treatment, pH adjustment, addition of complexing agents (EDTA *etc.*), chlorination, adsorption onto active carbon, chemical clarification, pre-microfiltration and pre-ultrafiltration. Fouling reduction starts in developing a proper pretreatment method. Sometimes very simple measures can be taken. For example, with a protein feed, pH adjustment is very important. In this case, fouling is minimised at the pH value corresponding to the isoelectric point of the protein. Normally, classical filtration or microfiltration methods can be used to prevent particles from entering the narrow fibres or channels on the feed side (Mulder, 1991).

Hugunin (1987) pointed out that calcium compounds in whey can foul or reduce the flux of water through the membranes. Processors have developed various techniques for reducing this problem, such as heating and adjusting the pH of the whey. They found that these pretreatment processes do have an effect on the final mineral content.

Changing the pH value or ionic strength of feed stream can greatly affect the retention of macromolecules, as they change the molecular conformation/aggregation or state of hydration of molecules. Also, pH and ionic strength may influence the protein adsorption to membrane surfaces. Al-Khamy (1988) reported that the permeability of both α-La and β-Lg can be increased by addition of NaCl into milk when ultrafiltering whole milk.

Study on the effect of fouling on rejection during microfiltration of protein solutions was carried out by Heinemann *et al.* (1988). Whey protein solutions of different pH in the presence or absence of salt were filtered to investigate the protein-membrane interaction. They found that addition of 100mM NaCl to water increased the water flux. In the absence of salt, transportation flux of protein solutions increases with decreasing pH, whilst the transmission of protein is highest at the isoelectric point. In the presence of salt, protein transportation is always high, but dense deposits tend to decrease the flux. The results can be explained by considering the interaction of charged molecules with the charges on the membrane surface.

**Membrane properties**

A change of membrane properties can reduce fouling. It was found that fouling with porous membranes (microfiltration, ultrafiltration) is generally much more severe than with dense membranes (pervaporation, reverse osmosis). Furthermore, a narrow pore size distribution can reduce fouling. The use of hydrophilic rather than hydrophobic membranes can also help reducing fouling. In general, proteins adsorb more strongly at hydrophobic surfaces and are harder to remove than at hydrophilic surfaces (Mulder, 1991). If colloids in the feed contains negative charge, the negatively charged membranes are suggested to be used to reduce the fouling. Thus, it seems feasible to reduce adsorption and fouling by selecting polymers with charges of equal sign to those of the foulants.

Trials on various coating techniques for the membranes have been carried out. It was assumed that adsorbed polymer molecules can inhibit electrostatic interactions between the protein and the surface, due to their non-ionic properties, as well as minimise the possibility of hydrophobic bonding due to their hydrophilicity. Furthermore, most non-ionic polymers also seem to prevent the formation of strong hydrogen bonds between protein molecules and the surface (Brink *et al.*, 1990).

**Module and process conditions**

It is known that efforts in reducing concentration polarisation will lead to a decrease in membrane fouling. Concentration polarisation can be reduced by increasing the mass transfer coefficient (high flow velocities) and using low flux membranes. Various kinds of turbulence promoters could be used to reduce fouling, although some systems, like fluidised bed etc., seem not very feasible from an economical point of view, especially for large scale applications. In MF, the membrane is susceptible to fouling and to the formation of a boundary layer by gelatinous material (Merin, *et al.*, 1990). It was suggested that one of the best way to prevent this phenomena from occurring is to increase the shear rate on the membrane surface, so as to sweep away the accumulating particlates retained by the membrane (Maubois *et al.*, 1987).

**Cleaning**

In practice, the cleaning method is most important and is always applied, although all the above methods can reduce fouling to some extent. Three cleaning methods can be distinguished: i) hydraulic cleaning, ii) mechanical cleaning and iii) chemical cleaning. A proper selection of cleaning method is very important. It mainly depends on the module configuration, the chemical resistance of the membrane and the type of foulant encountered.

## 2.1.5 Membrane Cleaning/Sanitising/Storing

After UF processing of milk, membranes are fouled mainly by proteins, to a different extent, depending on the concentration of product reached during processing. Whatever the amount of fouling, the membranes need to be washed by clean-in-place (CIP) and

used repeatedly. It is recognised that the cleaning of membranes is a tedious, but necessary process.

Highly alkaline detergents and chlorine are required to break down protein deposits, and acids to remove minerals. High pH levels with high temperatures bring about hydrolysis and oxidation of protein which will break up the deposits formed on the membranes. Sanitation of many UF membranes is commonly accomplished by the use of solutions of sodium hypochlorite containing 100-200ppm of available chlorine (Beaton, 1979). Chlorine released from sodium hypochlorite rapidly attacks proteins and amino acids. Since high temperature increases molecular movement, cleaning is commonly carried out at around 50°C. Furthermore, the purity of water is important and manufacturers often recommend the quality water as desirable for membrane cleaning. In the experiments performed by Bohner *et al.* (1992), the polysulfone ultrafiltration membrane system can be cleaned effectively and successfully, based on evaluation of the membrane surface by scanning electron microscopy. Cheap chemicals can be used for the cleaning and sanitising of the polysulfone ultrafiltration membrane system, which do not damage the membrane. Besides, the cleaned and effectively sanitised membranes need not to be stored in sanitised solution to maintain low microbial counts. Glover (1985) suggested that the water flux of the cleaned membrane should be measured under standard conditions of pressure, flow rate and temperature after processing. It should be restored to the level at which it stood before the processing operation.

### 2.1.6 Factors Affecting the Permeate Flux

There are many factors affecting the flux of permeate during UF/MF processes. Among those, pressure, flow rate, temperature and viscosity are considered to be important factors.

**Pressure**

Most investigators have found that increasing the operating pressure increases the flux up to a limiting value. It means that above the limiting value a further increase in the pressure causes no further increase in the flux, and also probably cause compaction of the gelled layer near the membrane (Lewis, 1982). If the feed material is too viscous, the

pressure drop over the module will be high, leading to ineffective separation of the material.

During UF of cheese whey with an M4 Carbosep membrane, it was found that the permeate flux was increased with an increase in transmembrane pressure (from 1-4 bar). However, further increase in transmembrane pressure to 5.7 bar did not result in further increases in the permeate flux since membrane fouling was also increased (Taddei *et al.*, 1991).

During MF of cell recovery and washing, it was concluded that increasing the transmembrane pressure caused the flux to increase initially but that it later accelerated flux decay (Defrise and Gekas, 1988). The permeate flux can decline to less than the flux at lower pressure. The reason could be as mentioned earlier that membrane fouling is more severe with increasing pore size. Therefore, to maximise the permeate flux, there is an optimum pressure, below which the driving force is too low and above which increased fouling causes a large reduction in flux (Marshall *et al.*, 1993).

**Flow rate**

Flow rate is also important factor in increasing the permeate rate. An increase in the flow rate increases the permeate flux owing to the increased turbulence. It is considered that increasing flow rate is more beneficial than increasing pressure for increasing permeate flux since this action is to assist the dispersion of the polarised layer (Glover, 1985). However, the flow rate may be limited by the maximum allowable pressure drop over the module (Lewis, 1982).

**Temperature**

It is well known that temperature has an important influence on permeate flux. It has been found by many investigators that increasing the operating temperature increases the permeate flux. The relationship between permeate flux and temperature is usually linear. This phenomena can be explained by the dual effects of temperature. Increase in temperature can lower the viscosity which assists flow rate and, on the other hand, can increase diffusivity which assists dispersion of the polarised layer (Glover, 1985).

Therefore, the highest temperature is recommended under the conditions that no protein denaturation and pump damage occur. Obviously, temperature control during UF is very important. If UF is carried out at a low temperature, serious concentration polarisation will occur. If temperature 35-45°C is used, a maximum bacterial growth will be encouraged (Lewis, 1982). If UF operation is over 60°C, the whey proteins will start to denature. Therefore, considering the above, the temperature of about 50°C is commonly suggested for milk products.

**Viscosity**

As concentration proceeds, flux decreases due to the increased viscosity. Glover (1985) commented that the decrease in the flux was due to the increase in protein rather than total solids. When the material is very viscous, the flux becomes very low and it is no longer economic to concentrate by ultrafiltration. The possibility of crystallisation or gelation of the product may exist. In consequence, this could be one of the worst situations arising during an ultrafiltration process.

### 2.1.7 Determination of Retention

The choice of the proper membrane depends primarily on the characteristics of the membranes, particularly the retention. Therefore, knowledge of retention for different components in milk is necessary. Retention of different components in milk by certain membranes can be determined by different methods.

The equation used for determining retention of milk components was reported by Peri *et al.* (1973). The equation was expressed as:

$$R = 1-Yp/Yr$$

Where R= Retention

Yp= percentage of any component Y in permeate

Yr= percentage of any component Y in retentate.

This equation does not rely on concentration factor. It accounts for retentate and permeate constituents, allowing retention of components in suspension (such as fat and micellar casein) to be determined. However, it does not consider that concentration occurs only by removal of the aqueous phase. This results in reports of negative retention. Therefore, Bastian *et al.* (1991) developed a more appropriate equation for determining retention of partially retained constituents.

$$R = 1-\{[(Yp)/(\%WATERp+Yp)]/[(Yr)/(\%WATERr+Yr)]\}$$

Where R = Retention

Yp = percentage of any component Y in permeate

Yr = percentage of any component Y in retentate.

WATERp = water content (%) of any component Y in permeate.

WATERr = water content (%) of any component Y in retentate.

They stated that this equation allows determination of instantaneous retention at several points during UF process. The advantage of this equation is that it can compare the movement of partially retained constituents with the movement of water through the membrane.

Total nitrogen in the permeate and concentrate can be determined by the Kjeldahl procedure, and the protein concentration calculated by multiplying by the appropriate conversion factor. However, it is known that the milk protein system is heterogeneous in nature and contains several proteins together with non-protein nitrogen. Thus the retention determined may well be lower than the true retention for the protein fraction, as most of the non-protein nitrogen may permeate through the membrane. In this situation, the problem can be overcome by precipitating the protein using the standard precipitating agents (e.g. trichloroacetic acid). Therefore, it is necessary to determine the retention of total nitrogen, true protein, non-protein nitrogen and individual proteins in the milk, respectively.

Most UF membranes show protein retention between 0.97 and 1 and lactose retention between 0 and 0.05. Information on the retention of the individual proteins in a mixture is very limited due to the analytical problems involved. Lewis (1982) reported that retention for β-Lg, α-La and bovine serum albumin by using polyacrylamide gel electrophoresis followed by densitometric scanning of stained gels were 0.76, 0.66 and 0.87, respectively, but increasing at different rates to 0.94, 0.92 and 0.97, respectively, by the end of the process. It was also observed that the retention levels were increased dramatically before reaching a 2-fold concentration and the retention of all substances increased as the concentration factor increased. The retention can change significantly during processing especially when the initial protein retention is low.

## 2.1.8 Changes in Milk Concentrates during UF

### 2.1.8.1 Physical Changes in Milk Concentrates during UF

As the protein concentration increases, the viscosity of the feed increases, particularly at low temperature (Setti & Peri, 1976). Increasing the temperature from 17 to 63°C reduces the viscosity which causes a 5-fold increase in flux (Renner & Abd El-Salam,

1991). This is apparently advantageous if high protein concentrations are required by UF.

When the protein concentration is up to 12%, as ultrafiltration proceeds, the product is becoming too viscous to handle and there may be a danger of gelling if high processing temperatures are used.

Hugunin (1987) pointed out that the agitation which causes foaming of protein solutions will denature proteins. System designs which cause excessive shear, pressure drops and air incorporation can contribute to protein denaturation and affect the functional properties of the proteins, e.g., WPC.

Abd El-Salam & Shahein (1989) found that when reconstituted skim milk is ultrafiltered, the flux is higher than when ultrafiltering fresh pasteurized milk. The increase in flux can be explained by the heat denaturation of whey proteins which are considered to be major foulants in UF in their native form. The flux is dependent on the different degree of heat treatment received during processing of the skim milk powder used, i.e. the highest heat treatment of skim milk powder gives the highest flux.

The retention coefficients of components of milk are independent on pressure, velocity and temperature, but not concentration. For example, the retention of protein started at 90% for milk at 1-fold concentration, retention reached 94% at concentration 2.2-fold. Different components in milk have different retention by the membrane depending on their molecular weights. Ultrafiltration membranes retain all the fat and almost all protein from milk. Retention coefficients for protein is 99%, but was 90% in some early reports (Glover, 1985). The lower retention values may have resulted from the inclusion of non-protein nitrogen (NPN) in the analysis of the protein. The NPN is difficult to measure due to such low concentration in the region of 0.02% and lower in the permeates. Retention coefficient of NPN are generally 20-40% and higher for the high concentration factors (Glover, 1985). Urea and amino acids are considered to be the main losses through the membranes.

Retentions of the components of skim milk are the same as for whole milk. But, it was reported by Pompei *et al.* (1973) that retentions of the components of skim milk were affected by temperature. A slight permeation of whey proteins appeared at 50°C, while there was no escape of these proteins at 5°C. The difference was explained by the changes in membrane structure with temperature and more severe concentration polarization at the lower temperature. There is an argument that heating induces an interaction between casein, β-Lg and calcium leading to the formation of aggregates. The aggregates are non-fouling since they pack less densely on the membrane surface than the smaller component particles and form less of a barrier to permeation. The main fouling of the membrane is considered to be β-Lg. In the combined treatment of heat and pH, some whey proteins such as β-Lg will denature (Hayes *et al.*, 1974).

In UF, skim milk behaves the same way as whole milk. The only difference is that permeate flux from skim milk is higher than from whole milk by about 20%. Although the fat in whole milk is present in the largest particle sizes, it seems to not be a great hindrance to filtration due to the fact that skim milk and whole milk have the same protein content, and the protein, being in smaller particle sizes than the fat, is more liable to denser packing at the membrane surface. Therefore, of all the components in milk, protein exerts the greatest control over the rate of UF (Glover, 1985).

### 2.1.8.2 Chemical Changes in Milk Concentrates during UF

Changes in the chemical composition of skim milk during UF have to be of concern. Premaratne & Cousin (1991) made an investigation of the changes in the chemical composition during concentration of skim milk to 5-fold by UF. Their results have showed that the changes in the concentrations of milk protein, fat, lactose, minerals, B vitamins and free amino acids during concentration of skim milk to 5-fold by UF were quite different.

Protein and fat concentrations increased proportionally as the volume of skim milk was decreased by approximately 2-, 4- and 5-fold. During the same time, total solids content increased from an initial 9.19% in skim milk to 23.91% in the 5-fold retentate. This increase was proportional to the concentration factor of the retentates but to a lesser

degree, indicating the loss of small molecular weight components such as lactose. The lactose content of skim milk decreased from an initial 5.06 to 4.06% in the 5-fold retentates.

During the UF process, a proportion of the small molecular weight components which are associated with proteins is retained with the proteins, while some components which are unassociated with proteins, such as free amino acids, soluble minerals and B vitamins are lost in the permeate.

Minerals, which are smaller than the membrane pores and are associated with proteins, do not permeate but gradually increase in concentration. However, the concentrations of these components increase to a lesser degree than those of proteins and fats. The degree of retention depends upon their relative degree of association with macromolecules. Retention of $Ca^{++}$ and $Mg^{++}$ is high due to their association with milk proteins. The association of ionic $Ca^{++}$ with colloidal milk proteins is also influenced by temperature. High temperature leads to a stronger association.

Minerals and trace elements which are partly associated with the casein micelles increase in proportion in the micellar phase as the concentration factor increases (Green *et al.*, 1984).

It has been found that changes in the chemical composition, such as increase in protein and mineral content of milk during UF, gave rise to an increase in the buffering capacity of the retentates (Brule *et al.*, 1981; Srilaorkul *et al.*, 1989). The high buffering capacity of the retentates, compared with skim milk, can contribute to body, texture and flavour defects and growth of pathogenic and spoilage bacteria (Patel *et al.*, 1986; St-Gelais *et al.*, 1992). This is a problem for hard cheese making. However, this problem can be overcome by reducing the mineral content by acidification of milk coupled with diafiltration (Brule *et al.*, 1981; Bastian *et al.*, 1991; St-Gelais *et al.*, 1992). Another problem associated with the high viscosity of UF retentates is that air bubbles in the retentate are not released quickly and become incorporated into the product, giving a sponge texture (Lelievre & Lawrence, 1988). In addition, in cheese production, a great

amount of acid production is necessary to overcome the strong buffering capacity of the retentates and to obtain the required pH. However, it was pointed out by Abd El-Salam *et al.* (1989) that the high viscosity can be reduced by addition of NaCl to UF retentates.

It is known that temperature and pH influence the salt system and the structure of casein micelles in milk (Dalgleish & Law, 1988). Viscosity of the retentate is inversely proportional to temperature and directly proportional to protein concentration (Setti & Peri, 1976). Ultrafiltration of acidified skim milk at low temperature (4°C) led to the dissociation of the casein micelles and a high solubility for the micellar minerals, causing an increase in viscosity. This effect results in membrane fouling due to adsorption and concentration polarization of milk components on membrane surfaces (Tong *et al.*, 1988).

Hallström *et al.* (1988) stated that formation of aggregates can be caused by UF of skim milk to 6-fold. These aggregates do not disaggregate on dilution even during prolonged storage and can only be disrupted by homogenization at pressure in excess of 200bar.

In experiments conducted by Green *et al.* (1984), the proportion of casein in the micellar form decreased by about 12% of total casein as the concentration factor increased. It was possibly due to an increased interaction with fat in the more concentrated milk.

Bastian *et al.* (1991) reported that changes in constituent retention during UF process depend on level of concentration, diafiltration and acidification. Retention of different components of skim milk during UF and diafiltration was determined by finding their permeate : retentate ratios at different points during the process.

#### 2.1.8.3 Changes in Casein Micelles during Ultrafiltration and Diafiltration

Investigation of the effect of ultrafiltration and diafiltration on casein micelles was carried out by Lonergan (1983b) using a hollow-fibre membrane with a MW cut off of 50,000 daltons in skim milk concentrated to 6-fold. The results showed that no changes occurred in the size distribution of casein micelles, the equilibrium between micellar and serum casein, or the hydration of the casein as a result of ultrafiltration or diafiltration.

Therefore, he confirmed that the changes in texture observed when ultrafiltered or diafiltered milk was used in cheesemaking were not caused by a change in the structure or composition of casein micelles during these two processes, but probably caused by the high concentration of casein in the retentate, which affects the agglomeration of micelles following the action of rennet. He also reported that there were no changes in the calcium or phosphorus content of micellar casein, although ultrafiltration and diafiltration removed substantial amounts of calcium and phosphorous from milk.

Green *et al.* (1984) investigated the changes in whey proteins and casein micelles during UF of skim milk. The whey proteins and casein micelles were examined for their solubilities using electronmicroscope and light scattering techniques. Their results showed no denaturation of whey proteins or disruption of casein micelles. However, some casein micelles in UF concentrated milk appeared to have non-spherical and fuzzy outlines, suggesting partial solubilization of casein.

Research on the effects of ultrafiltration of skim milk on casein micelle size distribution in retentate demonstrated (Srilaorkul *et al.*, 1991) that there was a significant change in casein micelle size distribution when the milk was ultrafiltered to high concentrations. This was a different finding to Lonergan (1983b). The highest proportion of casein micelles was changed from 80 to 100nm range in skim milk to 60 to 80nm in milk concentrated five times. It was considered that the change in the composition of casein and minerals, particularly Ca and P, as a result of UF may be responsible for the change in casein micelle size distribution, volume distribution and average diameter of the casein micelles. Their results of transmission electron micrographs of casein micelles obtained from skim milk and UF skim milk concentrate showed a roughly spherical shape, in various sizes, as expected. The appearance of casein micelles from UF skim milk concentrated three times or five times also exhibited nearly spherical shapes with a wide range of sizes.

## 2.2 Milk Protein Fractionation

### 2.2.1 Casein Fractionation

***Casein fractionation by ultrafiltration/microfiltration***

Although no studies on casein fractionation by ultrafiltration techniques have been reported, there are some studies on the combined processes of UF and other techniques i.e. cryodestabilization, diafiltration and so on for the fractionation of casein proteins.

The trial on the suitability of HPMP (high porosity membrane processing) in the manufacture of micellar whole casein, using a commercial pilot-scale microfiltration plant, has been carried out by Smithers *et al.* (1991). The results demonstrated that physical separation of whey from casein micelles in milk is commercially feasible. Analysis of both product streams by polyacrylamide gel electrophoresis indicated that the retentate was highly enriched in casein and substantially depleted in whey proteins, while the permeate was rich in whey proteins, including both major β-Lg, α-La (Smithers *et al.*, 1990). The separation of native micellar casein through cross flow membrane microfiltration was carried out by Pierre *et al.* (1992). They reported that native phosphocaseinate (PPCN) was separated from raw milk by tangential membrane microfiltration (pore diameter: 0.2μm) followed by purification through water diafiltration.

Pompei *et al.* (1973) and Barbano *et al.* (1988) have also reported that α-La and β-Lg passed into permeate using 10,000 and 20,000 MW cut-off membranes. The absence of casein in the gel electrophoretic patterns of the permeate showed that the membrane was not physically leaking retentate into permeate, but obviously, there were pores in the membrane large enough to allow some whey proteins to pass through (Bastian *et al.*, 1991).

***Casein fractionation by a combination process of UF and cool storage***

Lonergan (1983a) discovered an alternative way for producing casein by a combined process of ultrafiltration (4- or 6-fold volume concentration ratio (VCR)) and cryodestabilization (storage of the retentate at -8°C for 1-4wk.). He reported that the resulting casein isolate is dispersible in water and could be returned to its native state as a

casein micellar colloidal dispersion. This property is unique when compared to acid or rennet casein. He also found that the extent of ultrafiltration and storage time at -8°C could influence the extent of cryodestabilization of casein. This means that a greater extent of ultrafiltration (4 or 6 VCR) resulted in a greater extent of cryodestabilization of casein with 95% recovery of casein. Casein thus precipitated could be washed with water at 0°C without any significant loss of casein. He pointed out that, apart from solubility, the differences between the functional properties of cryocasein and acid or rennet casein, and the structure of resuspended cryocasein are not known and remain to be studied. The phenomenon of precipitation of casein isolate in milk held at high sub-freezing temperatures (cryodestabilization) is of interest here because the reaction is potentially reversible by the application of heat and agitation (Christianson, 1953; Rose, 1954).

***β-casein and $\alpha_s$/κ-casein fractionation***

Preparation of β-casein fraction from whole casein was done by Smithers *et al.* (1991). They exploited the substantial difference between dissociated β-casein and the modified micelles together with the use of high porosity membrane processing (HPMP) at low temperature to obtain isolation of small quantities of a β-casein-enriched whey permeate from non-fat milk. The feasibility of this enrichment process was confirmed by Love (1992).

***β-casein fractionation from sodium caseinate by ultrafiltration***

A method for the preparation of β-casein from sodium caseinate by ultrafiltration was developed by Murphy & Fox (1991). They fractionated sodium caseinate into β-casein enriched permeate and $\alpha_s$/κ-casein enriched retentate using ultrafiltration through 300,000 dalton cut-off membranes at 4°C. They reported that it was essential to dissolve the Na-caseinate in deionized or distilled water (pH in the range 7-9). A caseinate concentration of 1% was found to be optimal in the terms of yield and ease of operation, while processing with caseinate concentration 3% proved difficult due to membrane fouling. A method for the isolation of β-casein by microfiltration of calcium caseinate at 5°C was improved by Famelart *et al.* (1989) who purified β-casein from whole casein at 4°C and pH 4.2-4.6.

It is known that casein micelles are affected by pH and temperature in a number of ways. As the pH is decreased from that of normal milk (~6.7), the micellar calcium phosphate begins to dissociate (Van Hooydonk *et al.* 1986). At 4°C, all types of caseins were dissociated from the micelle, and not simply β-casein, especially in the case at lowered pH values (Roefs *et al.*, 1985). Further information on pH-induced dissociation of bovine casein micelles was given by Dalgleish & Law (1988). They reported that there are three processes that affect the dissociation of casein, namely (1) temperature: at low temperature, dissociation of casein is greatest, which favours a greater concentration of β-casein in the serum. Creamer *et al.* (1977) reported that when milk is cooled, β-casein initially moves from the surface of the micelle to the serum and the surface is replenished from the micelle interior. When cold milk is heated, the effect is reversed with β-casein moving from the surface into the micelle interior and with serum β-casein depositing onto the surface. (2) pH: as the pH decreases, more micellar calcium phosphate is dissolved, causing the dissociation of calcium and phosphate from the micelles and a loss of casein. (3) the isoelectric precipitation of the caseins: as the pH falls to a sufficiently low value, the dissociation of casein is considered both pH and temperature dependent.

At present, the physical-chemical-mediated fractionation of the total casein protein is based on the above theory. It involves cooling and slight acidification of skim milk to encourage dissociation of β-casein from the casein micelle, followed by membrane processing at low temperature. Under these conditions, the β-casein-depleted micelles remain in the retentate while the substantially smaller free β-casein appears in the permeate (Smithers & Bradford, 1991). It is suggested that the β-casein product can be used as an ingredient in the food industry to improve infant formulae and also can be used as source material in pharmaceutical industry to prepare biologically-active peptides, such as β-caso-morphines.

### 2.2.2 Whey Protein Fractionation

***Whey protein fractionation by ultrafiltration/microfiltration***

Processes which are used in the production of whey protein concentrates include heat coagulation, gel filtration, polyphosphate precipitation, lactose crystallization and separation, electrodialysis, ion exchange and ultrafiltration (Hugunin, 1987).

It is known that ultrafiltration separates molecules on the basis of size through the use of a semi-permeable membrane. Since the size of protein molecules is 100 times larger than lactose, minerals and short chain polypeptides, it is possible to select membranes with a porosity (or molecular weight cut off) which retain the whey proteins from cheese whey and allow the soluble minerals and lactose to permeate with water.

Ultrafiltration permits the fractionation of whey components without heat. However, the WPC solutions are generally concentrated by thermal evaporation and spray dried. Processes such as freeze concentration and freeze drying are technically feasible, but not economical. Heat denatures proteins, and there are indications that more purified whey protein solutions are more susceptible to heat denaturation than whole whey solutions.

***β-Lg and α-La fractionation from whey protein***

Efforts to fractionate the major whey protein components, i.e. β-Lg and α-La, to produce whey protein concentrates with improved and specific functional properties for particular food product applications have been reported (Pearce, 1983). In general, three major fractions from whey protein concentrate (WPC) can be produced, namely 'enriched-β-lactoglobulin', 'enriched α-lactalbumin' and 'enriched lipoproteins'.

Although several methods for separating individual whey proteins have already been proposed, most cannot be industrially scaled up because of their complexity, their cost in energy, their very low yield and the irreversible degradation of the joint-products due to the use of intensive heat-treatments (Nielsen *et al.*, 1973), of a very alkaline pH or of high amounts of salts (Kumata *et al.*, 1985). Furthermore, the purity and physico-chemical characteristics of the α-La fraction obtained were not satisfactory due to the high associating ability of whey lipoproteins and α-La (Pearce, 1983).

A pilot scale process for the fractionation of whey proteins has been established based on the reported fact that α-La aggregates more extensively than does β-Lg at >55°C in the pH range 4.0-4.5. Aggregation of α-La could be induced by heating at temperature

greater than 55°C in the pH range 4.0-4.5, while such conditions were ineffective for β-Lg at temperatures below 70°C (Pearce, 1987).

Maubois *et al.* (1987) developed a process which is likely to have a good industrial feasibility and can lead to the preparation of high purity whey protein concentrates and β-Lg and α-La fractions. Whey is cooled to 2°C at pH 7.3, and its Ca content is adjusted to 1.2g/kg. The whey is rapidly heated to 50°C for 8 min to precipitate the lipid fraction, which is removed by microfiltration. Ultrafiltration and diafiltration of the whey gives a 95% pure whey protein concentrate. Fig. 8 shows individual whey protein separation.

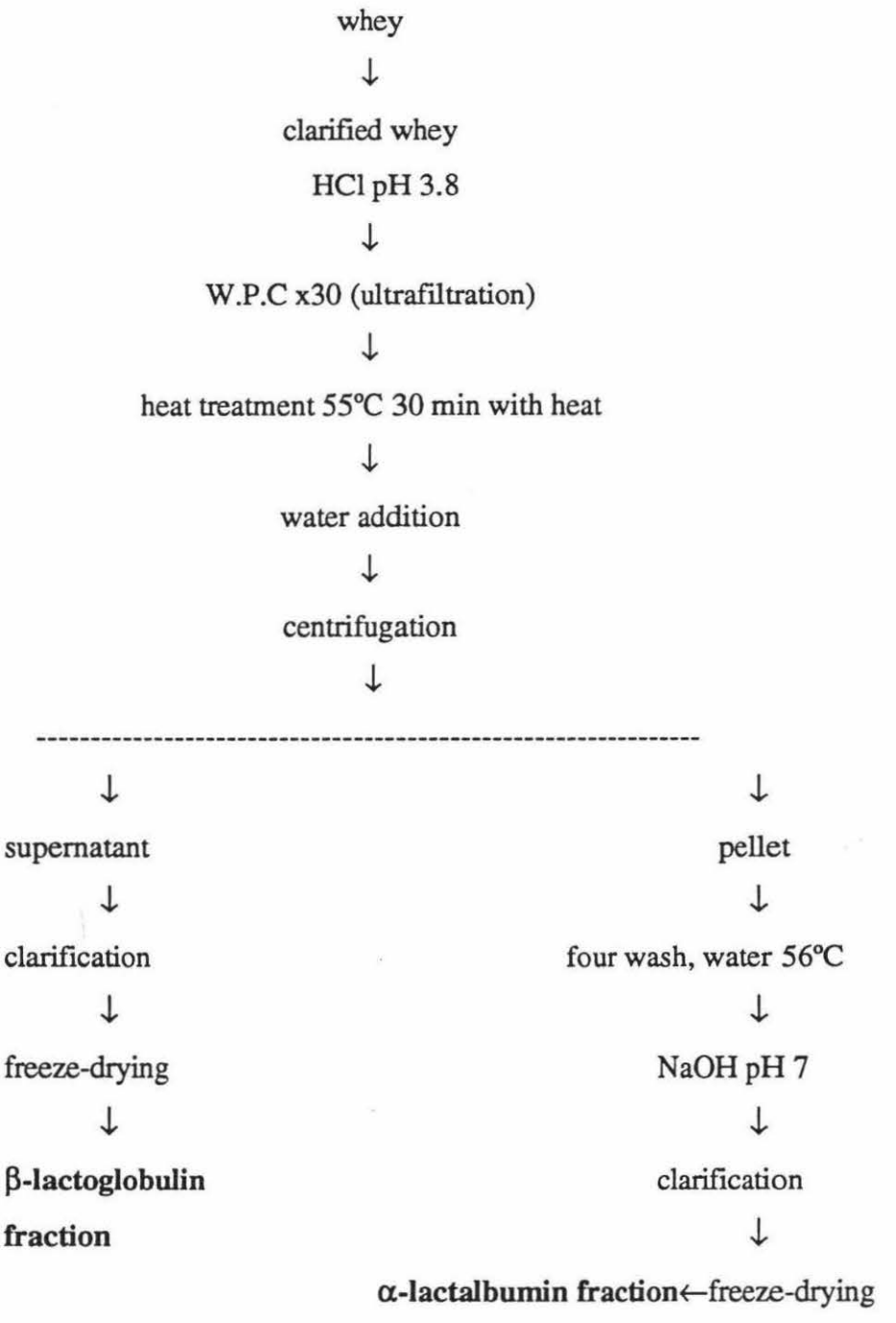


**Fig. 8.** Individual whey protein separation (Maubois *et al.*, 1987).

The individual proteins are obtained by adjusting the pH to 3.8, heating to 55°C for 30 min to aggregate the α-La, followed by centrifugation. α-La with a purity of 80 % is obtained by washing the precipitate in water at 56°C and pH 3.8, redissolving at pH 7 and spray drying. The supernatant is adjusted to pH 7, diafiltered and spray dried, to give β-Lg with >98% purity. Glover (1985) stated that a fraction enriched in α-La (MW 14, 000) and a fraction enriched in β-Lg (MW 37, 000) can be obtained by two stages of ultrafiltration using membranes with different pore sizes. A membrane with 20, 000 MW cut-off was used first to obtain β-Lg and then a membrane with a 2, 000 MW cut-off to obtain α-La. Fig. 9 shows the fractionation of whey protein by two stages of ultrafiltration.

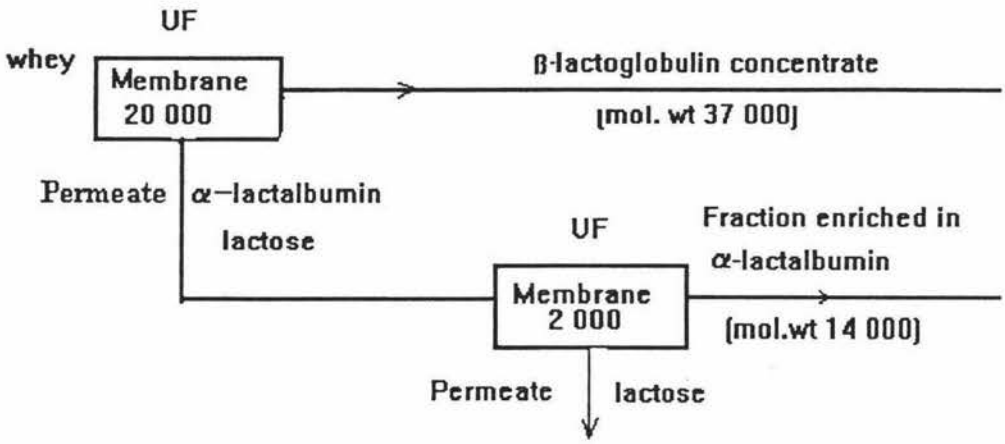


**Fig. 9.** Scheme for the fractionation of whey proteins (Roger & Maubois, 1981)

Fats and lipids are concentrated with the proteins during ultrafiltration. Fats can significantly affect the foaming properties of whey protein concentrates. In addition, the fats present in WPC are susceptible to oxidation and can cause flavour deterioration during storage (Hugunin, 1987).

Apparently, some work is still needed for optimising the process in order to improve the purity of the α-La. An investigation of the possibility of using membrane microfiltration for the separation of β-Lg from precipitated α-La and for washing the α-La precipitate was proposed by Maubois *et al.* (1987).

## 2.3 Functional Properties of Different Milk Protein Fractions by UF/MF

### 2.3.1 Functional Properties of β-casein and $\alpha_s$/κ-casein Enriched Fractions

Bovine casein is a mixture of four proteins, $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-casein, the functional properties of which differ significantly (Mulvihill & Fox, 1989).

The functional properties of a β-casein-enriched and $\alpha_s$/κ-casein-enriched fractions obtained from ultrafiltration of sodium caseinate are summarised in Table 4 by comparing to those of Na-caseinate (Murphy & Fox, 1991).

**Table 4. The functional properties of β-casein-enriched and $\alpha_s$/κ-casein-enriched fractions**

| | $\alpha_s$/κ-enriched | β-enriched | Na-caseinate |
|---|---|---|---|
| **1. solubility** | high | same | |
| **2. viscosity** | high | low | |
| **3. water sorption** | high | similar | |
| **4. foaming** | | | |
| **capacity** | low | high | |
| **stability** | low | high | |
| **5. emulsification** | | | |
| **activity** EA | low | high | |
| **capacity** EC | low | high | |
| **stability** ES | high | low | |
| **fat globule size** FGS | ? | ? | |

β-casein is the most surface active of the caseins due to its random structure, hydrophobicity and non-uniform distribution of hydrophobic and hydrophilic residues. The availability of bovine β-casein on an industrial scale would offer the possibility of developing improved infant formulae. The principal casein in human milk is β-casein,

which is highly homologous to bovine β-casein (Greenberg *et al.*, 1984), while α-like caseins which are the principal caseins in bovine milk, are present at low concentration, or absent, in human milk.

### 2.3.2 Functional Properties of β-Lg and α-La Enriched Fractions

Maubois *et al.* (1987) stated that whey proteins have excellent functional properties and a very high nutritional valve due to their exceptional content of sulphur amino-acids, lysine and tryptophan.

The whey protein concentrates (WPC) have protein purity ranging from 35% to 85%. The main functional advantages of WPC are:

- solubility all over the pH scale,
- high water retention capacity,
- gelification ability,
- foaming ability.

The functional properties of whey proteins may be modulated by variation of factors including pH, temperature, inorganic environment and methodology. The isolated or partially purified whey proteins have been showed to be more functional than WPC (Amundsen *et al.*, 1982).

# CHAPTER 3

# EXPERIMENTAL

## 3.1 The Pilot Ultrafiltration Equipment

The Koch UF/MF equipment consists of a feed tank, positive displacement pump, Koch separating module containing spiral wound membranes (type $S_2$-MFK), heat exchanger, pressure and temperature gauges and connecting pipework. The Koch UF/MF equipment is showed in Fig. 10. Fig. 11 shows the schematic diagram of the Koch UF/MF equipment.


**Fig. 10.** The Koch UF/MF equipment

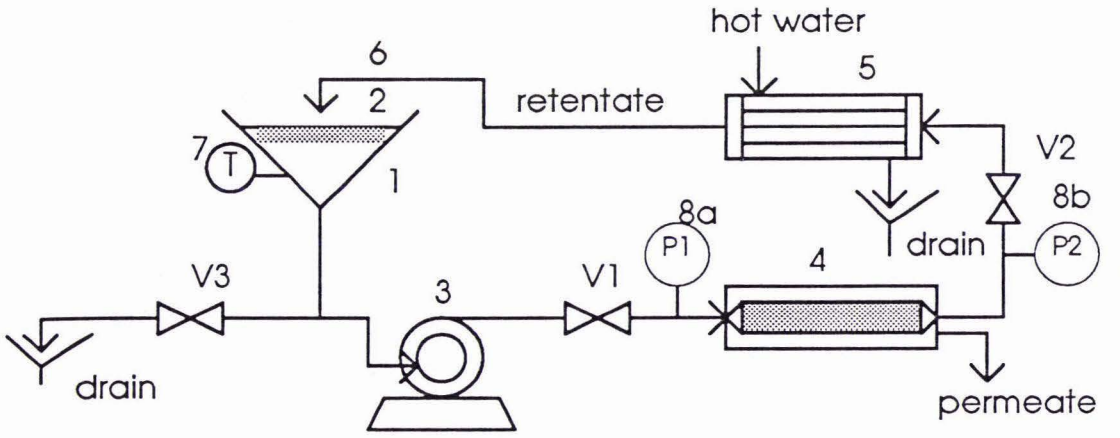


**Fig. 11.** The Schematic diagram of the Koch UF/MF equipment

1. Conical Balance Tank
2. 0.5mm Sieve Screen
3. Positive Displacement Pump (Evro Johnson)
4. Koch Separating Module
5. Shell and Tube Heat Exchanger
6. Flexible Hose Return Line
7. Temperature Gauge

8a, 8b. Pressure Gauge

V1, V2, V3. Butterfly Valve

## 3.2 Microfiltration/Diafiltration Process

### 3.2.1 Microfiltration Process

The microfiltration experiments were carried out using pilot plant equipment equipped with spiral wound membranes (type $S_2$-MFK) as showed in Fig.11. Three kinds of MF membranes with membrane area 0.18m² were used: MFK 603 (0030892), MFK 601 (0030894) and MFK 600 (0030895). The pore sizes of these MF membrane were 0.17μ, 0.85μ and 1.99μ, respectively (Koch, 1991).

The milks used in the experiments were commercial pasteurised skim milks (less than 0.1% fat) which were obtained from Capital Dairy Products, Palmerston North.

Prior to microfiltration process, the milk was preheated to 52°C and then transferred to the 20 litre, stainless-steel feed tank (1). The milk was pumped over the spiral wound membranes (type $S_2$-MFK) in the Koch separating module (4) by positive displacement pump (3). The milk permeate which came from permeate hose was collected, while the

milk retentate was pumped back to the feed tank through the heat exchanger (5) on the recycle line. A constant temperature of the retentate was maintained at 52±1°C by circulating hot water through the heat exchanger. This Koch UF/MF equipment was operated in a batch mode by recycling the retentate in the feed tank, until the desired level of concentration was attained by continuously collecting permeate. Permeate and retentate samples were taken, respectively, when a certain weight of permeate was collected which was equivalent to a certain concentration factor. The permeate weight collected was monitored continuously by an electronic scale (type ID2, Nr.-S/N 1851352, made in West Germany by August Sauter GmbH Albstadt 1).

During the process, valve V1 was kept fully open at all times. The pressure was obtained by throttling V2.

The operating conditions used were kept constant during the process for each trial (Pin = 120kPa, Pout = 60kPa, temperature = 52±1°C). Pump speed setting can be varied according to different pore size membranes used (the selection of operating conditions was based on the results obtained in preliminary experiments).

### 3.2.2 Diafiltration Process

At later stages of MF process, the flux is reduced to almost zero because of the increased viscosity. In order to achieve a better separation, a diafiltration (DF) process was utilised. A schematic drawing of diafiltration is given in Fig. 12.

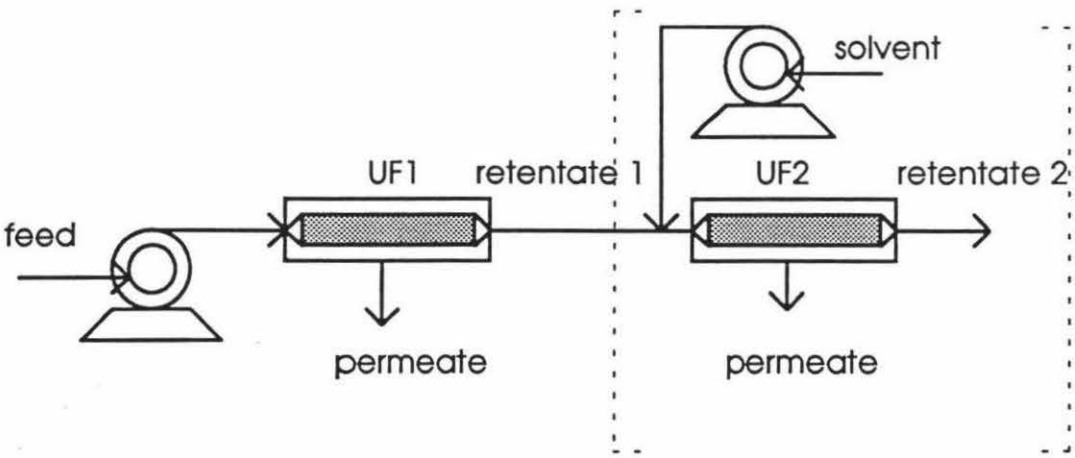


**Fig. 12.** Schematic drawing of diafiltration arrangement

Fig. 12 clearly shows that diafiltration is not another membrane process but just simply a continuation of the process of microfiltration. At the end of the microfiltration process, the retentate remaining in the system was mixed with water to wash out smaller components. An equal amount of deionised water at 52°C or 100mM salt solution as permeate removed from the system during the MF process was fed into the feed tank and the MF process restarted. The optimum point at which to add the water was just before the viscosity reached its critical level since the physical properties of the proteins might alter owing to the change in the ionic environment.

The operating conditions in the DF process were kept the same as that in the MF process. During the DF process, the collection of permeate and retentate samples was the same as in the MF process, i.e. the permeate and retentate samples were taken, respectively, when a certain weight of permeate was collected which was equivalent to certain concentration factor. An electronic scale was used to monitor the collected permeate weight.

### 3.2.3 Cleaning Procedure

Prior to each MF trial, a thorough CIP procedure was followed. The caustic wash and caustic/chlorine wash were the two main steps which must be taken for the preliminary cleaning. The system was circulated with 55°C caustic solution which had pH 10.5-11.0 for 20 minutes. Followed by rinsing for 10 minutes with deionised water at 60°C, the system was circulated with caustic/chlorine solution (55°C) which had pH 10.5-11.0, 200ppm Cl for 20 minutes.

Then the system was rinsed with about 60°C deionised water until the system pH returned back to 7. Water flux needed to be recorded before the process started. After processing, the residues left in the system needed to be flushed out completely with deionised water. Then the acid wash and caustic/UCII (Koch Ultra Clean II liquid) /chlorine wash were carried out. For the acid wash, the system was circulated with 55°C nitric acid solution with pH 1.8-2.0 for 20 minutes. Then the acid residue was flushed out with deionised water. Afterwards, 0.1%w/w UCII was added into the 55°C caustic/chlorine solution which had pH 10.5-11.0 and 200ppm Cl to make up

caustic/UCII/chlorine solution. The system was circulated with this solution for 20 minutes. Then the system was flushed out with deionised water until the pH returned back to 7 which normally take about 20 minutes. Water flux was determined after the cleaning to check the cleanliness of the membrane washing. The feed tank was filled up with deionised water to avoid the membrane dehydration. During the whole cleaning process, the temperature of the solutions was maintained using the heat exchanger. The process was operated under the normal process conditions, i.e. Pin = 120kPa, Pout = 60kPa, pump speed set = 45 (flow rate of water = 21L/min). The procedure for cleaning of Koch UF system was given in Appendix II.

According to the manufacturers' recommendation, the purity of the water is important and high quality water is desirable for membrane cleaning. Therefore, deionised water was used for membrane cleaning. After cleaning, the flux of water was measured under constant conditions of pressure, flow rate and temperature. Starting cleaning proceeded until the flux of water restored to the level at which it stood before the processing operation. Thus water fluxes were taken before and after every operation to record the state of the membrane. The feed tank was filled up with deionised water with valves V1 and V2 fully open to prevent membrane dehydration. The deionised water in feed tank was changed regularly to avoid the mould growth if not used.

## 3.3 Sample Collection during the Process

During the process, instant permeate and retentate samples were collected at the permeate and retentate recycling hoses, respectively, when a certain concentration factor was reached. The weight of permeate which was calculated according to their concentration factor was monitored using a scale. In the same time, permeate in the permeate collecting bucket was mixed well and then an accumulative permeate sample was also taken from the bucket and the weight of permeate was recorded. In order to minimise the growth of bacteria, the collected samples were placed in 4°C fridge immediately after being taken.

## 3.4 Sample Analysis

### 3.4.1 Total Protein Analysis

Samples of feed, accumulative permeates, instant permeates and retentates at different concentration factors were analysed for the contents of total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN), respectively, by the macro-Kjeldahl method using Tecator Digestion System 6 1007 Digester and KJELTEC System 1026 Distillating Unit. The procedure for the micro-Kjeldahl protein analysis is given in Appendix III.

***(a) Determination of non-casein nitrogen (NCN)***

Permeates and retentates samples were adjusted pH to 4.6 using 1M HCl solution to precipitate casein proteins. The precipitated casein proteins were filtered using Whatman filter paper No.2. About 3 grams of the filtrates were analysed for total nitrogen. The nitrogen in the filtrates is due to whey protein and non-protein nitrogen of milk.

***(b) Determination of non-protein nitrogen (NPN)***

For the determination of non-protein nitrogen (refers to DDM4 (1.11.5)), 15% trichloroacetic acid was added to 10ml volumetric flask containing 2ml well mixed milk sample. All proteins i.e. casein proteins and whey proteins were precipitated out. The filter paper, Whatmans No.542, 11cm, was used to remove the precipitate. The filtrate was analysed for total nitrogen. The nitrogen in the filtrates is due to non-protein nitrogen of milk.

***(c) Determination of casein nitrogen (CN)***

The content of casein nitrogen in the permeate and retentate samples was calculated by subtracting contents of NCN and NPN from the content of total nitrogen (TN), i.e.

% casein nitrogen = % total nitrogen- (% non-casein nitrogen + % non-protein nitrogen).

### 3.4.2 Polyacrylamide Electrophoresis Gel (PAGE)

#### 3.4.2.1 SDS-PAGE

Discontinuous polyacrylamide gels were used in these experiments, which consist of a resolving or separating (lower) gel and a stacking (upper) gel. The stacking gel acts to

concentrate large sample volumes, resulting in better band resolution. Molecules are then completely separated in the resolving gel.

Samples were diluted with sample buffer containing β-mercaptoethanol (a disulfide bond reducing agent) in different proportions for the permeate and retentate samples. Dilution for permeates and retentates was 1:40 and 1:100, respectively. The samples diluted with sample buffer were heated in a boiling water for 5-10 minutes.

Mini-Protein II cell (model 1000/500 power supply) was used for gels running. The procedure for preparation of gels and running conditions is given in Appendix IV.
The stained bands in the gels were scanned by LKB Ultroscan XL Laser Densitometer.

### 3.4.2.2 Native-PAGE

Alkaline PAGE (native-gel) was used to resolve only undenatured proteins. All procedures are the same as SDS-PAGE, i.e. in the aspects of preparations of SDS sample buffer, resolving gel, stacking gel, loading and running gels and staining/destaining the gels. The only difference with native gel from SDS-PAGE is that no SDS solution is used in the preparations of the sample buffer, resolving gel and stacking gel.

### 3.4.3 Lactose Analysis

The method of Lawrence (1968) was used to determine lactose content of retentates and permeates. The principle of this method is that sugar or related substances can produce stable colour with a peak about 490nm by phenol and concentrated sulphuric acid. The colour produced at a constant phenol concentration is proportional to the amount of sugar present.

A standard curve was made by preparing a series of standard lactose solutions which are from 0 to 100μg/ml. 1.0ml phenol 5% and 5ml concentrated sulphuric acid were added into the diluted samples to produce colour. The optical density of permeate and retentate samples was obtained using a spectrophotometer (CE 292 Digital ultraviolet

spectrophotometer) at 490nm and then their lactose contents were determined from the standard curve. The procedure for lactose analysis is given in Appendix VI.

### 3.4.4 Total Solids Analysis

The collected samples of feed and retentates at different concentration factors were analysed for the content of total solids (DDM4 (1.12.4a)). The principle of this method is that a known weight of a sample is dried under given conditions to a constant weight, the weight after drying constitutes the weight of total solids.

Drying conditions used were: 100°C vacuum oven for 20 minutes under 3kPa vacuum. The retentate samples were diluted with a small amount of distilled water after being weighed. Before the samples were put in the oven, they were evaporated on a boiling waterbath until dried. The procedure for total solids analysis is given in Appendix VII.

# CHAPTER 4

# PRELIMINARY EXPERIMENTS: SELECTION OF OPERATING PARAMETERS AND MEMBRANES

The objective of these preliminary experiments was to determine the optimum operating parametres for the Koch UF/MF equipment and consequently select the best membrane for the fractionation of milk proteins.

## 4.1 Selection of Operating Parameters

As reviewed previously, there are many variables affecting flux in the UF/MF process. In order to achieve a higher flux with a certain membrane, it is necessary to gain the knowledge of what operating parameters should be used. According to the literature reviewed previously, the following variables were selected for exploration of the operating parameters:

(a) Temperature;
(b) Pressure difference;
(c) Microfiltration time (Concentration polarisation);
(d) Concentration factor.

It should be noted that only one membrane ($S_2$-MFK-603) was used in these trials and the selection of variables was based on their effects on the flux.

**Temperature variation**

The temperature was varied from 40C° to 53C° in this experiment, while the operating pressures used were kept constant (Pin = 120kPa and Pout = 60kPa). The pump speed was set at 45 (flow rate of water was 21L/min).

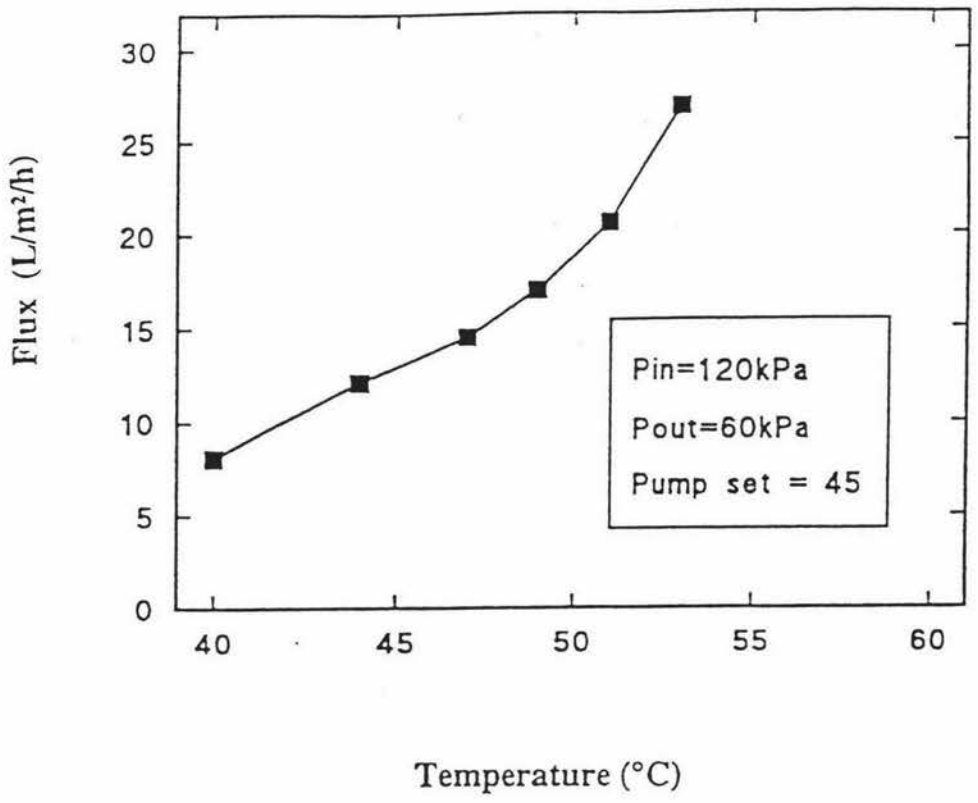


**Fig.13.** Effect of temperature on the flux of pasteurised skim milk during the microfiltration process using membrane 603 ( pore size 0.17μ).

From Fig. 13, it can be seen that the higher the temperature, the greater the flux. It is also found that the flux increases more rapidly when the temperature is increased above 50°C. This phenomena can be explained by the dual effects of temperature, i.e. increase in temperature can lower the viscosity which assists flow rate and, on the other hand, can increase diffusivity which assists dispersion of the polarised layer. Therefore, the highest temperature condition is recommended. However, a high temperature (>55°C) was not suitable because whey proteins in milk were liable to denaturation at temperature above 60°C (Glover, 1985). Therefore, the temperature 52±1°C is selected for this study.

**Pressure difference (flow rate) variation**

The pressure difference was varied from 20kPa to 100kPa by varying Pin while keeping Pout at 60kPa and temperature at 52 ±1°C, respectively.

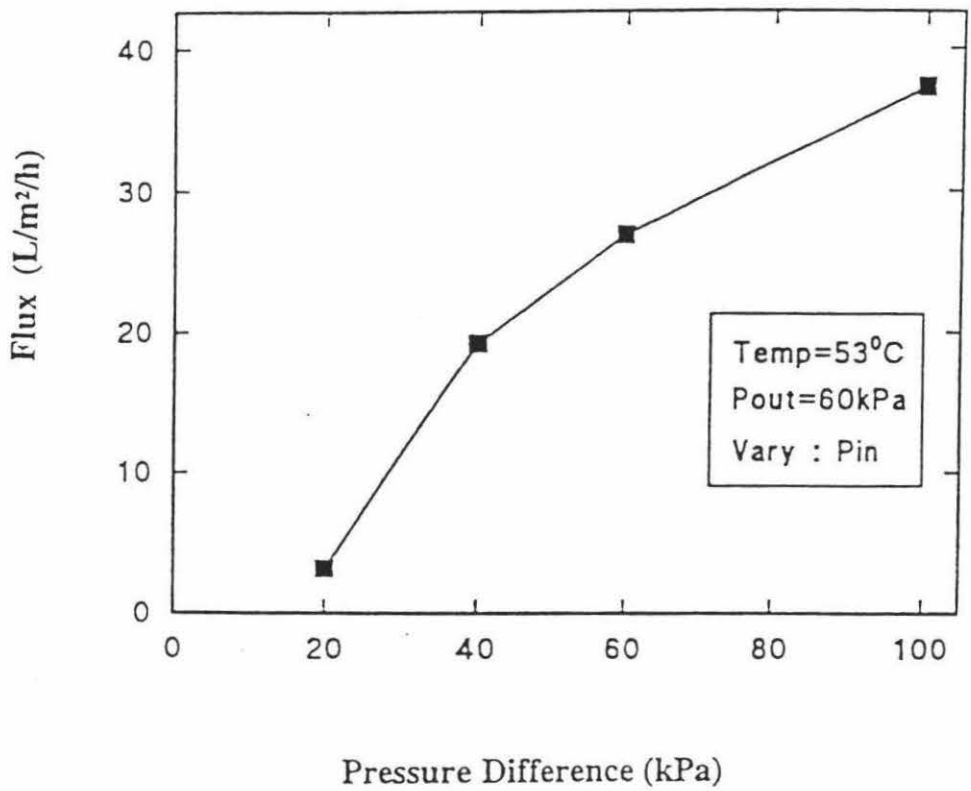


**Fig. 14.** Effect of pressure difference by varying Pin on the flux of pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

From Fig. 14, it can be seen that as the pressure difference increases, the flux increases. The higher the overall pressure difference, the greater the flux. As main pressure in the system was not kept constant during this trial, there is a compounding influence of system pressure and flow rate in these results. However, for the purpose of this trial, a standard condition of 120kPa inlet / 60kPa outlet was chosen because of adequate flux, membrane life and pump noise considerations.

**Microfiltration time (concentration polarisation)**

The microfiltration process was run from 5 to 40 minutes at a constant temperature (52± 1°C) and operating pressure (Pin = 120kPa, Pout = 60kPa) by recycling both retentate and permeate back to the feed tank.

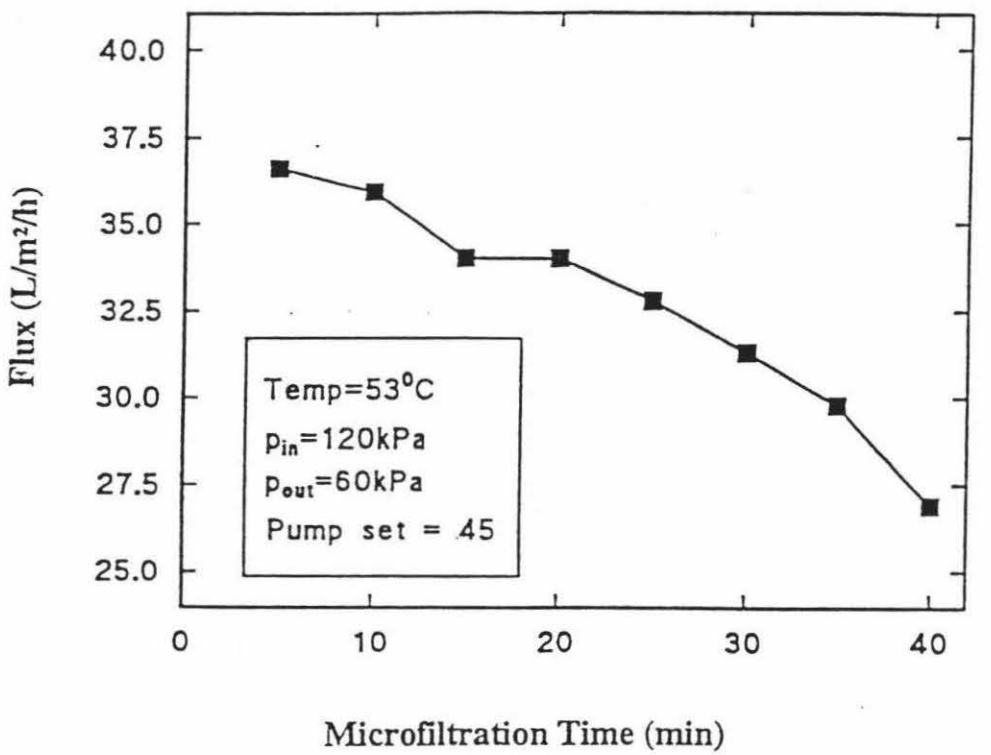


**Fig. 15.** Effect of the microfiltration time on the flux of pasteurised skim milk using membrane 603 (pore size 0.17μ).

Fig. 15 shows that as the microfiltration time increases, the flux of skim milk gradually decreases from 36 L/m²/h at the beginning to 26 L/m²/h after 40 minutes. This indicates that the concentration polarisation phenomenon occurred, which is probably caused by the accumulation of a protein layer on the membrane surface as the microfiltration time increases and retentate becomes more concentrated.

**Concentration factor**

The microfiltration process was run at a constant temperature (52±1°C) and operating pressure (Pin = 120kPa, Pout = 60kPa) by recycling retentate back to the feed tank and continuously collecting permeate at a certain concentration factor.

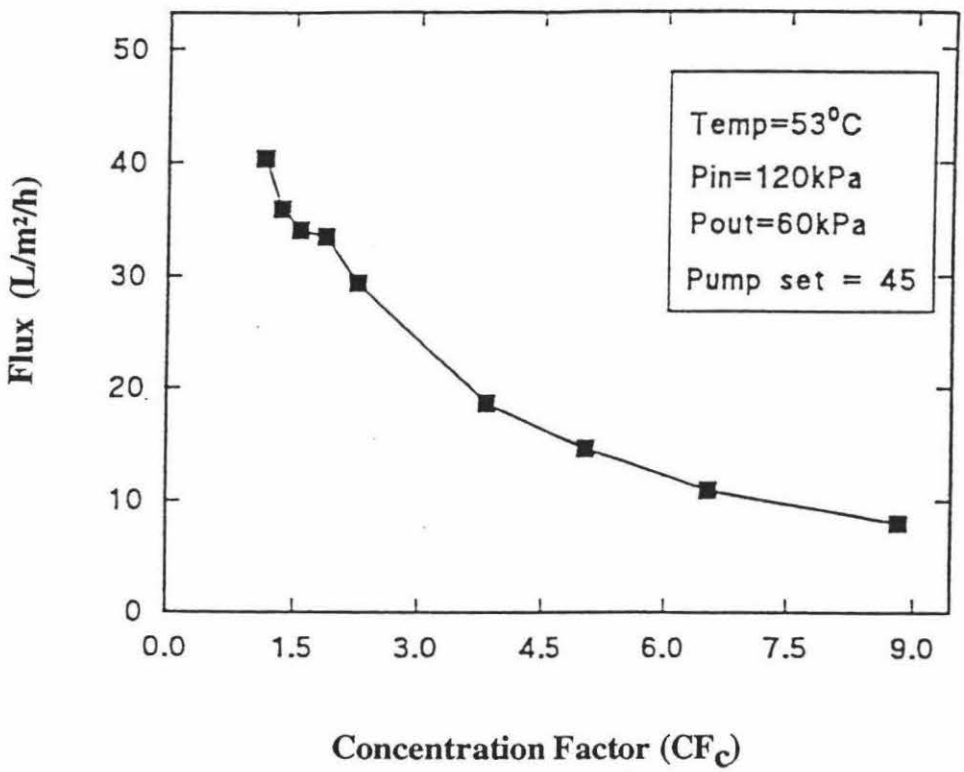


**Fig. 16.** Effect of concentration factor on the flux of pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

Fig. 16 shows that the higher the overall concentration factor, the lower the flux. The flux decreases dramatically from 40 L/m²/h ($CF_c$=1) to 15 L/m²/h with increase of $CF_c$ up to 4. Further increase of $CF_c$ up to 9 causes a slow decrease in the flux to <10 L/m²/h.

It is recognised that flux will decrease with time due to the concentration polarisation and fouling. These experiments (temperature, pressure difference) were conducted in a continuing series of trials on the same batch for each parameter and hence the compounding influence of time on flux was present, but was ignored in the selection of suitable operating parameters. The experimental plant was brand new and suitable operating parameters were being determined rather than a detailed optimisation of the plant, which would required a different approach.

To sum up the above results, the selected operating parameters are listed in Table 5.

**Table 5. Summary of selected operating parameters**

| Variables | Selected operating parameters |
|---|---|
| Temperature (°C) | 52±1 |
| Pressure difference (kPa) | 60 (Pin = 120, Pout = 60) |
| Concentration factor ($CF_c$) | 2-5 |
| Feed (skim milk) | commercial pasteurised skim milk |

## 4.2 Selection of Membranes

The membrane selection is basically based on 1) molecular sizes of the different constituents which need to be separated from milk. 2) membranes that were available at the time when the study was undertaken.

The composition of milk proteins and their molecular sizes is given in Table 6.

**Table 6. Composition of milk proteins and their molecular sizes** (Kessler, 1981)

| Milk Protein Components | Concentration in Milk (%) | Molecular Weight (M.W) | Size (nm) (Diameter) |
|---|---|---|---|
| Casein Micelles | 2.5 | $10^7$-$10^9$ | 25-150 |
| β-Lg | 0.25 | $36 \times 10^3$ | 4 |
| α-La | 0.14 | $14.5 \times 10^3$ | 3 |
| BSA | 0.03 | $69 \times 10^3$ | 5 |
| IgG | 0.06 | $160 \times 10^3$ | - |

It can be seen that there is a large difference between the sizes of casein micelles and whey proteins. Theoretically, the separation of casein micelles from the other proteins in milk is quite possible by using a proper membrane. However, since milk is a complex system, the judgment of using a proper membrane has to be based on the result of trials, instead of simply looking at the size differences of proteins fractionated and membranes

used. Duplicate trials were carried out for each membrane. The available membranes in the pilot plant are showed in Table 7.

**Table 7. Membranes available in the pilot plant**

| Type of Membranes | HF | | MF | | |
|---|---|---|---|---|---|
| | 100 | 131 | 603 | 601 | 600 |
| Pore Size | 45Å | 74Å | 0.17μ | 0.85μ | 1.99μ |
| Membrane Area | 0.28 $m^2$ | | 0.18 $m^2$ | | |

One trial was carried out on the HF membranes 100 and 131 which have pore sizes 45Å and 74Å, respectively, using 5% whey powder solution which was made from sweet whey powder. The total protein in the permeate and retentate samples with $CF_c$=2 was analysed using the Kjeldahl method. The results shows that the total protein content in the permeate and retentate was 0.29% and 2.95%, respectively. The protein content in the retentate was about 10 times higher than that in the permeate. This means that most of the whey proteins were retained by these two membranes. Therefore, it can be concluded that the HF membranes 100 and 131 are too tight for whey protein permeation and not suitable for fractionation of proteins in this study.

The next set of trials was done using the MF membranes (600, 601 and 603). Duplicate trials were done on membrane 603 and 600. One brief trial was done on the membrane 601. In order to compare the three membranes, all operating variables were kept constant. Permeate and retentate samples at $CF_c$=2 and $CF_c$=4 were collected, respectively. The total solids and N content in the retentates were analysed. The retention coefficient for total N was calculated based on the fraction of total N in the permeate and retentate samples. The results are summarised in Table 8.

**Table 8. Comparison of the results using three available MF membranes**

| MF Membranes . | Pore Size (μ) | Total Solids % (in Retentates) | | | Protein Content % (in Retentates) | | | Retention Coefficients (total N) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Feed | $CF_c$=2 | $CF_c$=4 | Feed | $CF_c$=2 | $CF_c$=4 | $CF_c$=2 | $CF_c$=4 |
| 603 | 0.17 | 9.40 | 12.40 | 15.53 | 3.73 | 6.64 | 10.82 | 0.95 | 0.97 |
| 601 | 0.8 | 8.86 | 8.71 | 8.91 | 3.82 | 3.20 | 3.42 | 0.02 | 0.06 |
| 600 | 1.99 | 9.04 | 8.84 | 8.85 | 3.35 | 3.26 | 3.41 | 0.01 | 0.06 |

It can be seen that, compared with feed milk, the total solids and protein content in the retentates are not significantly changed during the microfiltration process using membrane 600 and 601 at both $CF_c$=2 and $CF_c$=4. The retention coefficient of total N is only 0.06 for 600 and 601 at $CF_c$=4. This indicated that a proper fractionation of proteins could not be achieved by the MF membrane 600 and 601 since nearly all the milk protein components pass through the membranes.

SDS-PAGE was carried out on the permeate and retentate samples obtained from the microfiltration process using membrane 600. The results of the gel electrophoretic patterns of retentate samples and permeate samples are showed in Fig. 17 and Fig. 18, respectively.

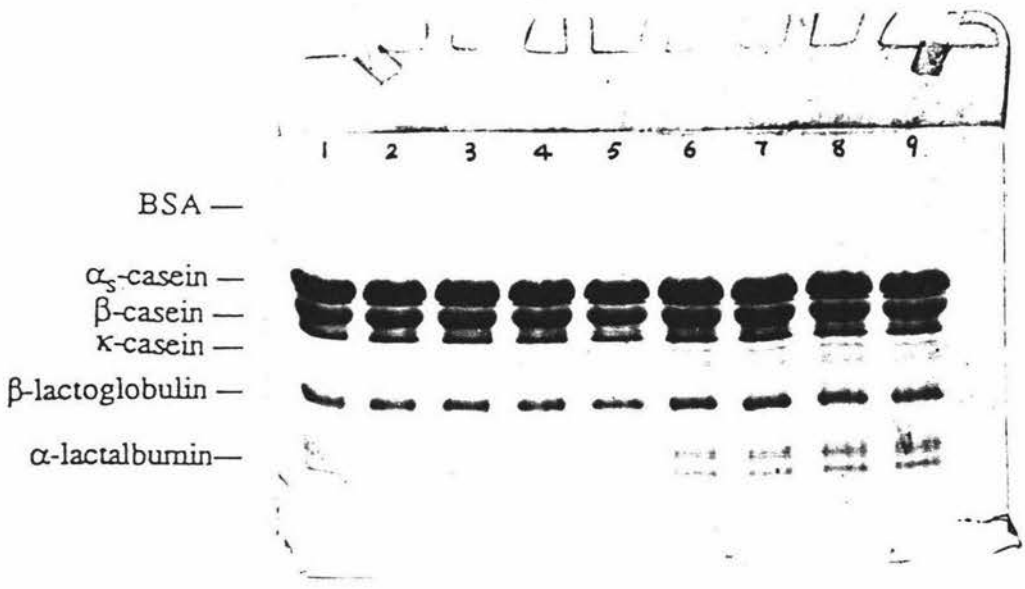


**Fig. 17.** Sodium-dodecyl-sulphate-polyacrylamide gel electrophoretic patterns of the retentate samples from skim milk microfiltration process using membrane 600. Lane 1 is skim milk feed for the microfiltration process as a control. Lane 2, 3, 4, 5, 6, 7, 8 and 9 are retentate samples with $CF_C$ 1, 1.5, 2.0, 2.5, 3.3, 4.2, 4.5 and 4.8, respectively.

Lane 1 shows a typical distribution of milk proteins in skim milk feed (Fig.17). Bovine serum albumin, $\alpha_s$-casein, β-casein, κ-casein, β-lactoglobulin, α-lactalbumin are seen clearly. By comparing the patterns in lanes 2, 3, 4, 5, 6, 7, 8 and 9 retentate samples with skim milk in lane 1, it can be seen that no significant differences exist in the concentration of all proteins, indicating that no significant concentration and fractionation of the skim milk occurs as the $CF_C$ increases. Similar observations were made on permeate samples (Fig. 18).


**Fig. 18.** Sodium-dodecyl-sulphate-polyacrylamide gel electrophoretic patterns of the permeate samples from skim milk microfiltration process using membrane 600. Lane 1 is skim milk feed for the microfiltration process as a control. Lane 2, 3, 4, 5, 6, 7, 8 and 9 are milky permeate samples with $CF_c$ 1, 1.5, 2.0, 2.5, 3.3, 4.2, 4.5 and 4.8, respectively.

Thus it is clear that nearly all milk protein components passed through the membrane 600.

The selection of membranes was based on the result of the above trials on the three available MF membranes. MF membranes 600 and 601 had similar retention coefficient for total N although the information given by the manufacturer about the pore sizes of these two membranes indicated a significant difference, i.e. membrane 601 = 0.85μ, 600 = 1.99μ. The milky colour of the permeates obtained from both membranes showed that most of casein proteins passed through the membranes as well. Therefore, the MF

membrane 603 was selected to further investigate the possibility of fractionating casein micelles and whey proteins from the milk.

# CHAPTER 5

# FRACTIONATION OF MILK PROTEINS USING MEMBRANE 603

## 5.1 Microfiltration Process

The objective of this experiment was to evaluate the MF 603 membrane. The separation effect was investigated by analysing whey proteins and casein proteins in the permeates and the retentates, respectively, using N analysis and gel electrophoresis. The retention coefficients of total nitrogen (TN), non-casein nitrogen (NCN) and casein nitrogen (CN) were calculated.

Trials were carried out in duplicate with 20 kg pasteurised skim milk for each trial.

**1) Effect of Concentration Factor ($CF_c$) on the Retention Coefficients of Different Protein Components**

The results of the effect of $CF_c$ on the retention coefficients of total N (TN), non-casein nitrogen (NCN) and casein nitrogen (CN) are showed in Figs. 19, 20 and 21, respectively. The equations used to calculate retention coefficients are showed in Appendix VIII.

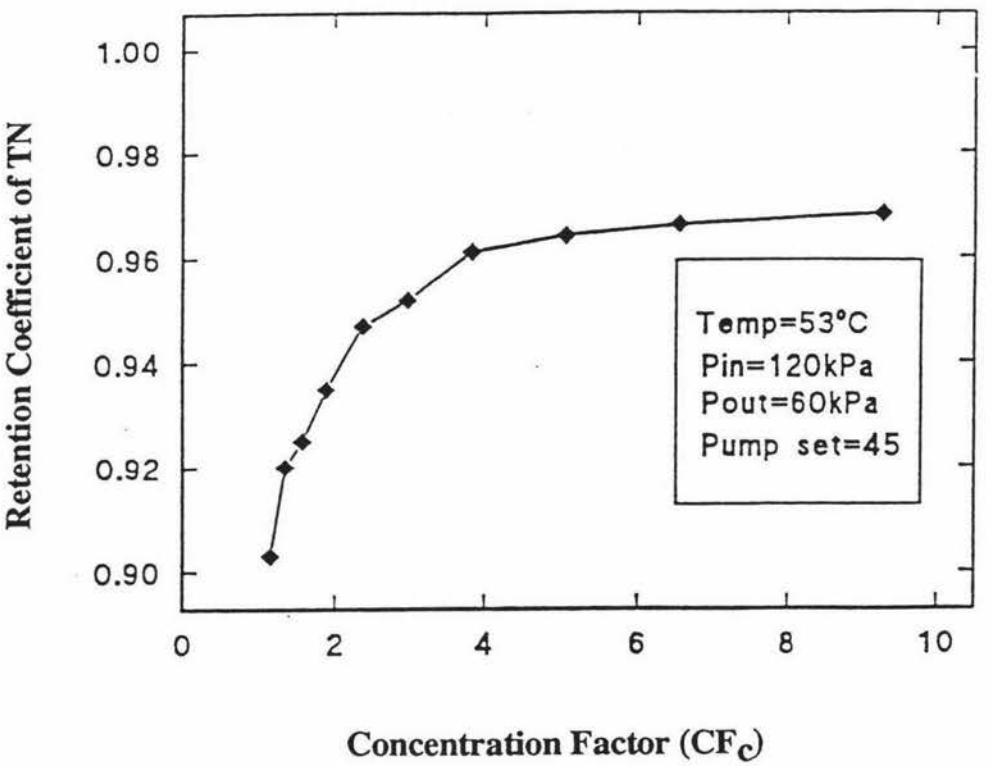


**Fig. 19.** Effect of concentration factor ($CF_c$) on the retention coefficient of total nitrogen (TN) for pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

From Fig. 19, it is observed that as the $CF_c$ is increased from 1 to 4, the retention coefficient of total N increases from 0.91 up to 0.96. Between $CF_c$ 4 and 9, the retention coefficient remains between 0.96-0.97 with no obvious change. This phenomenon is probably because the membrane pores are more open at the initial stages, thus some NPN and NCN, which consist of smaller molecules, easily pass through the membrane. However, as the $CF_c$ increases, more pores of the membrane have been filled up by some components in milk and possibly a secondary membrane is formed, leading to low flux .

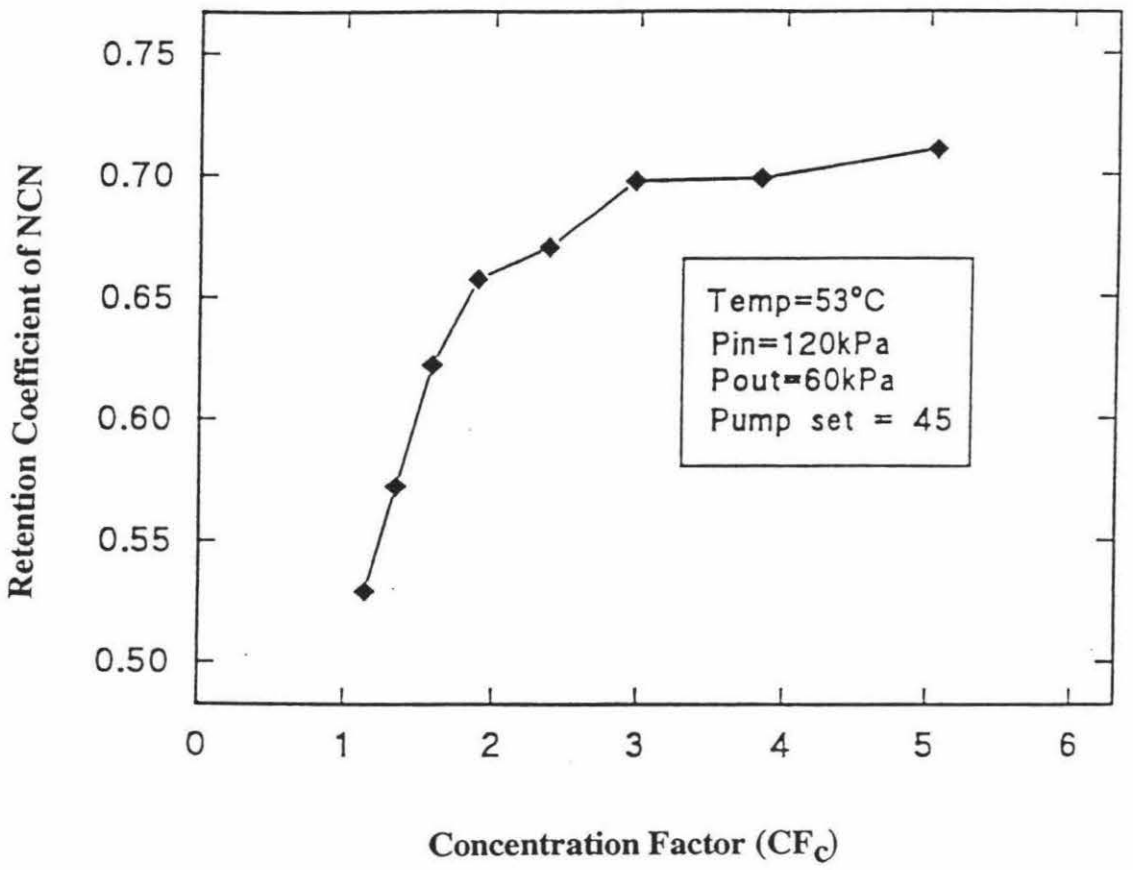


**Fig. 20.** Effect of concentration factor ($CF_c$) on the retention coefficient of non-casein nitrogen (NCN) for pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

In the same way, Fig. 20 shows that the retention coefficient of NCN, which includes NPN and whey proteins, is between 0.5 to 0.7 and it increases dramatically from 0.53 to 0.68 as the $CF_c$ is increased from 1 to 3. Between the $CF_c$ 3 and 5, the retention coefficient changes only slightly. This perhaps can be due to the formation of the secondary membrane at the later stages of the MF process.

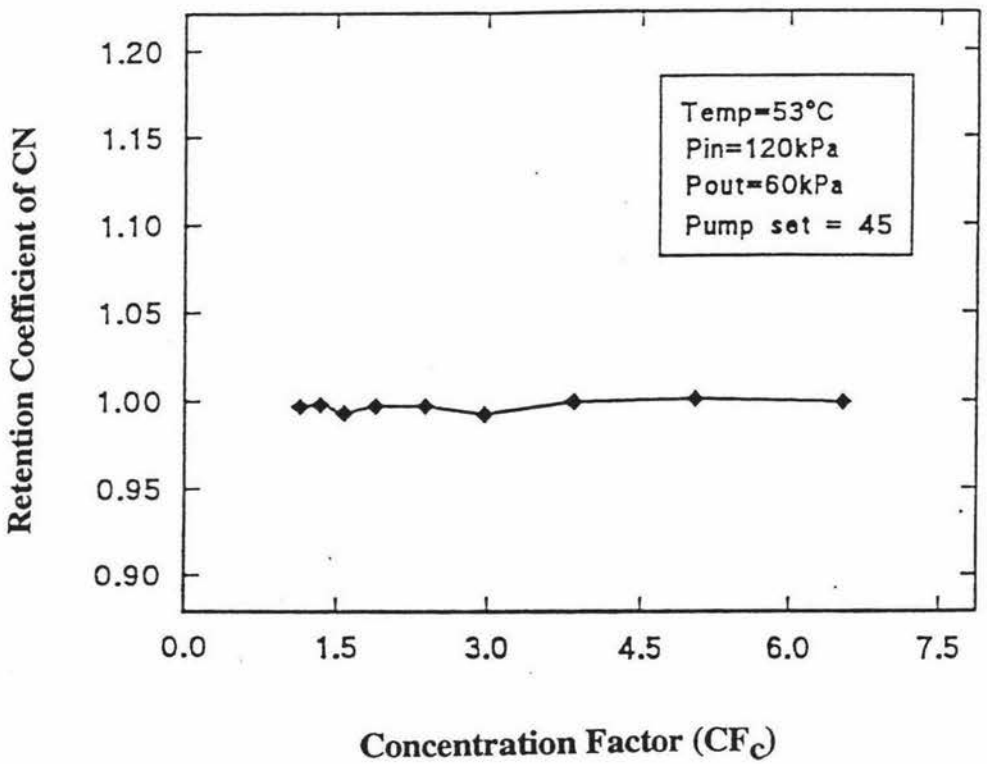


**Fig. 21.** Effect of concentration factor ($CF_c$) on retention coefficient of casein nitrogen for pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

Fig. 21 shows that the retention coefficient of casein N is essentially around 1 and no significant change occurs as the $CF_c$ is increased throughout the microfiltration run. It indicates that among the total caseins, over 99% of caseins have been retained after the MF process, even as the $CF_c$ goes up to over 6.

**2) Permeation (%) of Non-Casein Nitrogen (NCN)**

The results of the effect of $CF_c$ on the permeation of NCN are showed in Fig. 22.

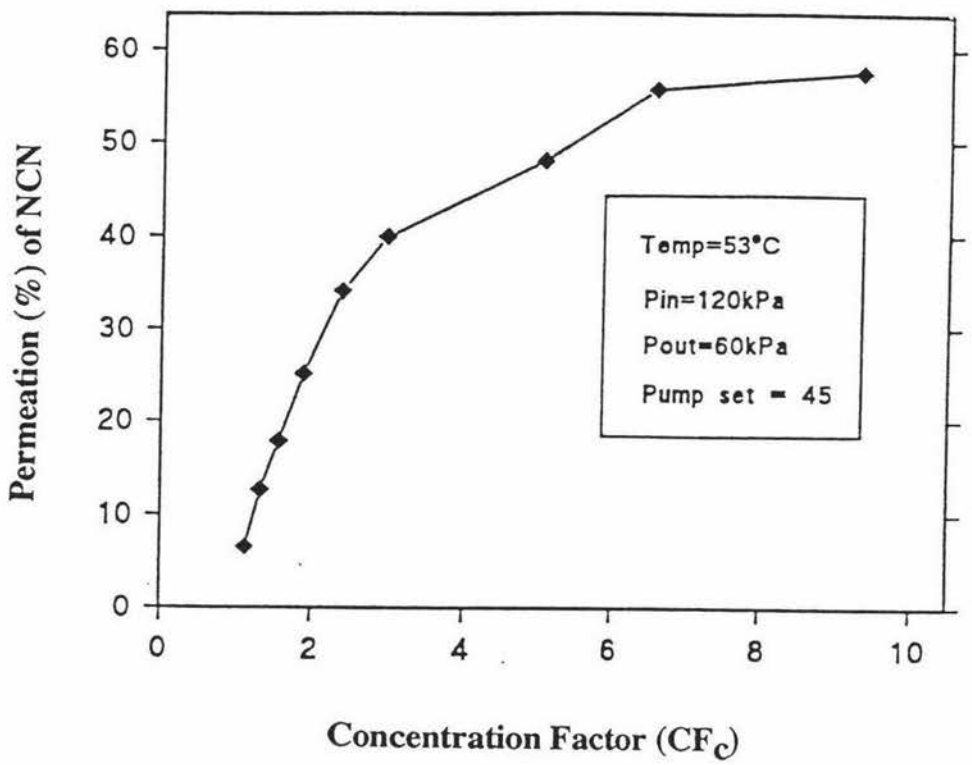


**Fig. 22.** Effect of concentration factor ($CF_c$) on the permeation (%) of non-casein nitrogen (NCN) for pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

Fig. 22 shows that NCN in the permeates increases as the $CF_c$ is increased. When the $CF_c$ is 1, i.e. the microfiltration process just starts, only about 8% of NCN is present in the permeate. However, when $CF_c$ is increased to 2-3, the permeation of NCN dramatically increases to about 30%-40% and then steadily increases up to about 60% when the $CF_c$ is increased up to 9. Although higher $CF_c$ (3 to 9) can give further about 20% more permeation of NCN, i.e. about 20% more NCN removal from the retentates, it probably will not be recommended as an effective processing method except only for the experimental purposes. The reason is due to the fact that the retentates become very

concentrated as the $CF_c$ increases, resulting in very low flux and a major extension of the processing period. At the same time, the system pressure tends to increase and become difficult to control. Consequently, membrane damage, protein damage and possibly aggregation of milk proteins could occur.

### 3) Changes in CN/NCN Ratio in the Retentates

In order to evaluate a separation effect of the casein nitrogen from the non-casein nitrogen, the changes in CN/NCN ratio in the retentates are assessed. Casein nitrogen (CN) content in the retentates is calculated from the content of total nitrogen (TN) and non-casein nitrogen (NCN) in the retentates. The results are showed in Fig. 23.

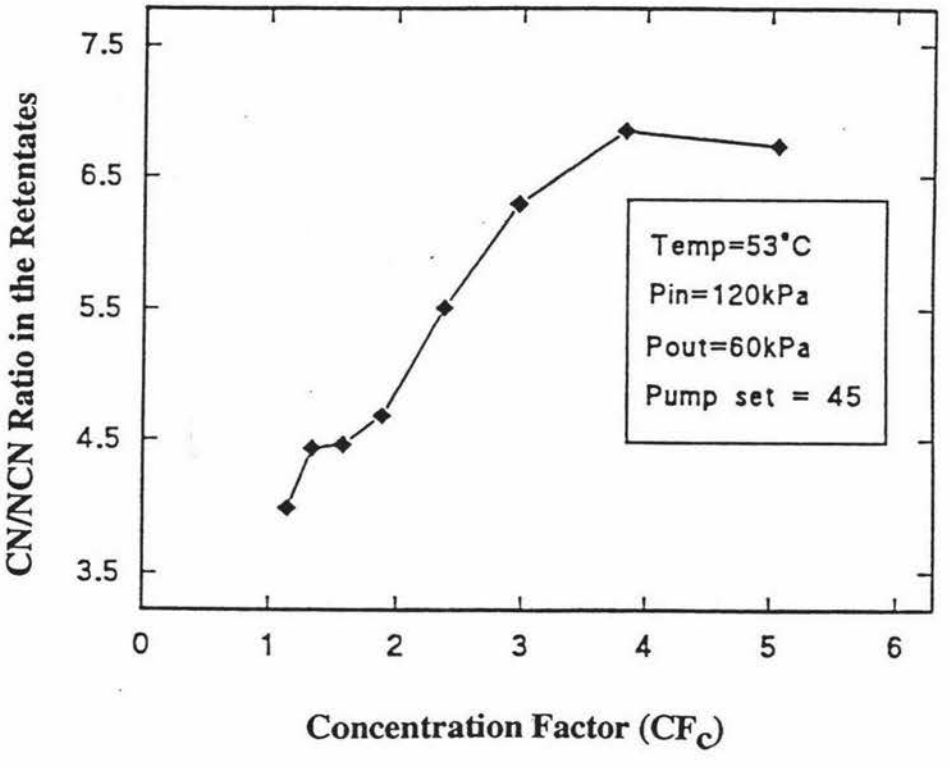


**Fig. 23.** Effect of concentration factor ($CF_c$) on the changes in CN/NCN ratio in the retentates for pasteurised skim milk during the microfiltration process using membrane 603 (pore size 0.17μ).

As the $CF_c$ is increased, CN/NCN ratio in the retentates changes greatly from around 4 to 7 at the $CF_c$ of 4. This result is similar to the results that Woychik *et al.* (1992)

reported. They reported that casein/whey ratio was between 0.7-0.9 in the permeates and 5.5-7.7 in the retentates when skim milk was concentrated by microfiltration using the membranes with porosities of 100nm and 200nm at $CF_c$ 4. In this experiment, the increased ratio in the retentates perhaps can be explained as discussed earlier that almost all casein nitrogen have been retained by the membrane, while non-casein nitrogen steadily pass through the membrane into the permeates as the $CF_c$ is increased, which results in this increased CN/NCN ratio. Therefore, the changes in CN/NCN ratio in the retentates can be considered as a good indication of protein separation performed by the MF process using the membrane 603.

**4) Permeation (%) of Lactose**

In order to determine lactose behaviour during the MF process, lactose content in the accumulative permeates and milk feed was analysed using a spectrophotometeric method (Lawrence, 1968). Consequently, permeation (%) of lactose was calculated (refers to Appendix VIII). The results are showed in Fig. 24.

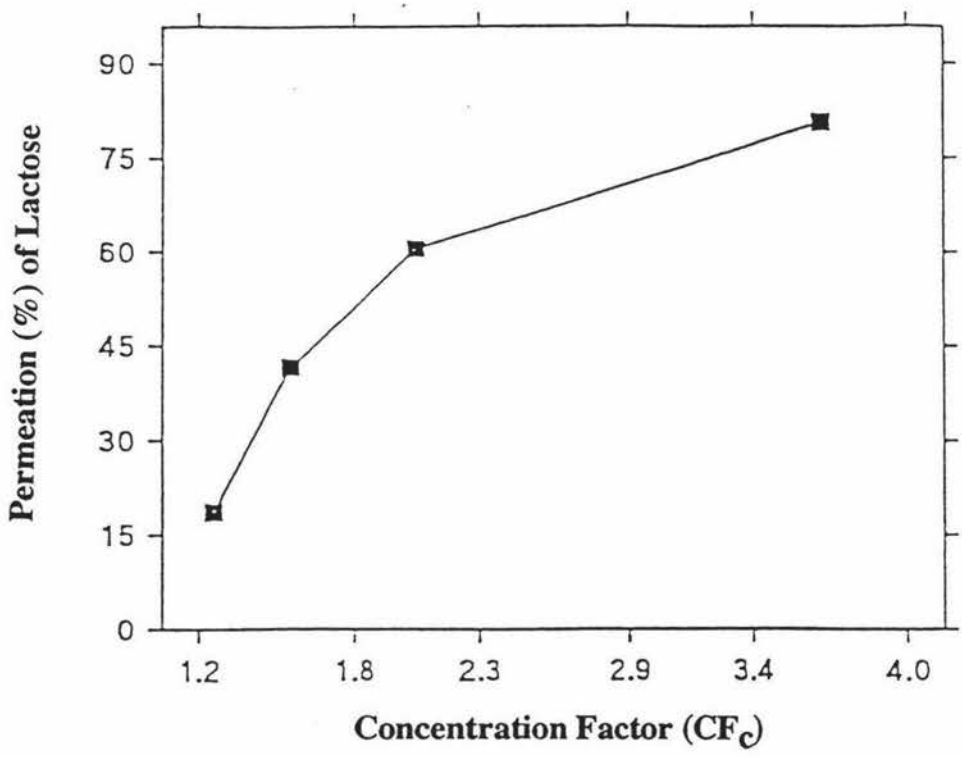


**Fig. 24.** Effect of concentration factor ($CF_c$) on the permeation (%) of lactose during the microfiltration process using membrane 603 (pore size 0.17μ).

At initial stage, i.e. when the $CF_c$ is 1.5, 41.5% of lactose is permeated through the membrane. And then the permeation of lactose dramatically increases to 60.5% when the $CF_c$ is increased to 2. As the $CF_c$ is continuously increased up to nearly 4, the permeation of lactose quickly increases to 80.6%. This high permeation of lactose is due to the fact that lactose are small particles which can almost freely pass through the membrane, especially for the MF membrane with high pore sizes.

## 5.2 Effect of Microfiltration and Diafiltration Processes

During the microfiltration process, as the $CF_c$ increases continuously, the flux is reduced to almost zero because of the increased viscosity. In order to achieve better protein separation, diafiltration was carried out after the MF process. For the diafiltration process, water is normally used. However, according to Abd El-Salam *et al.* (1989) & Al-Khamy (1988), in normal milk, addition of NaCl slightly increased viscosity, but addition of NaCl to the UF retentates reduced their viscosities. Al-Khamy (1988) also reported that the permeability of both α-La and β-Lg can be increased by addition of NaCl into milk when ultrafiltering whole milk. Therefore, based on above observations, the concept of diafiltration of the retentate from the MF process with deionised water or 100mM salt solution was utilised in this experiment to achieve a better protein separation.

The design of the diafiltration process was as follows: after the microfiltration process ended, the concentrate was kept in the MF system and mixed with deionised water or 100mM salt solution. Then the MF process restarted at 52±1°C. The DF process was operated under the same operating conditions as the MF process.

### 1) Effect of MF and DF Processes on the Retention Coefficients of Different Protein Components

Table 9 shows that during the MF process, when the $CF_c$ is up to 7, the retention coefficients are 0.628, 0.771, 0.997, 0.685 and 0.821 for total N, whey protein, casein nitrogen (CN), non-protein nitrogen (NPN) and β-Lg, respectively. The retention coefficients calculated were based on the protein content in the permeate and retentate samples (at fixed CF values).

**Table 9. Retention coefficients of different proteins (TN, WP, CN, and β-Lg) and NPN by the MF and DF processes with water or salt solution**

| Retention coefficients of different proteins in skim milk | | | | | |
|---|---|---|---|---|---|
| Processing Methods | Kjeldahl | | | | SDS-PAGE |
| | TN | WP | CN | NPN | β-Lg |
| MF($CF_C$=7) | 0.628 | 0.771 | 0.997 | 0.685 | 0.821 |
| MF+1DF with S (CF=63) | 0.874 (*0.246) | 0.900 (*0.129) | 0.999 (*0.002) | 0.893 (*0.208) | 0.917 (*0.096) |
| MF+1DF with W (CF=63) | 0.850 (*0.222) | 0.885 (*0.114) | 0.998 (*0.001) | 0.889 (*0.204) | 0.909 (*0.088) |

Note: MF = Microfiltration

"*"=Diafiltration effect

1DF = One stage of diafiltration process

$CF_C$ = Concentration factor during the microfiltration process

CF = Overall concentration factor

S = Salt (NaCl 100mM)

W = Deionised Water

The microfiltration process followed by the diafiltration with salt solution (100mM) increases the retention coefficient up to 0.246 for TN, 0.129 for WP, 0.208 for NPN and 0.096 for β-Lg, while the microfiltration process followed by diafiltration with water increases the retention coefficient up to 0.222 for TN, 0.114 for WP, 0.204 for NPN and 0.088 for β-Lg.

By comparison, in overall, the increases in the retention coefficients of TN, WP, NPN and β-Lg are slightly higher using the diafiltration with salt solution than that using the diafiltration with water. However, the difference is not significant. Nyström (1989) mentioned that increase in ionic strength of the polysulfone UF membranes by proper

modification can enhance the UF flux, as it increased repulsion by increasing the electric charge of the protein molecule or by lowing the electroviscous effects in the pores. Accordingly, diafiltration with salt solution is likely to have similar effect and could possibly enhance permeation of whey proteins. However, the results of this experiment have not showed this effect.

The increased retention coefficients by both diafiltration processes can be possibly due to the secondary membrane formation.

Retention coefficient of CN remains essentially about 1, through the MF and DF processes. This means nearly 100% casein proteins have been retained by the membrane 603. Further diafiltration with salt solution and deionised water can not force much casein protein through the membrane since that the pore sizes of the membrane 603 are small enough to retain all the casein micelles.

**2) Effect of MF and DF Processes on the Permeation (%) of NCN**

**Table 10. Permeation (%) of NCN by the MF and DF Processes with salt solution or water**

| Processes | Permeation (%) of NCN |
|---|---|
| MF ($CF_C$=7) | 46.5 |
| MF+1DF with S (CF=63) | 66.5 (*20) |
| MF+1DF with W(CF=63) | 63.3 (*16.8) |

Note: "*" = Diafiltration effect
MF = Microfiltration
1DF = One stage of diafiltration process
CF = Overall concentration factor
S = Salt solution (NaCl 100mM)
W = Deionised water
$CF_C$ = Concentration factor during the microfiltration process

Table 10 shows that after the microfiltration process to $CF_C$ 7, the permeation of NCN is 46.5%. After the diafiltration with salt solution and water, a further 20% and 16.8% of NCN is permeated from the retentates, respectively. By comparing between the

diafiltration with salt solution and deionised water, the permeation of NCN using diafiltration with salt solution is about 3% higher than that using the diafiltration with deionised water.

### 3) Effect of MF and DF Processes on the Ratios of CN/NCN and caseins/β-Lg in the Retentates

The ratios of CN/NCN and caseins/β-Lg in the retentates with different processing were determined. The content of CN and NCN in the retentates was analysed using micro-Kjeldahl method. The content of caseins and β-Lg in the retentates was determined by SDS-PAGE. Feed skim milk was used as a control. The stained bands which represent different proteins on the gel were scanned by a densitometer and the content of different proteins was obtained. The experimental results are summarised in Table 11.

**Table 11. The effect of MF and DF processes on the changes in the ratios of CN/NCN and caseins/β-Lg in the retentates**

| CN/NCN and caseins/β-Lg Ratios in the Retentates | | |
|---|---|---|
| Methods | By Kjeldahl | By SDS-PAGE |
| | CN/NCN in Retentates | Caseins/β-Lg in Retentates |
| Feed | 4.71 | 4.74 |
| MF ($CF_c$=7) | 5.62 (**0.91) | 5.54 (**0.80) |
| MF+1DF with S (CF=63) | 7.75 (*3.04) | 7.33 (*2.59) |
| MF+1DF with W (CF=63) | 7.35 (*2.64) | 6.55 (*1.81) |

Note: "**" = Microfiltration effect "*" = Diafiltration effect

MF = Microfiltration 1DF = One stage of diafiltration process

CF = Overall concentration factor S = Salt (NaCl 100mM)

W = Deionised water

$CF_c$ = Concentration factor during the microfiltration process

Table 11 shows that, comparing with original skim milk, the ratio of CN/NCN in the retentatesis increased from 4.71 to 5.62 and the ratio of caseins/β-Lg is increased from 4.74 to 5.54 after the microfiltration process. After the diafiltration process with salt solution, the ratio of CN/NCN and caseins/β-Lg in the retentates is increased from 4.71 to 7.75 and from 4.74 to 7.33, respectively. After the diafiltration process with water, the ratio of CN/NCN and caseins/β-Lg in the retentates is increased from 4.71 to 7.35 and from 4.74 to 6.55, respectively. These increased ratios of CN/NCN and caseins/β-Lg in the retentates indicate that protein separation does occur after the microfiltration and diafiltration processes using membrane 603. The above results also indicate that 1) diafiltration process apparently improves the permeation of NCN and β-Lg; 2) the diafiltration with salt solution is more helpful for the permeation of NCN and β-Lg than the diafiltration with water.

**4) Effect of MF and DF Processes on the Changes in Total Solids (%) in the Retentates**

The total solids (%) in the retentates with the increased CF during the microfiltration and diafiltration processes were analysed (refers to DDM4 (1.12 4a). The results are showed in Fig. 25.

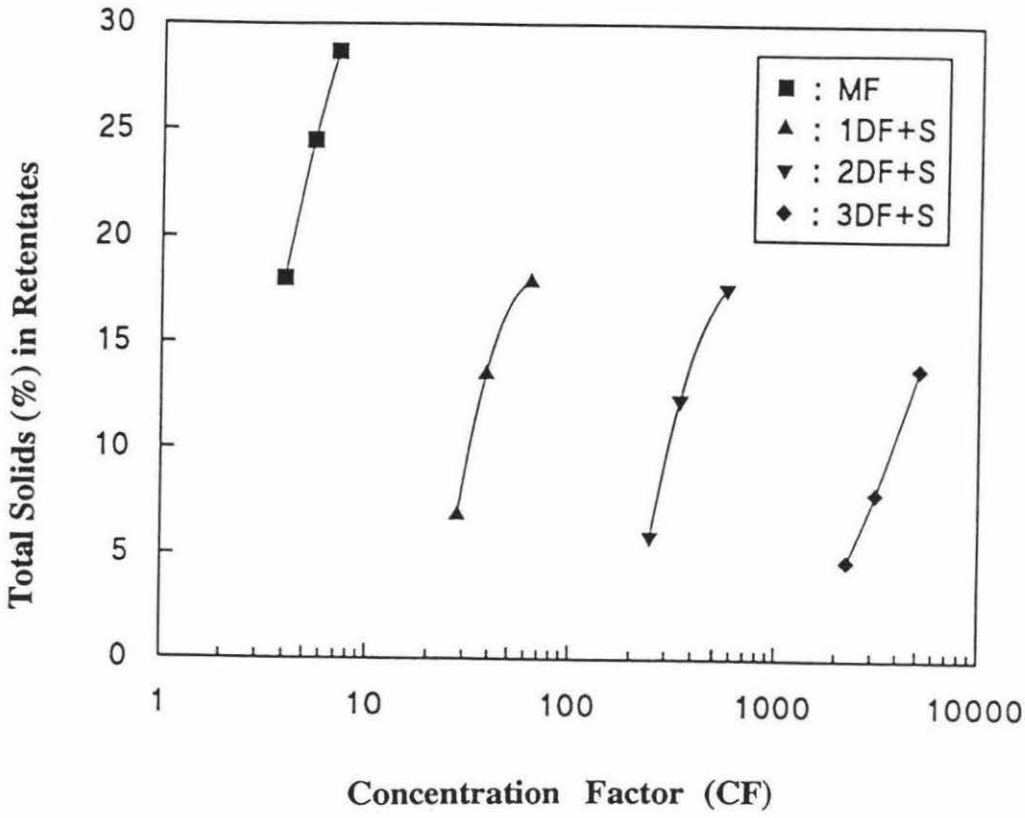


**Fig. 25.** Effect of CF on the changes in total solids (%) in the retentates during MF and three DF processes with salt solution using membrane 603 (pore size 0.17μ).

In general, total solids (%) in the retentates increases as the $CF_c$ increases during the microfiltration process and following 3 stages of diafiltration processes. However, by comparing the results of total solids obtained from these four different processes, the total solids (%) in the retentates from the microfiltration process with $CF_c$ 7 are highest and decrease in sequence from the first diafiltration with CF 63 and the second diafiltration with CF 567 to the third diafiltration with CF 5103. This decrease could be explained as that, wth diafiltration to the same final volume in each case, the total solids in the retentate will decrease as further low molecular components are removed (washed out) by the diafiltration water.

**5) Effect of MF and DF Processes on the Possibility of Protein Denaturation (Aggregation)**

The native-PAGE was performed to determine the undenatured whey proteins in the retentate samples from the microfiltration and three diafiltration processes.

The skim milk feed and retentate samples (diluted back to original total solids) were ultracentrifuged using 90,000 rpm for one hour. Then the supernatant samples of the skim milk feed and retentate were analysed by the native-PAGE.

The results of the gel electrophoretic patterns of retentate samples from the microfiltration and three diafiltration processes using membrane 603 are showed in Fig. 26.

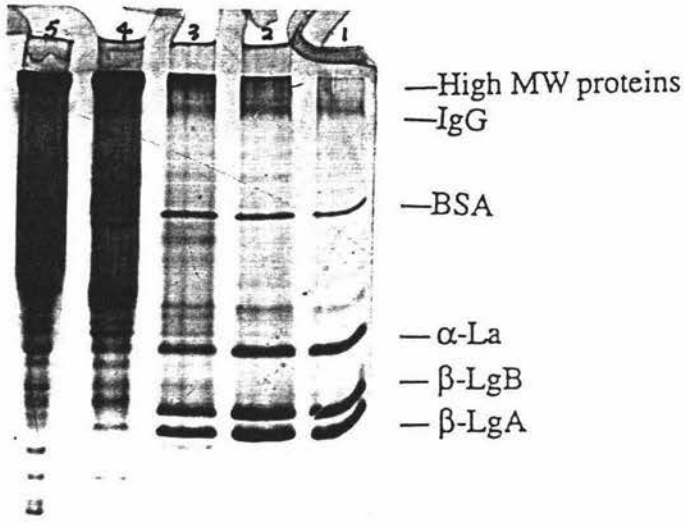


**Fig. 26.** Native-PAGE patterns of the ultracentrifugal supernatants obtained from retentate samples of skim milk microfiltration and three diafiltration processes using membrane 603. Lane 1 is the supernatant from a control feed. Lane 2, 3, 4 and 5 are supernatant samples of the retentates from the microfiltration process with the $CF_c$ 7 and three diafiltration processes with CF 63, 567 and 5103, respectively.

Lane 1 demonstrates a distribution of milk proteins in the supernatant sample of skim milk feed. High MW proteins, IgG, BSA, α-La, β-LgB and β-LgA are showed, respectively, from the top to the bottom on the lane 1. By comparison of the correspondent portions of high MW protein on lane 2, 3, 4 and 5 which represent the microfiltration process with $CF_C$ 7, first diafiltration process with CF 63, second diafiltration process with CF 567 and third diafiltration process with CF 5103, respectively, it is noticed that as the number of the diafiltration process increases, the corresponding portions of high MW protein on lane 2, 3, 4 and 5 are apparently increased in sequence. This is a indication that protein denaturation/aggregation possibly occurred. On the other hand, the bands of α-La, β-LgB and β-LgA dramatically decreased, especially for the lane 4 and 5, which perhaps can also support the above point that the denaturation of whey proteins or aggregation between protein components has occurred due to the high turbulence and pressure in the system that retentates were subjected to during the MF and the three DF processes. Therefore, the appropriate CF during each process and the proper number of diafiltration process used are very important to avoid or reduce this phenomena.

## 5.3 Effect of MF and DF with Membrane Cleaning between Each Run on the Permeation (%) of NCN

Due to the unavoidable concentration polarisation of the membrane during the process, the separation efficiency of casein micelles from milk with the MF 603 membrane still could not reach what was expected although diafiltration was performed after the MF process. Therefore, membrane cleaning after the MF process and before the DF process was utilised to improve protein separation. The membrane cleaning was performed by the standard membrane cleaning procedure (refers to Appendix II).

The results of the effect of the MF and DF processes with and without membrane cleaning between each run on the permeation (%) of NCN are summarised in Table 12.

**Table 12. Comparison of the effect of MF and DF processes with and without membrane cleaning on the permeation (%) of NCN**

| Processes | Permeation (%) of NCN | |
|---|---|---|
| | without membrane cleaned | with membrane cleaned |
| MF ($CF_C$=7) | 46.5 | 46.2 |
| MF+1DF with S (CF=63) | 66.5 (*20) | 68.3(*22.1) |

Note: "*"= Diafiltration effect
MF = Microfiltration
IDF = First stage of diafiltration process
S = Salt (NaCl 100mM)
CF = Overall concentration factor
$CF_C$ = Concentration factor during the microfiltration process

Table 12 shows that, after the microfiltration process with $CF_C$ 7, the permeation of NCN is about 46% for both membrane cleaning and without membrane cleaning. There is no significant difference between them. Afterwards, the diafiltration with salt, a further 20% and 22% of NCN are permeated from the retentates. By comparison, the diafiltration with membrane cleaning gives 2% more permeation of NCN than that without membrane cleaning. This indicates that membrane cleaning between microfiltration and diafiltration process is marginally beneficial for increasing NCN permeation. This marginal improvement reinforces the fouling mechanism of pore blocking in microfiltration.

## 5.4 Effect of MF and Double DF Processes on the Permeation (%) of NCN

In order to achieve a better separation effect, the microfiltration and double diafiltration processes were carried out. The results of the effect of MF and double DF processes with membrane cleaning between each run on the permeation (%) of NCN are summarised in Table 13.

**Table 13. Comparison of the effect of MF and double DF processes on the permeation (%) of NCN**

| Processes | Permeation (%) of NCN |
|---|---|
| MF ($CF_C$=7) | 46.2 |
| MF +1DF with S (CF=63) | 68.3(*22.1) |
| MF+2DF with S (CF=567) | 79.9(*11.6) |

Note: "*" = Diafiltration effect

MF = Microfiltration

1DF = First stage of diafiltration process

2DF= Second stage of diafiltration process

S = Salt (NaCl 100mM)

CF = Overall concentration factor

$CF_C$ = Concentration factor during the microfiltration process

Table 13 shows that, after the microfiltration process with $CF_C$ 7, the permeation of NCN is 46.2%. After the first diafiltration with salt solution, a further 22% of NCN is permeated from the retentates. The following second diafiltration process results in another further 12% permeation of NCN. Consequently, in total, about 80% of NCN is permeated through the microfiltration process with the $CF_C$ 7 and the double diafiltration processes with the CF 567, while 33% of NCN is permeated by the double diafiltration processes. This indicates that, the double diafiltration processes with the membrane cleaning after the microfiltration are obviously beneficial for increasing the permeation of NCN. However, possible damage or restructuring of milk protein components should be taken into consideration due to the consistent dynamic movement caused by the three stages of processes.

## 5.5 Gel Electrophoresis Analysis

### 5.5.1 Microfiltration Process Using Membrane 603

The results of the gel electrophoretic patterns of the permeates and retentates of the skim milk from the microfiltration process using membrane 603 are showed in Fig. 27.

It should be noted that for this SDS-PAGE, the dilution of milk, retentates and permeate samples was different. The milk sample and all retentate samples were diluted with sample buffer solution into 1:40, while all permeate samples were diluted with sample buffer solution into 1:10.

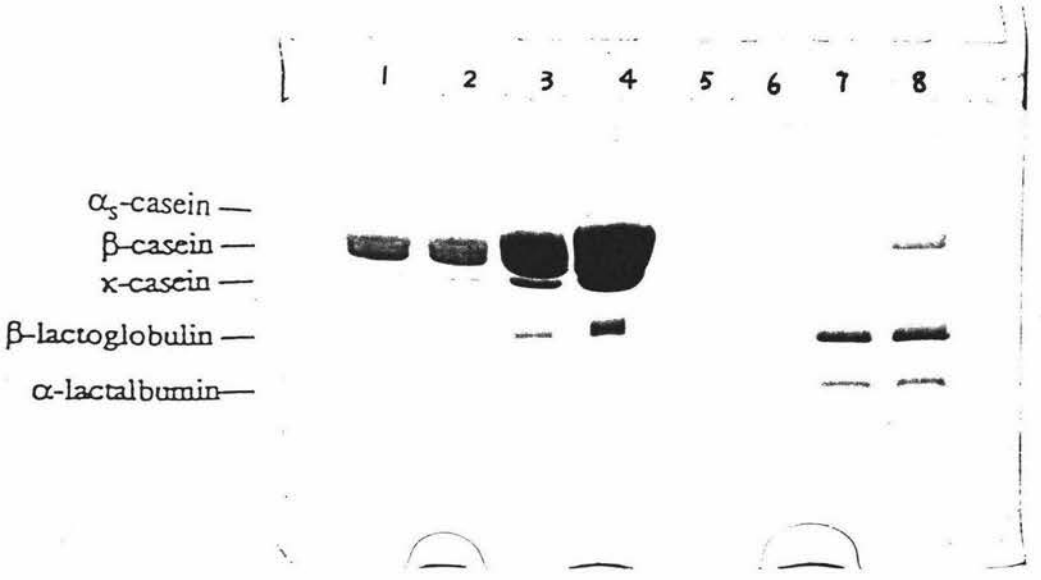


**Fig. 27.** Sodium-dodecyl-sulphate-polyacrylamide gel electrophoretic patterns of permeate and retentate samples from the skim milk microfiltration process using membrane 603. Lane 1 is the skim milk feed for the microfiltration process as a control. Lanes 2, 3 and 4 are retentate samples with $CF_C$ 1, 2 and 3, respectively. Lane 5, 6, 7 and 8 are permeate samples with the $CF_C$ 1, 2, 3 and 4, respectively.

Lane 1 demonstrates a distribution of milk proteins. $\alpha_s$-caseins, β-casein, κ-casein, β-lactoglobulin, α-lactalbumin are seen. By comparing the patterns of lanes 3 and 4 retentates with lane 1 milk control, it can be seen that all proteins become concentrated.

However, the caseins proportion in lane 4 is even much more concentrated although α-La and β-Lg also become slightly concentrated compared with that in lane 1 (milk control). Basically, no band of caseins appears on lane 5, 6 and 7 permeates samples, indicating that nearly all caseins have been retained by the membrane 603 and the separation of caseins from whey proteins does occur. While α-La and β-Lg bands on lane 7 permeate samples are obviously concentrated with the $CF_c$ 3. This indicates that more whey protein (mainly α-La and β-Lg) passes through the membrane 603 into the permeates with increase of the $CF_c$. This is beneficial to the separation of caseins from whey protein. Lane 8 is a cloudy permeate sample with the $CF_c$ 4. It is noticed that although whey protein become much more concentrated in the permeate samples compared with that in the milk control, some bands of caseins are also quite clearly showed up, which indicates that some caseins pass through the membrane as well. Therefore, it should be mentioned here that high $CF_c$ should be limited due to more air bubbles pumped in as the retentates become concentrated, which could cause the damage of casein micelles. As a result, some small casein micelles damaged could pass through the membrane, leading to the casein loses.

### 5.5.2 Microfiltration and Diafiltration Process using Membrane 603

The results of the gel electrophoretic patterns of the retentates from the skim milk microfiltration and diafiltration process using membrane 603 are showed in Fig. 28.

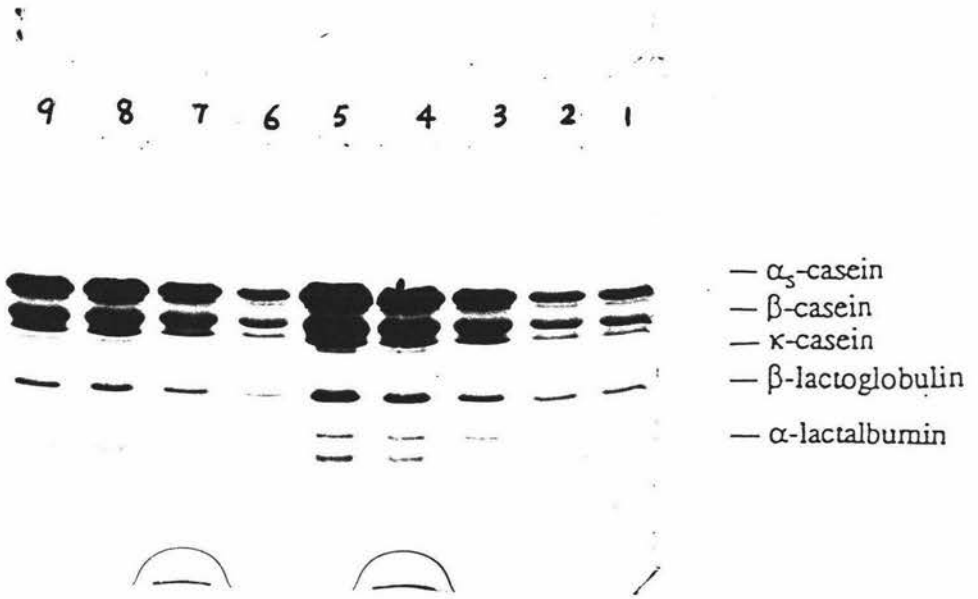


**Fig. 28.** Sodium-dodecyl-sulphate-polyacrylamide gel electrophoretic patterns of retentate samples from skim milk MF and DF process using membrane 603. Lane 1 is the skim milk feed for the MF process as a control. Lane 2, 3, 4 and 5 are retentate samples from MF process with $CF_C$ 1, 2, 3 and 4, respectively. Lane 6, 7, 8 and 9 are retentates samples from the DF process with the $CF_D$ 1, 2, 3 and 4, respectively. Milk sample and all retentate samples are diluted with sample buffer solution into 1:100.

Lane 1 demonstrates a distribution of milk proteins. $\alpha_s$-caseins, β-casein, κ-casein, β-lactoglobulin, α-lactalbumin are seen on the lane 1. In general, the relative proteins in lane 2 (MF) and 6 (DF) with the CF 1 are similar to that in the lane 1. The patterns in lane 3 and 4 retentates with $CF_C$ 2 and 3, respectively from the microfiltration process compared with that in lane 7 and 8 retentates with the $CF_D$ 2 and 3, respectively from the diafiltration process show no significant difference except that α-La and β-Lg in lane 7 and 8 become less obviously. This indicates that the consequent diafiltration with water

can cause more whey proteins (mainly α-La and β-Lg) to permeate from the retentates while still keeping caseins in the retentates. In contrast, caseins in lane 9 (DF retentate with $CF_D$ 4) become less by comparing with caseins in lane 5 (MF retentate with $CF_C$ 4). This indicates that some caseins are lost during the diafiltration process. This casein lose probably can be explained due to that (1) some casein solubilization after the diafiltration with water especially under the condition of high CF may occur. (2) the original spherical structure of casein micelles may be physically damaged due to the higher turbulence and pressure in the system that the retentates subjected to during the MF and DF processes.

# CHAPTER 6

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

It can be concluded from this experimental work that:

1. Among the available UF/MF membranes, only the membrane 603 with a pore size 0.17μ is suitable for the separation of caseins from the whey proteins. Over 99% casein is retained by the membrane 603. The MF membrane 600 with pore size 1.99μ and 601 with pore size 0.85μ is not suitable due to the high casein loses during the microfiltration process.

2. The retention coefficients of different protein components using the microfiltration process depend on the different MF membranes used. The retention coefficients (at fixed CF values) of TN, NCN, CN, β-Lg and NPN from the skim milk microfiltration process with $CF_c$ 7 using the MF membrane 603 are found as follows: TN 0.628, NCN 0.771, CN 0.997, β-Lg 0.821 and NPN 0.685. After the diafiltration process with water at CF of 63, the retention coefficients are as follows: TN 0.850, WP 0.885, CN 0.998, β-lactoglobulin 0.909 and NPN 0.889. The retention coefficients of TN, NCN were not constant; they varied with the different concentration factor during the microfiltration process. However, the retention coefficient of CN is not significantly influenced by the $CF_c$ during the microfiltration process, but influenced by the number of diafiltration process. The results of SDS-PAGE shows that some caseins are lost during the diafiltration process due possibly to some casein solubilization after the diafiltration with water, especially under the condition of high CF.

3. Permeation of NCN is about 30-40% using the microfiltration process at the $CF_c$ 3. However, the permeation increases with the increase of the $CF_c$. The permeation is up to 60% when the $CF_c$ is up to 9. The high $CF_c$ achieved by

concentration without diafiltration is not recommended due to the fact that the retentates become more concentrated as the $CF_c$ increases, resulting in very low flux and a major extension of the processing period.

4. In total, the permeation of NCN is about 80% using the microfiltration process at the $CF_c$ 7 and double diafiltration processes with a final CF of 567. The permeation of NCN by the double diafiltration processes is 33%. This indicates that the double diafiltration processes with the membrane cleaning after the microfiltration are obviously beneficial for increasing the permeation of NCN. However, the results of native-PAGE show that aggregation of milk proteins or restructure of milk protein components may occur due to the consistent dynamic movement caused by the three stages of processing.

5. Membrane cleaning after microfiltration and before diafiltration is beneficial for increasing the permeation of NCN. The marginal improvement suggests rapid fouling by pore blocking.

6. Diafiltration with water especially under the condition of high CF could possibly cause some modifications to whey protein structure.

## 6.2 Recommendations

In future work on this subject, it is recommended that:

1. It would be worthwhile to trial other MF membranes with different pore sizes, if they are commercially available, to assess their separation of casein micelles from skim milk.

2. The effect of the microfiltration and diafiltration processes on the structure (aggregation and denaturation) of milk proteins system could be explored.

3. It is likely that the casein micelles obtained by the microfiltration could have different functional properties compared with that prepared by traditional acid or rennet methods. Therefore, it is worthwhile to investigate the functional properties of the MF retentates to provide more information for future applications in the food industry.

# APPENDIX I

## UF/MF Terms and Expressions

| | |
|---|---|
| **Concentration Factor (CF)** | Indicates the extent of an UF/MF process.<br>CF = volume or weight of original feed/volume or weight of final concentrate<br>$V_F / (V_F - V_p)$ |
| **Concentration Polarization(CP)** | Means a protein-fat layer accumulates on the membrane surface and its thickness gets greater as the retentate becomes more and more concentrated. |
| **Diafiltration (DF)** | Restart UF/MF process by simply adding water to the retentate to remove more small molecules and purify the final products. |
| **Flux (permeation rate)** | Means the quantity of permeated liquid (kg or litre) per membrane area unit ($m^2$) and time unit (h).<br>Flux = volume permeate removed/unit area of membrane / unit time.<br>The flux is usually expressed in $L/m^2/h$. |
| **Membrane Fouling** | Means the accumulation of solids deposits on the membrane surface, i.e. a higher concentration of retained solute species adjacent to the membrane surface than in the bulk stream. Fouling reduces the flux substantially. |
| **Microfiltration (MF)** | This involves an more open membrane compared with ultrafiltration. Membrane pore sizes are in the range of 0.1-10μ. Microfiltration operates at low pressures-usually in the range of 1-4 bar and at a fairly high specific flux. |
| **Module** | The smallest practical unit containing membranes and supporting structure. |

| | |
|---|---|
| **Molecular cut-off** | Designates the smallest molecular weight component retained at a retention coefficient of 0.95. |
| **Permeate** | This is the filtrate passing through the membrane. |
| **Permeation** | This is the fraction of a solute passing through the membrane expressed in percentage. |
| **Retentate** | Same as concentrate<br>Means the material retained (held) by the membrane. |
| **Retention Coefficient (RC)** | The quantitative measure for the characteristic ability of a membrane to retain solute species under specific operation parameters. It is calculated as:<br>$RC=(C_F-C_n) / C_F = 1-(C_n/C_F)$<br>where, RC is the retention coefficient of a component; $C_n$ and $C_F$ are its concentration in permeate and in feed, respectively, at that point. Retention coefficient may be expressed as a fraction, range from 0 to 1 or percentage (P=100-R). |
| **Ultrafiltration (UF)** | A pressure-driven process that can be used in the separation and concentration of substances having a molecular weight between $10^3$-$10^6$ dalton (molecular size 0.001-0.02μ.<br>The membrane pore sizes are: 0.001-0.1μ. The operation pressure is in the range of 1-10 bar and with lower fluxes. |

# APPENDIX II

## Procedure for Koch UF Equipment Cleaning

**Caustic Wash :**

1. Make pH 10.5-11.0 caustic solution with temperature 55°C.
   Add approx. 30ml concentrated caustic solution (10%) into about 20L deionised water which was heated up to 55-58°C in a steam kettle and mix well. Check the final pH with pH meter. The reading should be between 10.5-11.0.
2. Circulate the cleaning solution at standard processing conditions for 15- 20 minutes. Carefully transfer about 10L to the conical feed tank, ensuring valve V1 and V3 were closed. Check the pH and turn on the heat exchanger. The normal processing conditions used were: Pin = 120kPa, Pout = 60kPa, pump speed = 45 (flow rate of water = 21L/min), temperature = 55°C.
3. Rinse the system with deionised water (60°C) for 5 minutes.

**Caustic/Chlorine Wash:**

1. Make caustic/chlorine solution
   Add 10%(w/w) Sodium Hypochlorite (NaOCl) solution to the remaining 10L of caustic solution with pH 10.5-11.0, mix well. Checking the chlorine concentration using the iodometric method. The chlorine concentration should be 200ppm, ensuring not over 200ppm which could damage the membrane.
2. Circulate the caustic/chlorine solution at the processing conditions same as caustic wash for 15-20 minutes. Carefully transfer the solution to the conical feed tank, ensuring valve V1 and V3 were closed. The temperature was maintained at 50-60°C during the circulation process by controlling the heat exchanger. The pressure and pump speed used were same as caustic wash.
3. Rinse the system with deionised water (60°C) until the system pH returned to 7 (about 20 minutes).
4. The water flux on the clean membrane was determined using additional 10L of fresh 53°C deionised water before the process started.

**Stopping Cleaning:**

Acid wash and caustic/UCII/chlorine wash were carried out after the system shut down.

**Acid Wash**

1. The process fluid in the system was flushed completely with deionized water at about 50°C.
2. Add 10L of nitric acid solution (55°C) with pH 1.8-2.0 into the conical feed tank, ensuring that V1 and V3 were closed.
3. Circulate the cleaning solution for 20 minutes with the heat exchanger on to maintain the temperature. Checking pH. It should not be below 1.8.
4. Drain the solution via V3.
5. Flush out the acid residue by rinsing the system with deionized water for a few minutes.

**Caustic/UCII/Chlorine Wash**

1. Make up 10L of caustic/UCII/chlorine solution.
   Follow the method as outlined in caustic/chlorine wash plus the addition of 0.1% (w/w) Koch Ultra Clean II liquid to the caustic/chlorine solution.
2. Circulate for 20 to 25 minutes at 55°C.
3. Flush the system thoroughly with deionised water until pH back to 7 (normally takes about 15-20 minutes).
4. The cleanness of the membrane was determined using additional 10L of fresh 53°C at normal process condition. The water flux should be same as that before the process started.
5. Fill in the conical feed tank with deionised water, ensuring the V1 and V2 were open to avoid the membrane dehydration.

**Iodometric Method- Chlorine**

**Reagents:**

1. 50% glacial acetic acid (CH3COOH).
2. saturated potassium iodide solution(KI)
3. 0.1N sodium thiosulphate solution.

**Method:**

1. Measure 5ml C.I.P. solution into a crucible and then add 3 drops of potassium iodide solution and 3 drops of glacial acetic acid into the crucible, respectively.
2. Mix them well.
3. Add dropwise 0.1N sodium thiosulphate solution, mixing between each drop. Count the number of drops required to remove yellow colour (until the solution is clear).
4. Calculation.

   Each drop of 0.1N sodium thiosulphate solution used = 30ppm chlorine.

# APPENDIX III

## Procedure for Micro-Kjeldahl Protein Analysis

**Principles of Micro- Kjeldahl Method**

A weighed sample is catalytically digested with sulphuric acid, converting the organic nitrogen into ammonical nitrogen. The ammonia is released by the addition of sodium hydroxide, which is distilled and absorbed in boric acid before being titrate with hydrochloric acid. The percentage nitrogen contents is multiplied by the factor 6.38 convert to percentage protein.

**The procedure for Macro-Kjeidahl Analysis:**

1. Weigh 4-5g sample into digestion tubes.
2. Put two Kjeldahl catalyst tablets which consist of $K_2S0_4$ and $CuSO_4$) and 12ml sulphuric acid (A.R density 1.84) into each tube.
3. Place all tubes on a digester (Tecator Digestion System 6 1007 Digester). Put lids on. Turn on condense water tap. Turn on power and put temperature gear in position 5.
4. Digestion. Start digestion until the solution in tubes turns from dark brown to clear, the digestion process has finished. For permeate sample, it took longer time than milk or retentates samples, normally about 2-3 hours to complete this process due to that permeate contains more whey proteins which form a lots of foams. The temperature should be turned up very slowly at the beginning stage.
5. Distillation. Cold the distilled solution down and add about 75ml distilled water. Turn on automatic distillation apparatus (KJELTEC System 1026 Distillating Unit) and circulating water tap. 25ml boric acid solution (4%) was used as a receiver solution to absorb the ammonia which released during the distillation. Place the tube on the tube holder of the apparatus. The distillation process started automatically once the door was closed. The time for distillation was set 36 seconds. When the time was over, the distillation process stopped automatically. The colour of boric acid solution changed from red to green.

6. Titration. 0.02M or 0.1M HCl was used to titrate the distilled solution until the colour changed from green to pink which was end point. The volume of HCl used was recorded.
7. Calculation. The percentage of protein in the samples can be calculated by converting percentage of nitrogen multiplied by the factor 6.38.

$$\% \text{ N} = 1.401 \times (\text{R-B}) \times \text{M/W} \qquad \% \text{ Protein} = \% \text{ N} \times 6.38$$

Where, R = Volume of HCl used for the titration of samples.

B= Volume of HCl used for the titration of blank.

M = Molarity of HCl used (0.02M or 0.1M).

W = Weight of samples (g).

# APPENDIX IV

## Procedure for the SDS-PAGE Preparation

SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) is used extensively in biochemistry to quantify and qualify proteins in the samples according to the bands of different proteins. Each protein has a specific charge that depends on its pH. SDS is a surface-active substance that has the ability to absorb to hydrophobic or positively charged sites on proteins. Therefore, if a mixture of proteins is placed in an electrofield, the protein migrate at a rate that depends on their charge. The larger the complex the harder it is for it to pass through the pores of the gel and the more it is retained.

Discontinuous polyacrylamide gels used in this experiment consist of a resolving or separating (lower) gel and a stacking (upper) gel. The stacking gel acts to concentrate large sample volumes, resulting in better band resolution. Molecules are then completely separated in the resolving gel.

β-mercaptoethanol was added in the SDS gels' sample preparation. The purpose of doing this is to reduce the sulfydryl groups, resulting in a better separation.

Samples were diluted with sample buffer in different proportion for the permeate and retentate samples.

The samples diluted with sample buffer were heated in boiling water for 5-10 minutes so as to inactivate the enzymes which could have some effects on the proteins present in the samples.

**1. Preparation of samples for gel electrophoresis**

**(a) Preparation of SDS sample buffer**

| | |
|---|---|
| Milli Q (deionised) water | 50ml |
| 0.5M Tris-HCl, pH6.8 | 12.5ml |
| Glycerol | 10.0ml |
| 10% (w/v) SDS | 20.0ml |
| ß-mercaptophenol | 5.0ml |
| 0.05 (w/v) Bromophenol blue | 2.5ml |
| | --- |
| Total volume | 100.00ml |

**(b) Preparation of resolving gel**

(1) Combine the following reagents together.

| | |
|---|---|
| Deionised miliQ water | 2.02ml |
| M Tris-HCl buffer | 2.50ml |
| SDS stock | 100µl |
| Acrylamide (30%) | 5.3ml |

(2) Deaerate the solution under vacuum for 15-20 minutes by circulating pump to remove any air bubbles in the solution.

(3) Add 10% freshly made ammonium persulfate 50µl and Temed 5µl to the deaerated solution and pour the solution smoothly along down the middle of the longer plate of the gel sandwich to prevent it from mixing with air.

(4) Immediately overlay the solution with deionised water.

(5) Allow the gel to polymerize for 45-50 minutes at about 30°C.

**(C) Preparation of stacking gel**

(1) Combine the following reagents together.

| | |
|---|---|
| MilliQ water | 3.05ml |
| M Tris-HCl buffer | 1.25ml |
| SDS stock | 50µl |
| Acrylamide (30%) | 0.65ml |

(2) Deaerate the solution under vacuum for 15-20 minutes to remove any air bubbles in the solution.

(3) Add 10% freshly made ammonium persulfate 25µl and Temed 5µl to the deaerated solution.

(4) Rinse off the water which is overlaid above the resolving gel and dry the area with filter paper, followed by placing a comb in the gel sandwich before pouring the stacking gel solution.

(5) Pour the stacking gel solution down the spacer until all the teeth have been covered by stacking gel.

(6) Allow the gel to polymerize for 30-45 minutes at about 30°C. Remove the comb by pulling it straight up slowly and gently.

(7) Rinse the wells completely with deionised water. The gels are ready to be loaded with samples and run.

**2. Loading samples and running the gels**

(1) Leave samples at room temperature until they are melt. Heat samples in the boiling water for 5-10 minutes to inactivate the enzymes and then cold them down.

(2) Loading samples. Inject 10µl samples into each slab using 20µl syringe, using original skim milk sample as a control.

(3) Make up 5X Electrode Buffer

To a 1 litre volume cylinder, add

9.0g Tris base

43.2g Glycine

3.0 SDS

Bring to 600ml volume with deionised water. Check pH. Should be 8.3. Store at 4°C. Dilute 60ml 5X stock with 240ml distilled water for one electrophoresis run.

(4) Running the gels. Running the gels with the loaded samples in the Mini-PROTEIN II cell with 300ml of diluted electrode buffer in. The recommended power condition for optimal resolution with minimal thermal band distortion is 200 volts, constant voltage setting. The run time is approximately 45 minutes.

**3. Staining and destaining the gels**

The staining solution used consists of 1g brilliant blue R, 200ml acetic acid and 500ml iso-propylalchohol in 2L of distilled water. Gels were put in the plastic container with above dye solution to be soaked for over 1 hour (sometimes over 4-5hrs, depending on the content of proteins and clearness of the bands appeared).

Destaining the gels with destaining solution which consists of 10% iso-propylalchohol and 10% acetic acid to remove background. After about over 4 hours, the different protein bands are clearly showed on the gels.

**4. Quality and quantity analysis of gels**

The resulting electrophoretigram can be placed in an automatic densitometer (LKB Ultroscan XL Laser Densitometer). The densitomatic tracings were used to resolve and quantify protein peaks from stained bands in the gels by showing the presence and quantity of protein components such as whey protein and casein proteins.

# APPENDIX V

## Procedure for Native- PAGE Preparation

Alkaline PAGE (native-PAGE) is resolve only undenatured proteins.

After diluted with sample buffer, the retentate samples were ultracentrifuged using 90,000 rpm for one hour. Then the supernatant of the milk feed and retentate samples were analysed by whey PAGE. The objective of performing the native PAGE was to determine undenatured whey proteins in the retentates.

Procedure for the native-PAGE preparation refers to the SDS-PAGE preparation which was given in Appendix IV.

All procedures are same as SDS-PAGE, including the aspects of the preparations of SDS sample buffer, resolving gel, stacking gel, loading and running gels and staining/destaining the gels. The only difference between the native-PAGE from the SDS-PAGE is that no SDS solution is used in the preparations of the sample buffer, resolving gel and stacking gel.

# APPENDIX VI

## Procedure for the Lactose Analysis

1. Pipette 1.0ml of milk samples into 1 Litre standard flask and make up with distilled water. Mix.
2. Pipette 1.0ml of samples from the standard flask into stopped test tube.
3. Add 1.0ml phenol solution (5%) into the test tube, shake and mix.
4. Add 5.0ml of concentrated sulphuric acid, mix and stand at room temperature till cool (colour appears stable for at least 1 hour).
5. Read optical density in 1 cm glass cell in a spectrophotometer (CE 292 Digital ultraviolet spectrophotometer) at 490nm.
6. The lactose content (μg/ml) was found out from a standard lactose curve.

**Preparation of a standard lactose solutions (0-100μg/ml) for a standard curve.**

1. Weigh 1.00g lactose into a 1 litre volume flask and make up to mark with distilled water. Mix.
2. Pipette 20ml, 40ml, 60ml, 80ml,100ml above solution into 1000ml volume flasks and make up to mark with distilled water.
3. Repeat steps 1-5 of above lactose analyses of samples.
4. Draw a lactose standard curve (optical density vis lactose content (μg/ml)).

# APPENDIX VII

## Procedure for Total Solids Analysis

1. Take dish out of oven and place in desiccator for 30 minutes.
2. Weigh dish (W1).
3. Weigh about 1 g of sample into dish (W2). (The retentate samples were diluted with distilled water after being weighted).
4. Place on boiling waterbath for 25 minutes- this is to dry the sample.
5. Transfer sample to vacuum oven and dry at 100°C and 3kPa for 20±5 minutes.
6. Place samples in desiccator for 30 minutes.
7. Weigh samples back (W3).

Calculations: Total Solids % = [(W3-W1) / (W2-W1)] x 100

# APPENDIX VIII

## Calculation Equations

### 1. Concentration Factor (CFc) during the Microfiltration Concentration Process

$$CFc = W_F / (W_F - W_P) = W_F / W_R$$

where, $W_F$ --- weight (kg) of the original feed,

$W_P$ ---weight (kg) of the permeate removed,

$W_R$ ---weight (kg) of the final concentrate,

Example: if the weight (kg) of the original feed ($W_F$) is 20kg,
and weight (kg) of the permeate ($W_P$) removed is 15kg,
then weight (kg) of the final concentrate ($W_R$) is 5kg,

then, $CFc = W_F / (W_F - W_P) = W_F / W_R = 20kg/5kg = 4$

### 2. Concentration Factor ($CF_D$) during the Diafiltration Process

$$CF_D = (W_{FD} + W_D) / (W_{FD} + W_D - W_{PD}) = (W_{FD} + W_D) / W_{RD}$$

where, $W_{FD}$ --- weight (kg) of the stage feed (from previous concentration),

$W_{PD}$ ---weight (kg) of the permeate removed,

$W_{RD}$ ---weight (kg) of the final concentrate after diafiltration,

$W_D$---before the diafiltration process, weight (kg) of water or salt solution added which is correspondent to the weight (kg) of the permeate removed in the previous concentration process.

**Overall concentration factor $CF = CF_C \times CF_D$**

Example: if the weight (kg) of the stage feed ($W_{FD}$) is 5kg, and the weight (kg) of diafiltration water added is 15kg,
and weight (kg) of the permeate ($W_{PD}$) removed is 15kg,
then weight (kg) of the final concentrate ($W_{RD}$) is 5kg,

**then** $CF_D = (W_{FD} + W_D)/ (W_{FD} + W_D - W_{PD})$
$=(5+15) / (5+15-15) = 4$

The concentration factor for the diafiltration stage 1 was 4. The concentration factor prior to DF was 4, therefore, the overall concentration factor is the product 4 X4 = 16.

### 3. Retention Coefficient of Total Nitrogen ($RC_{TN}$)

$$RC_{TN} = 1-(C_P / C_R)$$

where, $RC_{TN}$ --- retention coefficient of total nitrogen,
$C_P$ ---content of total nitrogen (%) in the permeate sample at correspondent $CF_C$ or $CF_D$
$C_R$ ---content of total nitrogen (%) in the retentate sample at correspondent $CF_C$ or $CF_D$

Example: if when the $CF_C$ =3,
the content of total nitrogen (%) in the permeate sample ($C_P$) = 0.5%,
and the content of total nitrogen (%) in the retentate sample ($C_R$) = 12.1%,

then, $RC_{TN} = 1-(C_P / C_R) =1-(0.5\%/12.1\%) = 0.96$

### 4. Retention Coefficient of Non-Casein Nitrogen ($RC_{NCN}$)

$$RC_{NCN} = 1-(C_{PF} / C_{RF})$$

where, $RC_{NCN}$--- retention coefficient of non-casein nitrogen,

$C_{PF}$ ---content of total nitrogen (%) in the permeate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

$C_{RF}$ ---content of total nitrogen (%) in the retentate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

Example: if when the $CF_C$=3,

the content of total nitrogen (%) in the permeate filtrate ($C_{PF}$) at correspondent $CF_C$ (pH was adjusted to 4.6) = 0.4%,

and the content of total nitrogen (%) in the retentate filtrate ($C_{RF}$) at correspondent $CF_C$ (pH was adjusted to 4.6) = 1.6%,

then, $RC_{NCN} = 1-(C_{PF} / C_{RF}) = 1-(0.4\%/1.6\%) = 0.75$

**5. Retention Coefficient of Casein Nitrogen ($RC_{CN}$)**

$$RC_{CN} = 1 - [(C_P - C_{PF}) / (C_R - C_{RF})]$$

where, $RC_{CN}$ -- retention coefficient of non-casein nitrogen,

$C_P$ ------ content of total nitrogen (%) in the permeate at correspondent $CF_C$ or $CF_D$,

$C_R$ ------content of total nitrogen (%) in the retentate at correspondent $CF_C$ or $CF_D$,

$C_{PF}$------content of total nitrogen (%) in the permeate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

$C_{RF}$----- content of total nitrogen (%) in the retentate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

Example: if when the $CF_C$ = 3,

the content of total nitrogen (%) in the permeate sample ($C_P$) = 0.5%,

and the content of total nitrogen (%) in the retentate sample($C_R$) = 12.1%,

the content of total nitrogen (%) in the permeate filtrate ($C_{PF}$) (pH

was adjusted to 4.6) = 0.4%,

and the content of total nitrogen (%) in the retentate filtrate ($C_{RF}$) (pH was adjusted to 4.6) =1.6%,

then, $RC_{CN} = 1 - [(C_P - C_{PF}) / (C_R - C_{RF})]$

$= 1-[(0.5-0.4)/(12.1-1.6)]$

$= 0.99$

**6. Casein Nitrogen / Non-casein Nitrogen Ratio in the Retentates**

$$CN / NCN = [(C_R \times W_R) - (C_{RF} \times W_R)] / (C_{RF} \times W_R)$$

where, **CN** --- casein nitrogen,

**NCN** --- non-casein nitrogen,

$C_R$ ---content of total nitrogen (%) in the retentate at correspondent $CF_C$ or $CF_D$,

$C_{RF}$ ---content of total nitrogen (%) in the retentate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

$W_R$ ---weight (kg) of the retentate at correspondent $CF_C$ or $CF_D$,

Example: if when the $CF_C = 3$,

the content of total nitrogen (%) in the spot retentate sample($C_R$)=12.1%,

and the content of total nitrogen (%) in the spot retentate filtrate ($C_{RF}$) (pH was adjusted to 4.6) =1.6%,

the weight (kg) of the retentate at $CF_C$ 3 = 5.4kg,

then, $CN / NCN = [(C_R \times W_R) - (C_{RF} \times W_R)] / (C_{RF} \times W_R)$

$= [(12.1x5.4)-(1.6x5.4))]/(1.6x5.4)$

$= 6.56$

**7. Permeation (%) of Non-casein Nitrogen**

$$\text{Permeation of NCN}(\%) = [C_{PF(ACUM)} \times W_P] / [C_{FF} \times W_F] \times 100\%$$

where: $C_{PF(ACUM)}$.....content of total nitrogen (%) in the acumulative permeate filtrate at correspondent $CF_C$ or $CF_D$ (pH was adjusted to 4.6),

$C_{FF}$.....content of total nitrogen (%) in the milk feed filtrate (pH was adjusted to 4.6),

$W_P$.....weight (kg) of the permeate at correspondent $CF_C$ or $CF_D$,

$W_F$.....weight (kg) of the milk feed,

Example: if when the $CF_C = 3$,

the content of total nitrogen (%) in the acumulative permeate filtrate ($C_{PF(ACUM)}$)(pH was adjusted to 4.6) = 0.32%,

the content of total nitrogen (%) in the milk feed filtrate ($C_{FF}$) (pH was adjusted to 4.6) = 0.76%,

the weight (kg) of the permeate ($W_P$) at $CF_C$ 3 = 10.88kg,

the weight (kg) of the milk feed ($W_F$) = 16.25kg,

then, **Permeation (%) of NCN** = $[C_{PF(ACUM)} x W_P] / [C_{FF} x W_F] x 100\%$

$= [0.32 x 10.88] / [0.76 x 16.25] x 100\%$

$= 28.2\%$

**8. Permeation (%) of lactose**

**Permeation (%) of lactose** = $[(C_{l\,(ACUM)} x W_P] / [C_{l\,f} x W_F] x 100\%$

where: $C_{l\,(ACUM)}$--- content of lactose (μg/ml) in the acumulative permeate at correspondent $CF_C$ or $CF_D$,

$C_{l\,f}$.....content of lactose (μg/ml) in the milk feed,

$W_P$.....weight (kg) of the permeate at correspondent $CF_C$ or $CF_D$,

$W_F$.....weight (kg) of the milk feed,

Example: if when the $CF_C = 2$,

the content of lactose (μg/ml) in the acumulative permeate

$C_{l\,(ACUM)} = 41.7$(μg/ml),

the content of lactose (μg/ml) in the milk feed $C_{l\,f} = 42.0$(μg/ml),

the weight (kg) of the permeate ($W_P$) at $CF_C$ 2 = 7.91kg,

the weight (kg) of the milk feed ($W_F$) = 16.25kg,

then, **Permeation (%) of lactose** = $[(C_{l\,(ACUM)} x W_P] / [C_{l\,f} x W_F] x 100\%$

$= [41.7x7.91] / [42.0x16.25] \times 100\%$

$= 48.3\%$

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