MEMBER PROCESSING APPLICATION FOR PRODUCTION OF WHEY POWDER AND WHEY PROTIEN CONCENTRATE

THESIS
SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
DAIRY TECHNOLOGY

BY
H. M. JAYAPRAKASHA

DIVITION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL-132001(HARYANA),INDIA

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DEDICATED

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CERTIFICATE

This is to certify that the thesis entitled "MEMBRANE PROCESSING APPLICATIONS FOR PRODUCTION OF WHEY POWDER AND WHEY PROTEIN CONCENTRATE" submitted by Mr. H.M. JAYAPRAKASHA in partial fulfilment of the requirement for the award of the degree of DOCTOR OF PHILOSOPHY in DAIRY TECHNOLOGY of the National Dairy Research Institute (Deemed University), Karnal (Haryana), India is a bonafide research work carried out by him under our supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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(JAYAPRAKASHA)

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 With the implementation of various dairy development programmes in general and operation flood in particular, the dairy industry of India has made remarkable progress during the last two decades. As a result, significant increase in the production of milk has been observed. The white revolution has ended our foreign dependency for dairy products and ushered in an era of surplus milk which albeit mythical has resulted in diversion of substantial quantities of milk for the production of paneer, cheese, chhana, casein and shrikhand, which are resulting in enormous quantities of whey as by-product.

1.2 The annual world production of whey is estimated to be 133 million tonnes, which represents about 5.50 million tonnes of whey solids (Tinbergen, 1988). Whey disposal has always been a problem since the advent of cheese and casein production. No wonder, even today 40 per cent of whey is being drained out into gutter. In our country, technologies available for processing of whey are still primitive. In the absence of reliable statistics, it is estimated that about 800 million kg of whey is annually derived as a by-product, which possesses about 52 million kg of nutritious whey solids. Unfortunately, almost all the whey produced in the country is being merely wasted.

1.3 Whey constitutes nearly 50 per cent of the nutritionally superior milk constituents. It contains 6 to 7 per cent dry matter of which, 70 to 75 per cent is lactose, 14 to 15 per cent is protein and 7 to 8 per cent ash and vitamins. Whey proteins are the best quality proteins available, which have high PER (3.6), biological value (104) and NPU (95)

and possess almost all the essential amino acids. Like protein, lactose acts as a major source of energy with high caloric value. Whey is also a rich source of important minerals such as calcium and phosphorus, and water soluble vitamins specially vitamin B_2 (Renner, 1983). Besides being nutritious, whey solids possess excellent functional properties, such as solubility, emulsifying, foaming, gel formation, water binding, flavour, viscosity, etc., which are in great demand for various food formulations (Morr and Foegeding, 1990; Patel and Kilara, 1990a).

1.4 Whey is very rich in organic matter content. The BOD of whey is as high as 35,000 to 50,000 mg O_2/l. Disposal of untreated whey, therefore, poses threat to environment and human health. Most of the industrially developed countries have stringent legal laws for the disposal of whey. In India as well, draining of untreated whey is legally not permitted. Conservation of whey solids by employing cost effective technologies, therefore, appears to be the best method/alternative for redeeming the problems associated with the draining of whey.

1.5 It is ironical to see that approximately one-third of our population falls below poverty line and suffers from malnutrition. The protein and calorie intake in India is far below compared to developed countries. At this juncture, any possible source of food should be exploited to fill the demand and supply gap of nutrients. In this regard, whey solids have a promising role and are appearing as a potential source. Recovery of whey solids offer dual advantages. Firstly, it solves the disposal problems and secondly it provides high quality whey solids for human use.

1.6 Some attempts have been made in our country in the past to utilize

whey in the form of beverages but with limited success. Perishable nature of the product, high transportation cost and seasonal demand appear to be the major reasons. An urgent need is, therefore, felt to develop strategy to harvest and conserve these precious solids. Conservation of whey solids in the form of whey powder and whey protein concentrate using membrane technology will certainly be a viable proposition. The powders owing to their high nutritional and functional characteristics may find use in a wide range of food formulations, such as bakery products, confectionaries, biscuits, beverages, gravies, soups, sauces, ice cream, yoghurt, processed cheese, infant food, etc. Attempts can also be made to utilize whey powder and whey protein concentrate in the formulations of some of the indigenous dairy products.

1.7 It is in this area that no noteworthy achievement has been made in our country due to technological and economical problems encountered while processing the whey powder and whey protein concentrate. Most of the processes developed for the production of whey powder are patented. Besides, the high lactose and mineral contents create severe problems of hygroscopicity and caking. Moreover, due to low solids in whey, removal of water by conventional evaporators may pose economical problems. In this connection, membrane technology has emerged as a boon to economize the process of production of both whey powder and whey protein concentrate. Though the technology is about two decade old and is being widely adopted abroad, still it is in its infancy in India.

Problems are being constantly faced in the processing of whey

powder and whey protein concentrate partly due to the lack of technical know-how and partly due to the compositional differences of various types of whey. Most of the whey obtained in western countries is from cheese and casein prepared from cow milk and the process developed for the manufacture of whey powder and whey protein concentrate are based on these types of whey. Composition of indigenously available whey, obtained from cheese, paneer, casein and other products made from buffalo milk, varies widely with respect to pH, ionic strength, mineral composition, such as calcium, phosphorus content and also in the content of various protein fractions, viz., alpha-lactalbumin, beta-lactoglobulin, serum albumin, immunoglobulin, proteose peptone and non protein nitrogen.

1.8 The processing of whey by membrane technology varies widely with pH, composition and types of whey. The economy of membrane processing is determined by the flux rate (rate of permeation per unit area of the membrane). Maximum flux has to be maintained in order to increase the efficacy of the process. Fouling and deposit formation on the membrane are the main hindrances for the optimum flux maintenance which vary widely depending on the composition of the feed and the degree of interactions of whey constituents with the membrane. However, the same can be optimized by employing various pretreatments to whey and by optimizing the processing parameters.

In order to explore this technology for sustainable and economical production of whey powder and whey protein concentrate, the processing conditions need to be optimized. This would help in maintaining maximum flux rate for efficient and economic operation. The compositional differences of whey not only pose problems in concentration and fractionation of whey by membrane technology, but also severely affect

the subsequent evaporation and drying. The varying degree of pH, acidity and mineral composition in the indigenously available whey may aggravate the problems of caking and hygroscopicity of whey powder. Further, the processes of evaporation and drying have to be optimized to get free flowing powders, free of hygroscopicity and caking problems.

1.9 Therefore, detailed investigation has been undertaken with regard to the concentration and fractionation of whey by membrane technology and subsequent evaporation and drying for the production of whey powder of high quality. This would make the process more efficient and economical as it solves the problem of whey disposal besides conserving whey solids for human consumption.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

With the significant progress in the dairy development of our country, presently huge quantity of milk is being diverted for the production of cheese, paneer, chhana and casein, which are resulting in enormous quantity of whey as a by-product. In spite of its excellent nutritional value as well as problems faced in the disposal, proper solution for utilization has not been worked out.

Lot of research has been carried out to explore the ways of utilization of whey in liquid as well as dried form. Several beverages were recommended, a number of uses in other foods were suggested. Many research institutions are still engaged in finding out ways of novel and economical utilization of whey. Scores of papers appearing in the literature every year are a testimony for this.

Conservation of whey solids in the form of whey powder and whey protein concentrate (WPC) is a timely solution. Now-a-days, applications for whey powder and WPCs are being found increasingly in the food industry abroad. Since bulk of the water has to be removed for concentrating whey solids, energy efficient processes need to be explored. In this context, membrane process has been projected as an energy saving technology, highly amenable for whey concentration. Much work has been done abroad on membrane processing aspects and production of whey powder and WPC from cow milk cheese whey, most of which are, however, patented. These aspects are reviewed in this chapter to gain an understanding of membrane processing, evaporation and drying aspects of indigenously available whey.

2.1 SIGNIFICANCE OF WHEY

Whey is the liquid left after removal of casein and fat from milk in the manufacture of cheese, paneer, casein, chhana and other coagulated products. Its composition varies according to the type of product from which it is derived. Whey comprises of lactose, protein, fat, minerals and vitamins (Kennedy, 1985). In the production of cheese or casein, about 80 to 90 per cent of original milk used yields whey as a by-product. This whey contains, however, 50 per cent of all the milk constituents but because of low concentration of these constituents (about 6.4 to 7.0% dry matter), it has not been commonly considered as a by-product but as a waste product (Sienkiewicz and Riedel, 1990). Whey which contains most of the milk sugar and about 20 per cent of the milk protein, water soluble vitamins and minerals should play a significant role in human nutrition as a source of energy, protein, vitamins and micronutrients (Jelen, 1978).

2.1.1 DISPOSAL PROBLEM

Whey constitutes the most potent of all dairy wastes (Orchard, 1972) and of the strongest waste of any kind. One hundred kg of liquid whey containing approximately 3.5 kg Biological Oxygen Demand (BOD) and 6.8 kg of Chemical Oxygen Demand (COD) has the polluting strength equivalent to sewage produced by 45 people (Webb and Whittier, 1970). Drainage of whey into streams without prior treatment is not permitted in most of the milk processing countries due to its high BOD value of 30 to 60 g O_2/l (Sienkiewicz and Riedel, 1990). The corresponding COD values must constitute 170 or 100 mg/l (Anon., 1981). It is observed that a dairy farm processing 100 tonnes milk every day produces about the same quantity of organic products obtained as effluent as a town with 55,000

residents (Jelen, 1978). Due to its high BOD and COD, the disposal of whey is of great economic concern to the dairy industry.

2.1.2 NUTRITIONAL AND FUNCTIONAL SIGNIFICANCE

Whey nutrients represent a huge quantity of nutritionally rich food. In spite of its high nutritional quality, whey is still being largely wasted in the sense it is not available for human consumption where it might be needed (Allum, 1980). With the world hunger situation as it is, we can not afford to ignore any possible food source. Although whey is obviously not the sole answer to the food shortage problem, it can improve the quality of foods in which it is used and free the ingredients it replaces (egg and milk) for direct consumption by those who need them (Kennedy, 1985). Nutritionally, the most valuable whey component is the whey proteins, one of the best proteins known (Humphreys, 1977). The whey proteins are one of the highest quality natural proteins available, and their excellent nutritional value can be utilized to a greater extent in the form of WPCs (Schingoethe, 1976; Aalbersberg, 1981; Patel et al., 1991). The high nutritional value of whey proteins is based on its higher concentration of essential amino acids, such as tryptophan, leucine, isoleucine, threonine and lysine than casein (Renner, 1983). Whey proteins also contain about 2.5 g of cystine and 2.8 g of cysteine per 100 g of protein. They have all the essential amino acids in excess of FAO standards, of particular importance are isoleucine, lysine, threonine and tryptophan (Irvine et al., 1984). Whey proteins have shown higher biological value (104) as against whole egg (100) and casein (77). They also have higher PER and NPU than casein. The PER of whey proteins is 3.6 as against 3.8 (whole egg) and 2.9 (casein), whereas NPU is 94 for whole egg, 76 for casein and 92 for whey proteins (Renner, 1983; Renner and Abd El-Salam, 1991).

Because of the nutritional significance of whey proteins, these proteins have long been recognised by the nutritionists as one of the best proteins available for human needs and is being looked upon seriously as a nutritional ingredient to improve nutrient profile of some foods, especially those popular with children (Jelen, 1978). An outstanding example of the human food use now being made of whey products is by nutritionists and medical profession, who specify whey products for specialized dietetic food needs of geriatric and convalescing patients whose digestive function requires special dietary management (Clark, 1979).

Whey and whey derived products besides being a nutritional ingredient in various foods can also be used as functional ingredient supplying flavour, texture, colour and aeration properties in variety of foods (Kinsella, 1985; Morr and Foegeding, 1990). Whey proteins possess very good functional properties, such as solubility, foaming, emulsifying, gelling and water binding (Matthews, 1984; Modler and Jones, 1987; Patel and Kilara, 1990b). WPCs which contain sufficiently high protein concentrations in a predominantly undenatured form, with minimal lactose and lipid contents, are highly acceptable as functional protein sources for the food industry. These products should be competitive with other major protein ingredients such as caseinates, soy and egg protein products, for numerous food applications (Morr, 1982). Their functional properties have encouraged attempts to use them in a great number of food products in replacing other more traditional additives such as milk powder and egg albumin (Moulin and Galzy, 1984). Because of excellent nutritional and functional properties of whey solids, the various possible food applications include: bread and baked foods, infant and dietetic foods, ice cream and frozen desserts, cereals, soups, sauces, toppings and dressings,

snack foods, confections and beverages. In addition, whey and modified whey have been utilized as food ingredients in many nutritional programmes where they play an important role in feeding malnourished people of developing countries (Clark, 1979). Various aspects of utilization of whey in the form of liquid whey, condensed and dried whey, demineralized whey, delactosed and partially delactosed whey, WPC and use of fractionated components of whey have been critically reviewed (Mann, 1977,1980,1982, 1985,1987,1988,1989).

2.2 NEED FOR ADOPTION OF MEMBRANE TECHNOLOGY

Attempts are being made to conserve whey solids in different ways. However, it is observed that conservation of whey solids in the form of whey powder and WPC is a better proposition (Graham et al., 1981). Although it is a suitable way for preservation of whey solids, the evaporation of large quantity of water to convert the whey to dried form is found to be uneconomical with the conventional evaporators due to high evaporational cost (Pepper and Orchard, 1982). In the manufacture of dried whey, more than 50 per cent of energy consumed is required at the evaporation stage. Therefore, the cost of energy required for the evaporation of whey is a major factor determining the production of whey powder (Jebsen and Iyer, 1991). Hence, it is important for the economic production of whey powder to ensure that evaporators are operating at their maximum capacity and efficiency.

Membrane technology provides a solution to this problem because it uses much less energy in removing bulk of the water from whey than an evaporational process, although evaporation has to be used for final concentration upto 50 per cent solids (Pepper, 1981). Reverse osmosis (RO) will be a better alternative for preconcentrating whey in the dairy

industry (Sandfort, 1987). With the current energy prices and recent design innovation in evaporators, RO would be best suited in upgrading energy efficiency of evaporators (Cheryan et al., 1990). Potential energy savings are offered by partially substituting RO for thermal evaporation in whey concentration. Besides saving substantial cost and energy, it also helps in increasing the capacities of the evaporators (Wright,1982; Cheryan et al., 1990). Moreover, by ultrafiltration (UF) process, high quality proteins can be obtained which is difficult by any other commercially available process (Marshall and Harper, 1988). Thus, emphasises the need for the adoption of membrane technology for the conservation of whey solids.

2.2.1 MEMBRANE TECHNOLOGY IN ALLEVIATING DISPOSAL PROBLEM AND CONSERVATION OF WHEY SOLIDS

RO and UF are the two main membrane separation techniques which are being widely used to concentrate and fractionate whey. These technologies can be used successfully to overcome the pollution and disposal problem and precious nutritious solids of whey could be conserved. In particular, RO reduces the disposal problem as well as increases the by-product recovery. Concentration of whey by RO to about 30 per cent TS reduced the BOD of whey to 500 mg O_2/l and even to 200 mg O_2/l from an original value of 50,000 mg O_2/l under certain operational conditions (Madsen et al., 1970; Nielson et al., 1970; Mans, 1992). Whey can also be subjected to both UF and RO. In first stage, it can be concentrated to 20 per cent proteins by UF and in the second stage the permeate is concentrated to 20 per cent lactose. By this process, BOD of cheese whey can be reduced to as high as 99 per cent (Horton et al., 1970) and of cottage cheese whey by 97 per cent (Goldsmith et al., 1971). Besides reduction in BOD, this combined process also helps in recovery of range of by-products according to the operational conditions.

2.2.2 REVERSE OSMOSIS

RO is a pressure driven membrane process, where the fluid products are concentrated by using semipermeable membranes, such as polyamide and cellulose triacetate membranes having pore size of about 4 A°. It works at an operating pressure of 20 to 40 Bar or 300 to 600 psi. RO offers an economic advantage over thermal evaporation in terms of lower operating costs. Furthermore, RO possesses the additional advantages that it concentrates without heat, thereby retaining the native properties of the original raw materials (Pal and Cheryan, 1987; Pepper and Pain, 1987; Jensen and Oxlund, 1988). The selective removal of water from a solution through the semipermeable membrane has the potential for being an effective low cost method for concentrating whey. This technology appears to be a promising method for concentrating whey with significant savings in the total energy and overall cost (Peri and Dunkley, 1970; Kulozik and Kessler, 1988a).

2.2.2.1 Cost and energy saving

RO offers an energy saving alternative to thermal evaporation for concentration process in the dairy industry (Cheryan et al., 1987; Cheryan, 1991). Substantial energy savings are possible by incorporating RO in certain evaporator and dehydration plants depending on the design of evaporation system (Cheryan et al., 1990). Costs are significantly lower for the concentration of whey by RO upto about 50 per cent water removal compared to concentration through thermal evaporation. It was observed (Boer et al., 1975) that costs of concentrating 200,000 kg whey/day is 20 per cent lower by RO than by evaporation at concentration factor 2. However, at concentration factor 3, Wright (1982) reported 45 per cent savings in total energy and 25 per cent in overall costs. On the contrary,

when the whey is concentrated by 4 times, the cost may increase by 10 per cent (Boer et al., 1975) and may lead to clogging problems and accumulation of viscous materials and insoluble solids on the membrane (Mc Donough and Mattingly, 1970). Yet, keeping in view the increasing energy cost, concentration factor 4 may still become attractive when RO is used as a preliminary stage to evaporation (Boer et al.,1975). Concentration upto a certain TS can be done more economically by RO than by most of the evaporational processes (Stabile, 1983; Cheryan et al., 1987).

Moreover, the capital and energy cost including the one for preheating and cleaning process of a RO plant would be less than 17 per cent of that used by conventional evaporators. Also RO is reported to be 3 times more efficient than either a 7 effect evaporator or a new evaporator fitted with mechanical vapour recompression (Pepper and Orchard, 1982). According to Kulozik and Kessler (1988a), RO with a primary energy requirement of about 80 kJ per kg of dehydration (i.e.1:3.7 concentration), is more energy saving than is evaporation using vapour ventilators (200 kJ/kg). Keeping in view of the benefits of RO process, this can be proposed as an economical process for concentration of whey to recover valuable products as well as to minimize the disposal problems.

2.2.2.2 Factors affecting the efficiency of reverse osmosis process

The efficiency of RO process is measured by the permeation per unit area of the membrane which is known as 'flux' and is generally expressed as $l/m^2/h$. The economy of the process of concentration of whey by RO is greatly dependent on the flux. The flux is affected by the operational parameters such as pressure, temperature and the flow velocities of the feed. It also depends on the type, composition, pH and ionic strength of whey and on the degree of concentration. Lim et al.

(1971) expressed flux (J) for RO as $J = K (\Delta P - \Delta\pi)/(R_m + R_P + R_f)$ where 'K' is constant, ΔP is the transmembrane hydraulic pressure drop, $\Delta\pi$ is the osmotic pressure difference across the membrane and R_m, R_P and R_f are resistance due to the membrane, concentration polarization and fouling respectively. Macbean and Smith (1977) estimated the osmotic pressure of whey to be about 0.65-0.70 MPa and usual operating ΔP for RO as 4-6 MPa. As the whey is concentrated, the increasing osmotic pressure at the membrane surface, which may be aggravated, by concentration polarization leads to such a low driving force that operating becomes impracticable over about 25 per cent soluble solids. The combined effect of R_P and R_f is the major force governing the efficiency of RO process (Muller and Harper, 1979; Muir and Banks, 1985). As the cost of RO concentration of whey is highly dependent on the flux, it is necessary to obtain maximum flux per unit area of the membrane for the efficient and economic operation (Boer et al., 1977; Muir and Banks, 1985). The major processing variables influencing permeation rate are operating temperature, pressure, flow rate through the unit and feed concentration level (Cheryan et al., 1990; Boxtel et al., 1992).

2.2.2.2.1 Temperature of operation

Temperature of feed has profound effect on the permeation rate. There is a linear relationship between flux rate and temperature of operation (Donnelly et al., 1974). Higher the temperature, higher is the flux rate. It is always desirable to operate RO system at the highest possible temperature from the stand point of flux consideration (Rovaleyn et al., 1971). Every 5°C rise in feed temperature is reported to be accompanied by an increase in permeate flux of approximately 40 per cent. Increase in temperature improves the flux by lowering the viscosity of

permeate and increasing the back diffusion of solids away from the membrane (Glover, 1985). Although it is desirable to operate the RO system at the highest possible temperature, the temperature of operation has to be selected by looking into compatibility with membrane stability, microbial proliferation and impairment of some of the functional properties through thermal denaturation (Donnelly et al., 1974).

2.2.2.2.2 Operational pressure

During RO process, no permeation is observed until the applied transmembrane pressure exceeds the osmotic pressure of the feed (0.70-0.76 MPa or 100-110 psi) and the flux increases linearly with applied transmembrane pressure upto about 2.1 to 2.8 MPa. Flux then becomes asymptotic and decreases at much higher pressures. The pressure at which maximum flux attains is higher with higher flow rate (Cheryan et al., 1990). Donnelly et al. (1974) observed a linear increase in flux with pressure upto 31.5 kg/cm^2 where it reached plateau and became pressure independent at 35-42 kg/cm^2. This pressure independence was attributed to the concentration polarization effects and was dependent on concentration of the retentate.

Although RO is a pressure driven process, the permeate flux does not continue to increase as pressure is increased indefinitely. As more pressure is applied, the flux passes through a maximum after which further pressure reduces flux. The interpretation of this is that an increase in pressure initially improves flux, which at the same time leaves more solids near the membrane surface, thus increasing the fouling. Eventually, the fouling layer is compacted by further pressure causing decline in flux (Kessler et al., 1982; Glover, 1985). Pressure and flow velocities of feed are interrelated with respect to flux. Flux rate is highly dependent

on flow velocity. This dependence increases with elevation in pressure and decreases with increasing concentration. At low pressures and high concentrations, flux rate becomes independent of flow rate. This independence is explained by the fact that rejection is very low at low pressures and high concentrations (Cheryan et al., 1990).

2.2.2.2.3 Flow velocity

While it is desirable to work at as high a flux rate as possible, it is preferable to use as high a velocity as possible. However, as pressure drop increases rapidly with flow rate, there is an upper limit for velocity. In practice, it would appear that flow rate of 15-18 l/min is acceptable (Donnelly et al., 1974) depending on design configuration. If a particular plant does not permit such a flow rate, it may then be necessary to use turbulence promoters or volume displacers to minimize concentration polarization effects. In the process of concentration of cottage cheese whey by RO in general increasing flow velocity increased the permeation rates. Turbulence promoters are said to be most effective in increasing permeation rate under conditions that, in their absence, permitted fouling of the membrane surface by macrosolutes from the feed (Peri and Dunkley, 1970). As reported by Pepper and Orchard (1982), RO using multistage recycle (MSR) can concentrate whey from 6 to 28 per cent TS with the same average flux, previously achieved when concentrating to 12 per cent TS by batch process. The shorter path length of MSR system enables velocities to be maintained above the level at which deposits form, giving longer continuous operation. As the flow velocity is increased the maximum flux is increased especially at high pressures. Higher flow rates generate great shear at the surface and higher turbulence in the module enhances the rate of back transport of polarized solute into the

bulk of the solution. Polarization occurs at a higher pressure and thus the effect of flow rate is more noticeable at higher pressure (Cheryan et al., 1990). Flow velocity along with pressures affects mainly the resistance of the fouling and polarization layers. Increase in flow velocity from 1.5 m s^{-1} to 2.0 m s^{-1} reduced the fouling considerably (Glover, 1985).

2.2.2.2.4 Concentration of feed

In the process of concentration of whey by RO, flux decreases with increasing solids content and a double concentration reduces the flux by approximately half of the initial flux (Fenton-May et al., 1972). An exponential decrease in flux with an increase in concentration is reported for many biological and food feed streams during RO concentration (Cheryan, 1986). At a given pressure the permeation rate decreases with increasing concentration ratio (Besik, 1971). The actual permeation rate decreases rapidly after about 3:1 concentration and it would not appear to be practicable to concentrate whey above 4:1 (i.e. 24 to 26% solids). Commercial RO whey treatment plants with running time of 5-19 h/day, 4-6 day/week have shown flux of 20-25 $l/m^2/h$ for 1:2 concentration of whey (Eriksson, 1977), whereas processing of cheese whey by RO tubular Paterson Candy International Limited (PCI) membrane module to a volume reduction of 70 per cent had shown an average flux of 30 $l/m^2/h$ (Short and Doughty, 1976). Similarly, Patel et al. (1992a) observed an average flux of 30.20 $l/m^2/h$ during RO concentration of cheddar cheese whey by tubular PCI membrane module. In another study (Spangler and Amundson, 1986), permeate fluxes at a concentration factor of 2 for sweet whey and acid whey appeared to be 29 and 36 $l/m^2/h$, respectively. During concentration of Gouda cheese whey by RO, continuous runs upto 10 h gave stable flux rate of about

24 $l/m^2/h$ for the first stage, concentrated to 18 per cent TS, and 8 $l/m^2/h$ for second stage concentrated to 26-28 per cent TS, whereas flux characteristics of cottage cheese whey were slightly lower (Pepper, 1981).

2.2.2.3 Fouling and deposit formation in RO membrane

Besides operational parameters of RO, permeate flux is also strongly affected by pretreatments and the processing conditions of whey during concentration (Hiddink and Boer, 1979). The major obstacle is membrane fouling and concentration polarization, due to higher concentration of components in the liquid at the membrane surface than in the bulk. During the process, the solubility limit of calcium salts may be exceeded, giving precipitation on the membrane, while proteins may form a gel layer which acts as a secondary membrane and reduces permeate flux. Osmotic pressure and fouling tendency are the flux limiting factors during concentration of whey by RO (Kulozik and Kessler, 1988b; Cheryan et al., 1990). Recently, Boxtel (1992) reported that long term fouling is one of the barriers to the application of membrane filtration in the food industry. As a result of membrane fouling the production capacities decrease and the production costs increase. Precipitation of calcium phosphate and protein on the membrane and the formation of protein gel layer have been attributed for the fouling.

Permeate flow characteristics of RO indicated that the fouling layer is formed rapidly and its resistance is nearly constant over reasonable processing intervals. These fouling properties permit correlation of permeate flow, average mass transfer coefficient and osmotic pressure difference in terms of energy required for permeate volume vs time (Stabile and Roger, 1985). The fouling components, their concentration in retentate and their critical membrane concentration are affected by

characteristics of whey and the concentration procedure. Hence, the flux varies accordingly and differs according to pH of whey and heat treatment (Fukuwatari et al., 1987). As observed by Hiddink et al. (1980) for cheese whey processed at 30°C, fouling of membrane is due to calcium phosphate precipitation, whereas for skim milk concentrate main fouling agent is protein. Glover (1985) has also reported that calcium phosphate precipitation is responsible for fouling of membrane during RO of whey.

Deposited layer formed on the RO membrane during processing constitutes an additional resistance to permeation. Permeation is seen as mass flow through two resistances, namely, laminar flow through the deposited layer and transport by diffusion through the membrane. Inorganic ion increased the resistance to flow of the deposited layer considerably (Kessler et al., 1982; Kulozik and Kessler, 1988c). During RO of cottage cheese whey, it was observed that whey solids including casein, beta-lactoglobulin, alpha-lactalbumin and non protein fraction accumulated on the surface, was of two forms, a gel like deposit that resisted removal by fluid shear and a viscous layer that was readily removed by flushing. The accumulated material reduced the permeation rate to a great extent (Lim et al., 1971; Glover, 1985).

Fouling reduces the flux rate to a great extent resulting in poor economy of operation and hence increases cost of processing. The effect of fouling can be mediated by proper feed stock pretreatments by this aspect has not been much investigated for concentration of milk and whey by RO (Muir and Banks, 1985). Combined effects of pH, heat treatment and addition of calcium have been studied to explain the mechanism of fouling (Muller and Harper, 1979; Hickey et al., 1980). When whey is heated above pH 5.6, reactive sites of proteins are exposed, the net charge

of the protein becomes minimal and calcium dependent interactions can take place, increasing the permeation rates. However, when heated at low pH values as is the case in the cottage cheese whey, calcium ions aggregate to form apatites large enough not to block membrane pores resulting in better flux rates. The increase of pH of whey to more than 6.5, and addition of extraneous calcium with subsequent heat treatment also result in reduced fouling. This is because of the effect of calcium on protein interactions and self-aggregation of beta-lactoglobulin.

These observations are significant for the RO of buffalo milk cheese whey and paneer whey as they contain considerably higher amount of calcium and phosphorus.

Since calcium ions are found to be mainly responsible for fouling (Kessler et al., 1982; Glover, 1985; Kulozik and Kessler, 1988b), many workers have tried to remove the calcium from whey by different means (Hiddink and Boer, 1979; Hiddink et al., 1980). Suitable preheat treatments of whey to mitigate fouling have also been suggested. For example, pasteurization of whey at 74°C may increase the flux rate by 25 per cent (Pepper and Orchard, 1982). Heating Gouda cheese whey to 72°C or 65°C/ 5 min was also found to be beneficial (Hickey et al., 1980). Partial removal of Ca by ion exchange and addition of sequestering agents are the other approaches attempted (Smith and Macbean, 1978). It is observed that Cheddar cheese whey causes much less fouling than does casein whey. This can be interpreted in terms of different ionic compositions of the surface layer on the membrane in the two situations (Smith and Macbean, 1978). Boxtel et al. (1992) have demonstrated the fouling of RO membrane by the membrane fouling model. The results revealed that the fouling rate depends on the applied settings of operation variables. Low permeate fluxes, low process pressures and high flow velocities result in reduced

membrane fouling rates. As a consequence, these operation variables can be used to control membrane fouling during production runs.

Though limited reports are available for reducing the fouling of RO membrane, these do not clearly spell out which treatments are better to reduce the fouling so as to increase the flux.

2.2.2.4 Cleaning of reverse osmosis membrane

The cleaning and maintenance of RO membrane is an important and critical aspect for the economy of operation. Membranes should be properly cleaned and sanitized in order to obtain a high level of flux as well as to have long life of membrane.

The most important processing variable affecting ease of removal of deposited layer are pressure difference across the membrane and shear stress at the walls. Product composition is important: The main component of deposits that formed during concentration of milk was protein, but low molecular weight soluble substances such as salt ions exerted a marked influence on the stability of the deposit and the ease of removal by flushing. The Ca ions ceases protein, particularly to form networks within the deposited layer and thus increased its cohesion and the resistance to the axial flow forces during rinsing (Kulozik and Kessler, 1988c). The present Pasilac new RO membranes can be cleaned with 0.5 per cent HNO_3 or 0.5 per cent NaOH, can be sterilized at 80°C at low pressure. In RO plant where 80 tonnes of whey concentrated to 20 tonnes/day in 24 h batch operation, the cleaning procedures involve rinsing with water followed by detergent solution and water, sterilization with a hypochlorite solution and final rinsing with water. Once a week, the system is cleaned with trypsin solution (Madsen, 1972). Non-cellulose acetate membrane (type ZF-99) now being used in RO, can be cleaned at 60°C with NaOH or HNO_3,

thus substantially reducing cleaning cost and time (Pepper and Pain, 1987; Patel et al., 1992b).

Glover (1985) suggested that RO membrane can be cleaned by using 0.1 to 0.3 per cent nitric acid or phosphoric acid at 45°C, 0.1 to 0.5 per cent sodium hydroxide at 50°C, 0.1 to 0.5 per cent alkaline detergent (Henkel, P_3 ultrasil-10) at 50°C or other cationic surfactants. Disinfection can be done with water at 60-70°C, 500 mg/l of a 35 per cent solution of H_2O_2 at less than 30°C or 0.5 per cent formaldehyde. Membranes can be stored with sodium metabisulphite (1 to 2.5 g/l).

For non-cellulose acetate membranes, PCI has recommended a cleaning cycle which consists of cleaning with 0.3 per cent HNO_3, followed by cleaning with 0.1 per cent sodium hydroxide with 0.25 per cent ultrasil-10 or 0.25 per cent ultrasil-11. After the process of cleaning, the membranes should be preserved with 0.1 per cent sodium metabisulphite.

Most of the detergents used for cleaning RO membranes are patented and the details of cleaning procedure and the composition of these detergents are not available.

2.2.2.5 Compositional changes by reverse osmosis concentration of whey

In RO process, there may be a certain loss of low molecular weight components and this will result in change in the relative composition of the concentrate manufactured exclusively by evaporation (Jensen and Oxlund, 1988). The results of Sloth Hansen et al. (1978) have shown that the concentration of whey by RO from 5.6 to 12 per cent TS and upto 59 per cent by evaporation, the contents of low molecular weight nitrogen compounds are reduced from 11.6 to 10.7 per cent and no changes occurred in the concentration of whey by traditional evaporation alone.

Losses of low molecular weight compounds specially NPN (urea) and minerals (sodium, chloride and magnesium) through RO membranes have been reported by many workers (Muir and Banks, 1985; Jensen and Oxlund, 1988; Morales et al., 1990b). However, losses of true protein, calcium and phosphorus through permeate during processing whey by RO were not observed. The per cent retention of these components were found to be 100 (Marshall, 1985; Morales et al., 1990b). Traces of lactose were observed in the RO permeate. Morales et al.(1990a) have reported the retention coefficient (%) of 99.589, 95.990 and 97.920 for TS, NPN and ash, respectively for the concentration of sweet whey by employing ZF-99 RO membranes. Slight variation in the retention of components with respect to various types of membranes and operating conditions was observed. Although there were slight losses of NPN and ash in the whey concentrated by RO, the overall retention of all the components of whey were very high and the loss of solids through permeate was negligible. Hence, it is a viable alternative to remove bulk of the water from whey prior to evaporation and drying for the production of dried whey (Morales et al., 1990b).

2.3 EVAPORATION AND DRYING OF WHEY

With the increased demand for ordinary and demineralized whey powders in the food industry and baby food formulations, the dairy industry has taken renewed interest in manufacturing a quality product. Because of difficulties encountered in handling of whey, many people still regard it as the waste product of the dairy industry.

The pretreatment of whey can have significant effect on the ability to handle whey concentrate and on final product quality. With careful pretreatments of whey, proper adherence to the crystallization procedure

and drying technique, a good quality product which will meet the most stringent demand of the food industry can be produced (Hynd, 1980).

2.3.1 PROCESS OF MANUFACTURE

Several reports are available on manufacturing aspects of high quality whey powder (Moore and Pinkel, 1970; Becker, 1971; Prohaska and Marosevics, 1976). Most of them are patented and details of manufacture are not available. With the available reports, it can be observed that the general steps for whey powder manufacture involve evaporation to a desired TS level, flash cooling, precrystallization and drying. As reported by Pisecky and Haugaard Sorensen (1976) and Jensen (1987), whey powder can be categorized into four groups, viz., ordinary whey powder, precrystallized whey powder, non caking whey powder from straight through process and non caking whey powder from belt process. For ordinary whey powder, the whey is evaporated to 42-45 per cent TS and directly spray dried at 180°C. For precrystallized whey powder, the whey is concentrated to 50-60 per cent TS, precrystallized for 4-16 h and spray dried at 200°C. In case of a straight through non-caking powder, the whey is evaporated to 50-60 per cent TS, precrystallized for 16-24 h and spray dried at 185°C, followed by fluid bed drying. The belt process of non caking whey powder preparation involves the same steps as above but spray dried at 130°C followed by crystallization and fluid bed drying.

2.3.1.1 Evaporation

Evaporation process precedes drying to increase the capacity and economical operation of driers. Whey needs large amounts of water to be evaporated before drying. This is the reason why conventional evaporator to remove water from whey is uneconomical in less developed countries.

The evaporation of milk or whey is an energy intensive operation. In the manufacture of dried whey or milk more than 50 per cent of the energy consumed is required at the evaporation stage. Therefore, the cost of energy required for the evaporation of whey is the major factor determining the production of powder (Jebsen and Iyer, 1991). Hence, it is important for the economic production of powders to ensure that evaporators are operating at the maximum capacity and efficiency. Several studies have indicated that considerable energy savings can be achieved by incorporating RO prior to an evaporator during the manufacture of concentrated and dried product (Horton, 1982; Stabile, 1983; Kosikowski, 1986). With the current energy prices and recent design innovations in evaporators, RO would be the best suited in upgrading energy efficiency of older concentration plants or in existing plant capacities (Cheryan et al., 1990). In this context, use of membrane technology to remove part of water from whey is important (Pepper and Orchard, 1982).

Whey is generally concentrated to 40-60 per cent TS depending on type of whey and quality of powder required. It should be noted that level of TS has a bearing on subsequent handling of the concentrate. When concentrating whey above 55 per cent TS, there may be spontaneous lactose crystallization in the evaporator, leading to severe problems during further processing and drying (Westergaard,1983). To avoid this phenomenon, a higher evaporation temperature has to be used in the last stage of evaporation where the lactose is most concentrated. Therefore, whey evaporators are coupled in such a way that the highest content of solids is reached at a higher temperature than that which prevails in the last stage (Sandfort, 1987). Normally, multistage evaporators coupled with thermal or mechanical vapour recompression are being used for whey powder

manufacture. Since TS content of whey is very low, single stage evaporators are obviously uneconomical (Graham et al., 1981). However, RO coupled with single stage evaporators appears to be economically viable (Jensen and Oxlund, 1988).

In a multistage evaporator, the heating medium for first stage is a mixture of vapour and live steam. From stage to stage there is a decreasing boiling temperature which is due to the existing vacuum differences. The product is pumped from stage to stage as a heating medium. An advanced concentration installation consists of a 5-7 stage falling film evaporator. By adding stages to the evaporator the specific steam consumption is reduced. The boiling temperature in the first stage is about 70°C and in the final stage about 45°C. The vapour from the last stage is condensed by means of cooling water and the concentrate is then ready either for spray drying or for crystallization (Westergaard, 1983; Sandfort, 1987). The evaporators are also required to be equipped with flow and density control devices as even small variations will result in unacceptable fluctuations in the final solids content in the concentrate (Jensen and Oxlund, 1988). After evaporation to a desired TS content, the concentrate is transported to double walled crystallization tanks which are equipped with slow running stirring devices (10-30 min^{-1}).

2.3.1.2 Lactose crystallization

To avoid the very undesirable caking properties of ordinary whey powder, it is of great industrial importance to get the major part of the lactose content in a crystalline form. The advantages of this lie both in energy savings and in improved powder properties. In the spray drier, it is possible to dry whey concentrate containing upto around 60 per cent solids, if the lactose content has been subjected to a crystallization

degree of 85-90 per cent. On the other hand, it is not possible to go higher in solids content than 42-45 per cent if the aim is to dry non-crystallized concentrate (Pisecky and Haugaard Sorensen, 1976). Obviously this low degree of concentration has a very negative effect on the process economics compared to the process which involves crystallization.

Whey concentrated to a solid content of 55 per cent (at 38°C) or above represents a supersaturated lactose solution(Sloth Hansen et al., 1978; Sandfort, 1987). It is important to avoid spontaneous crystallization in the evaporator (Westergaard, 1983). By changing the flow pattern for the last evaporation stages, the output concentrate can thus be obtained at a sufficiently high temperature to avoid crystallization. Controlled crystallization can then be initiated by immediate flash cooling to about 30°C and subsequent seeding. It is of significant importance to control the crystallization as far as possible. This must be done in order to create a maximum number of small crystals in the order of 200,000/mm^3 (Sienkiewicz and Riedel, 1990), giving a large total crystal surface which means a fast and efficient crystallization. According to Westergaard (1983), the guidelines for crystallization are as follows. As soon as possible (i.e. when the agitator is well covered) agitation should start and run at a speed that does not create foam in the concentrate. Immediately, fine grained alpha-lactose monohydrate at a level of about 1 kg per ton of concentrate should be added. The holding time under these conditions should be 3-4 h. Cooling of the concentrate should then start, the rate being about 3°C/h until 10°C is reached.

In the studies of Vilder (1975), whey concentrated to 40-61.7 per cent dry matter (DM) was precrystallized at 30°C. The content of alpha-lactose crystals was found to increase from 50-60 per cent at 40

per cent DM to 73-75 per cent at 61.0 per cent Dm. In another work (Moll and Damman, 1979), cheese whey concentrated to 48-67 per cent TS was precrystallized at different temperatures before spray drying. An adequate level of lactose crystallization (about 70% level is needed to avoid caking of whey powder) was achieved within a few hours, when the TS content of whey exceeded 60 per cent, but about 20 h is needed if the TS content is below 50 per cent. At high TS contents, the crystallization temperature has to be raised to produce large crystals and thus reduce the viscosity of the concentrate.

Tvorog whey was concentrated by Zaets et al. (1979) to 48, 51 or 54 per cent TS, cooled rapidly to 30°C and seeded with 0.3 per cent fine lactose crystals and cooled slowly to a final temperature of 17, 21 or 24°C, under constant stirring at 30 rpm. Whey concentrated to 51 ± 1 per cent TS and having a final temperature of 21 ± 1°C was found to be optimum and the crystallization obtained was 82.6 per cent.

As high as 95 per cent crystallization can be obtained by first spray drying precrystallized concentrate to have 10-14 per cent moisture and then further crystallizing in the conveyor before final drying. The product so obtained remained sufficiently free flowing even after exposure to air (Anon., 1973; Stork Friesland, 1977). Hynd (1980) used alpha-lactose monohydrate as a seeding material at a concentration of 0.03 per cent to bring about precrystallization in about 6-8 h in the whey concentrated to 37-40 per cent TS. It is reported that non-lactose components of whey concentrate such as mineral salts, proteins and lactic acid have some influence on the crystallization rate and on product properties. Mineral salts and lactic acid influence the mutarotation, it has been found that some cations and the hydrogen ion can act as positive catalysts of

mutarotation (Hynd, 1980). On the other hand, mineral salts are hygroscopic and thus contribute to the hygroscopicity of the final product. Lactic acid is a liquid being present in the final product also contribute to the hygroscopicity. The proteins have an important influence on the crystallization rate and product properties. They increase the viscosity of whey concentrate and thus negatively the crystallization rate, and they also contribute to the hygroscopicity of the product. These properties of proteins can vary considerably with the type of whey and all the heat treatment given during processing of whey (Hynd, 1980; Jensen, 1987; Sienkiewicz and Riedel, 1990).

2.3.1.3 Properties of whey concentrate

The viscosity of whey concentrate is a property of great importance for the manufacture of whey powders (Westergaard, 1983). The heat treatment before evaporation should normally be in a narrow range around 80°C. Higher preheating temperature normally results in higher viscosities and lower temperatures yield lower viscosities. Lower viscosities result normally in a more thermoplastic powder which means that the outlet temperature of the spray drier should be higher, leading to an inferior powder quality. Total solids content in whey concentrate is not always dissolved solids. The viscosity varies with the state of lactose crystallization in a way that the first part of the crystallization time is accompanied with a viscosity increase. The later stages of crystallization give a concentrate of declining viscosity (Sandfort, 1987; Jensen and Oxlund, 1988).

At the beginning of crystallization, the crystals are very small (large specific surface area). This results together with a relatively concentrated mother liquor in a high viscosity. Maximum viscosity is usually reached ½ to 1 h after seeding with crystalline lactose. As the

time passes the lactose crystals grow, and the solids content of the mother liquor decreases, resulting in a lower viscosity (Hynd, 1980).

The viscosity and density of whey concentrate depend on its temperature. When the temperature increased from 20-60°C, the viscosity decreased from 3.32 to 1.32 cpat 25 per cent TS and from 18.5 to 5.2 cp at 40 per cent TS and density correspondingly decreases from 1.10 to 1.085 g/ml and from 1.172 to 1.16 g/ml (Buma, 1980). Whey concentrated to 50-68 per cent DM was found to have a density of 1.280 to 1.330 kg/m^3 at 50°C (Sienkiewicz and Riedel, 1990). Preheating fresh whey at 76.7-87.8°C has no effect on the viscosity during condensing to 50 per cent TS, whereas whey fermented to pH 5.4 before preheating thickened during condensing (Cook et al., 1980). The viscosity has to be controlled by selecting the appropriate pretreatments of whey in order to obtain high quality product and to overcome the problems encountered during evaporation and drying (Boersen, 1990).

2.3.1.4 Drying of whey

The techniques of drying and especially of spray drying is of great importance due to the fact that final powder quality concern'ng for instance nutritional and functional properties is determined to a great extent by the conditions of drying including pre and post-treatments of concentrate and powder, respectively. Developments of equipments and new drying technologies are initiated and influenced by requirements in respect to production economy, reduced energy consumption and greater flexibility, product quality, product assortment, automation, pollution control, etc. (Jensen and Oxlund, 1988). There are 5 main processes currently in use for the spray drying of sweet and acid whey. These are spray drying, spray drying with crystallization, spray drying with

fluidized bed cooling, the straight through process and the low temperature process. The details of these processes have been reviewed by many authors (Jensen, 1987; Boersen, 1990; Milanovic and Caric, 1990).

Whey drying can be divided into two processes: single effect drying and double effect drying. Double effect drying in contrast to single effect drying involves re-wetting of the spray powder containing 10-15 per cent moisture, followed by a second drying stage in which the moisture content is reduced to below 5 per cent (Sienkiewicz and Riedel, 1990). In recent years advanced three-stage drying techniques are also available. In one stage drying process, the product is dried to its final moisture content in the spray drying chamber alone. The principle of two stage drying is a combination of spray drying as the first stage drying and fluid bed drying at the second stage. By this innovation it is possible to obtain agglomerated powders in a straight through process and also with advantage regarding product quality and drying economy in the manufacture of non-agglomerated products. Three stage drying or spray drying with integral fluid bed is an extension of the two stage concept by transferring the second drying stage into the spray drying chamber and having the final drying conducted in the third stage located outside the drying chamber (Jensen, 1987; Boersen, 1990; Milanovic and Caric, 1990).

2.4 PROPERTIES OF WHEY POWDERS

The properties of whey powder vary widely depending on the type, composition and various treatments given to whey during concentration and and drying process. An important characteristic of whey powder is to have major portion of lactose as alpha-lactose monohydrate, thus reducing powder hygroscopicity, stickyness and tendency to cake. Another advantage of having crystallized lactose is that the water of crystallization does

not contribute to the water activity of the powder which means that the powder is able to contain more moisture for the same water activity (Labuja and Saltmarch, 1981). This aspect plays a major role as far as the physical properties of the powder and storage stability are concerned.

Jensen (1987) reported that crystallized dried whey has much better physical properties and storage stability than non-crystallized types and the best quality dried whey is produced by double crystallization, i.e., precrystallization of lactose in the concentrated whey combined with post-crystallization in the final product. Initial drying to a high moisture content in the 3-stage systems results in post-crystallization, thus optimizing the non-hygroscopic properties of the dried whey.

Analysis of the dried whey samples obtained from different factories revealed (Mol, 1975a) that the degree of crystallization averaged 54.7 per cent (32.5-67.4%). By exposure to 75 per cent RH, precrystallized powders were virtually noncaking, depending on degree of crystallization whereas non-crystallized samples showed caking into a hard mass. It was observed (Mol, 1975b) that powder particles were damaged (when the samples were examined under microscope) caused by the pneumatic conveying system resulting in increased fines and increased product losses. When whey powders exposed to 75 per cent RH over 1 day at 20°C, amorphous sample absorbed 10.7 per cent moisture, whereas precrystallized powder 0.8 per cent.

The primary factors for the physical properties of powder are dependent on powder material density and content of air inside the particles, and the amount of air between the particles. Occluded air content is affected by air incorporated into feed, centrifugal atomization, pressure nozzles, properties and concentration of the feed, drying

conditions and temperature of drop lets. Control of air between particles depends on particle size distribution, agglomeration and flowability (Pisecky, 1978).

The bulk density values of whey powder as reported by Pisecky and Haugaard Sorensen (1976) vary from 0.55 to 0.70 g/cm^3, wettability from 5-10 sec. and the degree of crystallization 50-75 per cent. In their work, when whey was concentrated to as high as 60 per cent TS, the bulk density of resultant powder ranged from 0.68 to 0.88 g/ml, and the solubility was found to be very low.

German Agricultural Society has prescribed the following standards for whey powders. Moisture: Max. 5.0 per cent for sweet powder and 6.0 per cent for acid whey powder; solubility index: Max.0.5 ml (for spray dried sweet whey powder),not more than 1.2 ml (for spray dried acid whey powder); dispersibility: Min. 93.0 per cent; wettability: Max. 30 sec.

According to the American Standard for rennet whey powder, the product should be of the following characteristics. Solubility index, 1.2 ml max.; scorched particles, 15.00 mg max.; titratable acidity, 0.16 per cent max. and ash alkalanity, 225 ml 0.1 N HCl per 100 g, max.

2.5 CHEMICAL COMPOSITION OF WHEY POWDER

Composition of whey powders varies depending on the type of whey from which it has come, pretreatment given to the whey, and the various processing steps followed in the production.

Analysis of commercially produced sweet and acid type dry whey samples from 12-15 plants of various geographical area revealed the values for lactose, total protein, NPN, total ash and fat for sweet and acid type whey powder were 69.4 and 63.2 per cent, 13.0 and 11.7 per cent, 0.50 and

0.58 per cent, 8.3 and 10.6 per cent and 1.03 and 0.48 per cent, respectively. The moisture content varied from 3.7 to 6.0 (Karl Fischer), 5.08 and 6.2 per cent (Toluene), 3.0 to 3.1 (vacuum oven) for sweet and acid whey powder, respectively. The lysine content was 8.8 and 10.3 g/100 g protein, respectively (Glass and Hedrick, 1977).

Analysis of dried sample of commercial wheys either from single cheese types or blended types revealed (Cerbulis et al., 1972) an average nitrogen content of 2.03 per cent (range 1.82-2.40%) of which 75.2 per cent was nondialysable, giving an average protein content of 9.7 per cent. Average lipid content was about 1 per cent. Ash content of sweet whey powder averaged 8.23 but were 11.3 to 11.5 per cent for the two samples of cottage cheese whey. Lactose represents an average of 71.7 per cent of TS of whey.

In another study, 29 samples of roller dried and spray dried sweet whey sold in Germany were analysed (Rückemann et al., 1973). The water content of samples varied from 1.6 to 1.8 per cent; crude protein, 12.1 to 35.9 per cent; protein digestibility, 93.9 to 100 per cent; available lysine, 0.53 to 1.83 per cent DM or 3.8 to 7.2 per cent of protein; fat, 0.51 to 0.33 per cent and ash, 7.5 to 27.3 per cent. Some of the high values for ash were due to the minerals used for neutralization. Chloride content was 0.88-6.34 per cent or 1.4-10 per cent NaCl, solubility in water 75.8-100 per cent, the pH was 4.4 to 7.1. Lactose content was 32.0-78.1 per cent but the minimum as reported by them should be 60 per cent. It is also reported that pH should be < 7.0 in order to avoid damage to lysine.

Renner (1983) reported that HMF content of milk product is not considered to be a reliable indicator of heat damage to lysine. It was observed that during processing of UHT milk, dried skim milk and spray

dried whey only 2.5-6.4 per cent of available lysine was lost. Available lysine of these products ranged from 6.2 to 11.0 g/100 g protein. Roller dried powder lost about 35 per cent of its available lysine. The average lysine content of instant spray dried fresh samples was found to be 7.4 per cent and the roller dried 3.5 per cent (Huss, 1971).

As per Mair-Waldburg (1974), Ca and P contents were lower in dried whey with pH $\geq$ 5.7 (0.45-0.88% Ca; 0.50-0.70% P) than in dried whey with pH $\leq$ 5.1 (1.38-2.28% Ca; 0.82-1.23% P). Glass and Hedrick (1977) reported the following mineral composition for 100 g dried sweet and acid whey. Ca, 0.88 and 2.40 g; P, 1.1 and 1.59 g; Na, 1.29 and 1.09; K, 1.86 and 1.92 g; Mg, 0.18 and 0.29 g; Zn, 2.1 and 8.1 mg; Fe, 0.9 and 1.3 mg; Cu, 2.8 or 5.3 ppm; I, 6.79 and 8.64 ppm; Pb, 1.15 and 1.68 ppm; Cd, 0.11 and 0.14 ppm and Ag, 0.77 and 0.59 ppm.

2.6 STORAGE OF WHEY POWDER

Whey powders have received increased attention during the last 10 years as inexpensive and nutritious alternative ingredient in many food products. With this increased use, there has arisen a practical need to predict the shelf-life of both whey powders and WPCs.

Non-enzymatic browning via the Maillard reaction is one of the important modes of deterioration in whey powder which limit shelf-life (Saltmarch *et al.*, 1981). Whey powders contain relatively high concentrations of lactose and protein. In the presence of moisture, these components readily participate in the Maillard reaction. This interaction may result in a decrease in protein quality which is accompanied or followed by undesirable colour changes (Mauron, 1981; Ledl and Schleicher, 1990). Maillard reaction increases with increasing temperature in dehydrated

milk products (Ben-Gara and Zimmerman, 1972; Holsinger et al., 1973). In the dried milk product, mainly lactose and the ϵ-amino groups of protein bound lysine are involved in this Maillard reaction or nonenzymatic browning which leads to change in flavour, colour and severe loss in nutritive value (Henle et al., 1991). Maximum loss of lysine and brown pigment are observed when dried milk powder is stored at 40°C (Saltmarch et al., 1981). The rate of reaction is related to the state of the water. An increase in the water content (and thereby the water activity) above the monolayer value acts to dissolve and mobilize reactant species for the Maillard reaction, thereby increasing the reaction rate. As water activity and water content continue to increase further, however, the amount of water present eventually begins to decrease the reaction rate as a result of dilution (Labuja, 1972) and product inhibition.

Kehrber and Johnson (1975) examined rennet whey powders, after packing in polyethylene sachets, glass containers and open containers at room temperature or in cold storage after 30, 60 and 90 days, respectively. After 30 days at 20°C, there was already evidence of browning and reduced protein solubility. Rennet whey powders can be kept for a maximum of 60 to 80 days if intended for use in the foodstuffs industry (especially for pastries). When storing whey powders at 20°C and at a relative humidity of 35 to 40 per cent, the moisture content in the powder should not exceed 5.4 per cent if caking is to be avoided. Kessler (1976) on the other hand, suggested a relative humidity of only 5 to 15 per cent for storage of whey powder at 20°C. If whey powder is to be kept in an acceptable condition over a 3 month period or longer, the storage should be at the lowest possible temperature and under air tight conditions (Bunnies and Timm, 1983).

Available lysine content in dried milk was reduced by 12, 23 and 49 per cent respectively after 24 weeks storage at 4, 20 or 37°C. Equivalent change was found in HMF content. There was an induction period before the samples began to show defective colour changes (Renner and Kaboth, 1982). It was observed (Cheng, 1979) that available lysine decreased from an initial value of 9.84 g/100 g protein to values (after 30 and 90 days) of (i) 9.66 and 9.54 g, (ii) 9.42 and 9.23 g, (iii) 8.56 and 7.81 g, and (iv) 7.62 and 7.10 g/100 g protein, respectively when the freeze dried whey was stored in glass bottles at (i) 4°C, (ii) ambient temperature (Mean 20°C), (iii) 40°C or (iv) uncovered at ambient temperature.

It is reported that during storage of dried foods under the severe conditions of storage, odour and taste are affected more than the colour or consistency. Changes are more marked in samples stored in polyethylene/ paper laminate which is more permeable to O_2 and water vapour than the aluminium laminate. The composition of the product has marked influence on pattern of storage changes (Renner and Rommer, 1976).

2.7 ULTRAFILTRATION

UF is a pressure driven membrane process that can be used in the separation and concentration of substances having molecular weight between 10^3-10^6 daltons. The process is being used for fractionation and concentration of aqueous solution. With the introduction of this technology, it is possible at present to recover the whey proteins in native form. UF is a low energy consuming process and is being widely used in the dairy industry to recover whey proteins. Fractionation of whey into protein rich and lactose containing streams is one of the most successful industrial applications of UF. The economy of operation depends on the flux and rejection characteristics of membrane. Flux can be optimized

by appropriate manipulation of trans-membrane pressure, fluid velocity (recirculation rate) and temperature. Flux during UF as affected by these factors have been studied by many workers (Kuo and Cheryan, 1983; Cheryan and Kuo, 1984; Patel and Reuter, 1985a).

2.7.1 FACTORS AFFECTING THE EFFICIENCY OF ULTRAFILTRATION

The economy of the process is dependent on permeation rate of the membrane. For an efficient operation of UF, maximum flux should be maintained from a given area of membrane. The flux is affected by various operational parameters, such as pressure, flow velocity and temperature of feed. It is also influenced by type, composition, pH and ionic strength of whey. The influence of some of these factors on UF performance is being reviewed here.

2.7.1.1 Temperature of operation

Flux during processing of whey by UF generally increases with increasing temperature of feed. Flux was observed to increase by 3.5 times when temperature of whey was raised from 10°C to 50°C (Boer et al., 1973). It was also reported (Donnelly and Delaney, 1974; Patel and Reuter, 1985a) that permeate flux increases exponentially with an increase in temperature. Almost all the theories of UF predict higher flux with higher temperature due to lower viscosity and higher diffusivity resulting in improved mass transfer (Cheryan, 1977). However, Maubois (1980) observed greater fouling rates at higher temperature with milk which could be related to the fact that calcium salts have a decreased solubility with increase in temperature. Cheryan and Kuo (1984) observed that flux was not significantly different at 30° and 40°C, but flux at 50°C was significantly higher.

2.7.1.2 Operational pressure

According to concentration polarization theory, the flux increases with the increase of applied pressure until the gel formed reaches a concentration limit where flux becomes independent of pressure. Further increase in applied pressure results in a temporary increase in flux. However, the pressure increases the driving force for UF but does not affect transport of solutes back into the bulk stream. Consequently, a thicker and denser gel layer is formed which reduces flux until it reaches its initial steady state. Increasing pressure over a critical point results in lower flux due to the compaction of the gel layer formed and the increased hydraulic resistance (Glover, 1985; Cheryan, 1986; Renner and Abd El-Salam, 1991). Although at some stage pressure independence occurs, higher velocities make it profitable to use higher pressures and obtain higher fluxes. However, increasing flow velocities is more beneficial than increasing pressure (Glover, 1985). Donnelly and Delaney (1974) observed that inlet pressure of 0.5 kg/cm^2 and outlet pressure of 1.4 kg/cm^2 were optimum when cheese whey was ultrafiltered and it was undesirable operating at more than 4 kg/cm^2. However, the pressure to be used varies with the type of module and membrane configuration. At high pressures, high flow rate increases rate of fouling. After prolonged operating times, neither flow rate nor pressure appeared to have a significant effect, perhaps due to a change from surface fouling to a pre-deposition phenomenon (Kuo and Cheryan, 1983).

2.7.1.3 Flow velocity

In general, higher flow rate reduces the rate of fouling. But, there is a significant interaction between flow rate and trans-membrane pressure effects on fouling. Flux declines at relatively low trans-membrane

pressure of 240 KPa (35 psig). In general, flux improvement was observed at higher flow rates, at least in the initial stage. The same relative improvement with flow rate was observed at an intermediate trans-membrane pressure of 310 KPa (45 psig). However, in contrast to these pressures, at the highest trans-membrane pressure studied 485 KPa (70 psig), high flow rate did not benefit flux. In fact, high flow rate might actually have aggravated fouling (Kuo and Cheryan, 1983). Generally, higher shear rates at the membrane surface are very important factor in combating membrane fouling. At higher sear rates deposited materials are continuously removed and reduces the hydraulic resistance of the fouling layer (Cheryan, 1986; Renner and Abd El-Salam, 1991). Increasing flow velocity is more beneficial than increasing pressure. The action is to assist dispersion of polarized layer (Glover, 1985). It is reported (Daufin et al., 1992) that no marked limiting flux could be observed, particularly over a sufficient tangential flow rate (4.5 or 5.5 $m.s^{-1}$), so that membrane could operate at higher fluxes.

2.7.1.4 Type of feed and pretreatments

The flux during UF of whey widely varies with the composition, pH, ionic strength and the concentration and type of protein and also concentration of various inorganic materials in whey, as the chemical and physical state of whey components and their environment are significant factors affecting fouling and concentration polarization of UF membranes. Merin and Daufin (1990) pointed out that calcium ions, fat residues, casein fines and bacteria are obstacles to improve UF flux and quality of the WPC produced. The flux rate is different for different kinds of whey. The pretreatments such as alteration in pH, preheat treatment, decalcification, clarification, filtration, microfiltration may improve the flux

to a great extent (Muller and Harper, 1979; Merin and Cheryan,1980; Hiddink et al., 1981; Kessler et al., 1982; Kuo and Cheryan, 1983; Fauquant et al., 1985). The membrane and solute interactions contribute significantly to flux reduction, primarily by precipitation of calcium phosphate complexes and adsorption of proteins that result in narrowing of membrane pores (Hanemaaijer et al., 1989). Many pretreatments aimed at reducing such interactions have improved UF performance (Patocka and Jele.., 1987a). The extent of flux decline increases with the level of calcium and phosphorus deposition, precipitation of proteins by CMC, EDTA, chelation of calcium and reduction of 'Ca' by ion exchange during UF process of acid whey (Henz and Glatz, 1991).

During UF of Gouda cheese whey, decalcification and clarification were found to give higher flux rates (Hiddink et al., 1981). This is mainly because of removal of suspended casein fines during clarification which are considered to be one of the main foulants present in the whey (Marshall et al., 1974; Patocka and Jelen, 1987b), and removal of calcium by decalcification which is also one of the main foulant (Hayes et al., 1974; Delaney and Donnelly, 1975; Lee and Merson, 1976; Ennis et al., 1981). It is also reported that removal of lipids from whey enhances the flux rate to a great extent (Fauquant et al., 1985).

However, decalcification process is not generally employed. Instead the calcium present is rendered soluble by lowering pH. Calcium is present in whey in two forms, a permeable and impermeable fraction. The latter is present as colloidal phosphate and attached to beta-lactoglobulin of whey (Renner and Abd El-Salam, 1991). As pH is lowered the calcium phosphate becomes more soluble and soluble calcium is more likely to permeate freely through the membrane. At high pH, on the other hand, calcium phosphate is

in the insoluble colloidal form, which will probably precipitate out and deposit on the membrane under the hydraulic pressure, bulk movement of fluid to the membrane occurring in the module (Cheryan and Merin, 1980, 1981). It is observed that pH values $\lesssim$ 3.0, HCl casein and Cheddar cheese wheys gave higher flux rates than at their normal pH values (Muller et al., 1973). Similarly, when pH of Gouda whey was lowered to 3.0, the flux rate improved from 70 $l/m^2/h$ at pH 6.6 to 150 $l/m^2/h$ (Hayes et al., 1974). Increasing pH of whey may have little improving effect as with Cheddar cheese whey or may not have any effect as with HCl casein whey (Muller et al., 1973). However, increased pH and heat treatment together have a remarkably increasing effect. As reported by Hiddink et al. (1981), increasing the pH to about 7.5 and heating whey enhances the flux rate.

Moreover, changes in pH also affect the dispersion of the protein. The protein carries a higher electrical charge, when the pH is away from its isoelectric point. With higher charges, the dispersion of proteins will be greater, reducing the concentration polarization and increasing the flux (Glover, 1985).

Flux rate of HCl casein whey can be doubled by heating it at 80°C for 15 sec. and then adjusting pH to optimum between 5.2 and 5.9 (Hayes et al., 1974). Fifty per cent increase in flux can be obtained for cheddar cheese whey by heating at 85°C for 15 sec. at pH 6.0 or by reducing pH to about 5.2 heating at 80°C for 15 sec. and then increasing pH to an optimum of 5.9 (Hayes et al., 1974). Flux of cottage cheese whey can be enhanced by 30 per cent by heating whey at 95°C/15 min. after adjusting pH to 6.5. These preheat treatments minimize the fouling by causing agglomeration of a complex of casein and beta-lactoglobulin and by avoiding the formation of apatite gels. The manner in which the permeation rate changed with pH

and heating can be explained largely by the effects of calcium on the interactions and(or) self aggregation of beta-lactoglobulin (Roeper, 1970; Townend and Gyuricsek, 1974). Below pH 5.6, calcium ions do not appear to affect the permeation rate and the protein is either electrically charged or not sufficiently denatured to expose the necessary relative sites for the formation of aggregates large enough to reduce fouling or increase the permeability of the fouling layer. Above pH 5.6, the reaction sites are exposed, the net charge on the protein is minimal and calcium dependent interactions can now take place and permeation rates are increased (Hickey et al., 1980).

As reported by Hickey et al. (1980), when the calcium content is higher in a given whey, adjusting pH to above 6.5 followed by heating increased the flux to a great extent. Heat treatment preceded by pH adjustment to about 7.0 with added calcium also enhances the flux (Hayes et al., 1974).

2.7.2 RETENTION AND PERMEATION OF CONSTITUENTS DURING ULTRAFILTRATION

The retention or the rejection of various constituents during UF is influenced by various operational parameters as well as processing conditions. Experiments with membranes of different permeability and lactose retention showed that it is possible to concentrate whey to about 24 per cent solids (Khell-Wicklein, 1973). It was observed that maximum theoretical protein that could be achieved by UF of whey is about 20 per cent (80% on DM basis). However, because of very low flux rates at high protein concentration the practical limit is probably about 60 per cent protein (Donnelly and Delaney, 1974), a small amount of protein about 10 per cent passes through membrane. The newest development in the area concerned is processing of WPC with very high protein level

(upto 80%) and low fat content. The 80 per cent WPC product is already being made by a number of processors using UF (Mans, 1992). To reduce fat content in WPC, the European process uses microfiltration to remove fat from the whey before it is concentrated by UF.

In UF process generally most of the protein is retained and most of the lactose, ash and NPN pass through permeate depending on operational parameters and cut off value of membrane. The rejection of protein varies with the degree of volume reduction during the process. At the initial stages protein retention is less but as the concentration increases, there is an increase in the retention of protein. At 1.0 per cent protein level in the feed the retention coefficient is 0.972, but as the concentration of protein in retentate increases to 10 per cent, the retention coefficient also increases to 0.99 (Ennis, 1982). However, at higher concentration of protein in retentate, the loss of protein in permeate is reported to be high. The protein rejection is related to fouling type and operating condition. Hence, a compromise solution between high fluxes and high protein rejection is suggested to limit protein losses since the membrane is only partly retentive for whey proteins (Taddei et al., 1988). As reported by many workers (Khorshid, 1974; Mylius and Saier, 1974; Ceeland and Robinson, 1982), the retention of protein ranges from 95 to 99 per cent. In large experiments it was shown that (Mylius and Saier, 1974) the protein retention fell from an initial 90 per cent to about 73 per cent at the end of 1:30 concentration. In the UF-whey retentate, the content of alpha-lactalbumin reduces as compared with the initial whey, indicating that UF membranes are slightly permeable for alpha-lactalbumin (Tratnik and Krsev, 1991). The protein lost in permeate is about 0.25 g/l and 90 per cent of it is alpha-lactalbumin, proteose peptone and traces of beta-lactoglobulin are also reported in permeate (Khorshid, 1974; Matthews

et al., 1976; Barbano et al., 1988). As observed with rennet whey, the selectivity for alpha-lactalbumin, beta-lactoglobulin and immunoglobulin was found to be 70, 95 and 100, respectively (Boer et al., 1973). Whey nitrogen distribution of undenatured whey protein, proteose peptone and NPN was reported to change from an initial value of 9.0, 17.2 and 33.8 to 82.5, 9.8 and 7.7 per cent, respectively in the final retentate (Matthews et al., 1976).

As regards NPN, its retention increases with the concentration of retentate upto a certain volume reduction (deWit and Boer, 1975). It ranges from 12 to 18 per cent (Khorshid, 1974). The retention coefficients of NPN compounds in the initial stages are reported to be 0.34 and 0.39.

The rate of permeation of lactose is higher in initial stages, but at 50-95 per cent volume reduction, there is a decreasing trend of permeation. At higher level of volume reduction, the permeation rate again increases (deWit and Boer, 1975). Retention coefficient of lactose is found to be 0.178 (Ceeland and Robinson, 1982). According to Mylius and Saier (1974), the retentions at 1.0 and 10.0 per cent protein levels are 0.110 and 0.124 respectively. In actual terms, the lactose retention varies from 11.3 to 21.8 per cent (Khorshid, 1974). However, as low as 2.8 or 3.0 per cent retention values are also reported (Boer et al., 1973).

The retention coefficient of minerals is found to be significantly less than other components of whey. With the change in volume reduction, the behaviour of minerals with regard to retention is similar to that of other components (deWit and Boer, 1975). The retention coefficient of ash is reported to be 0.092 (deWit and Boer, 1975). But actually 3.4 to 13 per cent of total ash is retained during UF (Khorshid, 1974). It was observed that preheating of whey and UF temperature treatments affect

the constituents of UF retentate. The effect was most pronounced on calcium content. The preheating treatment of 68-72°C for 30-40 min before ultrafiltering whey at 50°C increased calcium/TS ratios of concentrate considerably and concentrates produced at higher UF temperatures had higher calcium/TS levels (Gupta and Reuter, 1987). Mircmev and Ivanov (1980) reported retention values for sodium, potassium, calcium and phosphorus as 9.6, 8.38, 12.32 and 3.4 per cent, respectively. When whey was ultrafiltered to have 19-20 per cent TS and 62-63 per cent protein on DM, they have reported the mean values for whey, permeate and retentate as 8.48, 9.06 and 3.99 per cent for ash; 0.876, 1.100 and 0.429 per cent for Na, 2.269, 2.573 and 0.964 for K, 0.720, 0.713 and 0.446 for Ca and 0.673, 0.437 and 0.290 per cent for P respectively.

UF permeate composition has been given by many workers. Its composition varies with type of whey, pH and preheat treatment given to whey and the operational conditions of UF. Average composition of sweet whey and acid whey permeate with respect to TS, lactose, ash, protein and lactic acid were found to be 5.8, 4.9 and 4.1; 0.5 and 0.7, 0.3 and 0.4, and 0.15 and 0.5 respectively (Sanderson and Reed, 1985; Modler, 1987; Zall, 1987).

2.7.3 DIAFILTRATION

Diafiltration (DF) is a process where UF retentate is diluted with water and reultrafiltered to effect further fractionation (Renner and Abd El-Salam, 1991). It means that water is added to the retentate thereby the viscosity is reduced, and the concentration of lactose, ash and NPN is decreased by further UF (Zall, 1982; Vuillemard *et al.*, 1989).

By UF varying of protein:lactose ratio can be obtained. This may range from 1:8 (raw whey) to 3:5 (a skim milk equivalent) to 2:1 or higher.

By 2 or 3 DF, a product with a protein lactose ratio of 20:1 could be obtained (Fenton-May et al., 1971). A maximum of 83.20 per cent protein (on DM basis) was obtained when whey was ultrafiltered followed by two step DF (Tratnik and Krsev, 1991) and the lactose was reduced to a minimum of 4.40 per cent DM. The exact protein to lactose ratio in the concentrate stream is a function of permeability and selectivity characteristics of the membrane and the system design and operating conditions. For the manufacture of end products with high protein and low ash contents UF at pH 6.6 followed by DF at pH 3 to 3.5 is recommended (Hiddink et al., 1978). DF and repeated UF of whey results in removal of largest quantity of K, Na and Mg and whereas P to a lesser extent (Tratnik and Krsev, 1991). The retention of 'Ca' appears to be highest and is dependent on pH of whey (Derham and Chanton, 1986; deWit et al., 1986). Copper becomes ultrafiltrable at pH 1.5-2.0. It was observed (Chojnowski et al., 1979) dried retentate (only by UF) had 53 per cent protein and 38 per cent lactose, while diafiltered dried retentate had 87 per cent protein and 6.7 per cent lactose and showed high solubility and stabilizing and fat absorption capacity. In order to achieve higher protein values (upto 90% of DM), one or more DF steps may be followed.

2.7.4 COMPOSITION OF WHEY PROTEIN CONCENTRATE

WPC represents a protein source with considerable potentials for blending with varieties of food proteins to improve their nutritional and functional properties (Morr, 1976) and these are defined by the U.S. Food and Drug Administration as follows: WPC is the substance obtained by the removal of sufficient non-protein constituents from whey so that the finished dry product contains not less than 25 per cent protein (Renner and Abd El-Salam, 1991). The average contents of lactose, ash and fat in

WPCs with a protein content of 30 to 85 per cent vary significantly (Towler, 1982; Schmidt et al., 1986; Marshall and Harper, 1988; Gupta and Reuter, 1992). The extent of variation of protein, fat, lactose, ash and moisture is found to be 30-80, 3-8, 5-55, 4-12 and 2-16 per cent respectively (Engl, 1982). In the US, the minimum reqirements for the composition of WPCs are fixed as follows: protein min. 25 per cent, fat 0.2-10 per cent, ash 2-15 per cent, lactose max. 60 per cent and moisture 1-6 per cent.

UF followed by DF and spray drying WPCs with minimum denaturation and more than 80 per cent of proteins can be attained in the dried product (Kinsella, 1985). By 95 per cent volume reduction of cottage or cheddar cheese whey by UF, it is possible to attain 18 per cent TS, which comprises of 55 per cent protein, 32 per cent lactose and 4 per cent ash on DM basis (McDonough et al., 1974). The maximum dry matter that can be obtained by UF is about 25 per cent which permits a protein content of 60 to 65 per cent in the powdered product (Lehmann, 1987).

At a protein level of 34 per cent of TS, WPC has an approximate composition equal to non-fat dry milk; difference lie in mineral profile of the ash content and in type of protein (Zall, 1982). If, for example, a 35 per cent protein powder is wanted, sweet whey with 6 per cent TS is ultrafiltered up to a dry matter content of 10 per cent, the nitrogen substances consist of 3.2 per cent protein and 0.3 per cent NPN. By subsequent evaporation and spray drying (upto 96% DM) a 35 per cent protein powder is obtained (Renner and Abd El-Salam, 1991). It was observed (Tratnik and Krsev, 1991) that increasing the protein content to a maximum of 35.96 per cent (on DM), the lactose and ash reduces to a minimum of 51.59 per cent and 5.4 per cent respectively. When protein content

increases to a maximum of 54.19 per cent TS, lactose and ash reduces to a minimum of 32.81 per cent TS and 4.7 per cent TS respectively. By two stage DF, protein content increased to a maximum of 75.65 per cent and 83.20 per cent and lactose decreased to a minimum of 10.30 per cent TS and 4.40 per cent TS respectively.

As the concentration of UF proceeds, the minerals gradually represent the small portion of TS. The amino acid content of WPC increased 5 fold, corresponding to the increase in the protein content, the vitamins content slightly higher in the WPC than in the whey. Similar amino acid pattern observed in whey and WPC (McDonough et al., 1974). During UF process, decreasing lactose and ash concentrations and the increasing fat content with increasing protein content became apparent with the varying protein-lactose/ash ratio (Delaney and Donnelly, 1975); the ratio in whey of about 1.0:5.3:0.72 changes to a 1.0:0.06:0.04 ratio in the WPC containing 80 per cent protein. As the percentage of NPN of total nitrogen is about 10 per cent in WPC containing 40 per cent protein which will decrease to about 6 per cent in the 70 per cent protein WPC (Renner and Abd El-Salam, 1991).

The process of concentration and drying of whey had no significant effect on protein solubility. However, pasteurization (at 78.2°C for 15 sec. or 62.4°C for 30 min) results in 20 per cent denaturation of WPC proteins. The extent of denaturation of WPC protein ranged from 14.3 to 21.67 per cent when UF concentrate having 9.2 per cent or 13.3 per cent protein was (23.5% TS and 20.5% TS) spray dried at air inlet temperature of 170°, 190° and 210°C (Delaney et al., 1973). It was observed that increasing outlet temperature of spray drier from 75-80°C to 120°C increased the protein reducing substance value (an index of non-enzymatic

browning) and reduced available lysine, especially at high lactose/protein (L/P) ratio, 1.36. The quality of spray dried powder at low outlet temperature and/or L/P ratio (0.28) resembled freeze dried product. The nitrogen solubility index was very high for all products irrespective of outlet temperature or composition (Autelli, 1974).

For manufacture of dried whey protein concentrate, the retentate obtained by UF can be directly spray dried. However, it is economical to further concentrate the UF retentate to have a protein content of about 20 per cent which helps in increasing the capacity of spray drier. The temperature of evaporation should be kept around 40°C to avoid deleterious effect on the quality of the product (Marshall and Harper, 1988).

2.7.5 FUNCTIONAL PROPERTIES OF WHEY PROTEIN CONCENTRATES

WPC may be produced with a very wide range of functional properties. It is this characteristic that can increase the attractiveness of WPC as an ingredient as functionality may be tailor-made to specific requirements (Zadow, 1986). Functional properties have been defined as those physical and chemical properties which affect the behaviour of proteins in food systems during processing, storage, preparation and consumption. An alternate definition is: any property of a food or food ingredient except its nutritional ones that affects its utilization (Kilara, 1984; Mangino, 1984). The functional properties of proteins as listed by West (1984) are divided into (i) major properties - solubility, emulsification, foaming, heat setting,fat binding, water holding, blandness; and (ii) minor properties - viscosity, dough formation, adhesion, texturization, browning, moisture retention (on storage). Some other functional properties mentioned by Evans (1980) are - whippability, colour, flavour, water activity, fibre formation, rheological properties and gel formation.

Proteins represent a most important class of functional ingredients because they possess a range of dynamic functional properties (Kinsella, 1989; Morr and Foegeding, 1990).

2.7.5.1 Solubility

Although solubility is itself a functional property of proteins, it is also a pre-requisite for other functional properties such as their foaming, emulsifying and gelling abilities (deWit, 1984; Nakai and Li-chan, 1985). Complete solubility of WPCs is necessary for optimum functionality in foams, emulsion, beverages and similar applications.

Protein solubility of 10 per cent protein dispersions of WPCs ranges from 90 to 100 per cent (Schmidt et al., 1986). The high degree of solubility of WPCs in acidic food products is one major advantage over casein products. Casein, caseinates, lactalbumin and co-precipitates, which are isoelectrically precipitated, exhibit minimal solubility and functionality in food products that have low pH or contain Ca ions (Morr, 1984).

Heat treatment has the strongest influence on the solubility of WPCs. Whilst pasteurization of whey does not have a significant effect upon WPC solubility; heating UF retentate causes a significant reduction (Morr, 1985,1987). Protein solubility of 10 per cent protein dispersions of WPCs was not affected by heating at 65°C for 30 min (Schmidt et al., 1986), but pasteurization of a retentate at 72°C for 15 sec. was found to decrease solubility (Mangino et al., 1987). Also a partial heat denaturation, e.g., caused by heat treatments during the production of WPC or in the processing of food products, makes protein solubility much more sensitive to the effects of pH and salts (deWit, 1984).

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WPCs have a good emulsifying capacity which has proved useful in emulsifying the oils present in many food systems (Hood, 1985). WPC has higher emulsification capacity than non-fat dry milk (Melachouris, 1984).

Reports on factors affecting the emulsifying properties of WPC are conflicting, and often reflect the differing systems used for examination of this characteristic (Hugunin, 1987). In general, conditions affecting emulsifying properties are similar to those controlling foaming and whipping, and are affected by the system used for emulsification, the energy input, the ionic strength of the environment and the type of WPC employed (Zadow, 1986). Excessive heat treatment, however, has a detrimental effect on the emulsification possibly due to interactions between the exposed hydrophobic groups (Hugunin, 1984). Also, Mangino et al. (1987) suggested that denaturation has a negative effect on the emulsifying properties of WPCs, but that under certain conditions, heating can increase the emulsion capacity.

Lipids and phospholipids are generally considered to be detrimental to the functionality of WPCs and whey protein isolates, but they are able to improve the emulsifying ability (Modler and Jones, 1987). Pretreatment of whey before UF (cooling to 0-5°C, adding $CaCl_2$, adjusting to pH 7.3, warming to 50°C, and removing insoluble precipitates) gives less functionality for emulsification, as lipids are removed to a larger extent by such a pretreatment (Kim et al., 1989). Patel and Kilara (1990b) observed that emulsifying capacity is negatively correlated with free fat content. Emulsion stability of WPC is negatively correlated with bound fat and negatively correlated with protein content, denaturation enthalpy, and

composition of WPC lipids pasteurization appeared to have the greatest effect, resulting in a reduction in lipid content, particularly in the neutral lipid fraction. These treatments have been reported to affect certain functional properties which could be related to the observed changes in the lipid components (Williams and Versteeg, 1991).

2.7.5.3 Foaming and whipping properties

Foaming may be defined as the creation and stabilization of gas (air) bubbles in a liquid. Factors essential for the formation of protein based foams are: a rapid diffusion of proteins to the air-water interface to reduce surface tension, followed by partial unfolding of the protein. This will result in the encapsulation of air bubbles and in the association of protein molecules, lead to an intermolecular cohesive film with a certain degree of elasticity (deWit et al., 1988).

Numerous investigations show that whey protein which has not been denatured has good whipping and foaming properties, second only to egg white provided the fat content is low, but compared with egg white whey protein requires a longer period of whipping in order tc give a stable foam (Evans, 1980; Bech, 1981).

Morr (1979) reported that the whipping properties of UF WPCs showed considerable variation: overrun 460-900 per cent (Mean 653%); whipping time 0.5-15 min (Mean 7.8 min) and drainage 10-36 m (Mean 23 m). Patel and Kilara (1990b) observed foaming capacity of WPCs to be varying from 330.0 to 648 per cent prepared from the whey obtained from different sources. They have observed that foaming capacity is negatively correlated with

whipping and foaming properties of whey protein products are affected by a number of compositional and processing variables that include: pH, calcium ion concentration, ash content, redox potential, residual lipid content, TS content, sulphydrals, protein solubility, heat treatments, fractionation technology, drying conditions, and the use of additives (Morr, 1982).

The presence of 1.5 per cent or more milk fat in WPCs effectively suppresses whipping and foaming properties. Since fat is concentrated by UF, high fat contents are common in WPC if whey has not been passed through a separator before UF (Hugunin, 1987). The removal of fat or a substantial reduction in the neutral and phospholipid content of WPC improves whipping ability and foam stability (Modler and Jones, 1987). Residual lipids may complex with the proteins, thus altering their exposure of hydrophobic and hydrophilic groups in the foam interface (Morr, 1976; Morr and Foegeding, 1990).

HTST pasteurization (72°C/15 sec) of UF retentate has no significant effect on maximum foam expansion of WPC dispersions (Morr, 1987). It has to be emphasized that a controlled denaturation by moderate heating of WPC solutions, prior to foam formation results in improved whipping and foaming properties (Schmidt et al., 1984). Maximum values are obtained with heat treatment of 55-60°C (30 min), thereby the maximum overrun could be increased from 800 to 1275 per cent (in comparison to egg white which has an overrun of about 900).

2.7.6 STORAGE STABILITY OF DRIED WHEY PROTEIN CONCENTRATE

Although WPC products generally exhibit a bland flavour immediately after drying, they develop typically stale flavour during storage due to a set of complex, interrelated chemical reactions which include lipid

oxidation and Maillard browning (Hammond, 1989). The off-flavours developed in WPCs during storage greatly limit their use in many bland food products (Hugunin, 1987). Factors that may have impact on the development of off-flavours in WPC include processing treatments which affect the composition of the whey and WPC, drying conditions which control moisture content of the WPC and initiate lipid oxidation and nonenzymatic browning reaction, and moisture and temperature conditions which affect the kinetics of the lipid oxidation and browning reactions during WPC storage (Morr, 1979; Morr and Foegeding, 1990). Although numerous factors may be responsible for the formation of off-flavours in stored whey and WPC, two major factors are usually involved, i.e., lipid oxidation and Maillard browning reaction (Ferretti and Flanagan, 1972; Min et al., 1990).

The Maillard browning reaction, which involves interaction of protein and lactose as its initial step, is believed to be important in the formation of stale flavours in dried milk and whey products (Morr and Richter, 1988). Aldehydes produced by the Strecker degradation of the amino acids undergo condensation and a series of further reaction to form furfurals, dehydration products and polymers that result in the brown pigments plus off-flavours (Hodge et al., 1972; Hammond, 1989). The amount of CO_2 formed, which is also a major product of the Strecker degradation of amino acids correlated to the intensity of browning (Min et al., 1990).

The factors affecting browning reaction rate in dried food products include temperature, water activity, pH and availability of reactants (O'Brien and Morrissey, 1989; Labuja and Saltmarch, 1981). The rate of the reaction at low temperature is quite low. However, it is the predominant reaction at temperature of above 35°C (Labuja and Saltmarch, 1981).

WPCs contain sufficient residual lactose, lipids, phospholipids, lipoprotein and copper ions and other prooxidants to render them

susceptible to lipid oxidation and Maillard browning reaction that result in development of aged, stale flavours during storage (Hugunin, 1987; Morr and Foegeding, 1990). The high contents of lactose and protein (high in lysine) in WPC are especially conducive to Maillard reaction which may result in undesirable changes in visual, organoleptic, nutritional and functional characteristics (Schneider and Fennema, 1989).

Li-chan (1983) observed that when WPC powder having 35 per cent protein was stored at 37°C and 75 per cent RH, after 42 days of storage, the soluble protein (at pH 4.6) decreased only slightly (14% loss) and no significant changes in sulphydryl or disulphide contents were noticed. HMF increased dramatically from 13 to 192 umol/100 g. Free lactose and dinitrobenzone sulphonate available and pepsin pancreatic digestible lysine content also decreased (17, 34, 57 and 72% losses respectively), and samples were darker after storage. Polyacrylamide gel electrophoresis revealed that upon storage, inter-molecular association between alpha-lactalbumin and beta-lactoglobulin molecules increased greatly, resulting in high molecular weight aggregates.

Changes in the functionality of dry WPC containing 52 per cent protein during storage (6 months at temperatures ranging from -40 to 40°C and water activities ranging from 0.15 to 0.41) have been investigated by Hsu and Fennema (1989), of the product attributes studied (protein solubility, foam stability, emulsifying capacity, browning) increase in browning was most important, it was concluded by them that the storage temperature should be no higher than 20°C and the water activity should not exceed 0.20.

CHAPTER 3

SCOPE AND PLAN OF WORK

3. SCOPE AND PLAN OF WORK

3.1 SCOPE

Growing demand for cheese and swelling industrial production of casein together with rising market for traditional dairy products, such as paneer, chhana and shrikhand, have generated enormous quantity of whey. Over a half of precious nutrients from milk appear in this by-product and yet it is drained into sewage non-chalantly. Nutritional importance of whey is generally not well appreciated as it is treated by many as just "waste" from milk. This attitude apart from causing colossal losses of whey solids is resulted in environmental vexation. The government naturally is compelled to put an end to this nuisance by imposing strict laws pertaining to drainage of industrial wastes. The industry is at a loss over the utilization of whey because not a single economically viable method of its usage is available at present.

The need of high quality proteins to combat protein calorie malnutrition and the prevailing stringent environmental pollution act emphasizes the necessity to utilize the whey. However, a major hindrance in the recovery of whey solids is the amount of energy involved in the process. A conventional way of evaporation and drying cannot be recommended to our dairy industry which is already ridden with economic problems. In this context, membrane processing may be a boon to us; it aids in removing considerable amount of water from whey with less energy inputs. It is obvious, therefore, that there is an urgent need to work out a commecially viable solution to utilize whey in a profitable manner.

This project envisages to develop a commercially viable solution for the utilization of indigenously available whey in the form of whey powder and WPC. By optimizing the processing conditions of membrane process and subsequent evaporation and drying, whey powder and WPC could be produced in an efficient and economic way. Optimizing the processing conditions of concentration of whey by membrane technology will pave a way for the successful adoption of this technology in the dairy industry of our country.

Today, the food industry is looking for ingredients which can provide good functional and nutritional properties in various food formulations. Whey powder and WPC will certainly provide good functional properties even if added in small quantities besides providing nutritional quality. Hence, there will be a good demand for whey powder and WPC from bakers, confectioners, and the manufacturers of beverages, soups, sauces, gravies, frozen desserts, yoghurt, cheese, cheese spreads, infant foods and from the manufacturers of indigenous dairy products. This aspect is of great importance for the economic development of the dairy industry. With the introduction of whey powder and WPC, the industry can certainly be benefited to a large extent.

3.2 OBJECTIVES

The proposed project was undertaken with the following objectives:

(a) To standardize processing parameters of reverse osmosis for the concentration of paneer and cheese wheys for the production of whey powder.

(b) To study the pattern of fouling of reverse osmosis membrane during during processing of whey in the context of cleaning schedule.

(c) To optimize the processing conditions for the manufacture of spray dried whey powder using reverse osmosis concentrate.

(d) To characterize physico-chemical and shelf-life attributes of spray dried whey powder.

(e) To optimize the processing conditions for the manufacture of spray dried whey protein concentrate by using ultrafiltration.

3.3 PLAN OF WORK

To accomplish the above mentioned objectives, the following approaches have been followed.

3.3.1 STANDARDIZATION OF PROCESSING PARAMETERS FOR THE MANUFACTURE OF WHEY POWDER

3.3.1.1 Analysis of whey

Paneer whey (PW), cow milk cheddar cheese whey (CW) and buffalo milk cheddar cheese whey (BW) procured from the Experimental Dairy Plant of National Dairy Research Institute were analysed for

a) pH, acidity, specific gravity and viscosity, and

b) Total solids, protein, non-protein nitrogen, lactose, ash, fat, calcium and phosphorus.

3.3.1.2 Concentration of whey by reverse osmosis

Processing conditions with respect to the following were standardized:

(i) Effect of clarification

(a) With clarification, and (b) without clarification.

(ii) Effect of operational pressure

(a), 25, (b) 30, (c) 35 and (d) 40 bar.

(iii) Effect of temperature of operation

(a) 40°C, and (b) 50°C.

(iv) Effect of pH and preheat treatment

(a) pH adjustments of whey

3,5, 4.5, 5.6, 6.4 (6.3 in case of CW), and 7.2

(b) Heat treatments

60°C/30 min, 70°C/15 sec and 80°C/15 sec.

3.3.1.3 Fouling and deposit formation

The fouling and deposit formation of RO membrane were studied with respect to the following:

i) Effect of various pH adjustments and preheating of different types of whey on the fouling pattern of RO membrane.

ii) The deposits formed on the membrane with the above processing conditions were separated and analysed for total mass, protein, ash, calcium and phosphorus.

3.3.1.4 Cleaning schedule for all the types of whey was optimized with respect to (a) temperature, and (b) duration for cleaning.

3.3.1.5 RO concentrate and permeate were analysed for total solids, protein, non-protein nitrogen, lactose, ash, fat, calcium and phosphorus.

3.3.1.6 Vacuum concentration

Concentrate obtained by RO was further concentrated in laboratory vacuum evaporator to different levels of TS, viz., 40, 45 and 50 per cent.

3.3.1.7 Precrystallization

Whey concentrated to above TS were flash cooled to 30°C and pre-crystallization process was standardized with respect to the following:

i) Levels of seeding (alpha-lactose monohydrate) material

(a) 0.00, (b) 0.001, (c) 0.03, (d) 0.05, and (e) 0.07 per cent.

ii) Duration of seeding

1 to 6 h.

iii) Effect of seeding material and duration on

(a) Extent of lactose crystallization, and (b) size distribution of lactose crystals.

3.3.1.7.1 Precrystallized concentrate was analysed for (a) specific gravity, (b) viscosity, and (c) size distribution of lactose crystals.

3.3.1.8 Spray drying

For large scale production, 800 l of whey was concentrated by RO followed by vacuum evanporation (50% TS) and precrystallized. The precrystallized concentrate was spray dried by adjusting inlet and outlet temperatures so as to obtain 3 to 4 per cent moisture in the final product.

3.3.1.9 Analysis of whey powder

Spray dried paneer whey powder (PWP), cow milk cheddar cheese whey powder (CWP) and buffalo milk cheddar cheese whey powder (BWP) were analysed for various physical and chemical characteristics along with the commercial whey powder procured from Germany.

(i) Physical characteristics

Solubility index, dispersibility, wettability, flowability, bulk density and sinkability.

(ii) Chemical characteristics

Moisture, lactose, protein, non-protein nitrogen, whey protein nitrogen, proteose peptone, ash, calcium, phosphorus, potassium, magnesium, sodium, lysine and extent of denaturation.

3.3.1.10 Storage studies of whey powder

Paneer whey powder, CWP and BWP were packed in polyethylene and metallized polyester laminates and stored at 20, 30 and 40°C temperature at a relative humidity of 60 ± 2 per cent. Similarly, commercial whey powder, Germany (WP-G) was also stored at these conditions. Changes occurring during storage with respect to (a) lysine content, (b) extent of denaturation, (c) colour, and (d) solubility were studied.

3.3.1.11 Energy requirements

Energy required for concentrating 1,000 l of whey by conventional evaporator to 50 per cent TS was calculated and compared with concentration of whey by RO followed by conventional evaporator to 50 per cent TS. The net energy savings were computed.

3.3.2 OPTIMIZATION OF PROCESS FOR THE PRODUCTION OF WHEY PROTEIN CONCENTRATE USING ULTRAFILTRATION

Buffalo milk cheddar cheese whey was used for the production of WPC.

3.3.2.1 Processing conditions of UF with respect to the following were optimized.

(i) Temperature of operation

(a) 40° and (b) 50°C.

(ii) pH of whey

(a) 3.0, (b) 4.5, (c) 5.6, (d) 6.4 and (e) 7.2.

(iii) Preheating temperature

(a) 60°C/30 min, and (b) 80°C/15 sec.

3.3.2.2 Effect of different levels of UF concentration of BW on the retention of following constituents were determined:

Protein, non-protein nitrogen, lactose, ash, calcium and phosphorus

3.3.2.3 Effect of diafiltration I and II on the following was studied:

i) Protein/TS ratio
ii) Protein/lactose ratio
iii) Protein/ash ratio
iv) Protein/NPN ratio

3.3.2.4 Spray drying

Ultrafiltered whey concentrate was spray dried to have 3 to 4 per cent moisture content in the end product.

3.3.2.5 Analysis

Buffalo milk cheddar cheese whey protein concentrate and commercial dried WPCs (Germany) were analysed for various physical, functional and chemical characteristics.

(i) Physical characteristics

Solubility index, dispersibility, wettability, bulk density and sinkability.

(ii) Functional characteristics

Solubility, emulsifying capacity, foaming capacity, and foam stability.

(iii) Chemical characteristics

Moisture, protein, non-protein nitrogen, whey protein nitrogen, proteose peptone, ash, minerals (calcium, phosphorus, potassium, magnesium and sodium), lysine, and extent of denaturation.

3.3.2.6 Storage studies

All the spray dried WPCs were packed in two types of packaging material and stored at 20, 30 and 40°C and relative humidity of 60 ± 2 per cent. The changes occurring during storage with respect to the following were measured.

(a) Lysine content, (b) extent of denaturation, (c) solubility, (d) emulsifying capacity, and (e) foaming capacity.

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS AND METHODS

In this Chapter various methods, materials and the analytical procedures used in the standardization of process for the manufacture of whey powder and WPC by employing RO and UF techniques are summarized.

4.1 MATERIALS

The particulars regarding materials used in the present investigation are delineated hereunder.

4.1.1 PANEER WHEY

Paneer whey (PW) was obtained from the Experimental Dairy Plant of National Dairy Research Institute, Karnal, prepared by following the procedure recommended by Sachdeva and Singh (1988). Buffalo milk was standardized to a fat and SNF level of 5.8 and 9.5 per cent and heated to 90°C and cooled to 70°C. Coagulation was brought about by the addition of 1 per cent solution of citric acid to milk slowly and with constant stirring till clear whey separated out. After complete coagulation the stirring was stopped and the curd allowed to settle down for 5 min and later on the whey was drained out. This whey was collected for the experiments.

4.1.2 COW MILK CHEDDAR CHEESE WHEY

Cow milk cheddar cheese whey(CW) was procured from the Experimental Dairy Plant of National Dairy Research Institute, Karnal prepared by following the procedure of Kosikowski (1982) with little modification. Cow milk was standardized to casein/fat ratio of 0.70, pasteurized at 63°C for 30 min, and cooled to 30°C. An active starter culture was added

at the rate of 1 per cent. After 45 min, Meito rennet (obtained from Mucor pusillus lindt. procured from Meito Sangyo Company, Tokyo, Japan) was added at the rate of 1.5 g per 100 l of milk with thorough mixing. After setting (30 min), the curd was cut with the help of knives. The stirring was started after 10-15 min of cutting and curd was cooked to 39°C for 60 min. The cooked curd was left undisturbed in the whey for 5-10 min and whey was drained out. This whey was used for the experiments.

4.1.3 BUFFALO MILK CHEDDAR CHEESE WHEY

Buffalo milk cheddar cheese was prepared in the Experimental Dairy of National Dairy Research Institute, Karnal by following the method suggested by Kanawjia (1987).

Buffalo milk was standardized to casein/fat ratio of 0.70, pasteurized at 63°C for 30 min, cooled to 28°C and an active starter culture (S. lactis and S. cremoris) was added at the rate of 2 per cent along with 0.50 per cent L. casei 300 to milk. After 30 min, Meito rennet was added at the rate of 1.5 g/100 l of milk with thorough mixing. After setting (30 min), the curd was cut with knives. The stirring was started after 10-15 min of cutting and curd was cooked to 36°C for 40-45 min. The cooked curd was left undisturbed in the whey for 5-10 min. Thereafter the whey was drained and collected for subsequent experiments.

4.1.4 Alpha-LACTOSE MONOHYDRATE

Alpha-lactose mohohydrate ($C_{22}H_{22}O_{11}.H_2O$) Mw 360.32 used in the precrystallization process was of edible grade procured from S.d. Fine Chemicals Private Limited, Biosar, India.

4.1.5 CHEMICALS

All chemicals used in this investigation were of AR grade.

4.1.6 PACKAGING MATERIAL

Two types of packaging materials were used for packing whey powder and dried WPCs.

4.1.6.1 Polyethylene

Low density polyethylene sachets of 300 gauge thickness and 12 x 15 cm size were used for packaging whey powder and dried WPCs.

4.1.6.2 Metallized polyester laminates

Metallized polyester laminates with the following specifications were used for packaging whey powder and dried WPCs.

Metallized polyester Low density polyethylene	- 350 gauge
Moisture vapour transmission rate (38°C/90% RH/24 h)	- 14-20 g/m^2
Oxygen permeability (at atmospheric temperature)	- 85-95 ml/m^2
Grease resistance	- Very good
Size of pouch	- 12 x 15 cm

4.1.7 COMMERCIAL WHEY POWDER AND WHEY PROTEIN CONCENTRATE

Commercial samples of whey powder and WPCs were procured from German factories.

4.1.7.1 Whey powder

Whole whey powder was procured from one of the leading whey powder manufacturing companies (Baldwildungen) of Germany. Samples were collected directly from the company soon after the production for further

analytical and storage studies at the Justus-Lie-big University, Giessen, Germany.

4.1.7.2 Dried whey protein concentrates

Fresh dried WPCs having 26, 70 and 80 per cent protein were procured directly from a whey protein concentrate manufacturing company (Meggle), Germany, for further analytical and storage studies at the Justus Lie-big University, Giessen, Germany.

4.2 METHODS

The various methods followed in the standardization of the process for the manufacture of whey powder and WPC and other related experiments are presented below.

4.2.1 PROCESS STANDARDIZATION FOR CONCENTRATION OF WHEY BY REVERSE OSMOSIS

The experimental methods followed in determining the effect of various processing parameters such as clarification of whey, various pH adjustments, preheating of whey, operational temperature and pressure on the efficiency of RO process for the concentration of PW, CW and BW have been delineated hereunder.

RO pilot plant (Photo Plate 1) installed at the Experimental Dairy of National Dairy Research Institute, Karnal supplied by the PCI, England was used to concentrate whey. The unit is having membrane area of 0.9 m^2 with tubular configuration, allowing turbulent flow within the membrane. This basic unit contains one PCI type B_1 module, 1.2 meters in length with a 316 stainless steel shroud. The module contains one set of AFC-99 membrane. A heat exchanger is fitted to maintain the temperature of the recycled liquid at the desired value. The heat exchanger similar in

construction to the module is 0.6 meters in length and is made of 316 stainless steel.

Process fluid is fed to the pump inlet pressure and supplied at pressure (via a flexible hose) to the 18 tube module. In the 18 tube module the separation process takes place. The permeate passes through the semipermeable membrane tubes into the stainless steel module shroud and is drained away via the lower ½ inch hose. The concentrate passes through the 'tube side' of the module and is piped (via flexible hose) to the pressure control valve which maintains to get pressure within the module and from there via hose to the balance tank.

In all the preliminary experiments, 60 l of whey was used for standardizing the process for the concentration of whey employing RO.

4.2.1.1 pH adjustment of whey

Paneer whey, CW and BW were adjusted to various levels of pH hanging from 3.5 to 7.2, either by the addition of 10 per cent NaOH or by 10 per cent HCl solution.

4.2.1.2 Heat treatment of whey

Heating whey to a temperature of 60°C/30 min was carried out by taking double jacketed cheese vat, whereas high temperature short time heating was done by employing Silkeberg (Denmark) plate heat exchanger supplied by Alfa-Laval Company.

4.2.1.3 Flux rate

Flux was determined by measuring the amount of permeate coming get of the membrane in a given time in the given area of the membrane and its expressed in terms of standard flux as $l/m^2/h$.

4.2.1.4 Volume reduction

Volume reduction was calculated by using the following formula

$$VR = \frac{VP}{VO} \times 100$$

where, VR = per cent volume reduction
VP = volume of permeate removed (ml)
VO = original volume of whey (ml)

4.2.1.5 Effect of clarification

All the three types of whey were heated to 70°C/15 sec and concentrated by RO with and without clarification. The clarification of whey was carried out at 50°C by employing a separator supplied by Modern Dairy Appliances Company, Delhi (575 l/h capacity). During the process of concentration, the flux was determined at a regular interval. The resultant flux of the two processes were compared and data were statistically analysed.

4.2.1.6 Effect of pressure

Concentration of whey was carried out at 4 levels of pressure, viz., 25, 30, 35 and 40 bar. The flux was recorded individually at each pressure. The results of flux were computed and analysed to select the optimum pressure required for the maximum efficiency of the process.

4.2.1.7 Effect of temperature

Clarified whey was heated to 70°C/15 sec and cooled to either 40° or 50°C. The RO concentrate was carried out at these temperatures at 35 bar pressure and the flux was recorded at a regular interval of volume reduction to determine the effect of temperature of operation on the flux rate. The flux data were compared and analysed.

4.2.1.8 Effect of pH and preheating of whey

Each type of whey was individually adjusted to various pH values, viz., 3.5, 4.5, 5.6, 6.4 (6.3 in case of CW) and 7.2. After the pH adjustments, individually each sample was given different heat treatments, namely 60°C/30 min, 70°C/15 sec or 80°C/15 sec. At each combination of these treatments, RO concentration was carried out at 50°C and 35 bar pressure. The resultant flux was recorded at every regular interval of volume reduction for different combinations of treatments. The flux results were tabulated and statistically analysed to select the best combination of treatment.

4.2.1.9 Fouling studies

The concentration of pH adjusted and heat treated whey was carried out by RO at 50°C and 35 bar pressure. The effect of various combinations of pH and heat treatment on the fouling was studied as per the method suggested by Patel and Reuter (1985b) by collecting the permeate at every 20 sec interval from the start of the process. During this process, constant volume of feed was maintained by recirculating the permeate. The permeate collection at a regular interval of 20 sec was continued till the end of 10 min. The decline in flux during the process was expressed as a function of time.

4.2.1.10 Cleaning schedule

Cleaning schedule was standardized by comparing with the standard water flux as reference. For the thoroughly cleaned RO membrane reference water flux was tabulated by measuring the flux at different temperatures and pressure combinations and is expressed as reference water flux. Water flux was recorded before and after every cleaning cycle.

After concentration of whey, the plant was flushed with water for 15 min at 40°C and 0.1 per cent ultrasil-25 detergent (40 ml in 40 l of water, pH 10.75, a combination of alkali, surface active agent and enzymes) was circulated for different periods of time (50°C). After cleaning with ultrasil-25, the detergent was removed and flushed out with water for 15 min. Subsequently, the plant was circulated with ultrasil-75, 0.1 per cent (40 ml of 50% HNO_3 in 40 l of water,pH 3.25) for different duration of time (50°C), followed by removal of detergent and flushing out with water for 15 min. After each duration of cleaning standard water flux was recorded. The time taken for attaining the standard water flux was considered as duration needed for cleaning. After every cleaning process the membrane was preserved by recirculating 0.1 per cent sodium metabisulphite solution (20 g in 20 l of water) for 10 min and kept under this till next use.

4.2.1.11 Deposit collection

Deposits left on the membrane after the concentration of whey by RO were collected as per the method suggested by Patel and Reuter (1985a). After the concentration process, the plant was flushed out with clean water for 15 min and the deposits left on the membrane were collected by recirculating the known quantity of ultrasil-25 and ultrasil-75 detergents for a definite period of time and the detergents were analysed before and after each recirculation. The constituents present in the solution were expressed as deposits on membrane in terms of g/m^2 area of the membrane.

4.2.2 VACUUM CONCENTRATION

In the preliminary trials, RO concentrated whey was further concentrated to different levels of TS, viz., 40, 45 and 50 per cent by

employing laboratory rotary vacuum evaporator (Buchi type) supplied by Metrex Scientific Instruments Pvt. ltd., New Delhi.

4.2.3 PRECRYSTALLIZATION PROCESS

Whey concentrated by RO followed by vacuum evaporation was flash cooled to 30°C and the precrystallization was carried out with various levels of alpha-lactose monohydrate ranging from 0 to 0.07 per cent and crystallization was continued for a period of 6 h in a thermostatically controlled water bath, which has been provided with an agitator of varying speed (supplied by Wurt Elektromotoren GmbH, Balingen, Germany). During precrystallization process, the degree of crystallization and size distribution of lactose crystals were measured for different levels of TS and seeding material at a regular interval of one hour.

4.2.4 PILOT SCALE CONCENTRATION AND PRECRYSTALLIZATION

Eight hundred litres of each type of whey was taken separately and concentrated by RO to 2.0 and 2.5 folds in case of paneer whey and cheese whey, respectively. Further, RO concentrate was directly taken into the vacuum pan and concentrated to 50 per cent TS at a vacuum of 635 mm Hg and a temperature range of 54-56°C. When the desired concentration was reached (estimated by hand refractometer) the concentrate was immediately cooled to 30°C and filled into the seeding tank, which had been maintained at 30°C. When the bottom of the seeding tank was filled up, the agitator was started. A known quantity of seeding material was added and seeding was continued. After 4 h of seeding, the seeding tank was cooled at the rate of 3°C/h till it attained a temperature of 10°C.

4.2.5 SPRAY DRYING

Precrystallized concentrate was spray dried by using anhydro spray

drier (Denmark), 35 kg/h water evaporation capacity by maintaining 180°C and 85°C inlet and outlet temperatures, respectively and at an atomizer speed of 25,000 rpm.

4.2.6 PACKAGING

Spray dried whey powder was cooled to room temperature and immediately packed in polyethylene and metallized polyester laminates sachets and sealed. These were further used for physico-chemical analysis and storage studies.

4.2.7 STORAGE STUDIES

Fresh samples of spray dried PWP, CWP, BWP and WP-G were kept for storage in sealed sachets of polyethylene and metallized polyester laminates (150 g each). Storage studies were carried out for a period of six months at 20, 30 and 40°C and a relative humidity of 60 ± 2 per cent. At a regular interval of one month, the samples were analysed for various attributes to determine the storage stability of the powders.

4.2.8 ENERGY REQUIREMENT FOR CONCENTRATION OF WHEY

Energy required for concentrating 1,000 l of PW, CW and BW by vacuum evaporation to 50 per cent TS was calculated. Similarly, energy required for concentrating whey by RO followed by vacuum evaporation to 50 per cent TS was also calculated. The net energy savings by the later process were computed (Annexure IV).

4.2.9 PROCESS STANDARDIZATION FOR WHEY PROTEIN CONCENTRATE EMPLOYING ULTRAFILTRATION TECHNOLOGY

Production of WPC from BW by employing UF technology was studied. UF was carried out by using a laboratory module hollow fibre membrane (Romicon, membrane type PM 50) plant by maintaining 1.80 bar (inlet) and

0.5 bar (outlet) pressures. The details of the principles of operation of UF plant are delineated hereunder.

UF plant supplied by Alfa-Laval, Denmark (Photo Plate 2) was used for the fractionation of BW for the production of WPC. The feed was pumped from an external tank by the feed pump and fed into the circulation loop. A circulation pump gives the liquid a high velocity on the inner side of the fibers in the cartridges. Part of the liquid permeates through the fibers, and is collected in the cartridges shell, from where it leaves through the upper permeate outlet. The remaining liquid inside the fibers, the concentrate is mixed with new feed and feed back to the circulation pump for another passage through the cartridge.

For all the preliminary trials, 150 l of BW was used and UF was carried out in hollow fiber PM 50 module of 1.4 m area. The process was standardized with respect to the following processing variables.

4.2.9.1 Effect of temperature

Buffalo milk cheddar cheese was clarified at 50°C and heat treated to 70°C/15 sec, cooled to either 40° or 50°C and UF was carried out at these temperatures. The flux (permeation rate) was measured at a regular interval of volume reduction. The flux data were computed and statistically analysed.

4.2.9.2 Effect of pH and heat treatment of whey

Clarified BW was adjusted to various pH levels, viz., 3.0, 4.5, 5.6, 6.4 and 7.2 (either by addition of 10% NaOH or by 10% HCl) and subjected to either 60°C/30 min or to 80°C/15 sec heating. At each combination of treatment, UF was carried out at 50°C and the resultant flux was recorded at a regular interval of volume reduction. The results were

PHOTO PLATE 1 : REVERSE OSMOSIS PLANT

PHOTO PLATE 2 : ULTRAFILTRATION PLANT

computed and statistically analysed to select the best combination of treatment.

4.2.9.3 Diafiltration

Ultrafiltration of 150 l of BW was carried out to a level of 95 per cent volume reduction. When 95 per cent volume reduction was attained, distilled water (50°C temperature) was added to the retentate (1:1 proportion). Filtration was continued till all the added water was removed in the form of permeate. Similarly, second diafiltration was also carried out. The effect of the diafiltration on the retention of various constituents was examined.

4.2.9.4 Retention and permeation of components

During UF, at different levels of volume reduction, the retentate and permeate were analysed for various components. The components retention was expressed on dry matter basis. Retention was expressed as a function of volume reduction, which indicated the desired level of any component in the WPC.

4.2.10 LARGE SCALE WHEY PROTEIN CONCENTRATE PRODUCTION

Six hundred litres of BW was clarified and its pH adjusted to 7.2. The whey was then heated to 80°C/15 sec and cooled to 50°C. UF was carried out using Romicon hollow fiber plant (PM 50 membrane effective area 2.5 m^2) at 50°C to a volume reduction of 85 per cent so as to obtain about 45 per cent protein on DM basis in the end product. The resultant concentrate was spray dried by using Anhydro (Denmark) spray drier, 35 kg water evaporation capacity per hour, by maintaining 180°C inlet and 80°C outlet temperatures, respectively.

4.2.11 PACKAGING

Spray dried BW protein concentrate was cooled to room temperature and immediately packed in polyethylene and metallized polyester laminates sachets and sealed.

4.2.12 STORAGE STUDIES

Fresh samples of spray dried BW protein concentrate and commercial dried WPCs were kept for storage in sealed sachets of polyethylene and metallized polyester laminates (100 g each). Storage studies were carried out for a period of six months at 20, 30 and 40°C and a relative humidity of 60 ± 2 per cent. At a regular interval of one month, the samples were analysed for various attributes to determine the storage stability.

4.3 ANALYTICAL METHODS

The analytical procedures followed in the investigation are delineated below.

4.3.1 PHYSICAL PROPERTIES

The physical properties of liquid and concentrated whey were measured as follows.

4.3.1.1 pH

pH of the samples was measured by using Elico digital pH meter, model LI-122 with comined glass electrode.

4.3.1.2 Acidity

Acidity was measured by titrating 10 g of sample against 0.1 N NaOH using phenolphthalein indicator and expressed in terms of per cent lactic acid as per the method described in IS:1479 (Part I) 1960.

4.3.1.3 Specific gravity

Specific gravities of whey, RO concentrate, vacuum concentrate and UF retentate were estimated at 20°C by using specific gravity bottle and taking distilled water as the standard liquid.

4.3.1.4 Dynamic viscosity

Viscosities of whey, RO and UF retentate as well as vacuum concentrate were measured by Hoppler's falling ball viscosimeter supplied by Veb Prufgerate-Wrk Medingen/Dresden.

Viscosity was measured at 20°C. The chamber of the viscosimeter as well as samples were maintained at 20°C. After instrument was levelled using spirit level, the measuring tube was filled with the experimental sample and the ball was inserted through the open end of the tube. Care was taken to avoid air bubbles in the tube and the open end was closed. Depending upon the falling time of ball and viscosities diffeent balls were used. The time taken by the ball to fall through a distance of 100 mm was measured accurately, in triplicate, by means of a 1/10 second stop watch and the mean time was calculated. The dynamic viscosity was calculated by using the formula:

$$n = t\ (Q_1 - Q_2)\ k$$

where,
- n = dynamic viscosity in centipoise (cp)
- t = fall time of ball in sec.
- Q_1 = density of the ball (g/cm^3)
- Q_2 = density of the liquid at the measuring temperature in g/cm^3
- k = ball constant

4.3.1.5 Lactose crystallization

The extent of lactose crystallization in the whey concentrate was determined by centrifugation of the suspension of lactose crystals at

5,000 rpm for 3 min resulting in the separation of solid and liquid phase. The fraction of lactose crystallized was calculated from the difference in lactose content of the original suspension and of supernatant as per the method suggested by Roetman (1982).

4.3.1.6 Size distribution of lactose crystals

Size and number of lactose crystals were measured under microscope by the following way.

4.3.1.6.1 Standardization of occular scale

Occular scale was standardized with the stage micrometer to get readings in terms of microns.

4.3.1.6.2 Preparation of slide

A small drop of well mixed concentrate was placed on a clean slide. The sample was spread evenly on the slide and covered with a cover slip. The cover slip was pressed till a uniform thin film is formed. This slide was observed under low power and high power.

4.3.1.6.3 Measurement and count of lactose crystals

On an average, 30 squares in the same field or just adjacent to the counted field were taken for measurement of lactose crystals. The number of crystals in each field and measurement according to their size as less than 5, 5-10, 11-20, 21-40 and over 40 μm was counted. Average of each field was taken and recorded.

4.3.2 CHEMICAL ANALYSIS

The analytical methods employed in various chemical analysis of whey, RO and UF concentrate, permeate and deposits are delineated below.

4.3.2.1 Total solids

Total solids content was estimated by gravimetric method as per IS:1479 (Part II) 1961.

4.3.2.2 Fat

Fat was estimated by Gerber method by using skim milk butyrometer as per the IMV Standard (1983).

4.3.2.3 Total protein

Total nitrogen content was estimated by standard Kjeldahl method as per AOAC method (1980). From the total nitrogen, total protein was obtained by multiplying with a factor 6.38.

4.3.2.4 Non-protein nitrogen

Samples were precipitated using 17 per cent trichloroacetic acid (Rowland, 1938) and filtered. The nitrogen content of the filtrate was analysed by Kjeldahl method (AOAC, 1980).

4.3.2.5 Lactose

Lactose content was estimated by phenol-sulphuric acid method as per the procedure recommended by Lawrence (1968).

4.3.2.6 Ash

Ash content was estimated by incinerating the samples at 550°C in a muffle furnace as per the ISI method described in IS:SP:18:Part XI (1981).

4.3.2.7 Calcium

Calcium was estimated by calmagnite method as recommended by Davies and White (1962).

4.3.2.8 Phosphorus

Phosphorus content was estimated by following the method recommended by Fiske and SubbaRao (1925).

4.3.3 ANALYSIS OF WHEY POWDER AND DRIED WHEY PROTEIN CONCENTRATE

Following analytical procedures were followed for the analysis of dried whey and whey protein concentrate.

4.3.3.1 Moisture

Moisture content was estimated by gravimetric method as per IS:1479 (Part II, 1961).

4.3.3.2 Fat

Fat content of dried whey and whey protein concentrate was determined by Rose-Gottlieb gravimetric method as per IDF (1987).

4.3.3.3 Lactose

Lactose content was estimated by enzymatic method by using the test combination supplied by the firm Boehringer Mannheim GmbH, Germany as per the method VDLUFA (1985).

One hundred mg of whey powder, weighed to ±0.1 mg was placed in a 100 ml volumetric flask and mixed with 50 ml bidistilled water (the lactose content of the sample should not exceed 100 mg). One ml of potassium hexacyanoferrate solution 15 g K_4 [Fe(CN)].$3H_2O$ dissolved to 100 ml with bidistilled water and 1 ml zinc sulfate solution (30 g $ZnSO_4.7H_2O$ dissolved to 100 ml with bidistilled water) were added to the mixture, which was well shaken after each addition. Neutralization was subsequently carried out by means of caustic soda (0.25 mol/l) and indicator paper. Filtration was done after filling up with water and mixing via a dry

folding filter. The clear filtrate was used for the enzymatic test. A parallel procedure was instigated for the lactose determination and the blank value. The following solutions were pipetted into cells:

	Blank value	Galactose sample	Lactose sample
NAD-citrate buffer solution (ml)	0.20	0.20	0.20
Beta-galactosidase suspension (ml)	0.02	-	0.02
Filtrate	-	0.10	0.10
About 15 min standing time after mixing, the addition of			
Phosphate buffer solution (ml)	1.00	1.00	1.00
Water (ml)	2.00	1.92	1.90
Mixing and, after 2 min, measuring of the solution extinctions(E1B, E1G, E1L). Then addition of			
Beta-galactose-dehydrogenase suspension (ml)	0.02	0.02	0.02

Renewed mixing and, after the reaction has stopped (about 15 min), measuring the extinction (E2B, E2G, E2L). The following differences were firstly calculated from the measured values:

Galactose value Δ EG = (E2G - E1G) - (E2B - E1B)

Lactose value Δ EL = (E2L - E21) - (E2B - E1B) - Δ EG

The following general formula is applied for calculation of the concentrations C in g/100 ml sample solution:

$$C = \frac{V.MG}{E.d.v.10,000} \Delta E \text{ (g/100 ml)}$$

where,

V = test volume (total volume of all solutions in a cell) in ml

MG = molecular mass of the substance to be determined (galactose 180.16; lactose 342.30; lactose monohydrate 360.31)

v = sample volume (filtrate) in ml

d = layer density in cm

E = extinction coefficient (1 $mmol^{-1}.cm^{-1}$)

For NADH, the following applies

at 340 nm, E = 6.3

at 365 nm, E = 3.4

at 334 nm, E = 6.18

The value Δ E should be substituted by Δ EG for the galactose calculation and by Δ EL in the case of lactose.

The results obtained from the above calculation gave the content in g per 100 ml of sample solution. They were converted on the basis of original weight in quantity by applying the following equation.

$$WL = \frac{C \times 100}{E} (\%)$$

where,

WL = lactose content of sample in per cent

E = weight of the sample in g

C = concentration as obtained from the above formula

4.3.3.4 Total nitrogen

Total nitrogen content of dried whey and WPC was estimated by Kjeldahl method by using Kjel-foss-automatic 16210 supplied by the A/S N Firm Elektrik Hillerod, Denmark. The total nitrogen content was multiplied by a factor 6.38 to obtain total protein content of the sample.

4.3.3.5 Non-protein nitrogen

Non-protein nitrogen content of the sample was determined by precipitating the proteins of the samples by trichloroacetic acid (Rowland,

1938) and the nitrogen content of the filtrate was estimated by Kjeldahl method using Kjel-foss-Automatic 16210 as per the recommendations of Rommel (1983).

4.3.3.6 Whey protein nitrogen

Reconstituted samples of powders were centrifuged at 6,000 rpm for 20 min and filtered. One ml of filtrate was analysed for the nitrogen content by Kjel-foss Automatic, which encompasses whey protein nitrogen, non-protein nitrogen and proteose peptone. From the total nitrogen, non-protein nitrogen was deducted which gave whey protein nitrogen and proteose peptone. From this amount, proteose peptone content was subtracted to obtain whey protein nitrogen content. Multiplying this with a factor 6.38 gave whey protein content of the sample.

4.3.3.7 Proteose peptone

Reconstituted samples of powder were heated to 95°C for 20-30 min and filtered. The filtrate was analysed for nitrogen content by Kjel-foss Automatic. From the total nitrogen content of the filtrate, non-protein nitrogen content was subtracted (Rowland, 1938; O'Sullivan, 1971) to obtain proteose peptone nitrogen. By multiplying with a factor 6.38, the content of proteose peptone in the sample was obtained.

4.3.3.8 Lysine

Lysine content of the samples was estimated by Carpenter (1960) method along with the modification suggested by Booth (1971).

Accurate quantity of sample was weighed and transferred into 100 ml conical flask (nitrogen content of the sample ranging from 20-40 mg). The samples were added with 8 ml, 8 per cent $NaHCO_3$ and were shaken for 10 min

in a mechanical shaker. Later on each flask was added with 12 ml of 1-fluoro-2,4 dinitrobenzene (FDNB) and ethyl alcohol mixture (for every 0.3 ml FDNB, 12 ml of ethyl alcohol), covered with aluminium foil and was again shaken in dark for a period of 2 h. After 2 h of shaking, conical flasks were transferred to a water bath maintained at 80°C.

After 15 min, samples were taken out, cooled to room temperature and added with 30 ml of 8.5 N HCl solution. Flasks were covered with 100 ml beakers and transferred to an autoclave. The samples were hydrolysed at 135°C and 2 atmosphere pressure for a period of two hours.

Flasks were taken out from the autoclave and were filtered hot into 250 ml volumetric flask through S and S 589-2 filter paper. The flask as well as filter paper was thoroughly rinsed with several aliquots of distilled water and finally the volume was made upto the mark by distilled water.

Exactly 2 ml of each sample was pipetted out in duplicate (A and B) into graduated test tubes and these were added with 5 ml diethyl ether and the extraction was done 3 times. After the extraction with diethyl ether samples were transferred into a water bath maintained at 50°C for a period of about 10-15 min (till all the diethyl ether escapes).

For the sample tube (A) volume was made upto 10 ml by the addition of 1 N HCl and were mixed properly and kept in dark till the absorption measurement.

For the tubes 'B' (Blank), a drop of phenolphthalein indicator followed by few drops of 10 per cent NaOH (till the permanent red colour appears) and immediately 2 ml of buffer solution ($NaHCO_3$ + Na_2CO_3, 6.8 pH), followed by 0.05 ml methylene chloroamino acids were added, mixed well and

concentrated HCl. Thereafter mixture was extracted 3 times with diethyl ether (5 ml each time) and was transferred to a water bath maintained at 50°C for 15 min till all the reaction was over.

The 'B' set of tubes was made upto 10 ml volume by the addition of distilled water and mixed well. Now the absorption of samples 'A' and blank 'B' was measured at 436 nm by using Eppendorf PCP 6121 spectrophotometer. The differences in the absorption of A and B were noted. The lysine content of the sample was calculated by the absorption values by using the following formula.

$$\text{Lysine g/100 g sample} = \frac{(A-B) \times 250 \times 5 \times 0.42 \times 100 \times F}{1000 \times \text{wt of sample(g)} \times 1000}$$

The factor 'F' is the slope of the standard curve obtained by using ϵ -DNP-lysine-HCl. Four concentrations of ϵ -DNP-lysine-HCl were prepared to have 20, 10, 5 and 2.5 ug/ml and absorption of these concentrations was measured. The absorption was plotted against concentration and the slope of the curve was obtained.

4.3.3.9 Extent of denaturation

Extent of denaturation was measured by estimating the whey protein nitrogen in sodium chloride filtrate by Kjeldahl method. Whey protein nitrogen was determined before and after sodium chloride precipitation to assess the extent of denaturation. The samples for the estimation were prepared as per the method suggested by Mahmoud et al. (1990). Accurately 1 g of powder was transferred to a test tube (25 x 150 mm size), 25 ml of 0.1 M phosphate buffer (pH 6.7) and 10 g of sodium chloride were added to the mixture and the tubes shaken vigorously and transferred to a water bath maintained at 37°C for a period of 30 min with intermittent shaking for about 15 min. Thereafter samples were cooled to 20°C

temperature and filtered through Whatman No.1 filter paper. The nitrogen content of the filtrate was determined by Kjel-foss Automatic 16210. The extent of denaturation was estimated by using the formula

$$\text{Per cent denaturation} = \frac{\text{Total whey protein nitrogen - Whey protein nitrogen after salt precipitation}}{\text{Total whey protein nitrogen before precipitation}} \times 100$$

4.3.3.10 Ash

Ash content was measured by incinerating the samples in a muffle furnace at 550°C as per the method IS:SP:18 Part XI (1981).

4.3.3.11 Minerals

Minerals such as calcium, magnesium, potassium and sodium were estimated by Atomic Absorption Spectroscopic Technique by employing Atomic Absorption Spectrophotometer, Perkin Elmer 430, whereas phosphorus was estimated by spectrophotometric method.

4.3.3.11.1 Sample preparation

About 1 to 2 g of sample was weighed accurately into thoroughly cleaned (in bidistilled water) and dried silica dishes. Samples were incinerated in a muffle furance at 550°C, cooled in desiccator and ash content was determined. To the ash of the dishes, 2 ml of 30 per cent HCl was added and transferred carefully into 100 ml volumetric flask. The dishes and funnels used for transferring the samples were rinsed thoroughly with several aliquots of bidistilled water and the volume of flask was made upto the mark by bidistilled water. The mouth of the flask was covered with parafilm and the contents were mixed thoroughly.

One ml of the above solution was transferred into a 50 ml volumetric flask by using Eppendorf micropipettes. These flasks were added with 2.5 ml of lanthan oxide and 0.5 ml of caesium chloride and the volume

was made upto the mark with bidisilled water, covered with parafilm and mixed thoroughly.

4.3.3.11.2 Blank preparation

2.5 ml of lanthan oxide and 0.5 ml of caesium chloride were transferred into a 50 ml volumetric flask and the volume was made upto the mark using bidistilled water, covered with a parafilm and mixed thoroughly.

4.3.3.11.3 Standard preparation

Standard solutions of calcium, magnesium, potassium and sodium having the concentrations of 1.0, 0.10, 1.0 and 0.10 g/l solution respectively were used to prepare the standard.

Into a 100 ml volumetric flask, 0.5 ml, 0.5 ml, 0.2 ml and 1.0 ml of calcium, magnesium, potassium and sodium standard solutions respectively were transferred by using Eppendorf pipettes. To this, 5.0 ml lanthan oxide and 1.0 ml of caesium chloride were added and the volume was made upto the mark, covered with a parafilm and the contents were mixed thoroughly.

4.3.3.11.4 Equipment standardization

The following parameters were selected while measuring different minerals.

Minerals	Lamp voltage (ampere)	Slit	Wave length
Calcium	15	0.7	422.0
Magnesium	15	0.7	202.5
Potassium	12	2.0	766.5
Sodium	12	0.7	586.0

Equipment was operated as per the instruction manual of Perkin Elmer 430. Proper wave lengths were selected in obtaining the values of the standard solutions. Equipment was standardized to the standard as well as to the blank solutions and thereafter samples were inserted for the measurement at their appropriate wave length and the concentration values were printed out. The values obtained were multiplied by appropriate dilution factor. The concentration was expressed in mg/100 g of product.

4.3.3.12 Phosphorus

Phosphorus was estimated by spectrophotometric method (Milupa, 1976). Appropriate quanity of sample was ashed in a silica dish and 1 ml of 30 per cent HCl was added. The sample was transferred carefully into a 100 ml volumetric flask and the volume was made upto the mark using bidistilled water.

Into a 25 ml volumetric flask, 2 ml of the above sample solution was transferred and added with 5 ml of Vandatmolybdat reagent (Merck Co., Germany), covered with parafilm mixed thoroughly and left for 10 min at 20°C and the absorption was measured at 405 nm in the Eppendorf PCP 6121 spectrophotometer against the blank (5 ml of Vandatmolybdat and 20 ml bidistilled water in 25 ml volumetric flask).

Different concentrations of phosphate standard solution were prepared and the absorption was measured as above. Concentration was plotted against absorption. The slope of the curve was determined and taken as factor for the calculation.

4.3.4 PHYSICAL PROPERTIES

Physical properties of the spray dried whey powder and WPCs were measured by the following methods.

4.3.4.1 pH

Exactly 10 g of powder was reconstituted with 100 ml distilled water and the pH was measured by using microprocessor pH meter Model 537 supplied by MAGv GmbH, Germany.

4.3.4.2 Colour characterization

Colour of the samples was measured by reflectance spectroscopy technique using reflectance meter, Model C1-28.

The reflectance meter was first standardized with the standard white magnesium oxide. The samples were taken in a clean glass petri dish (5 cm diameter), placed under the search unit and the reflectance value was recorded. At least three such observations were recorded on each sample at different places, and the average value was calculated. The reflectance of the sample was recorded in the coloured light using a blue filter (450 nm wave length) after adjusting the reflectance value with standard blocks to determine the extent of browning.

4.3.4.3 Solubility index

Solubility index of powders was determined by ADMI (1965) method. Thirteen grams of powder were reconstituted in 100 ml of distilled water at 24°C by blending in a solubility index mixer for 90 seconds. After allowing to stand for a while, the foam was removed and 50 ml of reconstituted liquid was centrifuged in a conical graduated tube for 5 min. The volume of sediment in ml obtained at the bottom of centrifuge tube was recorded and designated as solubility index.

4.3.4.4 Bulk density

Bulk densities were estimated as described by Beckett et al. (1962) since this test is not supposed to be influenced by the packing history

of the powder. A 100 ml graduated cylinder was filled with about 50 ml of hexane and stoppered. The volume of hexane (V_1) and the total weight (W_1) were recorded. Enough powder was added through a funnel to increase the volume to about 40 ml. The cylinder was placed on a level, vibration free surface. After one hour the volume of powder (V_3), the volume of powder and hexane (V_2) and the total weight (W_2) were recorded. The volume of the floating portions of the powder was added to V_3 when making calculations. Volume was estimated to 0.5 ml and weighed to 0.05 g. Calculation was made as follows.

$$\text{Bulk density} = \frac{W_2 - W_1}{V_3} \quad g/cm^3$$

4.3.4.5 Wettability

The wettability was tested by the method developed by Muers and House (1962) and was employed with some modifications. The modification was necessary due to the non-availability of satin drill specified by them (225 g/sq m having about 30 threads/cm in the wrap and 20 threads/cm in the weft). The fabric used in present investigation was satin drill of 221.6 g/sq m having about 28 threads/cm in the wrap and 19 in the weft. The second modification pertained to the dish. A tray of 21.2 x 16.3 cm (length x breadth) was used instead of the size 20 x 15 cm used by them. The method is as follows.

A piece of fabric (confirming to the above specifications) measuring about 10 x 10 cm was stretched over one end of the body of a metallic can (6.5 diameter and 4.5 cm height), open at both ends and was held on with a rubber band). Another open end can (5 cm diameter and 7 cm height) was placed as spacers to hold it in position centrally on the cloth. The tray (21.2 cm x 16.3 cm) was marked at a depth of 2.5 cm from the bottom

and filled with distilled water to this point. A triangle of 0.4 cm thick glass rod with sides about 8 cm long was placed in the dish and served to prevent close contact of cloth with the bottom of the dish.

With two cans assembled and the cloth resting clean on surface 1 g of powder was transferred to the inner can and spread over the 5 cm circle of cloth as evenly as possible with soft hair brush. The inner can was then removed and the outer can lowered into the dish on to the glass triangle and held in place until the water level in the can ceased to rise. A stop watch was started when the cloth touched the water and was stopped when the powder was completely wetted. The mean of 3 replicate tests was taken as the wetting time.

4.3.4.6 Dispersibility

In the present investigation, the method described by the American Dry Milk Institute (ADMI, 1965) was adopted with some modifications. ADMI specified the mixing of 52 g of powder with 100 ml of water at 24°C for for 20 sec using Hobert-Kitchen mixer of 192 ppm speed. In the present investigation, Hobert-Kitchen Mixer was replaced with general electrical mixer operating at 400 rpm.

A brass bowl (dimensions 13 cm height, 13 cm top diameter, and 24 cm inner diameter) fitted with an outlet of 0.5 inches diameter at the bottom was taken. To this a rubber tube fitted with a pinch cock was attached. This was clamped to a stand in such a way that it could be raised or lowered. Four hundred ml of water at 40°C was placed into the bowl and 50 g powder was transferred to the surface of water. The stirrer of the mixer which was earlier fitted in position in the centre of the bowl was operated at 400 rpm and stirring continued for 20 sec. The pinch cock at bottom outlet of the bowl was then opened to release the

contents to a standard 72 mesh sieve. The screened fluid was collected onthe flask and diluted to 500 ml. Two 10 ml portions were transferred to weighed aluminium dishes, evaporated to dryness in Mojonnier tester and cooled in a desiccator and TS was estimated. The weight of the solids cotained multiplied by 50 gave the dispersibility in gram.

.3.4.7 Sinkability

The sinkability of powder was measured by spectrophotometric method is described by Sammhammer (1966). In this test, 3.5 ml of distilled later at 20°C was taken in the spectrophotometric cuvette, and 10 mg sample of powder was dusted on the surface of water and the percentage transmittance was measured at 760 nm in a Beckman DU spectrophotometer. The headings were recorded after 2, 4 and 6 min interval after giving 6 wappings in the cuvette holder. The mean of the three replicate values was taken as the percentage transmittance.

1.3.4.8 Flowability

The angle of repose (as a static measure for flowability) was determined by the method of Sjollema (1963).

A plastic funnel with a narrow stem, cut at right angles, was nounted exactly 2 cm above a piece of butter paper, positioned on a horizontal table. A sieve with 16 mesh size was fixed to a shaker (100 shakings per minute). Powder samples were placed on a sieve and allowed to go through the funnel in a fine stream at controlled speed, so that a conical heap was formed. When top of the powder heap touched the end of funnel stem, the powder stream was stopped by switching off the shaker. The base of the powder heap was outlined with a pencil and powder was removed. The outlined paper circle was cut and weighed. From the

weight of paper per unit area (GSM), the area of paper circle was calculated. The radius was calculated and angle of the repose estimated.

$$\text{Tan } \theta = \frac{h}{(r - \frac{1}{2}a)}$$

where,

Tan θ = angle of repose
h = height of stem base (2 cm)
r = radius of the powder heap
a = diameter of funnel stem

4.3.4.9 Solubility

Solubility was determined by modified nitrogen solubility index procedure described by Morr et al. (1985).

About 500 mg of dry protein product was accurately weighed into separate 150 ml standard beakers and several aliquots of 0.1 M NaCl solution were added with stirring to form a smooth paste. Additional 0.1 M NaCl solution was then added to bring the total volume of the dispersion to about 40 ml. The beaker was placed on a magnetic stirrer. A 2.5 cm smooth, plastic coated stir bar was added and the dispersion was stirred at a rate that just failed to form a vortex. The pH of the dispersion was immediately determined and adjusted to 7.0 with 0.1 N solution. The dispersion was stirred for a total of 1 h under these conditions and the pH was intermittently monitored and maintained at the prescribed value throughout the stirring period. The dispersion was then transferred into a 50 ml volumetric flask, diluted to the mark with additional 0.1 M NaCl solution and mixed by inverting and swirling. An aliquot of the dispersion was centrifuged for 30 min at 20,000 x g and the resultant fraction was filtered through Whatman No.1 filter paper. The protein content of the filtrate was determined by micro-Kjeldahl method

by automatic Kjel-foss 16210, using appropriate aliquot volumes. The solubility of the protein product was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{Supernatant protein conc. (mg/ml)} \times 50}{\text{Sample wt. (mg)} \times \frac{\text{Sample protein content(\%)}}{100}} \times 100$$

4.3.4.10 Foaming properties

Foaming capacity and foam stability of whey protein product were determined using 5 per cent whey protein product dispersion at 7.0 pH and a whipping time of 10 min as described by Phillips et al. (1987).

Foams were produced by whipping 75 ml of whey protein product dispersion using a laboratory module Waring blender mixer. Foaming capacity was expressed as the percentage overrun in the resulting foam and was calculated as follows:

$$\text{Per cent overrun} = \frac{\text{(wt of 100 ml dispersion)} - \text{(wt of 100 ml foam)}}{\text{Weight of 100 ml foam}} \times 100$$

Foam stability was determined by monitoring weight of liquid drained from the resulting foam as a function of time and was expressed as time (min) taken for 50 per cent liquid drainage.

4.3.4.11 Emulsifying capacity

Emulsifying capacity of the whey protein product was determined using an emulsion system composed of 75 parts by volume of 2 per cent whey protein product dispersion at pH 7.0 as a continuous phase and 25 parts by volume of sunflower oil as a dispersed phase. The emulsions were prepared using laboratory module, Waring blender mixer at high speed for 3 min. Emulsifying capacity was expressed as emulsifying activity index (EAI) and was determined by a turbidimetric method described by

Pearce and Kinsella (1978) using diluted emulsion. It was calculated as follows:

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303 \times A\ 500}{\theta \times L \times C}$$

where,

A 500 = absorbance of diluted emulsions at 500 nm

θ = volume of fraction of dispersed phase

L = path length of cell (in meters)

C = wt of whey protein product per unit volume of whey protein product dispersion in g/m^3

4.4 STATISTICAL ANALYSIS

Data obtained from the various experiments during standardization process and storage studies were subjected to appropriate statistical analyses.

CHAPTER 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

Investigation was carried out to standardize the process for the manufacture of whey powder and whey protein concentrate by employing RO and UF technologies. The results obtained during process optimization for the concentration of different types of whey by RO, further optimization of process for vacuum concentration, precrystallization, spray drying and the effect of various storage conditions on the quality of the powder have been reported in this chapter. The results of process optimization for concentration and fractionation of whey by UF, its retention and permeation characteristics besides the effect of storage conditions on the quality of spray dried WPCs are also presented and discussed in this chapter with suitable tables, figures, illustrations and statistical analysis.

5.1 PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF DIFFERENT TYPES OF WHEY

Three varieties of whey, namely, paneer whey (PW), cow milk cheddar cheese whey (CW) and buffalo milk cheddar cheese whey (BW) have been used in the present investigation. It was imperative to determine the physical properties and chemical composition of whey as these properties have significant role to play in the processing of whey for the manufacture of whey powder and whey protein concentrate.

5.1.1 PHYSICAL PROPERTIES

The physical properties such as pH, acidity, specific gravity and viscosity of all the three types of whey were determined. The results are presented in Table 1.

5.1.1.1 pH

The mean pH values of PW, CW and BW were 5.60, 6.30 and 6.40, respectively. For different types of whey, the variation in pH appeared to be very less. The standard deviation (SD) of 0.040 for PW, 0.049 for CW and 0.029 for BW indicates that whey can be produced with a fairly constant pH.

The variation in the pH of paneer whey and cheese wheys is due to the difference in the methods employed in the product manufacture. In the production of paneer, coagulation is brought about by the combination of acid and heat treatment, whereas in the case of cheese coagulation is due to the rennet action. Within the cheese wheys, BW has a higher pH (6.4) than CW (6.3). This could be attributed to the overall differences in the compositional make up of buffalo milk and cow milk used in the manufacture of cheese.

5.1.1.2 Acidity

The acidity of PW was 0.231 per cent lactic acid (SD 0.006) as against 0.175 (SD 0.004) and 0.169 per cent (SD 0.005) of CW and BW, respectively (Table 1). The acidity of PW was significantly higher because of coagulation at lower pH during paneer making.

The variations in acidity and pH have to be noted as these might affect the efficiency of processing of whey by membrane technology and subsequent handling.

5.1.1.3 Specific gravity

Specific gravity of a product is dependent on the constitutional make up of a particular system, i.e., amount of dissolved, dispersed or suspended substances. In our investigation, PW had a specific gravity

Table 1. Physical properties of whey systems

Properties	Types of whey					
	Paneer whey		Cow milk cheddar cheese whey		Buffalo milk cheddar cheese whey	
	Mean*	S.D.	Mean*	S.D.	Mean*	S.D.
pH	5.60	0.040	6.30	0.049	6.40	0.029
Acidity (% lactic acid)	0.231	0.006	0.175	0.004	0.169	0.005
Specific gravity	1.024	0.002	1.025	0.001	1.027	0.002
Viscosity (cp)	1.066	0.004	1.083	0.003	1.097	0.005

SD = Standard deviation

* Mean of ten trials

of 1.024 (SD 0.002) as against 1.025 (SD 0.001) and 1.027 (SD 0.002) of CW and BW, respectively.

Though PW had lower total solids (6.06%), the specific gravity appeared to be nearer to cheese wheys. This may be attributed to the higher mineral content of PW compared to the other two types of whey as minerals contribute more to the density than the other components of the product. BW had a higher specific gravity than CW, which could certainly be due to higher solids of BW (6.87%) than CW (6.41%).

5.1.1.4 Viscosity

Viscosity is also one of the important physical properties of whey that has to be carefully considered during the manufacture of whey powder and whey protein concentrate.

The viscosity of PW was 1.066 cp, whereas the values obtained for CW and BW were 1.083 and 1.097 cp, respectively. The results illustrate that the viscosity of PW is lower than that of CW and BW and the reason for this could be its lower protein content. It is well known that proteins are the major contributors to the viscosity. As PW had less than half the protein content of cheese wheys its viscosity was lower. Thus, the variations in viscosity of these types of whey were effected by differences in their protein contents.

5.1.2 CHEMICAL COMPOSITION

The composition and the type of whey have a significant role to play in the concentration and fractionation of whey by RO and UF. In addition, they also affect further processing, final composition and quality of the end product. Therefore, all the three types of whey used were analysed for their chemical composition. The results obtained are depicted in Fig 1.

5.1.2.1 Total solids

The TS content of the three types of whey are represented in Fig. 1. The figure clearly indicates that BW had a higher TS content (6.87%) compared to PW (6.06%) and CW (6.41%). The variation in the TS was found to be less as indicated by respective SD values of 0.084, 0.051 and 0.068.

The lower TS in PW could be attributed to the method employed for the production of paneer, wherein coagulation of milk is brought about by higher heat treatment and acidification. As a result, most of the whey proteins get denatured and carried into the coagulum along with other solids, thereby reducing the solids content of whey. CW had also shown slightly lower TS than BW which could be ascribed to the compositional differences of milk being used. Higher fat, protein, lactose and ash contents led to higher TS content in BW (Srivastava, 1991).

5.1.2.2 Protein

It is clear from Fig 1 that BW had higher protein content (0.98%) than CW (0.78%) and PW (0.30%). The respective SD values were 0.037, 0.023 and 0.021.

Paneer whey had shown least protein content, i.e., less than half the protein content of cheese wheys. This is because during the production of paneer, when milk is heated to above 80°C, there is interaction of beta-lactoglobulin with the whole,alpha- or kappa-casein and the coagulated whey proteins get entrapped and go along with the casein in the coagulum. CW had shown slightly less protein content than BW because of lower total whey protein content in the original milk used. Higher whey protein content of buffalo milk cheese whey was reported by other workers (Mathur, 1975; Srivastava, 1991). The protein content of BW obtained in our study is in agreement with the findings of Mathur (1975) and Chandra Mohan (1977).

Protein content of PW is also in agreement with the findings of Mathur (1975). Similarly, protein obtained in CW is within the range of protein content of cheddar cheese whey reported by other workers (Moulin and Galzy, 1984; Sienkiewicz and Riedel, 1990).

5.1.2.3 Non-protein nitrogen

As depicted in Fig 1, the NPN content was 35.50 (SD 0.512), 28.05 (SD 0.546) and 37.55 mg/100 g (SD 0.905) for PW, CW and BW, respectively.

Higher content of NPN in PW and BW may be due to the higher initial NPN content of buffalo milk used in the product manufacture. The NPN content of BW obtained in our study was slightly lower than the values reported by Chandra Mohan (1977). The NPN of CW falls within the range reported by Sienkiewicz and Riedel (1990).

5.1.2.4 Lactose

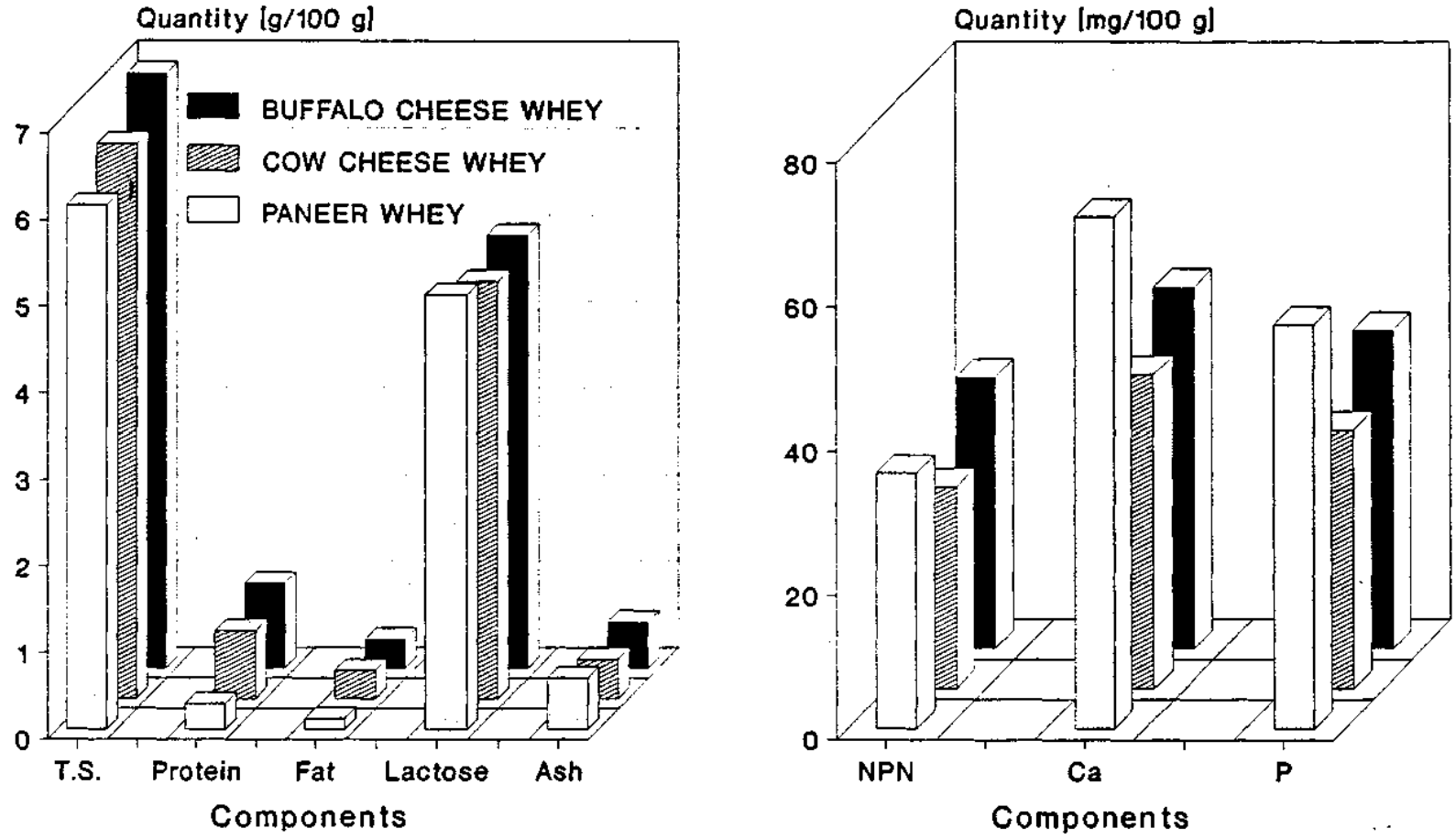
As can be seen in Fig 1, not much variation in the lactose content of BW and PW was noticed. However, slightly higher content of lactose was observed in case of PW (5.03% ± 0.044) than BW (5.01% ± 0.033). But the lactose content of CW was found to be lower (4.83% ± 0.024) compared to other two types of whey. This could be attributed to the compositional differences in lactose content of cow and buffalo milk used in the product manufacture.

5.1.2.5 Fat

Fig 1 reveals that PW had lower fat content (0.13%) than CW (0.33%) and BW (0.34%), the SD respectively being 0.017, 0.014 and 0.015.

This may be due to the fact that during paneer manufacture, there is rapid coagulation by heat treatment and acidulation, which entraps

FIG 1. CHEMICAL COMPOSITION OF WHEY SYSTEMS



most of the fat globules in the coagulum. On the contrary, during cheese manufacture due to slow coagulation some of the fat globules are not entrapped by the curd and are liberated into the whey, thereby, increasing the fat content of the resultant whey. Fat contents of CW and BW were observed to be almost similar. The fat content of cheese wheys obtained in our experiments are in agreement with the results of other workers (Moulin and Galzy, 1984; Sienkiewicz and Riedel, 1990).

5.1.2.6 Ash and minerals

As represented in Fig. 1, the ash content was 0.60 ± 0.020, 0.54 ± 0.016 and 0.46 ± 0.015 for PW, BW and CW, respectively. Similarly, calcium and phosphorus values were 71.05 and 56.05, 50.15 and 44.15, and 43.55 and 35.85 mg/100 g.

Mineral content of PW is distinctly higher than the cheese wheys. During the production of paneer, because of acidification process, the minerals bound to casein are released into the whey. Most of the casein bound calcium phosphate is converted to soluble form leading to increased calcium and phosphorus content in PW. The higher calcium and phosphorus content of BW than CW could be ascribed to the mineral make up of the milk used for the production of cheese. The calcium and phosphorus contents of BW obtained in our experiment are in close agreement with the findings of Chandra Mohan (1977). Similarly, calcium and phosphorus contents of PW were close to the values reported by Mathur (1975). Even the calcium and phosphorus contents of CW are within the range specified by other workers (Moulin and Galzy, 1984; Sienkiewicz and Riedel, 1990).

5.2 STANDARDIZATION OF FACTORS AFFECTING FLUX RATE IN REVERSE OSMOSIS

The economy of membrane process is highly dependent on the flux rate. As far as possible it should be aimed at attaining maximum

permeation per unit area of the membrane to run the plant efficiently. Various operational parameters and the pretreatments imparted to whey can alter the flux to a great extent. The selectivity of these pretreatments varies with the type of whey, pH, ionic strength and the other constitutive make up of the system. Hence, it was aimed in our investigation to study the various parameters such as clarification of whey, operational temperature and pressure, pH adjustments and preheat treatments of whey to enhance the flux and to run the plant effectively. The results obtained are presented and discussed below.

5.2.1 EFFECT OF CLARIFICATION

The effect of clarification on the flux of PW, CW and BW are depicted in Fig. 2.

5.2.1.1 Paneer whey

The flux of PW during RO as affected by clarification is presented in Fig. 2a. As indicated in the figure, at the initial stage, the flux was 37.00 and 37.40 $l/m^2/h$, respectively for unclarified and clarified whey. However, the flux declined to 9.20 and 9.80 $l/m^2/h$, respectively after 60 per cent volume reduction. The average flux was found to be 29.52 and 29.76 $l/m^2/h$, respectively for the unclarified and clarified whey.

The increase in flux noticed for the clarified whey was only marginal and statistically (Table 2) not significant ($P \leq 0.01$). Hence, it can be inferred that PW does not require clarification treatment for the processing by RO. Some reports, however, indicate that clarification of whey does improve the flux in case of cottage cheese whey and cheddar cheese whey (Hiddink *et al.*, 1980; Patocka and Jelen, 1987b). This may be attributed to the removal of casein fines which otherwise would cause

severe fouling of membrane. In case of PW no improvement in the flux rate by clarification was observed. This may be attributed to the fact that paneer coagulum obtained at 70°C is harder than cheese coagulum and hence casein fines are lower in PW compared to cheese wheys. Therefore, ordinary filtration through muslin cloth is good enough to remove casein fines. Also PW had low fat content of 0.13 per cent, which is nearly three times less than those of cheese wheys.

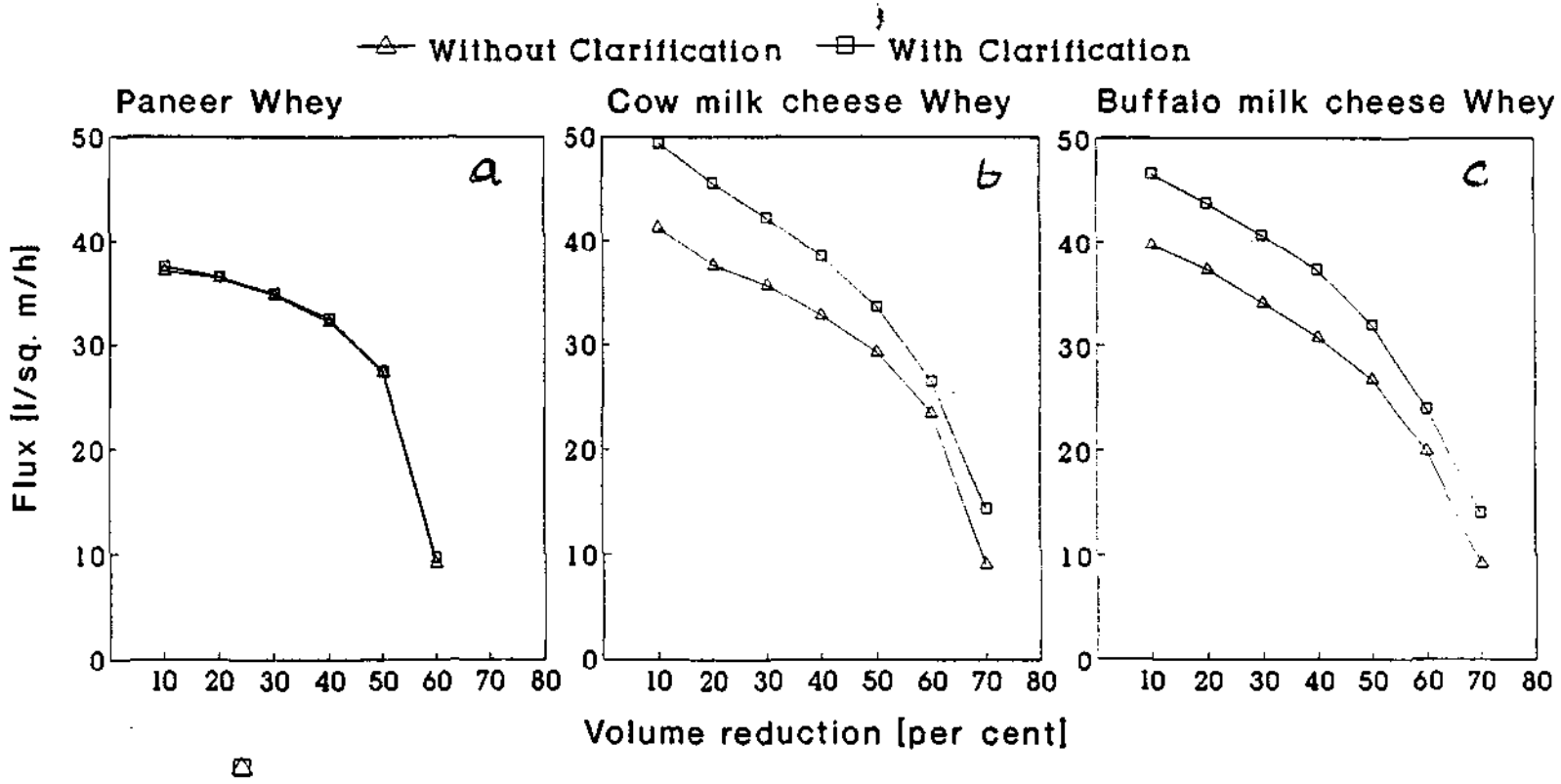
5.2.1.2 Cow milk cheddar cheese whey

The effect of clarification of CW on the flux is presented in Fig. 2b. The flux for the unclarified and clarified CW at the initial stage was found to be 41.30 and 49.40 $l/m^2/h$, respectively. However, these values were observed to be 9.10 and 14.40 $l/m^2/h$ after 70 per cent volume reduction. The average flux for 70 per cent volume reduction was 29.90 $l/m^2/h$ for the unclarified whey and 35.77 $l/m^2/h$ for the clarified whey.

From the results, it is apparent that clarification of CW improves the flux to a great extent. The statistical analysis (Table 2) also revealed that there was significant difference ($P \leq 0.01$) in the flux obtained for the unclarified and clarified whey. Significant improvement of flux by clarification could be attributed to the fact that clarification helps in the removal of casein fines as well as residual fat in whey which are considered to be major foulants during membrane processing (Patocka and Jelen, 1987b).

From the results it is evident that clarification of CW significantly improves the flux. Hence, the whey should be subjected to clarification before RO concentration.

FIG 2. EFFECT OF CLARIFICATION OF WHEY ON REVERSE OSMOSIS FLUX



5.2.1.3 Buffalo milk cheddar cheese whey

The results pertaining to the effect of clarification of BW on the flux are presented in Fig 2c.

From the figure it is clear that there is a distinct demarcation between the flux of clarified and unclarified BW. At the initial stages the flux values were 39.70 $l/m^2/h$ for unclarified whey and 46.60 $l/m^2/h$ for the clarified whey. However, the flux reduced to 9.20 and 14.10 $l/m^2/h$, respectively after 70 per cent volume reduction. Similarly, mean flux was 28.27 $l/m^2/h$ for the unclarified BW and 34.07 $l/m^2/h$ for the clarified buffalo milk cheddar cheese whey.

Buffalo milk cheddar cheese whey behaved in a way similar to CW. As could be seen from the results, there is substantial improvement in the flux of BW by clarification. Statistical analysis (Table 2) has also shown that there is significant difference ($P \leq 0.01$) between the flux of clarified and unclarified whey at all the stages of volume reduction. This may be attributed to removal of casein micelles and residual fat which are considered mainly responsible for reduction in flux (Glover, 1985; Patocka and Jelen, 1987b).

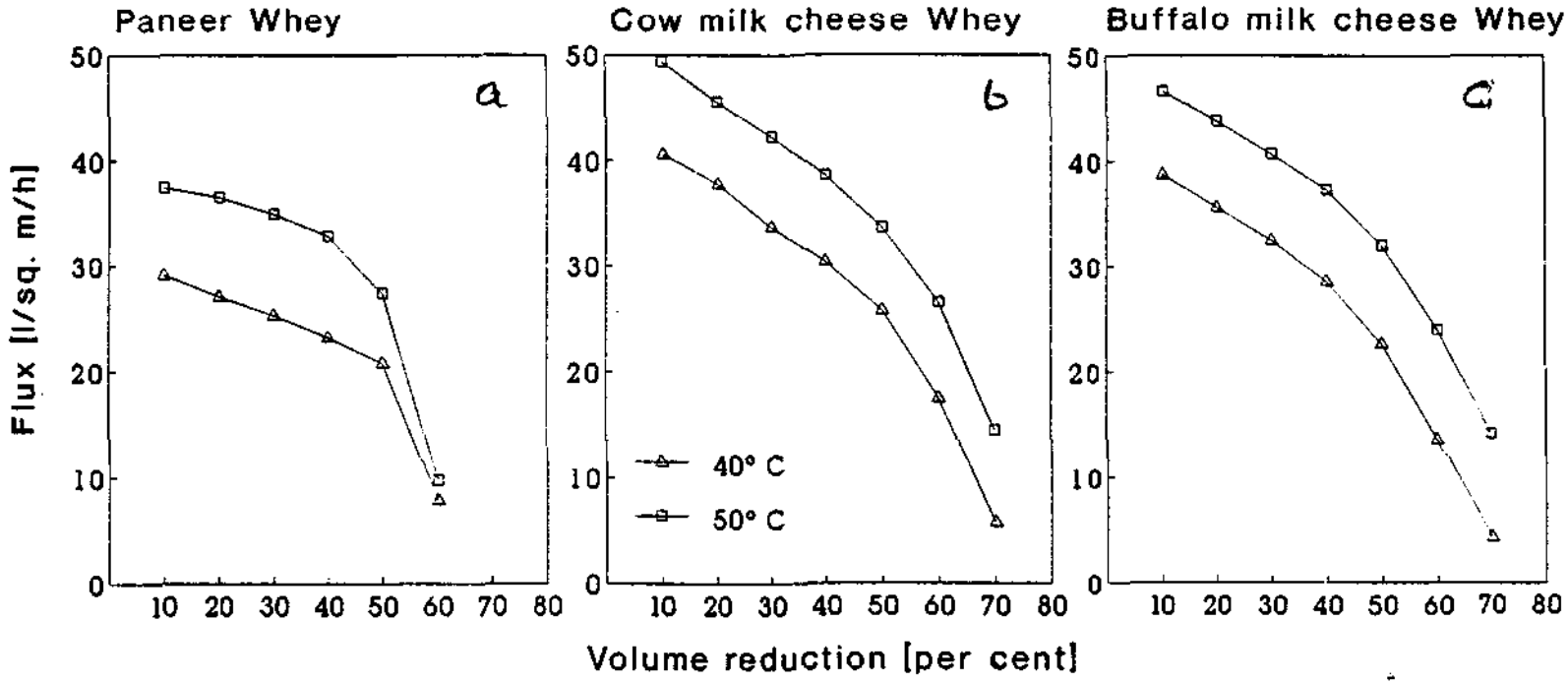
From the above observations it can be inferred that BW should be subjected to clarification before processing by RO in order to maximize the flux.

5.2.2 EFFECT OF OPERATIONAL TEMPERATURE

The effect of temperature of operation on the flux rate of PW, CW and BW is depicted in Fig 3.

As indicated in the figure, it is clear that as the temperature of whey was raised from 40 to 50°C, the flux rate increased all along the

FIG 3. EFFECT OF TEMPERATURE OF WHEY ON REVERSE OSMOSIS FLUX



run during RO concentration regardless of whey. Initial flux was 29.10, 40.60 and 38.70 $l/m^2/h$ at 40°C as against 37.10, 49.10 and 46.40 $l/m^2/h$ at 50°C for PW, CW and BW, respectively. The average flux of PW for 60 per cent volume reduction was 22.23 $l/m^2/h$ at 40°C as against 29.80 $l/m^2/h$ at 50°C. CW and BW had shown an average flux of 27.35 and 25.14 $l/m^2/h$ at 40°C and 35.76 and 34.07 $l/m^2/h$ at 50°C, respectively for a volume reduction of 70 per cent.

Statistical analysis (Table 3) indicated that the increase in flux was highly significant ($P \leq 0.01$). Thus, it can be concluded that operation at 50°C is more beneficial than at 40°C for all the types of whey. The results are in agreement with the reports of Glover (1985), who observed that for every 5°C rise in temperature, there was increase in permeate flux by 40 per cent. Donnelly et al. (1974) also reported that there was improvement in flux on increasing the temperature. Higher temperature of operation aids in increasing the back diffusion of solids away from the membrane and decreasing the viscosity of whey to a great extent, thus improving the flux substantially.

The inference can thus be drawn that the RO concentration of whey should be carried out at 50°C in order to maximize the efficiency of the process.

5.2.3 EFFECT OF OPERATIONAL PRESSURE

In order to select the optimum pressure for the RO concentration of whey, four levels of pressures were studied, viz., 25, 30, 35 and 40 bar. The effect of pressure on the resultant flux of PW, CW and BW are presented in Table 4a.

The results represent the average flux obtained for 70 per cent volume reduction of PW, CW and BW by RO concentration at an operating

Table 2. ANOVA for effect of clarification of whey on reverse osmosis flux

Source of variation	Paneer whey		Cow milk cheese whey		Buffalo milk cheese whey	
	d.f.	'F' value	d.f.	'F' value	d.f.	'F' value
Replication	2	0.688^{NS}	2	0.309^{NS}	2	0.994^{NS}
Clarification (A)	1	4.100	1	50642.754	1	40501.625**
Volume reduction (B)	5	181158.250**	6	110231.156**	6	108725.256**
Interaction A x B	5	2.600^{NS}	6	743.451**	6	628.360**
Error	22	-	26	-	26	-

Table 3. ANOVA for effect of temperature of whey on reverse osmosis flux

Source of variation	Paneer whey		Cow milk cheese whey		Buffalo milk cheese whey	
	d.f.	'F' value	d.f.	'F' value	d.f.	'F' value
Replication	2	0.059^{NS}	2	0.509^{NS}	2	2.902^{NS}
Temperature (A)	1	98642.49**	1	242199.73**	1	221592.10**
Volume reduction (B)	5	92976.18**	6	290466.81**	6	230418.23**
Interaction A x B	5	2596.46**	6	122.36**	6	346.24**
Error	22	-	26	-	26	-

NS = Non-significant
** Significant at 1 per cent level ($P \leq 0.01$)

pressure of 25, 30, 35 and 40 bar. The average flux was found to be 18.50, 23.30, 26.40 and 26.90 $l/m^2/h$ for PW, 23.90, 30.20, 33.80 and 34.70 $l/m^2/h$ for CW and 22.30, 28.60, 32.30 and 32.90 $l/m^2/h$ for BW at 25, 30, 35 and 40 bar pressure, respectively.

It is evident from the results that as the pressure increased from 25 to 40 bar, the extent of increase in flux was observed to be higher upto a pressure of 35 bar in all the three types of whey. Thereafter, the flux became independent of pressure and the extent of increase in flux from 35 to 40 bar was found to be marginal.

Statistical analysis (Table 4b) has also revealed that there is significant effect ($P \leq 0.01$) of pressure on the flux in all the three types of whey. However, the flux increase from 35 to 40 bar operational pressure was non-significant. The marginal increase in flux after 35 bar pressure could be attributed to the increase in the polarized layer resistance due to its compaction at high pressures. The results obtained in our experiments are in agreement with the results of Kulozik and Kessler (1988b) and Cheryan et al. (1990).

From the results it can be concluded that an operational pressure of 35 bar is optimum for the concentration of all the three types of whey by reverse osmosis.

5.2.4 EFFECT OF pH AND HEAT TREATMENT OF WHEY

Effect of various levels of pH adjustment and preheating of PW, CW and BW on the resultant flux during concentration by RO was evaluated. Preheated whey was concentrated by RO at a temperature of 50°C and a pressure of 35 bar. During concentration, at every regular interval of volume reduction the flux was recorded. The efficacy of flux as affected by various pretreatments is presented and discussed hereunder.

Table 4a. Effect of operational pressure on reverse osmosis flux

Pressure (bar)	Average flux ($l/m^2/h$)		
	Paneer whey	Cow milk cheese whey	Buffalo milk cheese whey
25	18.50	23.90	22.30
30	23.30	30.20	28.60
35	26.40	33.80	32.30
40	26.90	34.70	32.90

Table 4b. ANOVA for effect of operational pressure on reverse osmosis flux

Source of variation	d.f.	'F' value	CD
Replication	2	0.2519^{NS}	-
Type of whey	2	185.77**	-
Pressure	3	785.89**	1.09
Error	28	-	-

NS = Non-significant

** Significant at 1 per cent level ($P \leq 0.01$)

5.2.4.1 Paneer whey

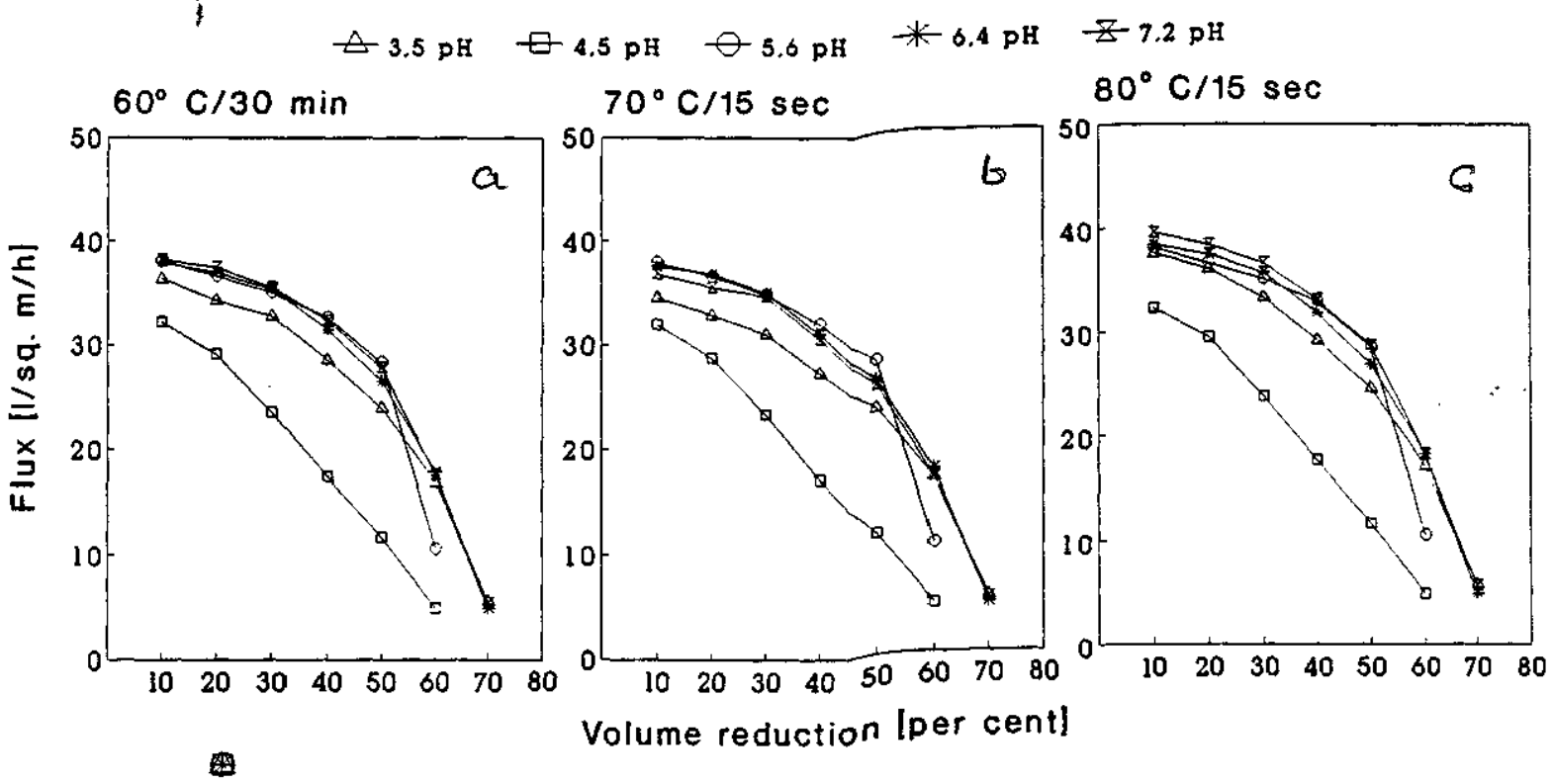
Paneer whey was adjusted to pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 and heat treated at temperatures of 60°C/30 min, 70°C/15 sec and 80°C/15 sec. At each combination of these pretreatments RO concentration was carried out and flux was computed at regular interval. The results are presented in Fig 4.

It was observed that when pH adjusted PW was preheated to 60°C/30 min, 70°C/15 sec and 80°C/15 sec, the initial flux rates were 36.40, 34.60 and 37.60 $l/m^2/h$ for pH 3.5; 32.20, 32.00 and 32.30 $l/m^2/h$ for pH 4.5; 38.10, 38.00 and 38.10 $l/m^2/h$ for pH 5.6; 37.90, 37.60 and 38.40 $l/m^2/h$ for pH 6.4 and 38.20, 36.90 and 39.60 $l/m^2/h$ for pH 7.2. Whereas, the average flux for above pH values was 28.83, 19.82, 30.25, 31.05 and 31.53 $l/m^2/h$ for 60°C/30 min, 27.68, 19.62, 30.02, 30.73 and 30.05 $l/m^2/h$ for 70°C/15 sec and 29.65, 19.97, 30.30, 31.43 and 32.43 $l/m^2/h$ for 80°C/15 sec heating.

Among the three heat treatments, 80°C/15 sec showed the highest flux rate at all pH levels. The flux rate decreased with decrease in pH upto 4.5, but further decrease to 3.5 improved the flux slightly. At a normal pH of 5.6, the initial flux was 38.10 $l/m^2/h$ (with 80°C/15 sec preheating) but it improved to 39.60 $l/m^2/h$ when the pH of whey was raised to 7.2. Though the difference between these flux rates is little in the initial stages, it became significantly larger at later stages of volume reduction. At pH 5.6, after about 50 per cent volume reduction, the flux was 28.50 $l/m^2/h$ and it declined sharply to 10.60 $l/m^2/h$ after about 60 per cent volume reduction. Adjusting the pH of whey to 7.2 was beneficial only when heated to 80°C/15 sec and hence these pretreatments will maximize the flux yield of PW compared to any other combination of treatment.

Statistical analysis (Table 5) has also shown that there is

FIG 4. EFFECT OF pH AND PREHEATING OF PANEER WHEY ON REVERSE OSMOSIS FLUX



significant ($P \leq 0.01$) effect of pH, preheating temperature and their interaction on the resultant flux at all the levels of volume reduction.

Hence, it is evident from the results that the flux rate of PW can be improved by altering pH and heat treatment. Therefore, PW should be adjusted to pH 7.2 and heated to 80°C/15 sec before processing through RO in order to obtain maximum benefit per unit area of membrane.

5.2.4.2 Cow milk cheddar cheese whey

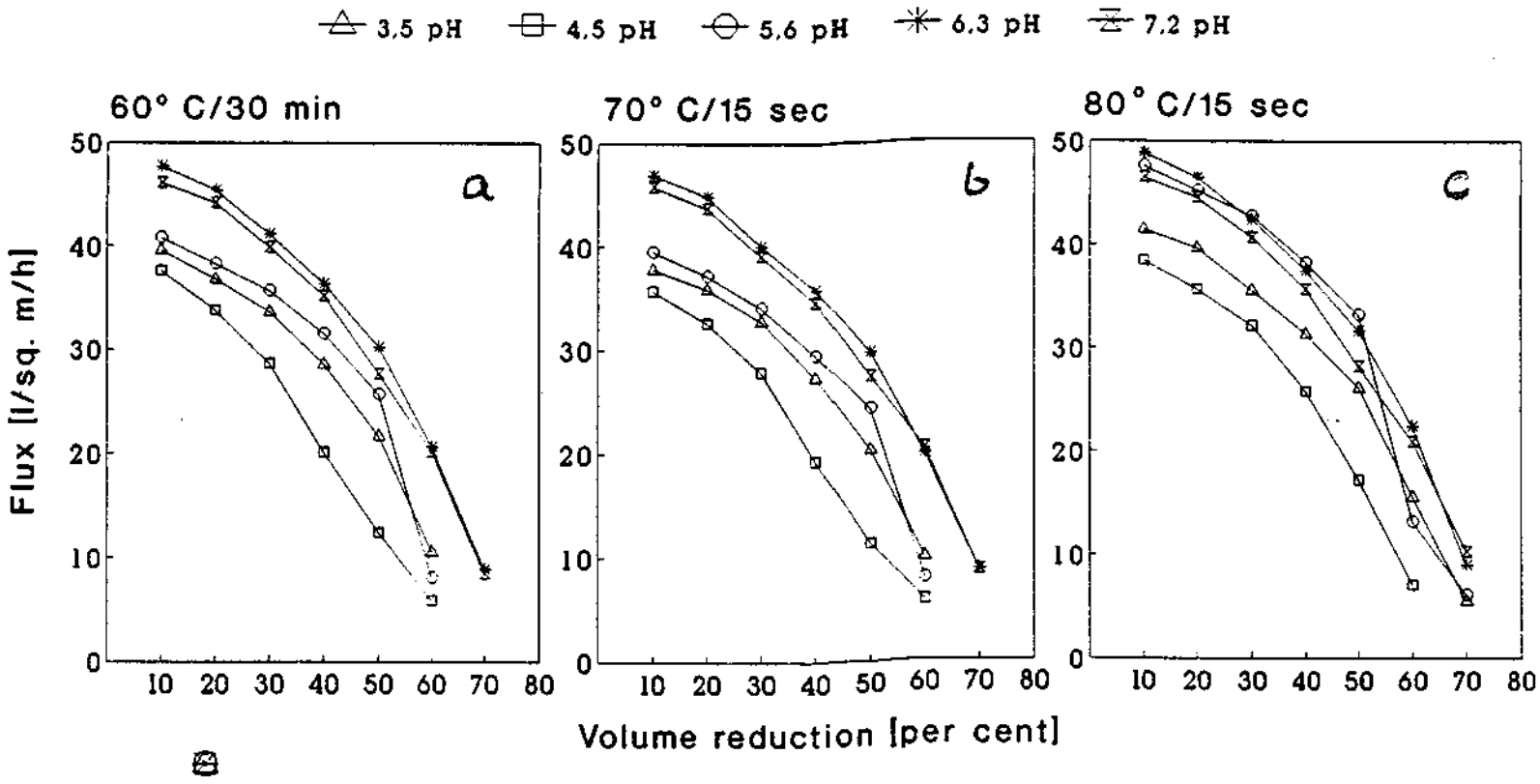
The effect of heating CW to different temperature of 60°C/30 min, 70°C/15 sec and 80°C/15 sec at pH values of 3.5, 4.5, 5.6, 6.3 and 7.2 on the resultant flux is shown in Fig 5.

The CW yielded initial flux of 39.60, 37.60, 40.80, 47.70 and 46.60 $l/m^2/h$ at 60°C/30 min, 37.60, 35.70, 39.50, 46.90 and 45.80 $l/m^2/h$ at 70°C/15 sec and 41.50, 38.50, 47.60, 48.90 and 46.50 $l/m^2/h$ at 80°C/15 sec, respectively for pH values of 3.5, 4.5, 5.6, 6.3 and 7.2. Similarly, the average flux values for 70 per cent volume reduction were found to be 25.20, 20.40, 26.44, 32.94 and 31.68 $l/m^2/h$ for 60°C/30 min, 24.15, 19.63, 25.40, 32.27 and 31.37 $l/m^2/h$ for 70°C/15 sec and 27.95, 23.12, 32.38, 34.09 and 32.44 $l/m^2/h$ for 80°C/15 sec.

It can be observed from the figure that as the concentration or volume reduction increased, the flux decreased irrespective of pretreatment given to whey. However, the extent of decline varied with the pH of whey and temperature of preheat treatment. It is interesting to note that a sharp decline was observed in flux at pH 5.6 after a certain volume reduction.

Heating CW at 80°C/15 sec yielded maximum flux whereas at 70°C/15 sec flux was minimum. Preheat treatment at pH values of 3.5 and 4.5 showed lower flux rates, but higher flux values were obtained at pH values of 5.6,

FIG 5. EFFECT OF pH AND PREHEATING OF COW MILK CHEESE WHEY ON REVERSE OSMOSIS FLUX



6.3 and 7.2. When whey was heated to 80°C/15 sec, the maximum flux was shown at normal pH (6.3).

Statistical analysis (Table 5) showed that pH and preheat treatment of whey significantly ($P \leq 0.01$) affected flux rate all volume reduction levels. The interaction effect (pH and heat treatment imparted to whey) was also observed to be significant.

Thus, it can be concluded that with regard to CW only preheat treatment of 80°C/15 sec without any pH adjustment will suffice for obtaining maximum permeation per unit area of the membrane.

5.2.4.3 Buffalo milk cheddar cheese whey

Buffalo milk cheddar cheese whey was heated to 60°C/30 min, 70°C/15 sec and 80°C/15 sec at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2. At each combination of these treatments RO concentration was carried out. The resultant flux at various levels of volume reduction was computed. The results obtained are delineated in Fig 6.

It can be seen from the figure that as the concentration or volume reduction increased, the flux decreased irrespective of the pH and heat treatment imparted to whey. However, the rate of decline varied from one combination of treatment to another.

The initial flux rates at pH 3.5, 4.5, 5.6, 6.4 and 7.2 were 40.80, 36.60, 45.20, 47.10 and 47.70 $l/m^2/h$ for 60°C/30 min, 37.60, 35.90, 44.30, 46.60 and 46.90 $l/m^2/h$ for 70°C/15 sec and 42.60, 37.10, 46.60, 48.00 and 48.50 $l/m^2/h$ for 80°C/15 sec treatment, respectively. Similarly, the average flux values for 70 per cent volume reduction at these pH values were found to be 28.14, 21.58, 30.40, 34.68 and 35.22 for 60°C/30 min, 26.98, 21.11, 29.78, 34.07 and 34.38 $l/m^2/h$ for 70°C/15 sec and 29.15, 22.08, 31.40, 35.28 and 36.24 $l/m^2/h$ for 80°C/15 sec heating.

FIG 6. EFFECT OF pH AND PREHEATING OF BUFFALO MILK CHEESE WHEY ON REVERSE OSMOSIS FLUX

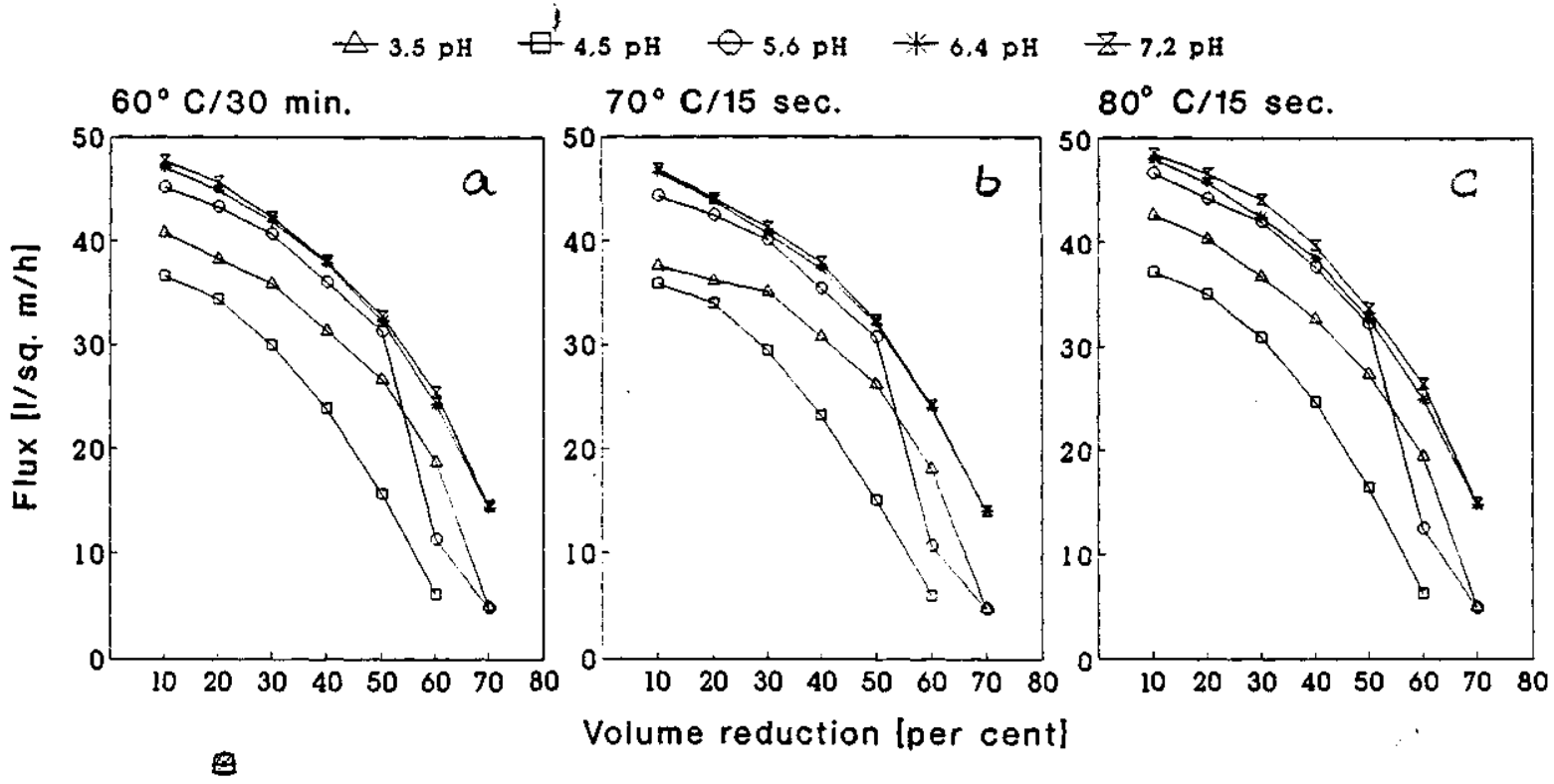


Table 5. ANOVA for effect of pH and heat treatment of whey on reverse osmosis flux

Source of variation	Paneer whey		Cow milk cheese whey		Buffalo milk cheese whey	
	d.f.	'F' value	d.f.	'F' value	d.f.	'F' value
Replication	2	3.009^{NS}	2	1.004^{NS}	2	2.980
Heating temperature (A)	2	10772.302**	2	17340.275**	2	22041.113**
pH (B)	4	458968.938**	4	103800.723**	4	670526.813**
Volume reduction (C)	5	1213303.250**	6	3578700.420**	6	2600973.750**
Interaction						
A x B	8	1590.774**	8	128.736**	8	447.369**
A x C	10	127.977**	12	968.120**	12	595.100**
B x C	20	16422.924**	24	12680.570**	24	21196.977**
A x B x C	40	96.882**	48	101.830**	48	165.845**
Error	178	-	208	-	208	-

NS = Non-significant

** Significant at 1 per cent level ($P \leq 0.01$)

At all the heating temperatures, the flux rate was lower at pH values of 3.5 and 4.5 than at other pH values. At 80°C/15 sec heat treatment, an increase in pH from a normal pH of 6.4 to 7.2 increased the average flux to 36.24 from 35.28 $l/m^2/h$. Reduction in pH to 5.6, 4.5 and 3.5 resulted in low average flux rate of 31.40, 22.08 and 29.15 $l/m^2/h$ respectively. The preheating at 80°C/15 sec at a pH of 7.2 resulted in higher average flux rate of 36.24 l/m /h than at 60°C/30 min (35.22 $l/m^2/h$) and 70°C/15 sec (34.38 $l/m^2/h$).

It was also observed from the statistical analysis (Table 5) that pH and heat treatments imparted to whey before RO processing have significant effect ($P \leq 0.01$) on the resultant flux at every stage of volume reduction. The interactions of various pH and heat treatment on the permeation were significant.

The statistical analysis and average flux data revealed that whey heated to 80°C/15 sec at a pH 7.2 resulted in the highest flux rate as compared to other treatments.

It is evident from the above results that during RO processing of PW, CW and BW as the concentration or the volume reduction increased, the flux rate decreased irrespective of the type of whey or the pretreatment imparted to whey. The decrease in flux with the increase in concentration could be attributed to the osmotic pressure exerted by the retentate (Kulozik and Kessler, 1988b; Cheryan et al., 1990; Boxtel, 1992). As the process progresses, the low molecular weight compounds such as lactose and soluble salts get concentrated, resulting into increased osmotic pressure. Hence, the net driving force decreases. The decline in the resultant flux with the progress of concentration could be ascribed to the fouling of RO membrane (Spangler and Amundson, 1986; Cheryan et al., 1990; Boxtel et al., 1992). The fouling decreases the flux either by

increasing the hydrodynamic resistance to permeate flow and/or increasing the effective osmotic pressure (Boxtel et al., 1992). As a result of membrane fouling, the permeation rate decreased due to growing protein gel layer, the precipitation of calcium phosphate on the membrane surface or in the pores, and the protein adsorption on the surface of the membrane.

By and large, PW had resulted in lower flux compared to CW and BW. This could be attributed to the nature of fouling caused during processing of PW. Fouling varies with type, composition, pH and ionic strength of whey (Hickey et al., 1980). As observed by Hayes et al.(1974), the nature of fouling depends on the calcium and phosphate concentration, the pH of the feed solution and the state of aggregation of protein after heat treatment or the prolonged exposure to conditions leading to intermolecular or ionic interactions. Hence, higher calcium and phosphate concentrations and varying ionic strengths could have caused lower flux in PW as compared to the other type of whey.

The pH and heat treatment given to whey were found to have a significant effect on the resultant flux rate. For instance, low pH values such as 3.5 and 4.5 resulted in lowest flux rates. But when pH was increased to near neutral and heated to 80°C/15 sec, the flux improved significantly as in the case of PW and BW. At low pH values, calcium phosphate, which is bound to protein will be partially converted into soluble form (Patocka and Jelen, 1987a; Renner and Abd El-Salam, 1991). Also at lower pH values even the reactive sites of proteins are not exposed to form large arregates. Hence, there will be blocking of membrane pores by soluble calcium and adsorption of proteins on the surface of the membrane.

At a pH value of 5.6, proportionately higher flux was observed in all the three types of whey compared to pH 3.5 and 4.5. This could be

because of the fact that at or above pH 5.6 the reactive sites of whey proteins are opened (Hayes et al., 1974). Hence, when pH is adjusted to 5.6 and heated, there is calcium induced protein interaction, which aids in forming apatite, large enough not to block the pores of RO membrane. This results in enhanced rate of flux. These results are in agreement with the results of Glover (1985). However, at later stages there is sharp decline in the flux at pH 5.6. This may be because of the fact that as the concentration of whey by RO proceeds, there is gradual reduction in the pH of retentate. Hence, when the pH reaches near the isoelectric point of beta-lactoglobulin, it leads to increase in viscosity of the feed. Due to increase in viscosity and severe adsorption of protein on to the surface of membrane, there is a drastic decrease in the flux.

In case of PW and BW, when pH was adjusted to 7.2 and heated to 80°C/15 sec, better flux was observed. This could be ascribed to the precipitation of calcium phosphate and resultant calcium related protein-protein interaction. As reported by Pauliot et al. (1991), addition of external calcium to whey and alkalinization induces the greater precipitation of calcium phosphate. As in the case of PW and BW, the calcium content is already higher, adjusting pH to 7.2 and heating to 80°C/15 sec induces calcium related protein-protein interaction which forms larger apatite, which are large enough not to block the pores of RO membrane. Similar observations, i.e., enhanced flux of cheddar cheese whey on addition of calcium to whey followed by adjustment of pH above 6.5 and subsequent heating have been reported by Hickey et al. (1980). The flux of CW did not improve when pH was adjusted to 7.2 followed by heating. The reasons for this could be the low calcium content of CW, which is not sufficient to form larger apatite to interact with protein to form larger aggregates.

In all the three types of whey, heating at 80°C/15 sec had shown higher flux compared to 60°C/30 min or 70°C/15 sec, because higher temperature is needed to stabilize the calcium phosphate complex formation (Pauliot et al.,1991) and to form larger apatite by calcium related protein interaction (Glover, 1985).

Our results are in agreement with the recent report of Boxtel (1992). According to him reduction in flux during RO of whey is based on precipitation of calcium phosphate. Protein, membrane and solute interaction which contribute significantly to the reduction of flux. Any treatment which can overcome these interactions may help in reducing fouling and increasing the flux.

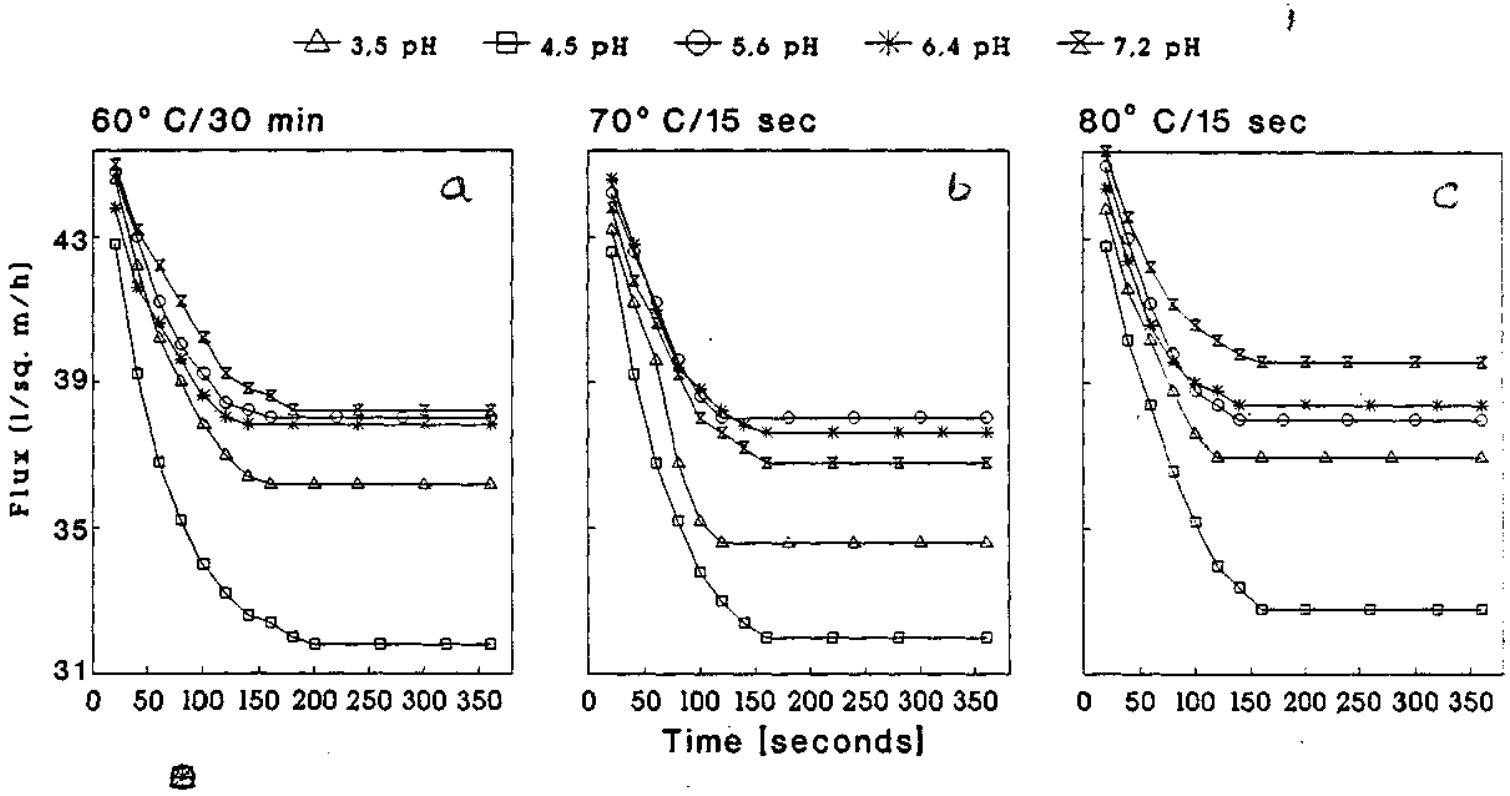
5.2.5 FOULING OF REVERSE OSMOSIS MEMBRANE

A major limiting step in the exploration of RO process, particularly with multi component streams, is membrane fouling. Membrane fouling is due to the deposition and accumulation of submicron particles on the membrane surface and/or the crystallization and precipitation of smaller solutes on the surface and within the pores of the membrane itself. This process occurs to a large extent at very beginning of the operation and continues as the concentration progresses. Attempts were made to mitigate the fouling of RO membrane during concentration of whey by various pretreatments. The results pertaining to the effect of various levels of pH adjustment and preheating on the fouling pattern are presented in Figs 7, 8 and 9, respectively for PW, CW and buffalo milk cheddar cheese whey.

5.2.5.1 Paneer whey

The effect of heating PW at 60°C/30 min, 70°C/15 sec and 80°C/15 sec at various levels of pH on the fouling pattern of RO membrane are presented in Figs 7a, 7b and 7c, respectively.

FIG 7. EFFECT OF pH AND PREHEATING OF PANEER WHEY ON FOULING OF REVERSE OSMOSIS MEMBRANE



As it can be seen from Fig 7a, when PW was heated to 60°C/30 min at various pH levels prior to RO concentration, the fouling was severe at pH values of 4.5 and 3.5 and this severity reduced drastically when the pH of whey was raised to pH 7.2. In almost all the combinations of pretreatments fouling occurred within a span of 3 min and thereafter remained constant. At pH 3.5, the flux reduced from an initial value of 44.60 to 36.20 $l/m^2/h$, whereas at pH 4.5 from 42.80 to 31.80, at pH 5.6 from 44.80 to 38.00, at pH 6.4 from 43.80 to 37.80 and at pH 7.2 from 45.00 to 38.20 $l/m^2/h$.

Perusal of Fig 7b indicate the effect of heating PW to 70°C/15 sec at various pH levels. It was observed that heating PW at this temperature aggravates the fouling. The intensity of fouling was severe at all the pH levels. The initial flux values were reduced to a great extent within 3 min. Even adjusting the pH to 7.2, followed by heating could not reduce the severity of fouling. In contrast to 60°C/30 min heating, this heat treatment at pH 5.6 was found to be slightly better than the other pH levels. The severity of fouling was higher at pH 4.5 followed by pH values 3.5, 7.2, 6.4 and 5.6, respectively.

Fig 7c clearly depicts the effect of heating PW at 80°C/15 sec on the fouling pattern. The fouling was limited at this treatment compared to other two heat treatments at all levels of pH. Least fouling was noticed at pH 7.2 followed by 6.4, 5.6, 3.5 and 4.5. The flux reduced from an initial value of 45.40 to 39.60 at pH 7.2, 44.40 to 38.40 at pH 6.4, 45.00 to 38.00 at pH 5.6, 43.80 to 37.00 at pH 3.5 and 42.80 to 32.80 $l/m^2/h$ at pH 4.5 within a period of 3 minutes.

From these results it can be inferred that the fouling of RO membrane during processing of PW could be reduced to a great extent by heating PW to 80°C/15 sec at a pH of 7.2.

5.2.5.2 Cow milk cheddar cheese whey

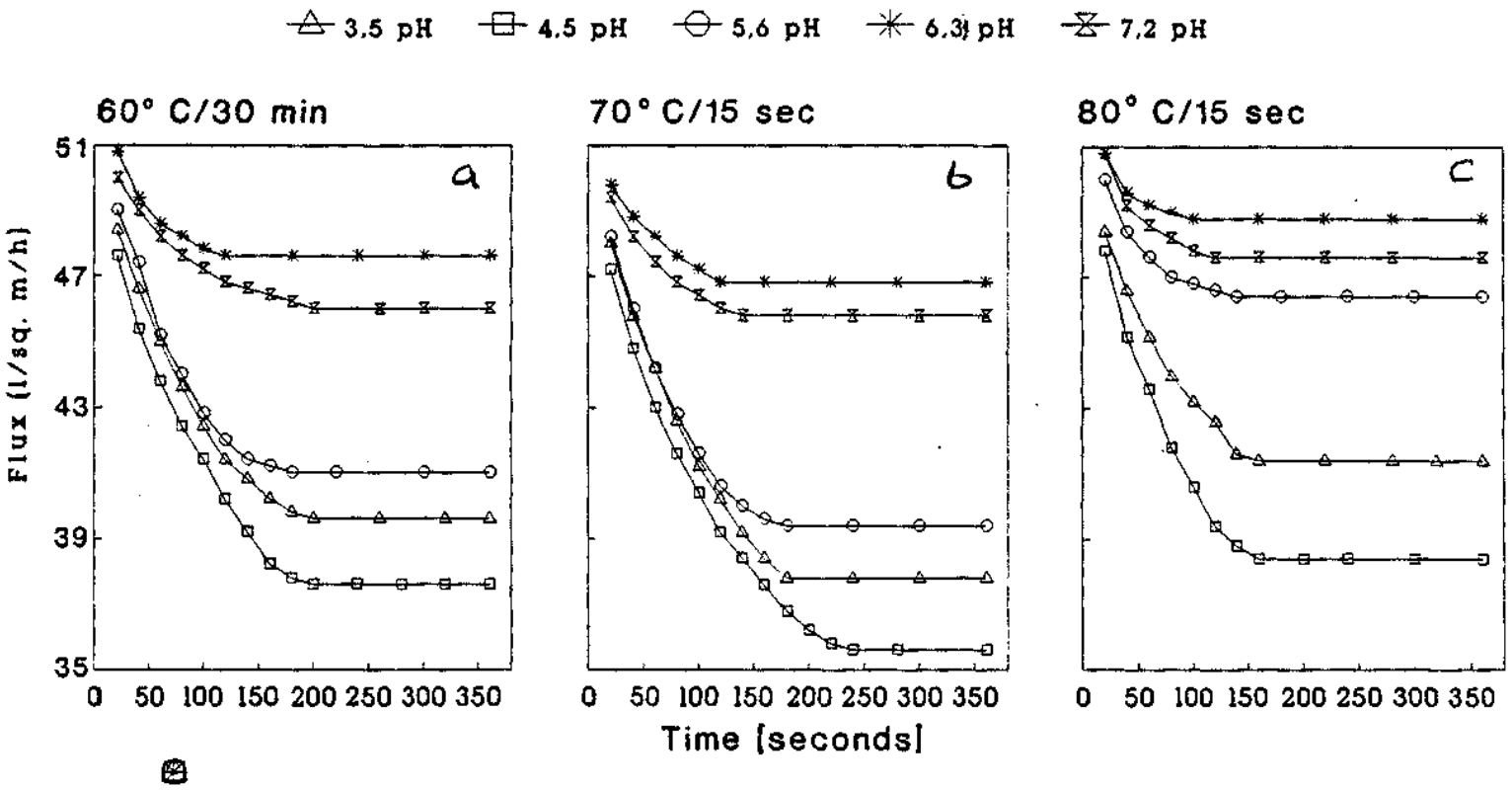
The results pertaining to the effect of heating CW at 60°C/30 min, 70°C/15 sec and 80°C/15 sec at various levels of pH on the fouling pattern are delineated in Fig 8.

As it can be noticed from Fig 8a, when CW was heated at 60°C/30 min at various levels of pH, the greater fouling was observed at pH values 4.5 and 3.5. The intensity of fouling reduced to a great extent at pH 6.3. The fouling at pH 5.6 and 7.2 was lower than at pH of 4.5 and 3.5. At pH 3.5, the flux reduced from an initial value of 48.40 to 39.60, at pH 4.5 47.60 to 37.60, at pH 5.6 49.00 to 41.00, at pH 6.3 50.80 to 47.60 and at pH 7.2 50.00 to 46.00 $l/m^2/h$. In most of the cases fouling occurred within a span of 3 minutes.

Effect of heating CW at 70°C/15 sec at different pH values on the fouling is described by Fig 8b. It is clear from the figure that the extent of fouling was high compared to other two heat treatments (60°C/30 min and 80°C/15 sec). Greater fouling was noticed at pH 4.5 and 3.5. The intensity of fouling was less at pH 6.3 than at pH 5.6 and 7.2. The fouling intensity was in a decreasing order from a pH value of 4.5 followed by pH 3.5, 5.6, 7.2 and 6.3.

However, Fig 8c shows that the fouling was drastically controlled by heating at a temperature of 80°C/15 sec compared to other two temperature treatments. Lowest fouling was observed at pH 6.3 followed by pH values of 5.6, 7.2, 3.5 and 4.5. The permeation rate decreased greatly within 3 min and became constant at all pH levels. The flux decrease was from 48.40 to 41.00, 47.80 to 38.40, 50.00 to 47.60, 50.80 to 48.80, and 50.00 to 46.40 $l/m^2/h$, respectively at pH values of 3.5, 4.5, 5.6, 6.3 and 7.2.

FIG 8. EFFECT OF pH AND PREHEATING OF COW MILK CHEESE WHEY ON FOULING OF REVERSE OSMOSIS MEMBRANE



From the results, it is evident that the fouling of RO membrane by CW was minimum when CW was heated to 80°C/15 sec at its normal pH of 6.3. No other treatments were found to be effective in reducing the extent of fouling.

5.2.5.3 Buffalo milk cheddar cheese whey

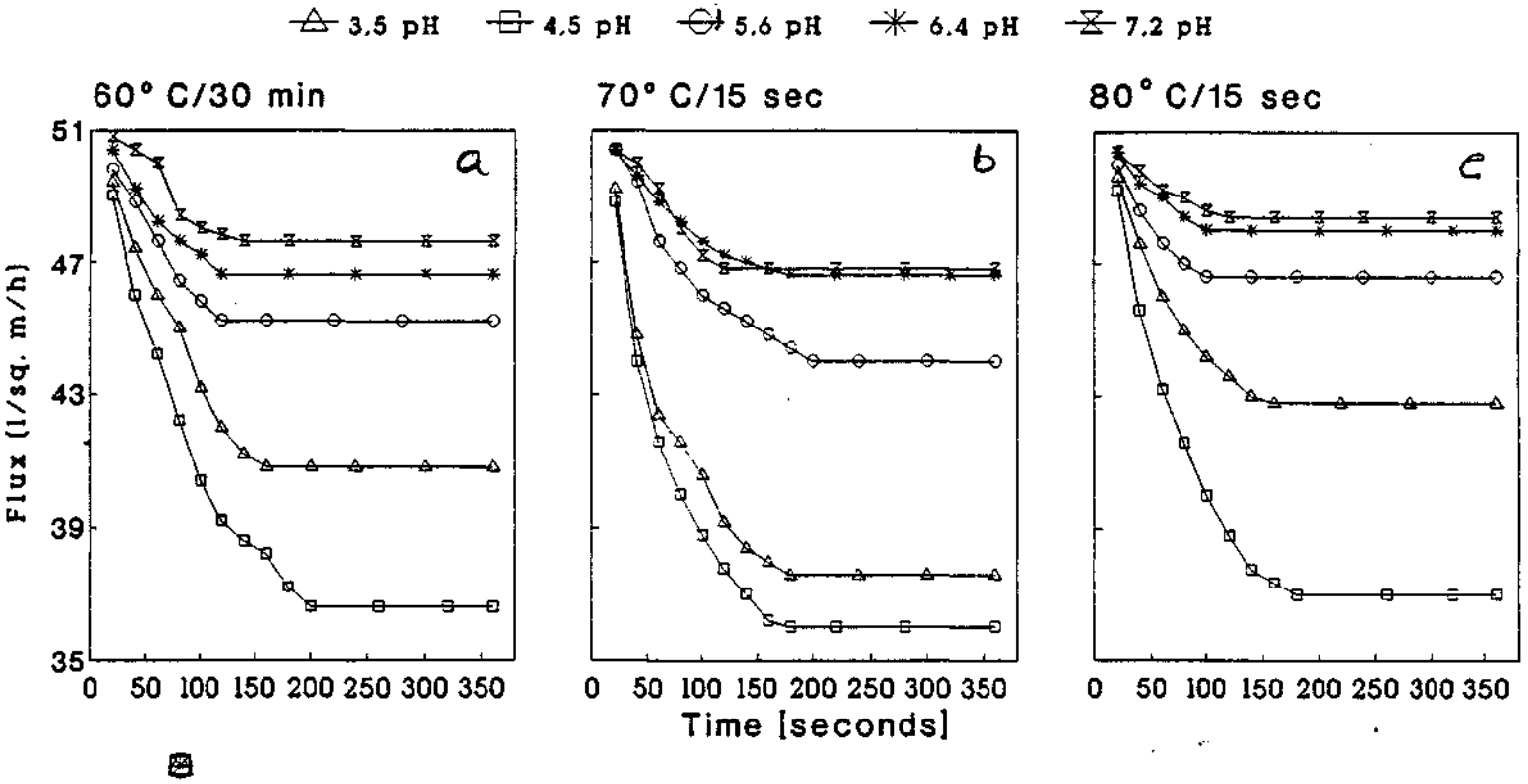
Results with respect to the fouling behaviour of BW are depicted in Fig 9..

It can be comprehended from Fig 9a that the rate of fouling was less at pH 7.2 followed by pH values of 6.4, 5.6, 3.5 and 4.5, when BW was heated to 60°C/30 min. Higher fouling as in other types of whey was observed at lower pH values. At pH 3.5, the flux decreased from an initial value of 49.40 to 40.80, at pH 4.5, from 49.00 to 36.60, at pH 5.6, 49.80 to 46.60, at pH 6.4, 50.40 to 46.60 and at pH 7.2 from 50.80 to 47.60 $l/m^2/h$.

Similarly, Fig 9b indicates that heating BW at 70°C/15 sec resulted in greater fouling than heating at 60°C/30 min at all pH levels. At this treatment greater fouling of the membrane was observed at lower pH values than at higher pH values. At pH values of 3.5, 4.5, 5.6, 6.4 and 7.2, the respective extent of decrease in permeation rates were from 49.20 to 37.60, 48.80 to 36.00, 50.40 to 44.00, 50.40 to 46.60 and 50.40 to 46.80 $l/m^2/h$

It is interesting to observe from Fig 9c that the fouling was greatly reduced compared to other heat treatment at all the pH values when BW was heated to 80°C/15 sec. The lowest fouling was noticed at pH 7.2 followed by 6.4, 5.6, 3.5 and 4.5. At pH 7.2, permeation rate reduced from 50.40 to 48.00, at pH 5.6, 50.00 to 46.60, at pH 4.5, 49.20 to 37.00 and at pH 3.5 from 49.60 to 42.80 $l/m^2/h$.

FIG 9. EFFECT OF pH AND PREHEATING OF BUFFALO MILK CHEESE WHEY ON FOULING OF REVERSE OSMOSIS MEMBRANE



From the fouling studies of BW, it can be clearly inferred that fouling can be greatly reduced during processing of BW by RO when BW heated to 80°C/15 sec at a pH of 7.2 prior to RO concentration.

It is evident from Figs 7, 8 and 9 that there is a drastic reduction flux within 2 to 3 min, though there was no change in concentration the feed. This could be attributed to the fouling of RO membrane caused the whey constituents. As reported by many workers (Kulozik and Kessler, 1988b; Cheryan et al., 1990; Boxtel et al., 1992), membrane fouling is one of the barriers in the RO concentration of whey besides osmotic pressure. As a result of membrane fouling, the flux rate decreases to a great extent. Though fouling of membrane was observed in all the three types of whey processed with various combinations of treatments, the degree of fouling varied with the type of whey and the pretreatments imparted to it. This can be ascribed to the fact that the fouling of membrane during processing of whey caused due to the interaction between membrane and solute, which is primarily by precipitation of calcium phosphate and adsorption of protein which resulted in narrowing of membrane pores Hanemaaijer et al., 1989). Any pretreatment aimed at reducing such interaction improves performance. Whey proteins and mineral salts have been identified as severe foulants during the processing of whey (Hanemaaijer et al., 1989; Henz and Glatz, 1991). The extent of fouling varies with type, pH, ionic strength and various pretreatments imparted to whey (Hickey et al., 1980). Hence, the degree of fouling widely varied with the type of whey and the treatments imparted.

It is evident from the results that maximum fouling was observed in the case of PW. This could be due to inherent nature of the PW, which possesses higher soluble salts than the other two types of whey. Cheryan

and Merin (1980,1981) have also reported that higher the soluble salts greater is the fouling.

It is noteworthy to observe that greater fouling occurred at pH values of 3.5 and 4.5 in all the 3 types of whey irrespective of heat treatment. This may be because when whey is acidified, more and more of calcium becomes soluble (Patocka and Jelen, 1987a) and when it is subjected to heat treatment thus fail to form larger apatite or aggregates as the reactive sites of proteins are not available for the calcium related protein interaction. Hence, the soluble calcium phosphate causes greater fouling by blocking the pores of RO membrane. It is also evident from the reports of Cheryan (1986) that calcium salts can interact with and bind the negatively chaged groups of membrane by electrostatic or charge effects. This could result in a salt bridge between the membrane and proteins which will lead to faster fouling of membrane. The results of Hickey et al. (1980) also revealed that the state of aggregation of inorganic constituents have a direct effect on the fouling. Also, the pH and ionic strength of solution affects the conformation and dispersion of protein and consequently the fouling of the membrane. The mineral salts have a profound influence on the fouling of RO membrane has been confirmed by the results of Kulozik and Kessler (1988b).

When pH was adjusted to 5.6, the fouling rate was considerably reduced compared to pH 3.5 and 4.5. This can be ascribed to the interaction of beta-lactoglobulin and calcium salts. As reported by Hayes et al. (1974) at or above pH 5.6, the reactive sites of proteins are exposed and when it is heated there will be self aggregation or calcium induced protein interaction leading to formation of larger aggregates, which are large enough not to block the pores of RO membrane, hence fouling is reduced.

In case of PW and BW, fouling was greatly reduced at pH 7.2 when heated at 80°C/15 sec. This can be attributed to the higher calcium content of these whey, which by alkalinization gets precipitated as calcium phosphate. Heating induces calcium related protein-protein interaction which leads to formation of larger apatites. These apatites are large enough to be incapable of blocking the pores of membrane thus reducing the fouling. Similar mechanism has been reported by Hickey et al. (1980). The results obtained by Hiddink et al.(1981) about the mechanism of calcium phosphate precipitation and aggregation also confirm our observations. However, in case of CW, adjusting pH to 7.2 and heating to 80°C/15 sec was not found to be effective, which could be ascribed to the low levels of calcium content of CW. Fouling was minimum when CW was heated to 80°C/15 sec at its native pH (6.3).

5.2.6 DEPOSITS FORMATION ON REVERSE OSMOSIS MEMBRANE

During the concentration of whey by RO, deposits are formed on the membrane which provide an additional resistance to permeation. The formation of a deposited layer on the membrane during RO has a deleterious effect both on the permeation rate and on the ease of cleaning of membrane. Hence, efforts were made to reduce deposit formation by subjecting whey to various pretreatments.

The effect of various pretreatments on the extent of deposit formation on the RO membrane was studied and the results are depicted in Figs 10 and 11.

5.2.6.1 Paneer whey

When the PW was adjusted to pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 and preheated at 60°C/30 min, the respective amount of deposit formed was 75.70, 100.40, 55.10, 44.40 and 34.20 g/m^2 whereas at 70°C/15 sec

heating, 78.92, 103.72, 55.41, 47.60 and 38.50 g/m^2. At 80°C/15 sec heating the respective deposits were 70.50, 97.60, 54.60, 38.80 and 31.40 g/m^2. It can be observed that as the pH of whey increased the amount of deposit decreased. However, the deposit increased when the pH was raised from 3.5 to 4.5. Heat treatment at 80°C/15 sec yielded lowest deposit at all levels of pH.

The major contributors for deposit formation were found to be proteins and ash. The extent of their deposition depended on the pH and the heat treatment given to whey before processing. The amount of protein accumulated on membrane when the whey was heated and processed at pH 3.5, 4.5, 5.6, 6.4 and 7.2, respectively was 17.60, 38.16, 31.95, 21.53 and 20.80 g/m^2 for the heat treatment of 60°C/30 min, 19.62, 34.23, 34.09, 26.55 and 20.53 g/m^2 for 70°C/15 sec and 11.79, 40.99, 30.10, 19.40 and 19.43 for 80°C/15 sec. Similarly, the amount of ash deposited was 58.10, 62.24, 23.15, 19.87 and 13.40 g/m^2 for 60°C/30 min, 59.30, 69.49, 31.41, 20.98 and 17.97 g/m^2 for 70°C/15 sec and 58.71, 56.61, 24.50, 18.93 and 11.97 g/m^2 for 80°C/15 sec. These results indicate that ash is the major contributor to the deposit at lower pH of processing, but at higher pH though the total mass was less, proteins were the major contributor.

Analysis of ash showed that calcium and phosphorus content in the deposit increased with decreasing pH. Minimum calcium and phosphorus were observed when PW was heated to 80°C/15 sec at a pH of 7.2. The values were found to be 4.30 and 1.30 g/m^2. At pH 4.5, these values increased to 8.30 and 3.30 g/m^2.

Statistical analysis as shown in Table 6 clearly indicated that pH and heat treatment of whey prior to Ro have significant effect ($P \leq 0.01$) on the total mass of deposits on membrane and its protein, ash,

calcium and phosphorus content. Statistical analysis also revealed that the interaction of pH and heat treatment had significant effect ($P \leq 0.01$) on the degree of deposit formation on the RO membranes. The total mass as well as contribution of various constituents vary significantly with pH and heat treatment imparted to whey.

From the results it can be inferred that by heating PW at 80°C/15 sec at a pH of 7.2 the deposits on the membrane could be reduced drastically.

5.2.6.2 Cow milk cheddar cheese whey

It was observed that when CW was processed by RO at a pH of 3.5, 4.5, 5.6, 6.3 and 7.2 the respective amount of deposit formed was 63.70, 64.80, 42.60, 23.10 and 24.10 g/m^2 for a temperature of 60°C/30 min, 64.90, 69.70, 43.10, 26.40 and 27.40 g/m^2 for 70°C/15 sec and 59.30, 62.80, 39.20, 20.30 and 23.10 g/m^2 for 80°C/15 sec. Thus, highest amount of deposit was observed at pH 4.5 followed by pH values of 3.5, 5.6, 7.2 and 6.3. The deposit reduced to a minimum when CW was heated to 80°C/15 sec at its normal pH. Compared to other two levels of heating, unlike PW, adjusting CW to a pH of 7.2 and subsequent heating did not reduce the deposit to a considerable extent compared to its normal pH.

The extent of protein and ash in the total mass at pH values of 3.5, 4.5, 5.6, 6.3 and 7.2 were 16.56 and 47.17, 26.31 and 38.49, 22.15 and 20.45, 14.74 and 8.66, and 16.12 and 7.98 g/m^2, respectively for 60°C/30 min. For CW processed at 70°C/15 sec protein and ash contents were 15.02 and 49.87, 23.49 and 46.21, 20.43 and 22.67, 15.57 and 10.82, and 17.16 and 10.24 g/m^2. Whereas for samples heated to 80°C/15 sec, the respective values were 29.12 and 30.18, 23.23 and 39.56, 20.34 and 18.85, 14.25 and 6.05, and 15.20 and 7.90 g/m^2. Hence, at lower pH values the

contribution of ash to the total mass was higher, whereas at higher pH values proteins were the main contributors. As the pH increased from 3.5 to 7.2, the contribution of ash to the total mass decreased whereas those of proteins increased irrespective of the heat treatment imparted to whey.

Similarly, calcium and phosphorus content was high at low pH values. As the pH increased from 3.5 to 6.3, the calcium and phosphorus content showed declining trend. However, a slight increase was observed at a pH of 7.2. Lowest calcium and phosphorus contents of 1.80 and 0.65 g/m^2 were observed when CW was heated to 80°C/15 sec at its normal pH.

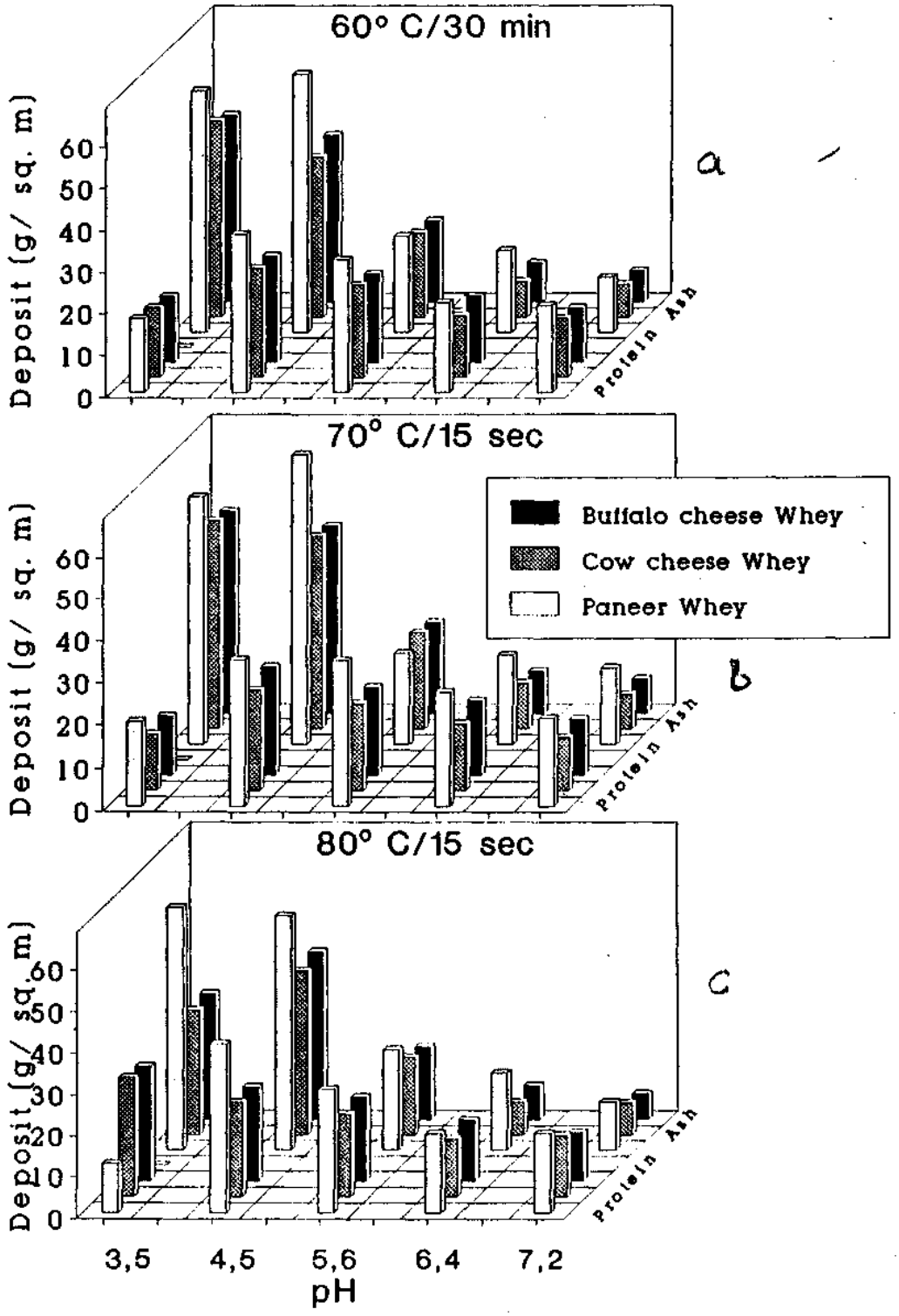
Statistical analysis (Table 6) also indicated that there is significant effect ($P \leq 0.01$) of pH and heat treatment on the deposit formation. The deposit formation significantly varied with respect to total mass, protein, ash, calcium and phosphorus content when CW was processed with various pretreatments. The interaction effect of pH and heat treatment also showed significant effect ($P \leq 0.01$).

From the results obtained it can be concluded that deposit formation during processing of CW by RO could be reduced to as low as 20.30 g/m^2 if CW is preheated to 80°C/15 sec at its native pH (6.3) prior to RO concentration.

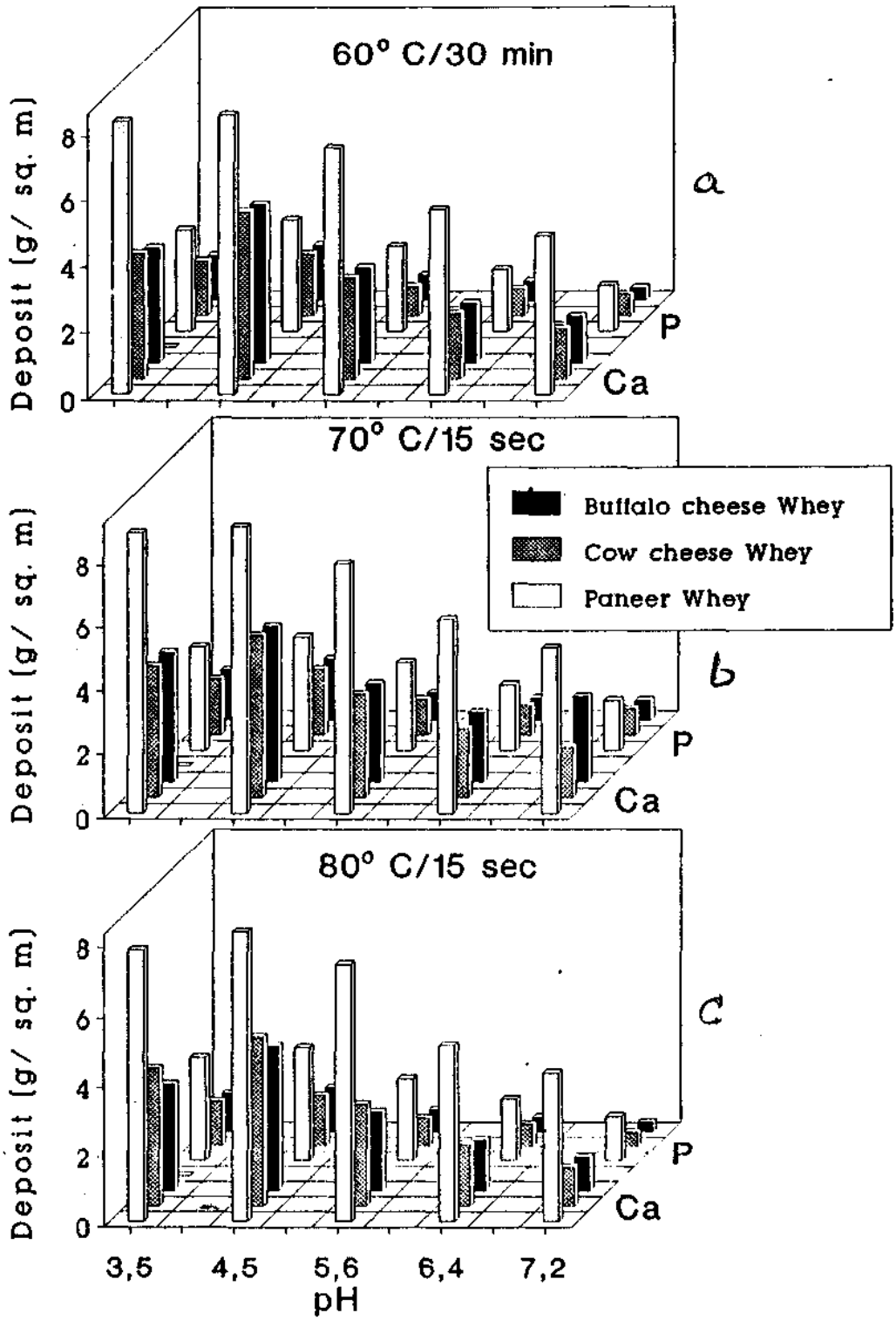
5.2.6.3 Buffalo milk cheddar cheese whey

When the BW was adjusted to pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 and preheated, the corresponding amounts of deposits formed were 60.80, 65.90, 41.10, 25.60 and 20.70 g/m^2 at 60°C/30 min, 70.50, 62.40, 42.20, 27.60 and 21.30 g/m^2 at 70°C/15 sec and 63.40, 58.20, 38.40, 23.50 and 18.40 g/m^2 at 80°C/15 sec. It was observed that as the pH of whey increased, the amount of deposits decreased. Contrary to this, the deposits

Fig 10. EFFECT OF pH AND PREHEATING OF WHEY ON DEPOSIT FORMATION



G 11. # EFFECT OF pH AND PREHEATING OF WHEY ON DEPOSIT FORMATION



increased when the pH was raised from 3.5 to 4.5. The amount of deposit reduced when BW was subjected to 80°C/15 sec heating prior to RO at all pH values compared to other two heat treatments.

The whey proteins and ash constitute the mass of deposit formed on the membrane during processing. pH of whey and pretreatment significantly alter the mass of deposit. The contribution of protein and ash to the above total mass of deposit at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 was 15.80 and 45.00, 25.70 and 40.19, 21.37 and 19.73, 15.87 and 9.73, and 13.04 and 7.66 g/m^2, respectively for 60°C/30 min. At 70°C/15 sec heating, protein and ash contents were 13.73 and 48.67, 25.38 and 45.12, 20.68 and 21.52, 17.53 and 10.07, and 13.07 and 8.21 g/m^2. Whereas for samples heated to 80°C/15 sec, the respective values were 28.00 and 30.20, 23.14 and 40.26, 20.74 and 17.66, 15.16 and 8.34, and 11.99 and 6.41 g/m^2. As in the case of other two types of whey, BW processing had also shown the same trend with respect to contribution of protein and ash to the total deposit. At lower pH values the deposit was mainly contributed by ash. Whereas at higher pH values, though the total mass of deposit was small, the major contribution was by proteins.

High amounts of calcium and phosphorus were observed at lower pH values. Whereas at pH 7.2, the contents of these minerals were found to be minimum. The amounts of calcium and phosphorus were less at 80°C/15 sec than at any other preheat treatment. The deposits had highest calcium and phosphorus contents of 4.90 and 1.95 g/m^2 when BW was adjusted to a pH of 4.5 and preheated to 70°C/15 sec. Whereas lowest values of 0.98 and 0.28 g/m^2 were observed when BW was heated to 80°C/15 sec at a pH of 7.2.

Statistical analysis (Table 6) revealed that pH and preheat treatments of BW have significant influence on the deposit formation during

Table 6. ANOVA for effect of various pretreatments on deposit formation

Source of variation	d.f.	'F' values		
		Total mass	Protein	Ash
Replication	2	3.0812^{NS}	2.9857^{NS}	2.6230^{NS}
Heat treatment (A)	2	1327.6730**	1560.3500**	1150.4800**
pH (B)	4	70374.6950**	65618.4700**	58374.5200**
Type of whey (C)	2	27534.2890**	30490.5200**	24580.3300**
Interaction				
A x B	8	11266.5712**	13575.3200**	14640.4200**
A x C	4	483.7520**	498.4750**	376.5990**
B x C	8	223.9900**	340.3506**	278.4206**
A x B x C	16	97.9680**	78.4520**	63.5670**
Error	88	-	-	-

NS = Non-significant
** Significant at 1 per cent level (P 0.01)

RO concentration of BW. pH and heat treatment combination showed a significant ($P \leq 0.01$) effect on the total mass per unit area of the membrane and its protein and ash contents. The interaction of the pH and heat treatment was also found to be significant ($P \leq 0.01$) with respect to total mass of deposit, protein, ash and mineral content.

From the results obtained, it can be inferred that the deposits formation on the RO membrane during processing of BW could be reduced to a minimum value of 18.40 g/m^2 by heating BW to 80°C/15 sec at a pH of 7.2 prior to RO concentration.

Deposit formation on RO membrane during processing of whey, reduces the flux to a great extent and it also has a deleterious effect on the ease of cleaning of the membrane (Kulozik and Kessler, 1988c). Thus, it affects the economy of the RO process to a large extent.

It was observed from the results that processing of PW by RO resulted in higher deposits at all the pH levels and heat treatments as compared to processing of CW and BW. This could be attributed to the inherent nature of the PW. The composition, pH and ionic strength of PW varied widely with those of CW and BW. PW possess greater amount of mineral salts and its higher ionic strength results in alteration in conformation of proteins leading to greater fouling by salts-membrane and salts-membrane-protein interaction resulting in higher deposit formation.

Processing at lower pH values of 3.5 and 4.5 resulted in higher deposits for all the three types of whey. This can be ascribed to the greater fouling of the membrane by mineral salts. At pH 3.5 and 4.5, the ash content of the deposits was higher than the protein content. When the pH of whey is adjusted to lower values, more of calcium phosphate

gets converted to soluble form. On heating, they may get precipitated on or into the membrane pores thus resulting into higher mineral deposits. At these two pH values, along with mineral deposits, greater amount of protein was also observed in the deposits. It could be possible that calcium salts interact with and bind to negatively charged groups in the membrane by electrostatic or charge effects which may result in salt bridge formation between the membrane and proteins leading to faster protein fouling of the membrane. Hence, higher protein deposits were also observed on the membrane (Cheryan,1986; Hiddink et al., 1981).

At a pH of 5.6, the total mass of deposits was less compared to pH values of 3.5 and 4.5. At this pH, all the reactive sites of proteins are exposed (Hayes et al., 1974). When whey is subjected to heating, there may be self aggregation of beta-lactoglobulin or calcium related protein-protein interaction leading to formation of larger apatite or aggregates which are large enough not to foul the membrane. Hence, the amount of deposit was found to be less. However, it can be observed from the results that the total mass of deposit was minimum at 80°C/15 sec heating. Probably high temperature of heating is necessary to induce the protein-protein or calcium related protein interaction.

The deposit was relatively low when whey was processed at pH 7.2, in case of PW and BW, when preheated to 80°C/15 sec. This is certainly due to decreased extent of fouling. As the calcium content of these two types of whey is high, when it is subjected to heat treatment it enhances the process of apatite formation (section 5.2.5). Hence, deposit formation is reduced. However, CW had shown lesser deposit at a pH of 6.3 than at a pH of 7.2. This can be ascribed to the lower calcium content of CW, which is not sufficient enough to form larger apatite when CW was subjected to heat treatment at a pH of 7.2.

From the results, it is evident that by heating PW and BW (pH 7.2) and CW (pH 6.3) to a temperature of 80°C/15 sec prior to concentration of whey by RO results in drastic reduction of deposit formation on RO membrane.

5.2.7 CLEANING OF REVERSE OSMOSIS MEMBRANE

Effective cleaning and sanitization of RO membrane is utmost important for the efficient operation and for long life of the membrane. After every processing schedule, the plant has to be thoroughly cleaned and sanitized. Soon after the processing of whey, the membrane has to be cleaned with detergents till the standard water flux is restored. Complete restoration of flux is used as an index of proper cleaning.

After processing of whey, the membrane was flushed with water followed by ultrasil-25 (alkali based) and ultrasil-75 (acid based) cleaning. Preliminary trials have indicated that after ultrasil-25 cleaning, 90-92 per cent of original water flux should be retained and after ultrasil-75 cleaning complete standard water flux should be restored. The time taken for restoration of standard water flux is an index of efficiency of cleaning and this depends on the pretreatments given to whey, type and concentration of detergent and the temperature of cleaning.

In this study, ultrasil-25 and ultrasil-75 were used as detergents as prescribed by manufacturers. In the preliminary trials, it was observed that the cleaning efficiency was lower at 40°C. The time taken to restore the standard flux was too long, more than 1½ h, and in most of the cases when pretreatment of whey varied, the flux could not be restored to the original value at all. At 50°C, the cleaning efficiency was better and it was possible to restore the original flux at all combinations of pre-

Hence, this study was conducted with a cleaning temperature of 50°C to determine a suitable combination of pH and heat treatment to be given to each type of whey where the time required to attain the standard flux is minimum.

5.2.7.1 Paneer whey

Paneer whey was concentrated by employing RO process at various combinations of pH and heat treatment. After processing, the time taken for cleaning of the membrane by ultrasil-25 and ultrasil-75 are presented in Table 7.

The time required for cleaning of RO membrane at various combinations of pretreatments varied from 40-60 min for ultrasil-25. The longest duration of 60 min was needed to restore the flux when PW was processed at pH 4.5, at all the three levels of heating temperature. The time taken for cleaning at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 was found to be 55, 60, 50, 45 and 45 min respectively for both 70°C/15 sec and 60°C/30 min heating whereas at 80°C/15 sec heating, the time taken at all the pH levels were observed to be the same except at a pH of 7.2. At this pH of processing, the time needed was only 40 min.

Similarly, for ultrasil-75 the time required for cleaning varied from 35 to 45 min. Longer duration was needed at lower pH values. The time required at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2 were 45, 45, 40, 35 and 35 min, respectively.

5.2.7.2 Cow milk cheddar cheese whey

After processing CW at various pH and heat treatments, the time required for restoring the original flux by ultrasil-25 and ultrasil-75 cleaning are summarized in Table 7.

As indicated in the table, the time required for ultrasil-25 cleaning, when whey was processed at various pretreatments varied from 40 to 55 min. Preheating whey to 60°C/30 min or 70°C/15 sec at pH values of 3.5, 4.5, 5.6, 6.3 and 7.2, respectively resulted in cleaning time of 50, 55, 45, 40 and 45 min, respectively for ultrasil-25. Whereas processing of whey, which was preheated to 80°C/15 sec at pH values of 6.3 and 7.2, needed a cleaning time of 35 and 40 min, respectively.

Similarly, ultrasil-75 cleaning had to be done for 40, 40, 35, 30 and 30 min when CW was processed at pH values of 3.5, 4.5, 5.6, 6.3 and 7.2, respectively at all the three levels of preheating to restore the flux.

5.2.7.3 Buffalo milk cheddar cheese whey

The duration of cleaning, after processing BW at various combinations of treatments is represented in the Table 7.

When BW was preheated to 80°C/15 sec at a pH of 7.2 and processed by RO, the cleaning time required was minimum. Whereas at lower pH values the time required for cleaning was higher. When whey was preheated at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2, the corresponding time required for ultrasil-25 cleaning was 50, 55, 45, 45 and 40 min when BW was heated to 60°C/30 min or 70°C/15 sec whereas at 80°C/15 sec preheating at pH values of 6.4 and 7.2, the time required for cleaning reduced to 40 and 35 min, respectively.

However, it was observed that ultrasil-75 cleaning need to be done for 45, 45, 40, 30 and 30 min at all the three levels of heating for restoring the flux when whey was processed at pH values of 3.5, 4.5, 5.6, 6.4 and 7.2, respectively.

Preheat treatment (temp.)	Types of whey														
	Paneer whey					Cow milk cheese whey					Buffalo milk cheese whey				
	Duration of cleaning (min)					Duration of cleaning (min)					Duration of cleaning (min)				
	3.5	4.5	5.6	6.4	7.2	3.5	4.5	5.6	6.3	7.2	3.5	4.5	5.6	6.4	7.2
							ULTRASIL-25*								
60°C/30 min	55	60	50	45	45	50	55	45	40	45	50	55	45	45	40
70°C/15 sec	55	60	50	45	45	50	55	45	40	45	50	55	45	45	40
80°C/15 sec	55	60	50	45	40	45	50	40	35	40	45	55	40	40	35
							ULTRASIL-75**								
60°C/30 min	45	45	40	35	35	40	40	35	30	30	45	45	40	30	30
70°C/15 sec	45	45	40	35	35	40	40	35	30	30	45	45	40	30	30
80°C/15 sec	45	45	40	35	35	40	40	35	30	30	45	45	40	30	30

* After Ultrasil-25 cleaning, 90 to 92 per cent standard water flux should be retained

** Subsequent Ultrasil-75 cleaning, complete standard water flux should be retained

Concentration of whey by RO inevitably leads to fouling, and deposits formed on the membrane must be removed by alkali/enzyme/acid detergents action.

At lower pH values of processing of whey, the time taken for cleaning was longer in all the three types of whey which can be ascribed to the higher fouling and deposit formation while processing at these pH values. Both ultrasil-25 and ultrasil-75 cleaning had taken more time at these combination of treatments. This is probably due to higher levels of both protein and ash deposits on the membrane. These results are in agreement with the results of Kulozik and Kessler (1988c), where they have indicated that the higher the total mass of deposits, the longer was the time taken for cleaning of the RO membrane.

The cleaning duration needed for PW was found to be longer than the other two types of whey. This can be ascribed to the higher ionic strength and mineral content of PW which must have caused higher fouling of the membrane leading to higher deposit. In the salt containing protein solutions, the influence of dissolved ionic constituents on the structure of the deposited layer becomes evident. Hence, the residual resistance to removal is considerably higher. At lower pH values as the calcium content is high which has a marked influence on the power of adhesion of the deposited layer to the membrane and on the network formed within the deposited layer (Kessler et al., 1982). Hence, longer time was found to be necessary.

In case of CW processing, the time taken for cleaning was less when whey was processed at pH 6.3. This may be due to lower amount of deposits on the membrane compared to processing CW at other pH values. Time taken for ultrasil-75 cleaning was also less at the above pH, which

could be ascribed to lower mineral deposits on the membrane while processing whey at this pH.

When PW and BW were heated to a temperature of 80°C/15 sec at a pH of 7.2, prior to RO, the time taken for cleaning was less compared to any other pretreatments which could be again attributed to lower amount of deposits on the membrane.

From the results, it is evident that there was direct relationship between fouling/deposit formation and the time taken for cleaning. The higher the deposits on the membrane, the longer was the time taken for cleaning to restore the original flux.

5.2.8 CHANGES IN CHEMICAL COMPOSITION OF WHEY IN REVERSE OSMOSIS PROCESS

During RO concentration it was observed that there was no loss of true protein, calcium and phosphorus through permeate and their retention was 100 per cent. There were, however, slight losses of NPN, ash and lactose in the permeate (Table 8).

Paneer whey permeate contained 0.0018 per cent NPN, 0.007 per cent lactose and 0.0125 per cent ash. The TS content was 0.0213 per cent. The respective retention were 94.99, 99.86, 97.92 and 99.65 per cent. The retentate had a TS content of 20.50 per cent which was the maximum to which PW could be concentrated. The retentate possessed 0.254 per cent true protein, 0.118 per cent NPN, 17.580 per cent lactose, 2.096 per cent ash, 0.240 per cent calcium and 0.188 per cent phosphorus.

The retention coefficients (per cent) of NPN, lactose and ash of CW and BW were not significantly different from those of PW. The retention values for NPN, lactose, ash and TS, respectively were 95.60, 99.88, 97.96 and 99.74 for CW and 95.57, 99.85, 97.94 and 99.71 for BW. The retentate

Table 8. Retention and permeation of various constituents of whey in reverse osmosis process*

Constituents (%)	Types of whey								
	Paneer whey			Cow milk cheese whey			Buffalo milk cheese whey		
	Retentate	Permeate	Retention value (%)	Retentate	Permeate	Retention value (%)	Retentate	Permeate	Retention value (%)
Total solids	20.500	0.0213	99.65	22.100	0.0164	99.74	23.200	0.0203	99.71
True protein	0.254	-	100.00	2.139	-	100.00	2.580	-	100.00
Non-protein nitrogen	0.118	0.0018	94.99	0.094	0.0012	95.60	0.127	0.0017	95.57
Lactose	17.580	0.0070	99.86	16.890	0.0058	99.88	17.510	0.0075	99.85
Ash	2.096	0.0125	97.92	1.577	0.0094	97.96	1.850	0.0111	97.94
Calcium	0.240	-	100.00	0.153	-	100.00	0.176	-	100.00
Phosphorus	0.188	-	100.00	0.125	-	100.00	0.154	-	100.00

* Average of five trials

and permeate composition, however, varied slightly which could be because of difference in composition of whey rather than any difference in permeation behaviour. The BW retentate had a TS content of 23.200 per cent and the permeate had 0.0203 per cent TS which comprised of 0.0017 per cent NPN, 0.0075 per cent lactose and 0.0111 per cent ash. The CW retentate could be concentrated to a maximum of 22.10 per cent TS. At this level of TS, the permeate had 0.0164 per cent TS, 0.0012 per cent NPN, 0.0058 per cent lactose and 0.0094 per cent ash.

It is thus clear that all of the whey constituents have very high retention coefficients and permeation losses are negligible. High retention values for these constituents during RO of whey have also been reported by Morales et al. (1990b). Several other workers (Marshall, 1985; Jensen and Oxlund, 1988) have also observed slight losses of low molecular weight substances such as NPN and ash during concentration of whey.

Reverse osmosis membranes are slightly permeable to low molecular weight compounds. The actual basis of separation is not clearly understood (Marshall, 1985). For example, though the molecular weight of urea, acetic acid and ethanol is more than that of calcium they pass through the membrane, whereas calcium is fully retained. The mechanism of permeation might be explained by the incorporation of water molecules into interstices on the high pressure side of the membrane in an ice like state and melt away from the lower pressure side. Only those molecules, which can enter tetrahedral crystal structure of ice without producing excessive distortion of the lattice and are thus able to pass through RO membranes. These include some low molecular weight organic compounds such as urea, acetic acid, ethanol and smaller inorganic ions like sodium and chloride (Marshall, 1985).

5.2.9 STANDARDIZATION OF PRECRYSTALLIZATION PROCESS

Reverse osmosis concentrate was further concentrated to various levels of TS, precrystallized with different levels of seeding material for different duration in order to standardize the process for the production of whey powder, free of hygroscopicity and caking problems.

It is necessary to concentrate whey to as high TS as possible in order to run drier economically. Initial trials have shown that it is difficult to concentrate whey to 55 per cent TS as the viscosity was very high and at this concentration whey appeared as supersaturated solution. Hence, the lactose got crystallized in the pan itself and it was difficult to handle the concentrate further.

Though it is economical to operate whey concentrate with maximum TS content, other factors need to be considered as well. The high content of lactose in whey concentrate poses problem of hygroscopicity and caking in the resultant powder. It is hence always necessary to convert most of the lactose to alpha-monohydrate form which is non-hygroscopic. An optimum level of TS has to be aimed at in order to get maximum crystallization.

Hence, three levels of TS were chosen to study the extent of lactose crystallization. TS levels of 40, 45 and 50 per cent were attained in RO retentate by concentrating it further in a laboratory rotary vacuum evaporator.

Paneer whey, CW and BW were concentrated to three levels of TS, viz., 40, 45 and 50 per cent. When the desired TS was attained, it was immediately cooled to 30°C. The precrystallization was carried out by the addition of alpha-lactose monohydrate at four different levels ranging from 0.01 to 0.07 per cent and the crystallization was continued for a

period of 6 h at 30°C. At every 1 h interval, the degree of crystallization of lactose was measured. The effect of levels of TS, rate of seeding material used and the duration of seeding on the degree of lactose crystallization and size distribution of lactose crystals are presented in Figs 12 to 17.

5.2.9.1 Paneer whey concentrate

Paneer whey concentrate was precrystallized at 30°C by using alpha-lactose monohydrate at the rate ranging from 0.01 to 0.07 per cent for a period of 6 h. The extent of lactose crystallized was measured at a regular interval of 1 h. The rate of lactose crystallization and the size distribution of lactose crystals are presented in Figs 12 and 13, respectively.

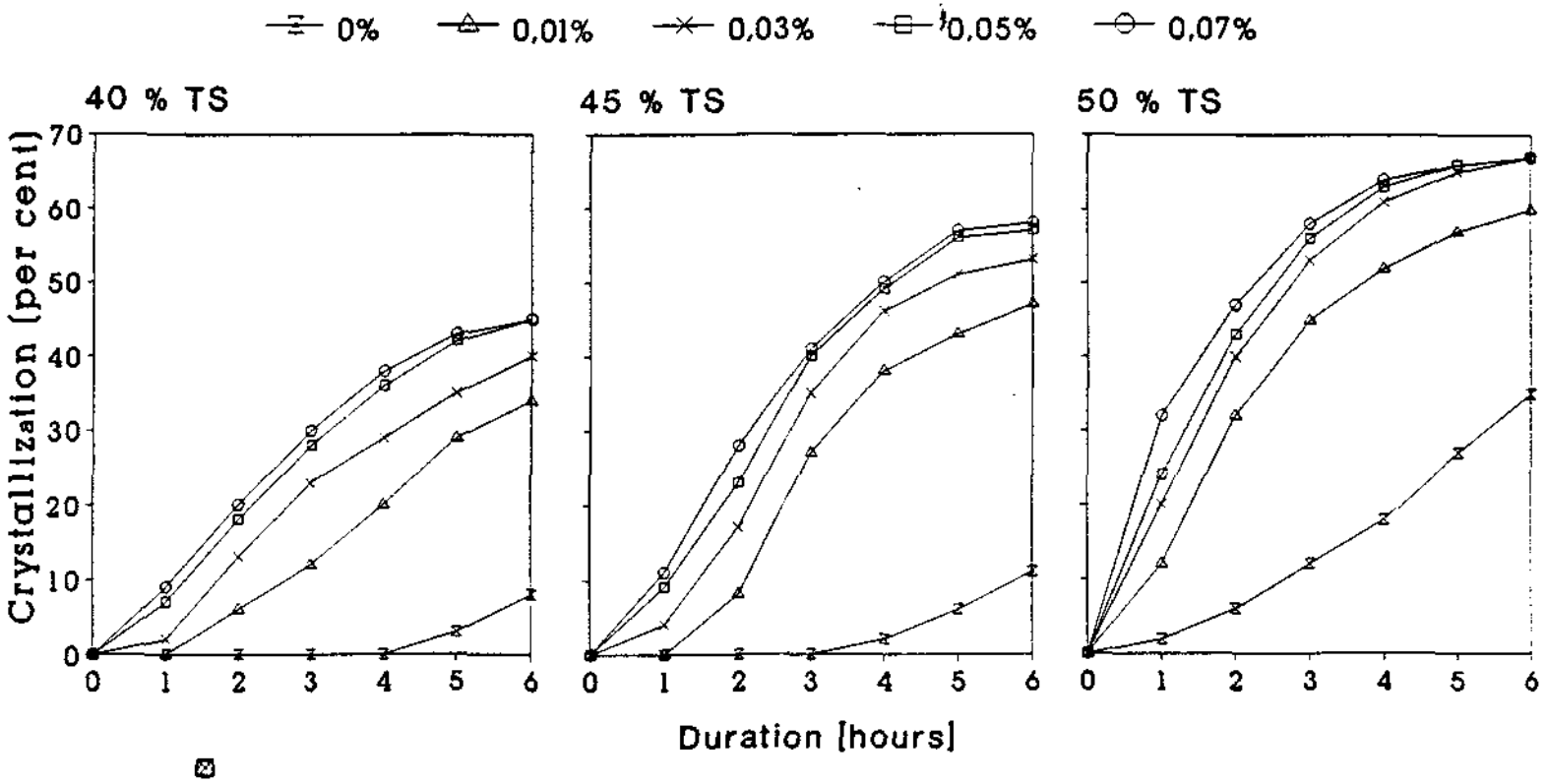
5.2.9.1.1 Lactose crystallization

It can be observed (Fig 12) that the extent of crystallization increased with the duration of seeding. In general, after 4 h, the increase in crystallization was slower. The extent of crystallization after 4 h of precrystallization was 20, 29, 36 and 38 per cent at 40 per cent TS, while 38, 46, 49 and 50 per cent at 45 per cent TS and 52, 61, 63 and 64 per cent at 50 per cent TS, respectively for 0.01, 0.03, 0.05 and 0.07 per cent seeding material. The extent of lactose crystallization was highest in the concentrate with 50 per cent TS. As the amount of seeding material increased, the degree of crystallization also increased but to a varying extent at three levels of TS. At 50 per cent TS the improvement in crystallization was less after 0.03 per cent level of seeding material.

5.2.9.1.2 Size distribution of lactose crystals

It is desirable to obtain large number of crystals of small sizes

FIG 12. EFFECT OF SEEDING ON THE CRYSTALLIZATION OF LACTOSE IN PANEER WHEY CONCENTRATE



in order to have maximum crystallization and to avoid further problems during drying. As the amount of seeding material increased, the size of smaller crystals also increased. For example, when alpha-lactose monohydrate added increased from 0.01 to 0.07 per cent, the frequency of 5 µm crystals increased from 23 to 55 per cent at 40 per cent TS, 25 to 61 per cent at 45 per cent TS and 33 to 65 per cent at 50 per cent TS, respectively. Marginal increase was observed in the occurrence of crystals in the frequency range of 0 to 10 µm, whereas there was drastic decrease in 11 to 20 µm crystals size from 34 to 15, 39 to 8 and 34 to 3.0 per cent. Formation of more than 20 µm size crystals was not observed when 0.05 and 0.07 per cent seeding material was used (Fig 13).

The significance of forced crystallization is emphasized by the frequency of size distribution obtained without seeding material. As high as 49 per cent crystals were above 20 µm size and about 22 per cent above 40 µm size as at 50 per cent TS which may invite handling problems besides very less extent of crystallization.

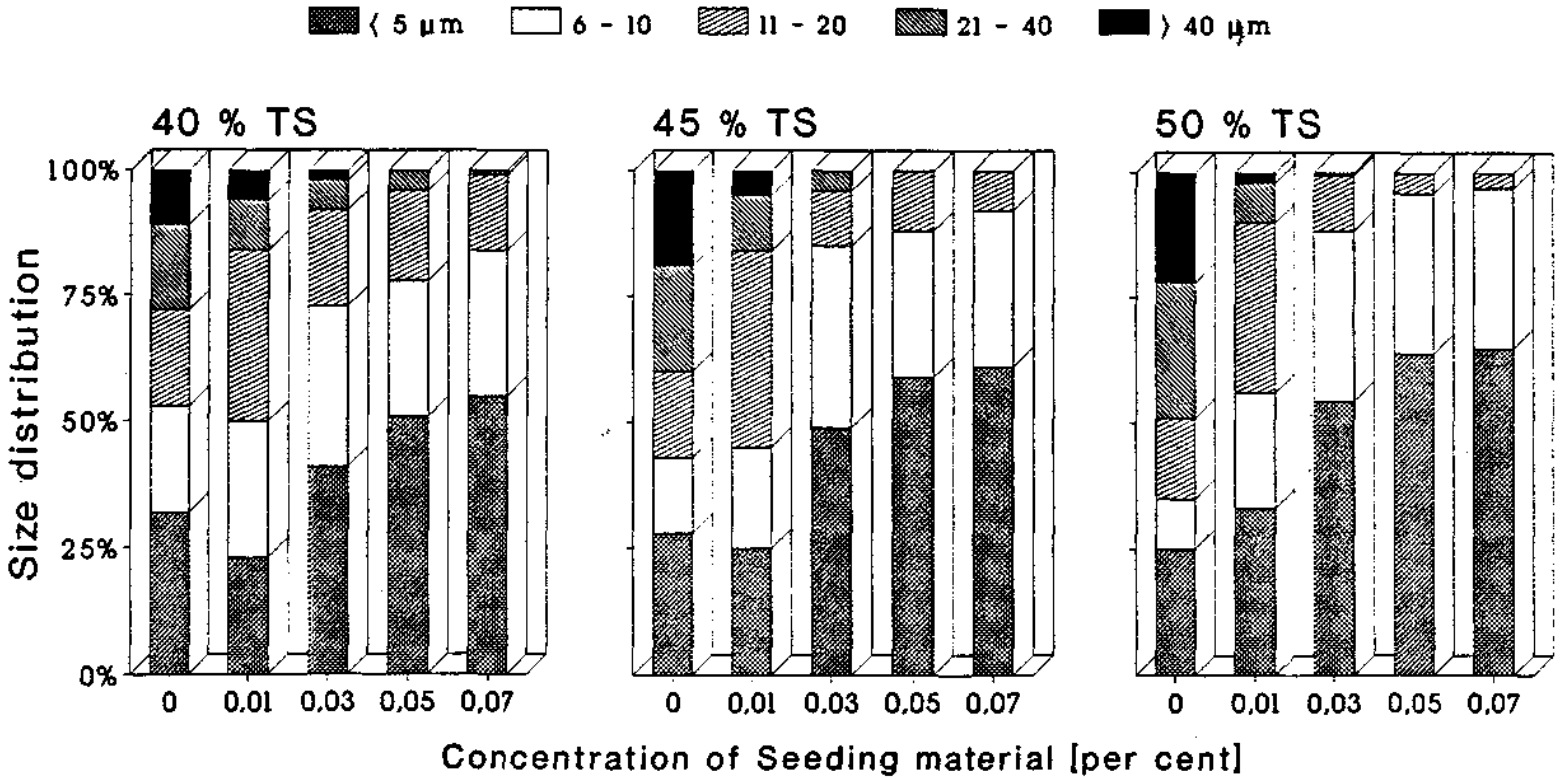
5.2.9.2 Cow milk cheddar cheese whey concentrate

Cow milk cheddar cheese whey concentrated to 40, 45 and 50 per cent TS was precrystallized at 30°C for a period of 6 h. The extent of lactose crystallization and the size distribution of lactose crystals as affected by TS, seeding material and duration of seeding are depicted in Figs 14 and 15.

5.2.9.2.1 Lactose crystallization

The extent of lactose crystallization at various levels of TS of CW concentrate as affected by the seeding material and duration of precrystallization is shown in Fig 14.

FIG 13. EFFECT OF SEEDING ON THE SIZE DISTRIBUTION OF LACTOSE CRYSTALS IN PANEER WHEY CONCENTRATE



It illustrates that as precrystallization progressed, the extent of crystallization increased. In general, as in the case of PW concentrate, after 4 h duration, the extent of increase in crystallization was slower. The lactose crystallized, after 4 h of precrystallization was 16.0, 26.0, 33.0 and 38.0 per cent at 40 per cent TS, 33.0, 42.0, 46.0 and 49.0 per cent at 45 per cent TS and 48.0, 54.0, 62.0 and 64.0 per cent at 50 per cent TS, respectively for 0.01, 0.03, 0.05 and 0.07 per cent seeding material. The lactose crystallized was highest in the concentrate with 50 per cent TS. As the amount of seeding material increased, the degree of crystallization also increased at all the levels of TS, but to a varying extent. At 50 per cent TS levels of CW concentrate, the crystallization increased from 16 to 62 per cent when the seeding material was increased from 0 to 0.05 per cent. However, after 0.05 per cent, further increase in seeding material to 0.07 per cent, increased crystallization only marginally. It was observed that the maximum crystallization of 63.0 per cent could be attained in CW concentrate at 50 per cent TS by pre-crystallizing for 4 h with 0.05 per cent alpha-lactose monohydrate.

5.2.9.2.2 Size distribution of lactose crystals

It can be observed from the Fig 15 that when seeding material was not used during precrystallization, the size of crystals was found to be very large. For instance, at 50 per cent TS about 38 per cent of the crystals were in the frequency of above 20 µm size. This emphasizes the need for forced crystallization. As the amount of seeding material increased from 0.01 to 0.07 per cent, the size of crystals decreased drastically. As for example, when per cent lactose added increased from 0.01 to 0.07, the percentage of 5 µm crystals increased from 10 to 45 at 40 per cent TS, 15 to 46 at 45 per cent TS and 25 to 63 at 50 per cent

FIG 14. EFFECT OF SEEDING ON THE CRYSTALLIZATION OF LACTOSE IN COW MILK CHEESE WHEY CONCENTRATE

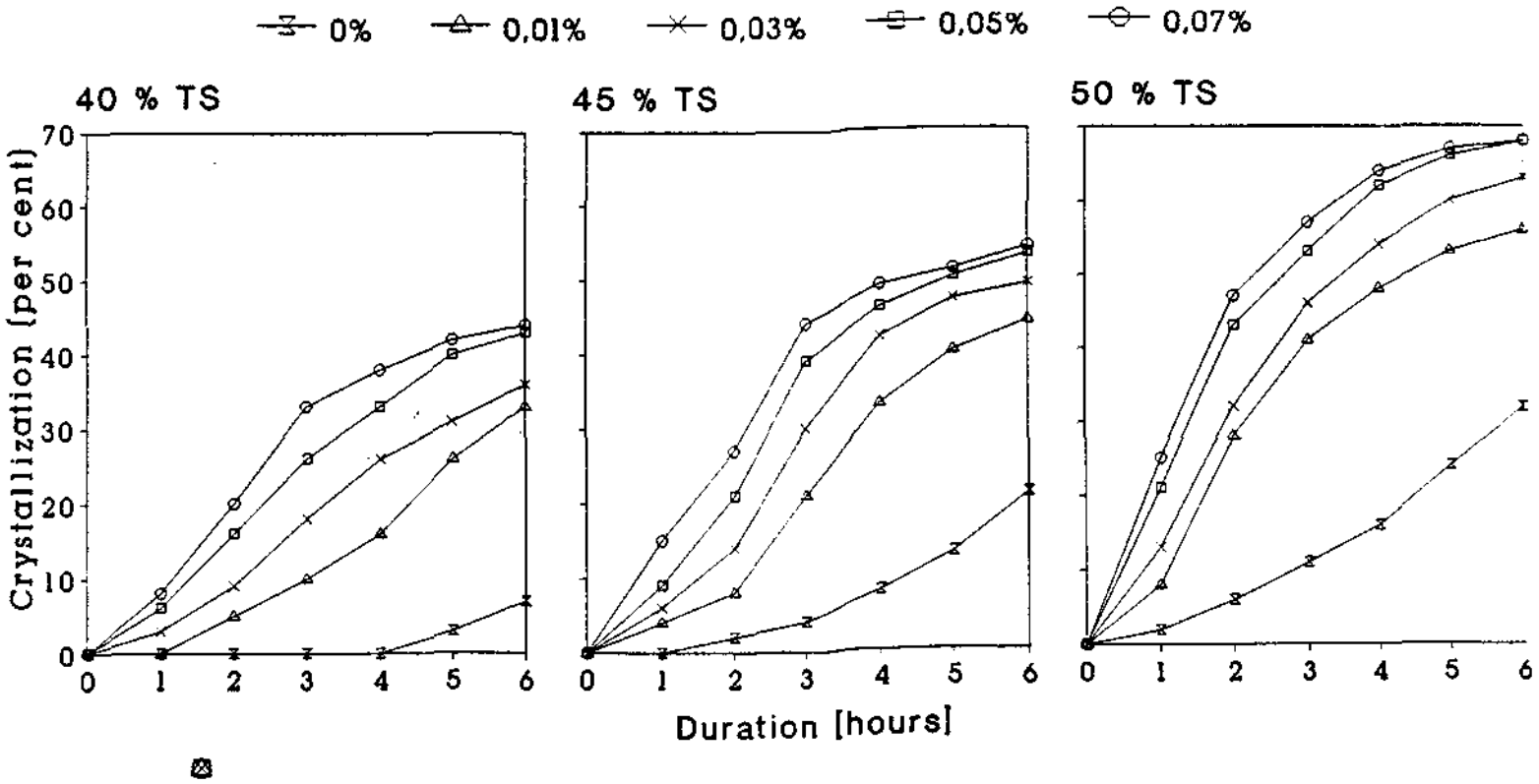
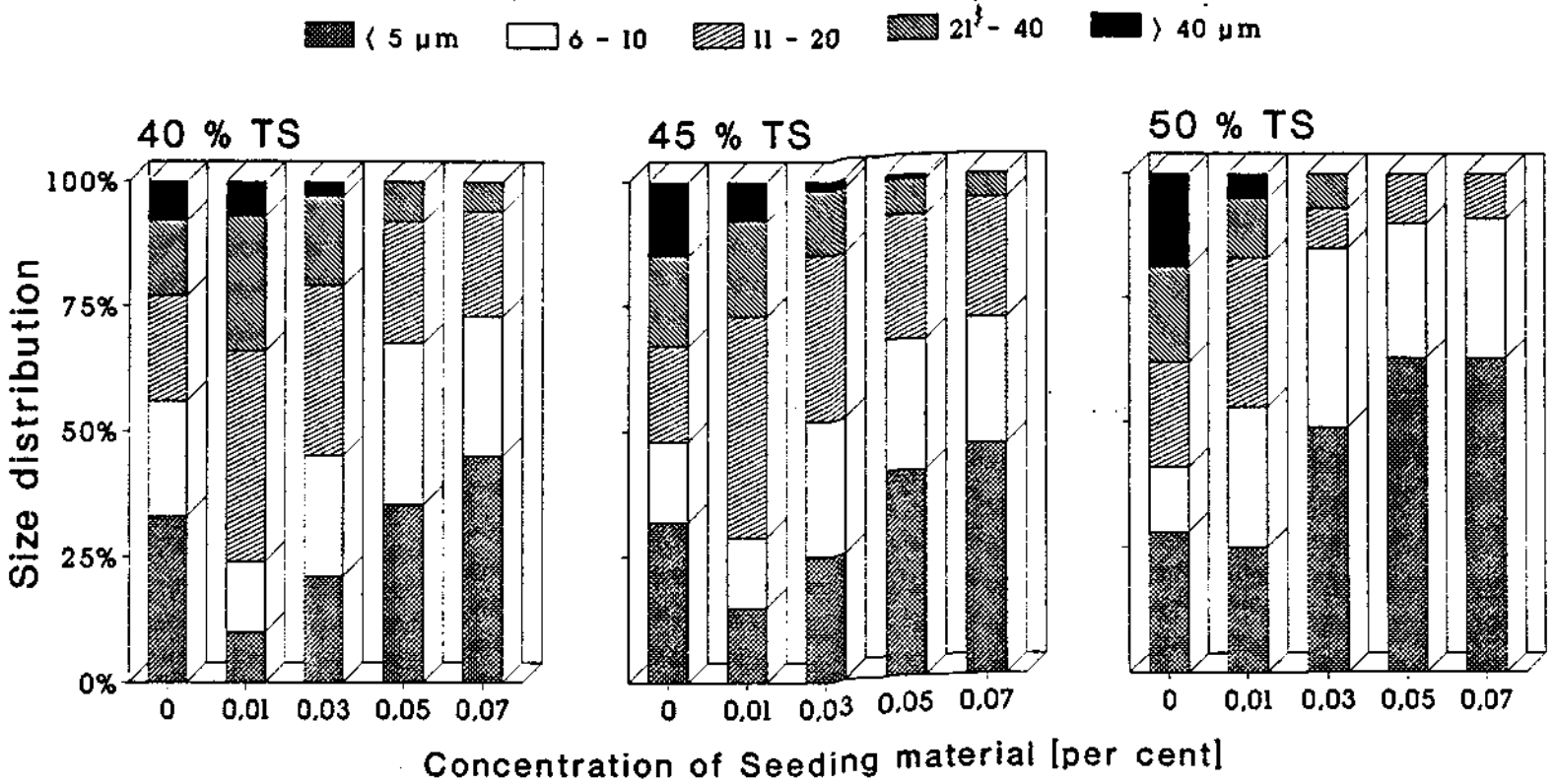


FIG 15. EFFECT OF SEEDING ON THE SIZE DISTRIBUTION OF LACTOSE CRYSTALS IN COW MILK CHEESE WHEY CONCENTRATE



TS, respectively. The extent of decrease in the size of crystals was found to be marginal, when seeding material increased from 0.05 to 0.07 per cent. Marginal increase was observed in the occurrence of crystals below 10 um size, whereas there was drastic decrease in 11 to 20 µm crystals from 42 to 41, 44 to 24 and 30 to 9 per cent at 40, 45 and 50 per cent TS, respectively. Formation of crystals above 20 µm size was drastically reduced when 0.05 and 0.07 per cent seeding material was used as shown in Fig 15. At 50 per cent TS of CW concentrate and 0.05 per cent seeding material, none of the crystals were found above 20 µm size. As high as 63.0 per cent crystals were below 5 µm size.

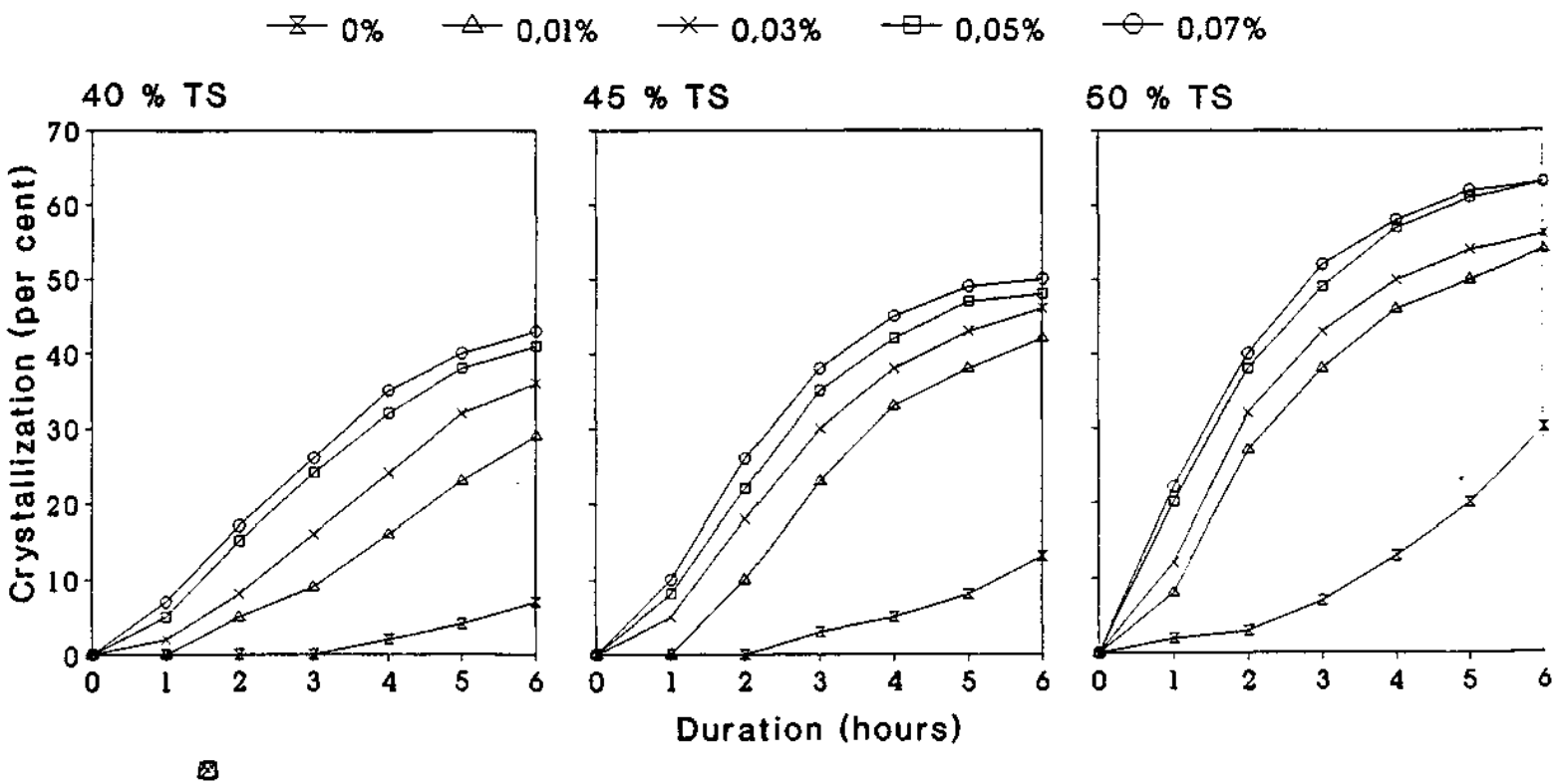
5.2.9.3 Buffalo milk cheddar cheese whey concentrate

The effect of levels of TS, extent of seeding material used and duration of seeding on the degree of lactose crystallization and the size distribution of lactose crystals of BW concentrate are delineated in Figs 16 and 17.

5.2.9.3.1 Lactose crystallization

It can be seen from Fig 16 that the extent of crystallization increased with the duration of seeding. In general, as in the case of PW and CW, after 4 h duration, the increase in crystallization was slower at all the levels of TS. The crystallization attained after 4 h of seeding was 16, 24, 32 and 35 per cent at 40 per cent TS, 33, 38, 42 and 45 at 45 per cent TS and 46, 50, 57 and 58 per cent at 50 per cent TS, respectively with 0.01, 0.03, 0.05 and 0.07 per cent seeding material. Highest crystallization was attained at 50 per cent TS. As the amount of seeding material increased, the degree of crystallization also increased but at a varying extent at three levels of total solids. At 50 per cent solids improvement in crystallization was found to be less after 0.05 per cent

FIG 16. EFFECT OF SEEDING ON THE CRYSTALLIZATION OF LACTOSE IN BUFFALO MILK CHEESE WHEY CONCENTRATE



seeding material. At this condition as high as 57 per cent was attained.

5.2.9.3.2 Size of lactose crystals

It is evident from Fig 17 that as the amount of increased, the size of smaller crystals also increased. material increased from 0.01 to 0.07 per cent, the free crystals increased from 11 to 46 per cent at 40 per cent per cent at 45 per cent TS and 26 to 64 per cent at 50 per tively. The crystals in the frequencies of 11-20 µm decr 19, 45 to 22 and 33 to 8 per cent, respectively at 40, 45 TS of concentrate. At 50 per cent TS with 0.05 and 0.07 material levels none of the crystals were above 20 µm si the crystals were below 5 µm size (64.0%). From the resu that at 50 per cent solids of BW concentrate using 0.05 material, it is possible to attain all the crystals below 2

Statistical analysis (Table 9) has indicated that t cant difference in the degree of crystallization among the concentrate. It can also be seen from the table that there ($P \leq 0.01$) effect of TS, extent of seeding material us

FIG 17. EFFECT OF SEEDING ON THE SIZE DISTRIBUTION OF LACTOSE CRYSTALS IN BUFFALO MILK CHEESE WHEY CONCENTRATE

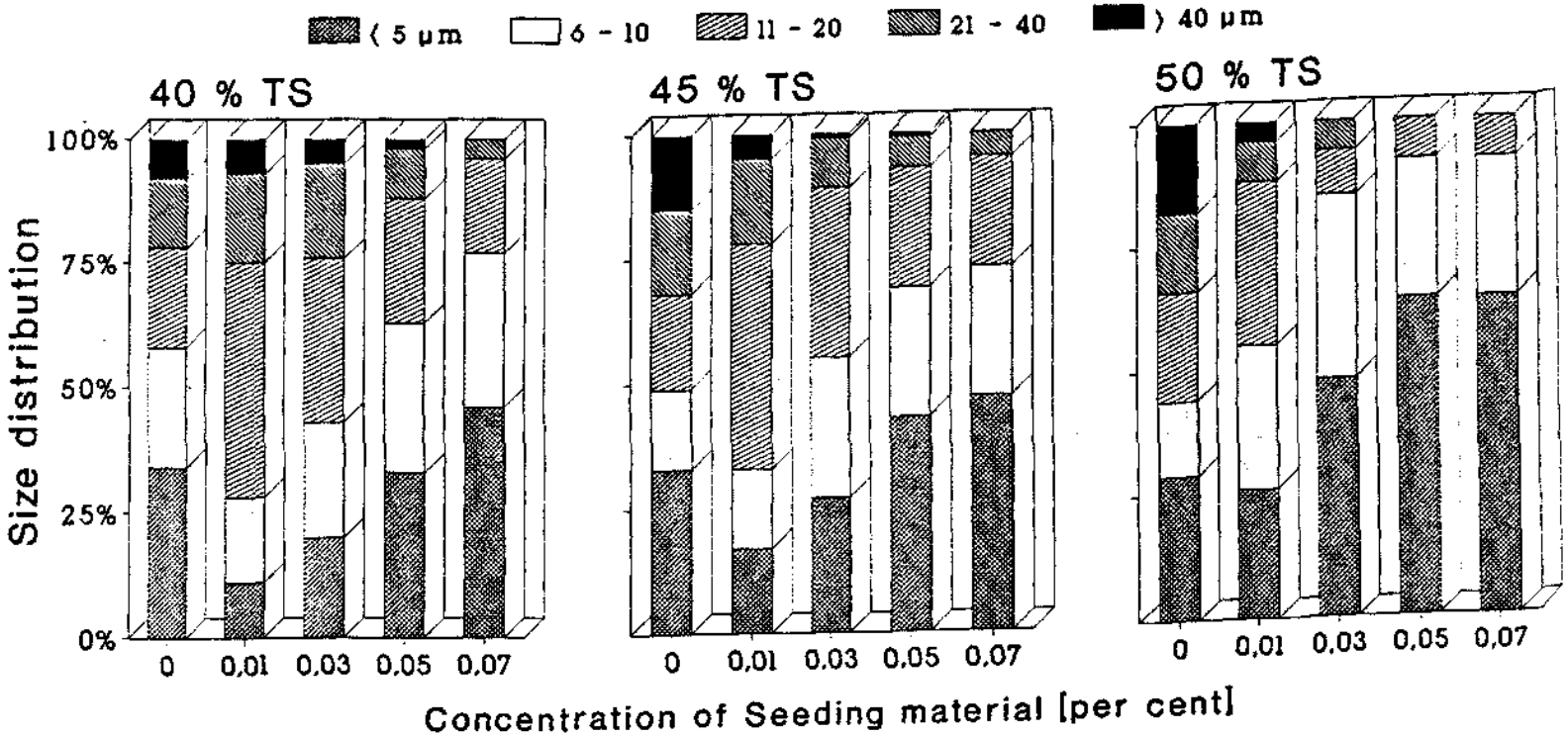


Table 9. ANOVA for the effect of seeding on the lactose crystallization

Source of variation	d.f.	'F' values		
		PW concentrate	CW concentrate	BW concentrate
Replication	2	2.8065^{NS}	3.0146^{NS}	2.3680^{NS}
Levels of seeding material (a)	4	12995.607**	20607.492**	16645.617**
Levels of total solids (b)	2	14864.772**	24054.494**	17414.004**
Duration of seeding (c)	5	10963.903**	19204.373**	15213.761**
Interaction				
a x b	8	148.255**	243.974**	267.051**
a x c	20	284.340**	442.804**	372.698**
b x c	10	142.268**	342.332**	197.812**
All effect interaction	40	36.112**	33.761**	31.837**
Error	178	-	-	-

NS = Non-significant

** Significant at 1 per cent level (P 0.01)

To avoid the very undesirable caking properties of ordinary whey order, it is of great industrial importance to get the major part of the lactose content into a crystalline form. The advantage of this lie h in energy savings and in improved powder properties (Hynd, 1980; sen and Oxlund, 1988).

It is of significant importance to control the crystallization so far as possible. This must be done in order to create maximum number of small crystals so as to get a large total crystal surface which facilitates best and efficient crystallization (Sienkiewicz and Riedel, 1990).

The rate and extent of crystallization depend on the TS in the ıcentrate, seeding material used and duration of crystallization (Westergaard, 1983). In our preliminary trials, when whey was concentrated 55 per cent TS it was difficult to handle the product. This could be attributed to the spontaneous crystallization of lactose and increased by scosity in the evaporator which results in difficulties in handling the product further (Jensen and Oxlund, 1988). Among three levels of the tried in this investigation, maximum crystallization was attained at per cent TS in all the three levels of whey concentrate. The higher the content the higher is the degree of supersaturation and greater is the crystallization (Hynd, 1980; Westergaard, 1983; Sandfort, 1987).

It is evident from the results that when the seeding material was not added to the concentrate, the degree of crystallization attained was very less and the size of crystals was found to be very large. Many of the crystals were more than 40 μm size. These results indicated that it is assential to add seeding material in order to obtain maximum number of small crystals and higher degree of crystallization.

It was observed in the present study that as the duration of seeding

increased the degree of lactose crystallization also increased upto 4 h. At the beginning there was sharp increase in the rate of crystallization, but at later stages the increase was found to be at a decreasing rate. This is because the crystallization process continues only as long as the solution is supersaturated. Once the supersaturation point is reached, crystallization stops. If, however, the solution is cooled, a supersaturated solution again results and crystallization can continue. In our experiment in most of the cases after 4-5 h, concentrate had probably reached saturation. Hence, further duration of seeding did not improve crystallization.

In all the three types of concentrate, as the level of seeding material increased the degree of crystallization also increased and the size of lactose crystals decreased to a minimum. This is because of the addition of external alpha-lactose monohydrate, which provides more nuclei for higher crystallization. Since the lactose crystallizes on the surface of already existing crystals, the rate of crystallization is proportional to the surface area of the crystals. Consequently, it means that the seeding material which is alpha-lactose monohydrate should have the smallest possible crystals and be added in sufficient quantity. Hence, as the level of seeding material increased, the rate of crystallization increased. However, it was observed that at 50 per cent TS in PW concentrate the seeding material above 0.03 per cent did not improve the lactose crystallization. Whereas in CW and BW concentrate above 0.05 per cent seeding material there was not much enhancement in the degree of crystallization. This could be ascribed to the higher lactose content of PW concentrate than CW and BW concentrates. In case of PW concentrates, 0.03 per cent and in CW and BW concentrates 0.05 per cent of seeding material was found to be optimum and further increase in the amount of

seeding material could not bring much improvement. Probably this amount of seeding material is sufficient to provide maximum number of nuclei to induce maximum crystallization.

Among the three types of concentrate, maximum crystallization was attained in PW concentrate at 50 per cent TS (67%) followed by CW concentrate (63%) and BW concentrate (58%). This could be attributed to the variation in the protein content of these concentrates which has great influence on the viscosity of the concentrate. Higher viscosity has negative influence on the crystallization rate (Westergaard, 1983). As the protein content of PW concentrate was very low compared to CW and BW concentrate, it had lower viscosity (10.50 cp) than CW concentrate (22.45 cp) and BW concentrate (25.34 cp) (Annexure III). Therefore, probably PW had shown higher degree of crystallization than CW and BW concentrates. This is also true in case of CW concentrate which has shown higher crystallization than BW concentrate.

From the results it can be inferred that maximum lactose crystallization can be attained in whey concentrate by crystallizing the concentrate at 30°C for a period of 4 h at a TS content of 50 per cent using 0.03 and 0.05 per cent seeding material (alpha-lactose monohydrate), respectively for PW and cheese whey concentrates. Hence, for further studies thse combinations of treatment were selected.

5.2.10 PROCESS FOR PRODUCTION OF SPRAY DRIED WHEY POWDER

Whey powder was manufactured from PW, CW and BW by a process of partial removal of water by RO followed by vacuum concentration, pre-crystallization and spray drying. The details of the processing steps followed are delineated below.

5.2.10.1 Reverse osmosis concentration

Reverse osmosis concentration of whey was carried out by using tubular RO plant installed at the Experimental Dairy Plant of National Dairy Research Institute, Karnal. The whey was concentrated at 50°C and an operating pressure of 35 bar.

Profiltered PW was adjusted to a pH of 7.2 and heated to 80°C/15 sec, cooled to 50°C and RO concentration was carried out to a level of 2 fold (50% volume reduction). In case of CW, it was subjected to clarification and heated to 80°C/15 sec and Ro concentration was done at 50°C to a level of 2.5 fold (60% volume reduction). Whereas clarified BW was adjusted to a pH of 7.2 heated to 80°C/15 sec, cooled to 50°C and concentrated by RO to a level of 2.5 fold (60% volume reduction).

5.2.10.2 Vacuum concentration

Paneer whey, CW and BW retentates obtained from RO concentration were further concentrated in a single effect evaporator. The concentration was done to 50 per cent TS at 635 mm Hg vacuum and a temperature of 54 to 56°C. The concentrate so obtained was flash cooled to 30°C and drawn into a crystallization tank, which had been maintained at 30°C.

5.2.10.3 Precrystallization

Paneer whey, CW and BW concentrated by RO followed by single effect evaporator to a TS content of 50 per cent was precrystallized at 30°C in a seeding tank by using alpha-lactose monohydrate.

Paneer whey concentrated to 50 per cent TS was seeded with 0.03 per cent seeding material at 30°C for a period of 4 h with continuous agitation and further cooled at the rate of 3°C/h to 10°C, which resulted in 79 per cent lactose crystallization. Whereas CW and BW concentrated

o 50 per cent TS were crystallized as above with 0.05 per cent seeding material. This yielded 73 and 75 per cent crystallization, respectively.

5.2.10.4 Spray drying

Precrystallized PW, CW and BW concentrates were spray dried at 80°C inlet temperature and 85°C outlet temperature with a atomizer speed of 25,000 rpm so as to get 3-4 per cent moisture in the resultant powder.

5.2.10.5 Packaging

Spray dried whey powders were cooled to room temperature and packed in polyethylene and metallized polyester packaging materials.

The complete flow diagrams of the process for production of whey powder using PW, CW and BW are given in Figs 18, 19 and 20, respectively.

5.2.11 PHYSICAL PROPERTIES AND CHEMICAL COMPOSITIONS OF WHEY POWDERS

The information on physical and chemical composition of dried whey is helpful in promoting their utilization especially for food product formulations. The data generated could provide basis for formulation of specifications for dried whey in our country. The whey powders prepared from three sources of whey were, hence analysed for their chemical composition and physical properties. These samples were compared with that of commercial whey powder (Germany) in order to assess the quality attributes of whey powders prepared in our investigation.

5.2.11.1 Physical properties

Whey powders prepared from PW, CW and BW were analysed for their detailed physical properties along with the popular commercially sold whey powder of Germany. As the physical properties of powders play a major role in the acceptability of various food formulations, the

FIG.18. FLOW DIAGRAM FOR THE MANUFACTURE OF PANEER WHEY POWDER

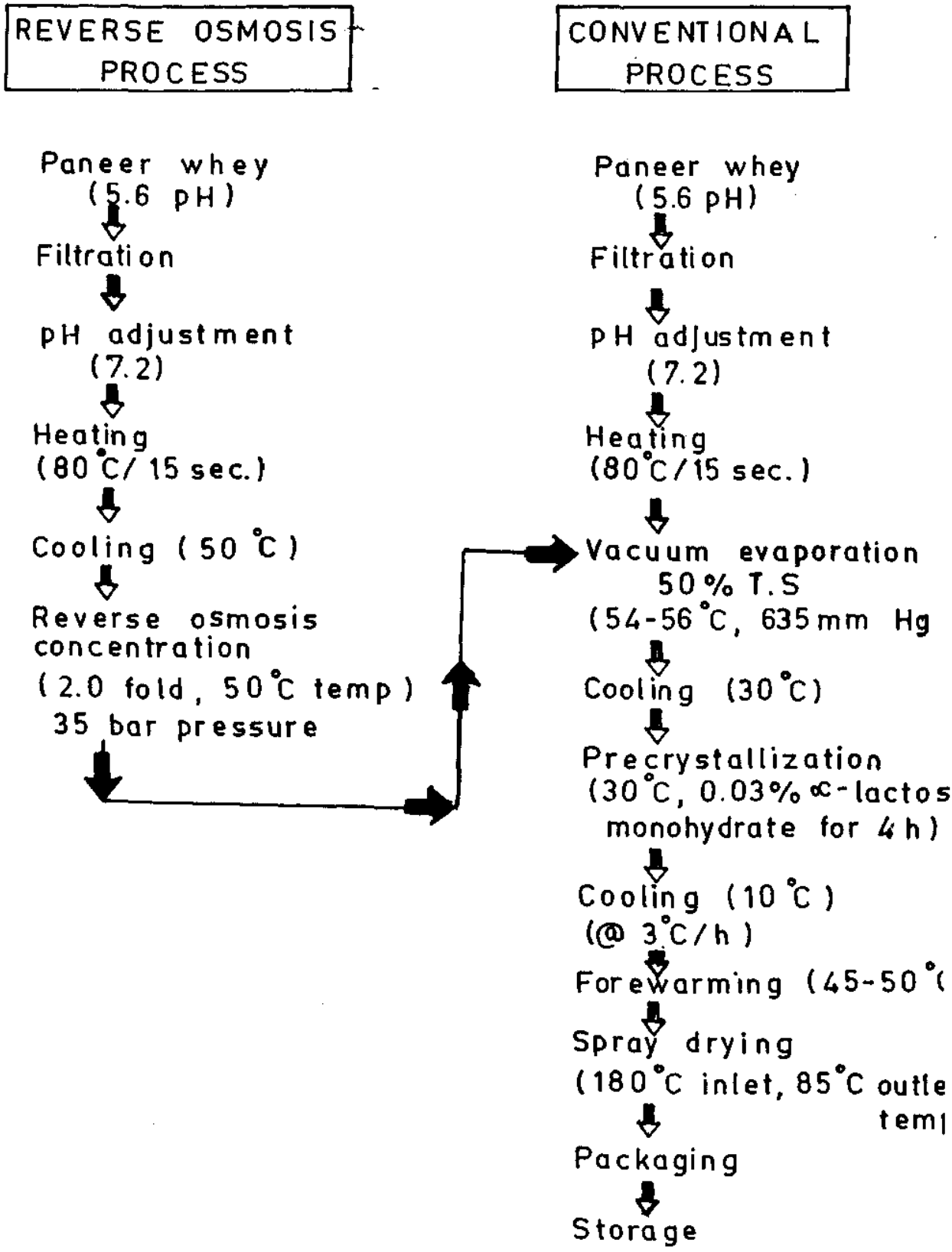
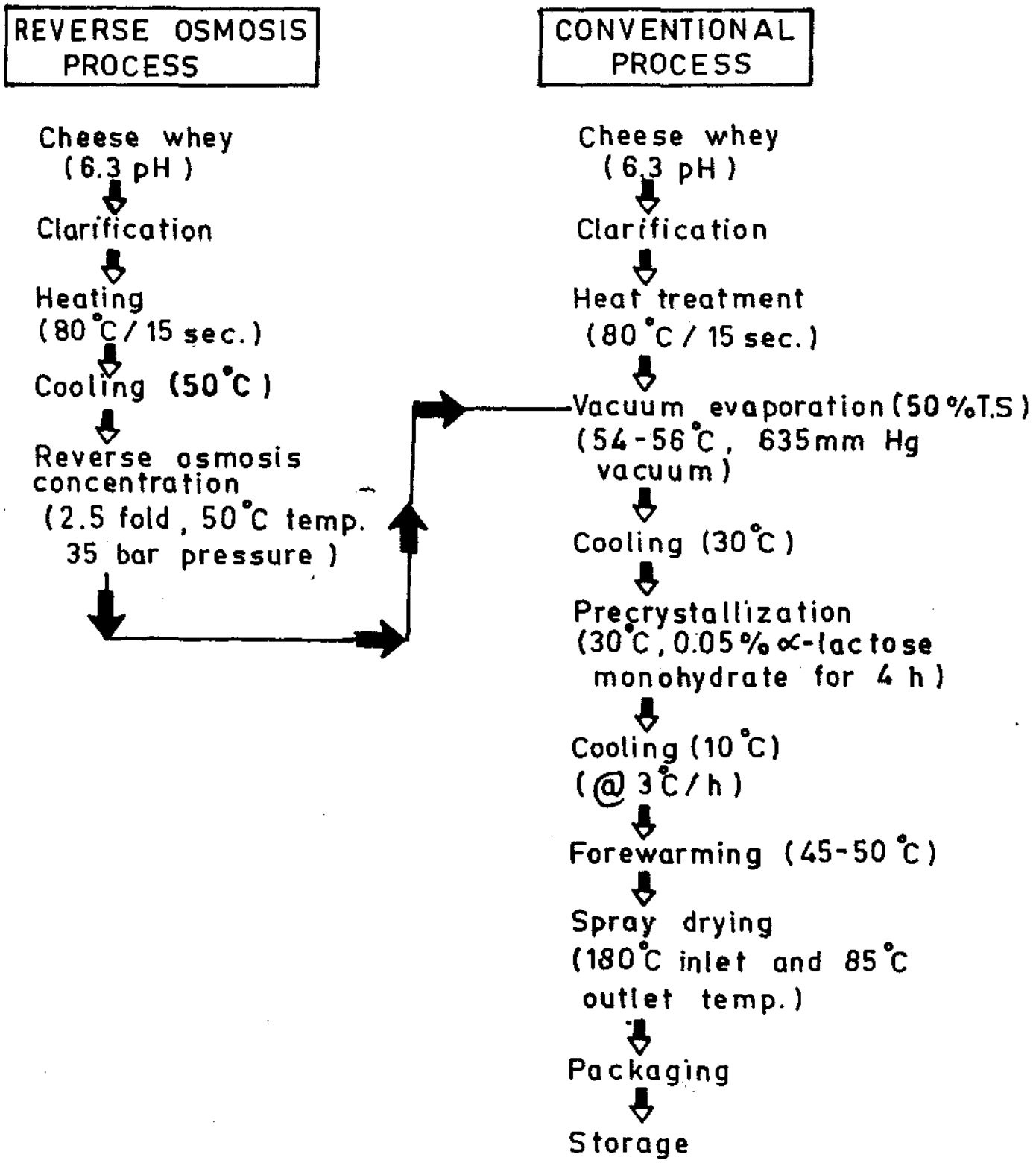
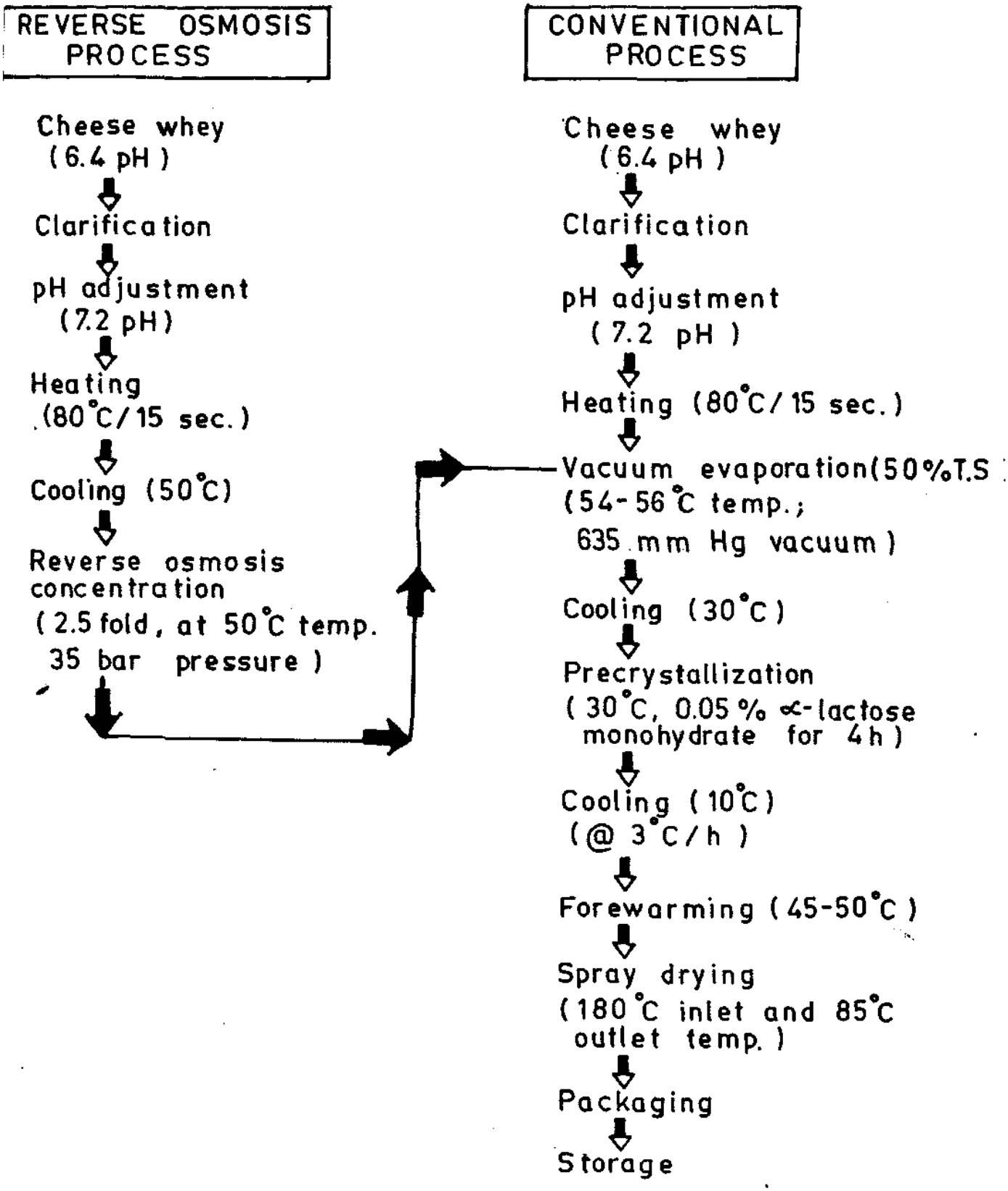


FIG.19. FLOW DIAGRAM FOR THE MANUFACTURE OF COW MILK CHEDDAR CHEESE WHEY POWDER



20. FLOW DIAGRAM FOR THE MANUFACTURE OF BUFFALO MILK CHEDDAR CHEESE WHEY POWDER



properties such as bulk density, solubility index, dispersibility, wettability, sinkability and flowability of the powders were studied. The results with respect to these properties are summarized in the Table 10.

5.2.11.1.1 Bulk density

Bulk density of whey powders varied from 0.508 g/cm^3 to 0.579 g/cm^3. Highest bulk density was observed in case of paneer whey powder (PWP) followed by buffalo milk cheese whey powder (BWP), cow milk cheese whey powder (CWP) and commercial whey powder (WP-G). The respective bulk density of the powders were found to be 0.579, 0.520, 0.510 and 0.508 g/cm^3. The highest bulk density of PWP can be attributed to the high ash content of the powder. The BWP had shown high bulk density than CWP and WP-G. This could be ascribed to the high mineral content of BWP than CWP and WP-G. The bulk density of all the powders are in agreement with the bulk density range reported by Jensen and Oxlund (1988).

5.2.11.1.2 Dispersibility

This is the property of powders which permits it to be distributed uniformly throughout the water. It is related to the ease with which lumps and agglomerates fall apart. Dispersibility is linked with particle size and the type of atomization. Small particles tend to reduce dispersibility due to over drying in the spray drier. Lowest dispersibility was observed for PWP which could be ascribed to the high heat treatment given to milk during production of paneer. The respective dispersibility for PWP, CWP, BWP and WP-G was found to be 90.44, 95.50, 94.90 and 95.80 per cent. German Agricultural Standard has recommended a minimum dispersibility of 93.0 per cent for whey powder. All the three types of whey powders except PWP were found to be within the limits of standards prescribed.

Table 10. Physical properties of whey powders *

Properties	Types of whey powder			
	Paneer whey powder	Cow milk cheese whey powder	Buffalo milk cheese whey powder	Commercial whey powder (Germany)
Bulk density (g/cm^3)	0.579	0.510	0.520	0.508
Dispersibility (%)	90.44	95.50	94.90	95.80
Wettability (seconds)	8.0	15.0	22.0	17.0
Sinkability (% transmission)	67.5	64.0	62.0	63.0
Flowability (Tan θ)	37°	32°	34°	33°
Solubility index (ml)	1.10	0.60	0.70	0.75

* Average of five trials

5.2.11.1.3 Wettability

The wettability of powders is primarily a measure of hydrophilic property, that is, the ability of the powder particles to be wetted by water. It is measured as the time necessary for a given amount of powder to pass through the water surface at specified conditions. The tendency of powders to form lumps on adding water indicates lack of wettability.

The wettability of PWP, CWP, BWP and WP-G were found to be 8.0, 15.0, 22.0 and 17.0 sec respectively. PWP had shown a very good wettability of 8.0 sec, probably due to its high lactose and low protein content compared to other powders. Slightly poor wettability of BWP could be ascribed to its high protein content. However, all the four types of whey powders were well within the limits of wettability standards. The German Agricultural Standard has prescribed wettability for dried whey to be less than 30 sec. For instant whey powders, wettability was observed to be 5-6 sec (Jensen and Oxlund, 1988).

5.2.11.1.4 Sinkability

This property permits a powder to overcome the surface tension of water and to sink into the water after passing through the surface. The rate of sinking depends upon the particle size, the surface area and density of the powder. Sinkability is related to wettability in many respects.

The sinkability of whey powder was 67.5, 64.0, 62.0 and 63.0 per cent for PWP, CWP, BWP and WP-G, respectively. All the four types of whey powder had shown very good sinkability.

5.2.11.1.5 Flowability

The flow property of a powder refers to the ease with which powder

particles move with respect to one another. The flowability of whey powder was measured by the angle of repose (Tan θ). Higher the angle of repose, lower is the flowability. The flowability of whey powder was found to be 37°, 32°, 34° and 33° for PW, CW, BW and WP-G. It is very interesting to observe that all the four types of whey powder had shown better flowability than skim milk powder (44-45°), whole milk powder (65°) and khoa powder (64.1°) (Ranganadham, 1988). Good flowability of whey powders could be attributed to the high degree of crystallization of lactose which probably aids in enhancing flowability of powders.

5.2.11.1.6 Solubility index

Solubility index is an indication of degree of denaturability and status of proteins in the powders which varies with the severity of heat treatment given during various processing steps.

The solubility index of PWP, CWP, BWP and WP-G appeared to be 1.10, 0.60, 0.70 and 0.75 ml, respectively. The values for the solubility index of PWP was high compared to other types of whey powder which is certainly due to high degree of heat treatment given to milk during the production of paneer. The solubility index of all the whey powders were within the limit of standards prescribed. According to German Agricultural Standards and US Standards, solubility index of whey powders should not exceed 1.20 ml.

5.2.11.2 Chemical composition of whey powders

The whey powders were analysed for their major components such as moisture, protein, fat, lactose and ash. The results are diagramatically presented in Fig 21.

5.2.11.1.3 Wettability

The wettability of powders is primarily a measure of hydrophilic property, that is, the ability of the powder particles to be wetted by water. It is measured as the time necessary for a given amount of powder to pass through the water surface at specified conditions. The tendency of powders to form lumps on adding water indicates lack of wettability.

The wettability of PWP, CWP, BWP and WP-G were found to be 8.0, 15.0, 22.0 and 17.0 sec respectively. PWP had shown a very good wettability of 8.0 sec, probably due to its high lactose and low protein content compared to other powders. Slightly poor wettability of BWP could be ascribed to its high protein content. However, all the four types of whey powders were well within the limits of wettability standards. The German Agricultural Standard has prescribed wettability for dried whey to be less than 30 sec. For instant whey powders, wettability was observed to be 5-6 sec (Jensen and Oxlund, 1988).

5.2.11.1.4 Sinkability

This property permits a powder to overcome the surface tension of water and to sink into the water after passing through the surface. The rate of sinking depends upon the particle size, the surface area and density of the powder. Sinkability is related to wettability in many respects.

The sinkability of whey powder was 67.5, 64.0, 62.0 and 63.0 per cent for PWP, CWP, BWP and WP-G, respectively. All the four types of whey powder had shown very good sinkability.

5.2.11.1.5 Flowability

The flow property of a powder refers to the ease with which powder

BWP and CWP. The fat content of all the whey powders except PWP are within the range of limit prescribed by German Agricultural Standard, which has prescribed a maximum 1.5 per cent fat in the dried whey.

5.2.11.2.4 Lactose

The lactose content of PWP, CWP, BWP and WP-G was 80.68, 75.49, 72.99 and 74.77 per cent, respectively. The high lactose content of PWP is due to the higher proportion of lactose and less protein in dry matter content of the whey. Lower content of lactose in BWP could be attributed to higher levels of proteins and other components in whey.

5.2.11.2.5 Ash

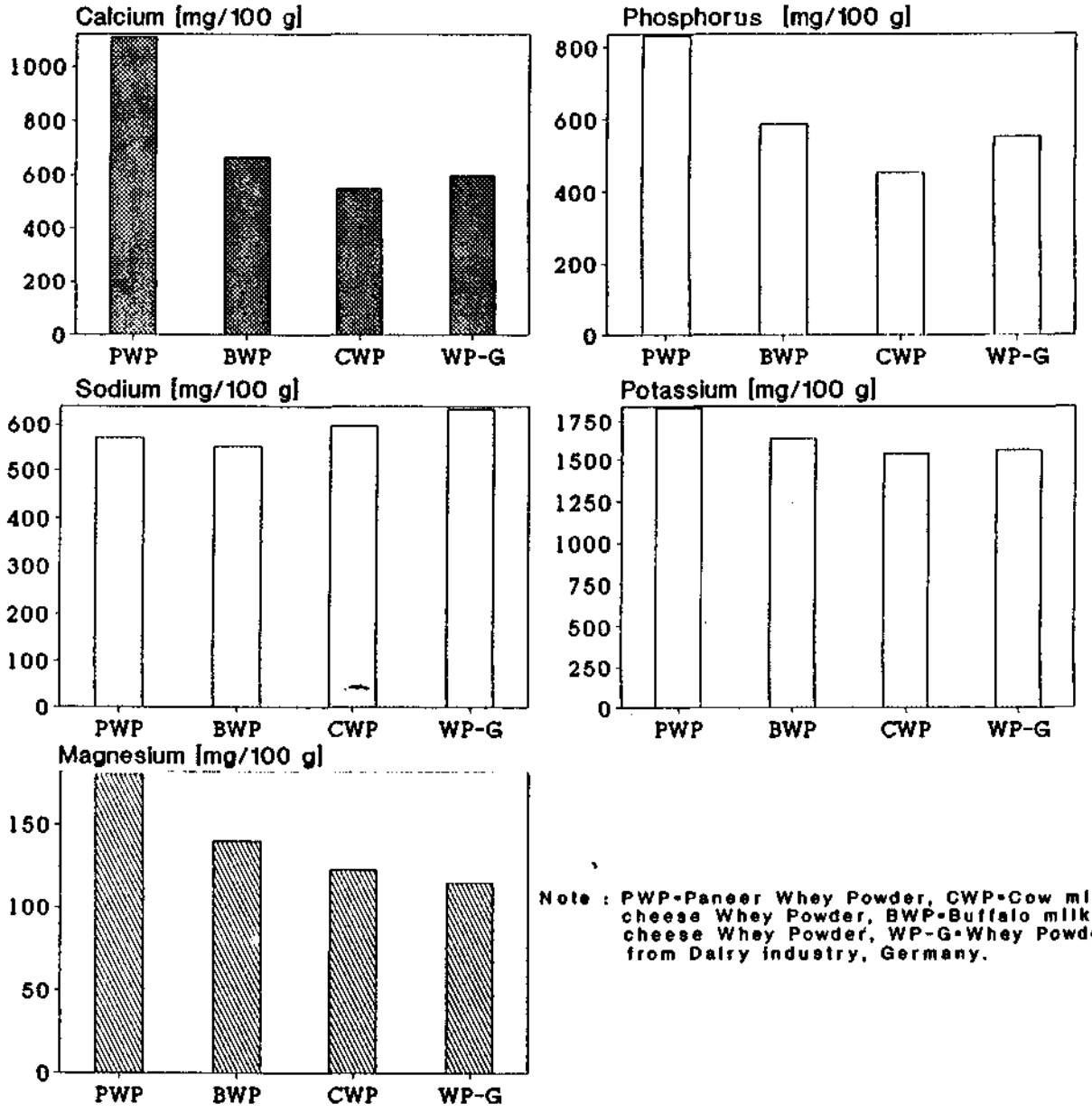
Ash content of dried whey samples varied from 7.34 per cent to 8.74 per cent. A maximum of 8.74 per cent was noticed in PWP followed by BWP (8.01%), WP-G (7.85%) and CWP (7.34%). Higher ash content of PWP was due to higher initial content of ash in the whey, which was due to lower pH of coagulation during paneer production. BWP had shown slightly higher ash content than CWP and WP-G. Ash content of all these samples was within the maximum limit reported by many workers (Cerbulis et al., 1972; Glass and Hedrick, 1977).

5.2.11.2.6 Mineral content

Whey powders were analysed for mineral composition. The minerals such as calcium, phosphorus, potassium, sodium and magnesium contents of PWP, CWP, BWP and WP-G are diagramatically presented in Fig 22.

The calcium content was found to be 1109, 667, 549 and 598 mg/100 g powder, whereas phosphorus was 831, 587, 454 and 553, potassium 1887, 1634, 1540 and 1563, sodium 570, 551, 559 and 633, and magnesium 180, 140, 123 and 115/100 g powder, respectively for PWP, BWP, CWP and WP-G.

FIG 22. # MINERAL COMPOSITION OF WHEY POWDERS



Note : PWP-Paneer Whey Powder, CWP-Cow milk cheese Whey Powder, BWP-Buffalo milk cheese Whey Powder, WP-G-Whey Powder from Dairy industry, Germany.

Paneer whey powder possessed high mineral load than the other three types of whey powders which could be attributed to pH of coagulation during production of paneer. During paneer production due to acidulation colloidal minerals get solubilized and go along with whey. Hence, high mineral load was observed in whey and resultant powder.

Calcium and phosphorus contents were found to be higher in PWP as well as BWP which could be ascribed to the inherent nature of buffalo milk used in the manufacture of these products. High content of calcium and phosphorus in BW has been reported by Srivastava (1991).

Sodium content of WP-G was found to be high compared to the other three types of whey powders, which could be due to the method of manufacture of whey powder followed in our investigation. In this study whey powders were manufactured by concentrating whey through RO followed by vacuum concentration and drying. Probably during RO concentration low molecular weight minerals must have been passed through the permeate. Hence, the resultant powders were found to contain less sodium. Permeation of sodium through RO membrane is reported by several workers (Marshall, 1985; Jensen and Oxlund, 1988).

Slight variation in the content of phosphorus, potassium, sodium and magnesium were observed which could be ascribed to the type of whey used, method of manufacture and various treatments employed during the production of whey powders. Analysis of several varieties of commercial whey powders by Rückemann et al. (1973) revealed that calcium content of whey powder ranged from 395-1600, whereas phosphorus varied from 563-1900, potassium 1460-2520, sodium 529-2910 and magnesium 125-160 mg/100 g powder. In general, the minerals of 4 samples in our study were found found to be within these ranges. Several other workers also reported

the values for calcium, phosphorus, potassium, sodium and magnesium in this range.

5.2.11.2.7 Protein quality of whey powders

Results with respect to various attributes pertaining to quality of dried whey are presented in Fig 23. These attributes comprised of total nitrogen (NT), non-protein nitrogen (NPN), proteose peptone nitrogen (PP), whey protein nitrogen (WPN), lysine content and the extent of denaturation.

(a) Nitrogen distribution

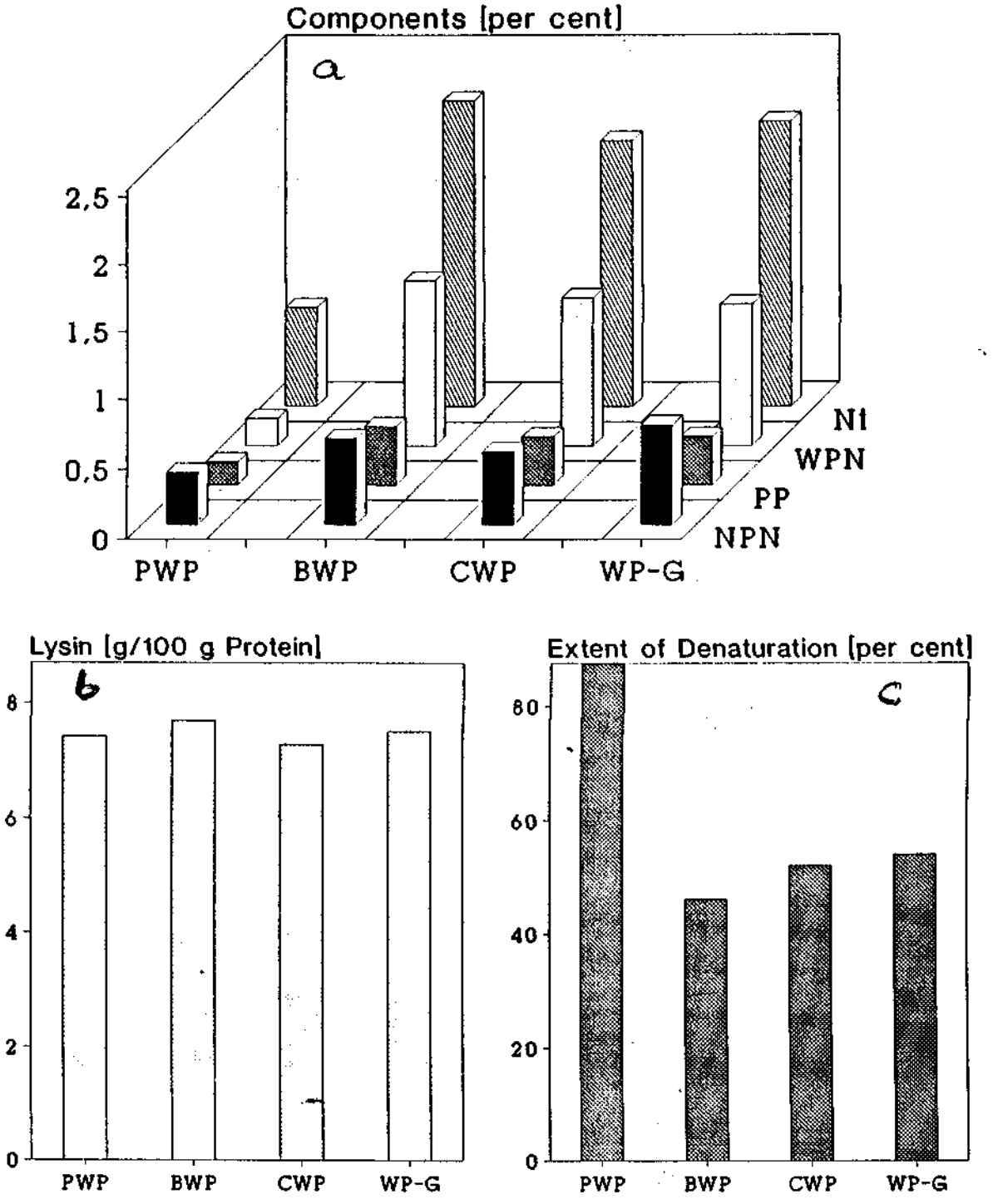
The nitrogen distribution of PWP, CWP, BWP and WP-G is diagramatically presented in Fig 23a.

Paneer whey powder was found to have 0.73 per cent NT which comprised of 0.37, 0.164 and 0.20 per cent NPN, PP and WPN, respectively. Whereas BWP was found to have 2.25 per cent NT which included 0.62, 0.42 and 1.21 per cent NPN, PP and WPN, respectively. CWP and WP-G were found to contain 1.96 and 2.10 per cent NT, respectively which consisted of 0.53, 0.36 and 1.06 per cent, and 0.64, 0.39 and 1.06 per cent NPN, PP and WPN, respectively.

From the results it is observed that PWP had NPN, PP and WPN in the ratio of 50.93:22.50:26.57, BWP 27.40:18.70:53.90, CWP 26.80:18.80:54.40 and WP-G 32.10:18.60:49.30, respectively.

It is to be noted that in the case of BWP and CWP the per cent NPN of NT was observed to be less which could be attributed to the permeation of these constituents along with the permeate during RO. Jensen and Oxlund (1988) also observed slight loss of NPN in whey powder manufactured using whey concentrated by RO. PWP had less WPN, which could

FIG 23. # PROTEIN QUALITY OF WHEY POWDERS



Note : PWP=Paneer Whey Powder, CWP=Cow milk cheese Whey Powder, BWP=Buffalo milk cheese Whey Powder, WP-G=Whey Powder from Dairy Industry, Germany.

be ascribed to the high heat treatment given to milk during production of paneer, which carried most of the whey proteins along with the coagulum. Hence, proportion of NPN to NT was high.

(b) Lysine content

The lysine content of whey powders is presented in Fig 23b. The PWP, CWP, BWP and WP-G had 7.40, 7.28, 7.70 and 7.52 g/100 g protein, respectively. Rückemann et al. (1973), after a wide range of analysis of commercial samples, reported wide variation in lysine content ranging from 3.8 to 7.2 per cent of protein. However, Renner and Kaboth (1982) observed an average of 7.0 g/100 g of protein in commercial sweet whey powders. Whereas, Huss (1971) reported an average lysine content of 7.4 per cent in commercial spray dried fresh samples of whey powders. All the four samples of whey powder were found to possess considerably good amount of lysine.

(c) Extent of denaturation

Whey proteins are known to be more heat susceptible than caseins. During whey powder manufacture, whey undergoes various heat treatments and hence presence of denatured proteins in the final whey powder is naturally expected. The extent of denaturation of whey proteins in PWP, CWP, BWP and WP-G are diagramatically represented (Fig 23c).

The extent of denaturation in PWP, CWP, BWP and WP-G was 87.5, 52.0, 46.0 and 54.0 per cent, respectively. PWP had shown significantly high extent of denatured proteins owing to high heat treatment that milk receives during paneer manufacture. In case of cheese making the milk is only pasteurized and at pasteurization temperatures, the extent of whey protein denaturation is less. Hence, the denaturation in BWP, CWP and

WP-G was found to be less compared to PWP. The lowest denaturation value in BWP may be because of higher alpha-lactalbumin content of BW which is more heat resistant than beta-lactoglobulin (Srivastava, 1991).

5.2.12 STORAGE STABILITY OF WHEY POWDERS

Dried whey powders have assumed an increasingly important role as an inexpensive, versatile and nutritional ingredient in many food products abroad. These powders will certainly become popular in our countries as well and could be used in varieties of food formulations. With the increasing demand for these products, there is a need to understand adverse storage effects on the physico-chemical properties and nutritional aspects of the powder. Hence, storage studies were undertaken in order to characterize the physico-chemical changes during storage at different temperatures and in different packaging materials.

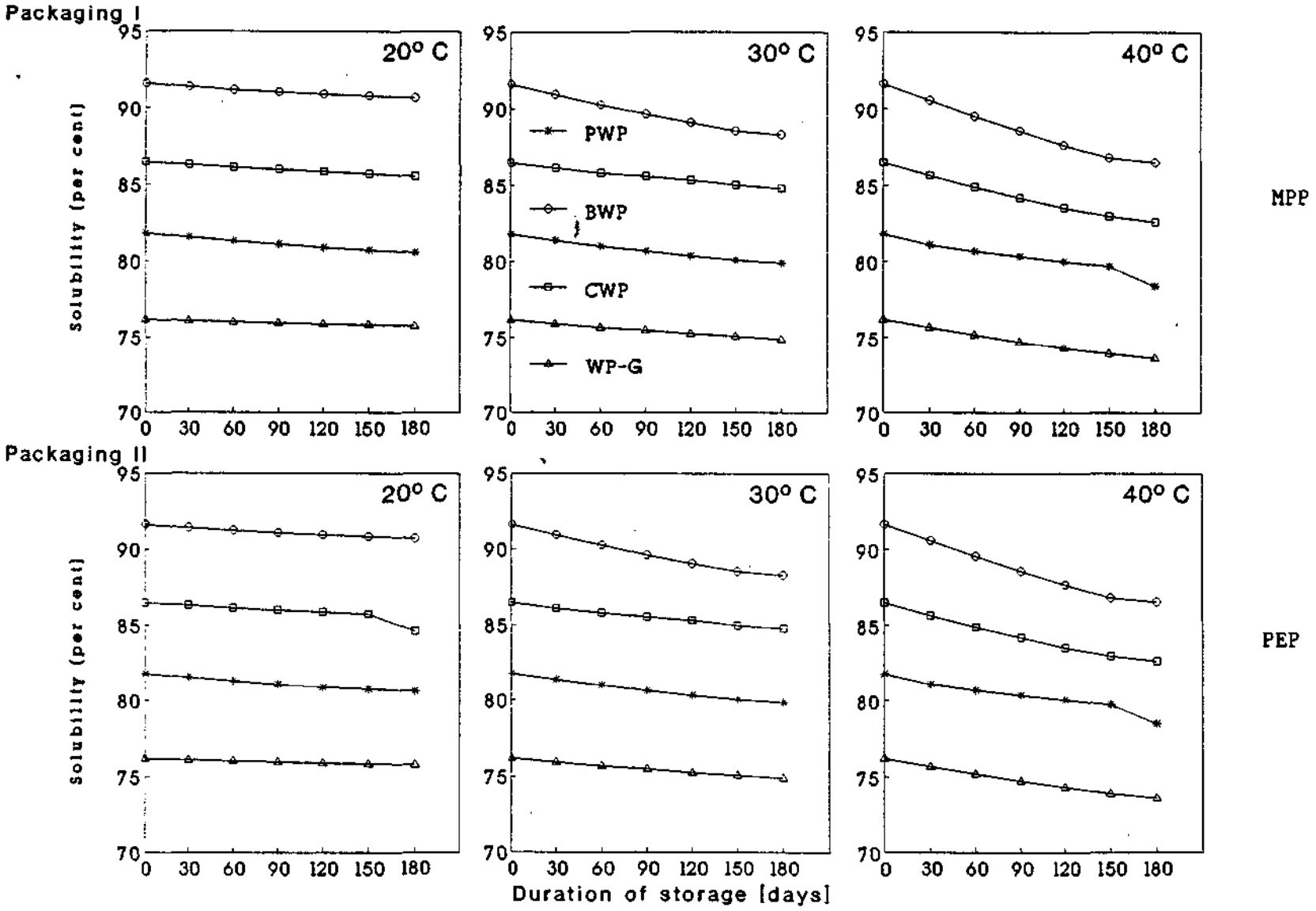
Paneer whey powder, CWP, BWP and WP-G were stored at 20, 30 and 40°C in two types of packaging materials. The samples were drawn at a regular interval of one month till the end of 6 months in order to characterize the changes occurring with respect to solubility, lysine content, extent of denaturation and colour.

5.2.12.1 Solubility

Solubility of whey powders was determined as total nitrogen solubility. Changes in solubility of PWP, CWP, BWP and WP-G as affected by temperature of storage and packaging material are depicted in Fig 24.

Initial solubility of PWP, BWP, CWP and WP-G were found to be 81.80, 91.60, 86.48 and 76.19 per cent, respectively. It can be observed from the figure that as the storage duration increased the solubility decreased to a slight extent in all the types of whey powder. However, the rate of

FIG 24. CHANGES IN SOLUBILITY OF WHEY POWDER DURING STORAGE



adsorption of beta-lactoglobulin as the pH is near to its isoelectric point (Hiddink et al., 1981). It is surprising to note a drastic increase in the flux at pH 7.2. This pronounced increase in the permeate flux may be due to heating of whey at elevated pH. Under such conditions, a considerable precipitation of calcium phosphate is expected in such a form (probably as hydroxy apatitie) that, results in reduced fouling of membrane. Hayes et al. (1974) observed that addition of calcium to whey and adjusting pH above 6.5 and heating, increased the flux. As the calcium content of BW is higher, heating at pH 7.2 probably resulted in calcium induced protein interaction which aided in larger aggregate or apatite formation, which were found to be non-fouling.

Higher temperature of heating 80°C/15 sec had given better flux than 60°C/30 min. Probably higher temperatures are required for the apatite formation or for the calcium induced beta-lactoglobulin self aggregation or for the protein-protein interactions. Muller and Harper (1979) also observed an increase in flux by 50 per cent when cheese whey was heated to 80°C/15 sec instead of pasteurization temperature.

From the results and the statistical analysis, it can be concluded that maximum flux during UF processing of BW could be attained when pH of whey is adjusted to 7.2 followed by 80°C/15 sec heating.

5.3.2 COMPOSITIONAL CHANGES DURING ULTRAFILTRATION OF BUFFALO MILK CHEDDAR CHEESE WHEY

Preliminary trials have shown that it is possible to obtain a maximum of 26.5 per cent solids (97.5% volume reduction) from BW by fractionating through UF. However, due to low flux rate caused by high viscosity, the concentration was restricted to 95 per cent volume reduction (21.60% TS).

solubility did not exceed 6 per cent even at 40°C in either of the packaging material.

Slight decrease in solubility during the storage of whey powder at higher temperatures could be attributed to the Maillard reaction, which must have led to protein-protein interaction, thus causing slight decrease in solubility. However, solubility is a complex function of various characteristics including charge and molecular weight. Denaturation often preceeds aggregation and loss of solubility. Probably the denaturation of protein that occurred during storage at higher temperatures was reflected by a loss of solubility (Li-chan, 1983).

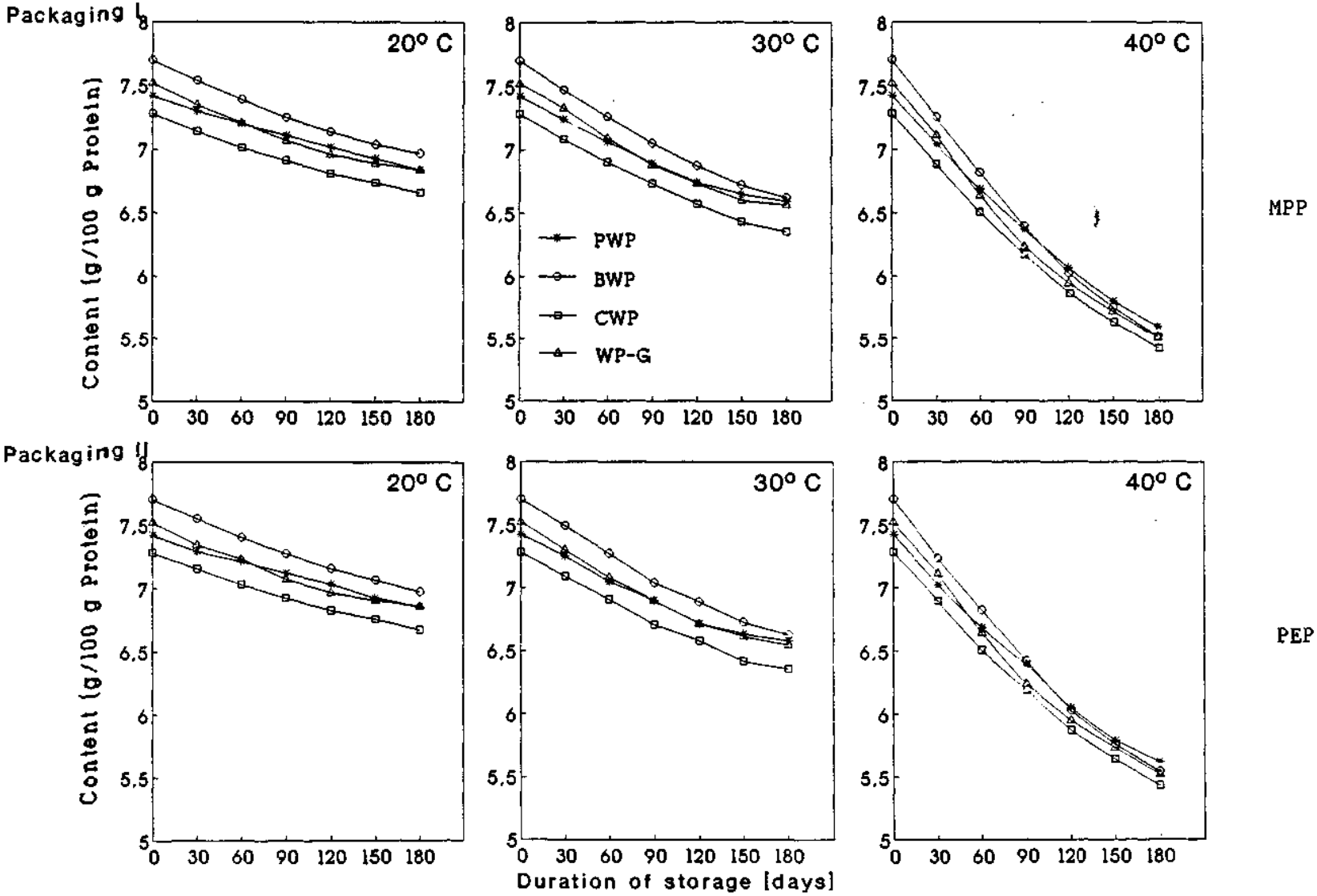
5.2.12.2 Lysine

The changes in lysine content during storage of PWP, CWP, BWP and WP-G at various storage conditions are depicted in Fig 25.

The initial lysine content of PWP, BWP, CWP and WP-G was observed to be 7.42, 7.70, 7.25 and 7.52 g/100 g of protein, respectively. It is apparent from the results that as the period of storage progressed there was decrease in lysine content of whey powders. The extent of loss was higher during the initial storage periods than at later stages.

The actual lysine content of whey powders after different storage periods is depicted in Fig 25. After 6 months of storage period in MPP, the loss of lysine was recorded to be 8.87, 13.08 and 28.04 per cent for PWP, 8.69, 12.27 and 25.10 per cent for CWP, 9.40, 13.89 and 28.05 per cent for BWP and 8.77, 12.89 and 26.59 per cent for WP-G, respectively at 20, 30 and 40°C storage temperature. Similarly, in PEP the extent of loss was 9.03, 12.92 and 28.34 per cent for PWP, 8.13, 12.41 and 25.24 per cent for CWP, 9.48, 14.02 and 28.31 per cent for BWP and 9.04, 12.76 and 26.73 per cent for commercial whey powder.

FIG 25. CHANGES IN LYSIN CONTENT OF WHEY POWDER DURING STORAGE



The results of changes in lysine content during storage of whey powder was computed by regression analysis. Reaction rate constants were derived for the effect of packaging material, temperature of storage and types of whey powder. ANOVA table 12 has shown that there is significant effect of temperature of storage ($P \leq 0.01$) on the extent of loss of lysine during storage. Effect of packaging material was found to be non-significant. The rate of loss of lysine was 0.2009 and 0.1998 for PEp and MPP, whereas for the temperature of storage the reaction rate constant was 0.1080, 0.1624 and 0.3306, respectively for 20, 30 and 40°C, indicating that higher the temperature of storage, greater is the loss of lysine regardless of the packaging material. The values for the rate of change in lysine in PWP, BWP, CWP and WP-G were 0.1806, 0.2243, 0.1903 and 0.2062 indicating that the extent of losses were almost uniform.

From the results it is evident that temperature of storage of whey powder has profound effect on the loss of lysine content. Higher the temperature of storage, greater is the loss of lysine in all the types of whey powder irrespective of packaging material. The loss of lysine in whey powder could be ascribed to the Maillard reaction. In the process of Maillard reaction, ϵ-lysine and lactose are involved which bring about losses in lysine. The Maillard reaction is faster and enhanced at higher temperatures. Therefore, greater losses of lysine were observed at 40°C than at 20 or 30°C. Loss of lysine during storage of whey powder via Maillard reaction leading to loss of nutritional quality has been reported by many workers (Labuja and Saltmarch, 1981; Hsu and Fennema, 1989). The effect of higher temperature of storage in enhancing the extent of Maillard reaction has also been documented by many workers (Ben-Gara and Zimmerman, 1972; Holsinger et al., 1973). In one of the recent report, Henle et al. (1991) observed change in colour, flavour and severe losses in nutritive

value in terms of lysine by Maillard reaction during storage. A loss of about 20.63 per cent lysine was observed by Cheng (1979) when whey powder was stored in glass bottles for a period of 3 months at 40°C, whereas at 20°C the loss was found to be 6.19 per cent. Renner and Rommer (1976) noted a loss of 23 and 49 per cent lysine, respectively at 20° and 37°C after 6 months of storage.

5.2.12.3 Extent of denaturation

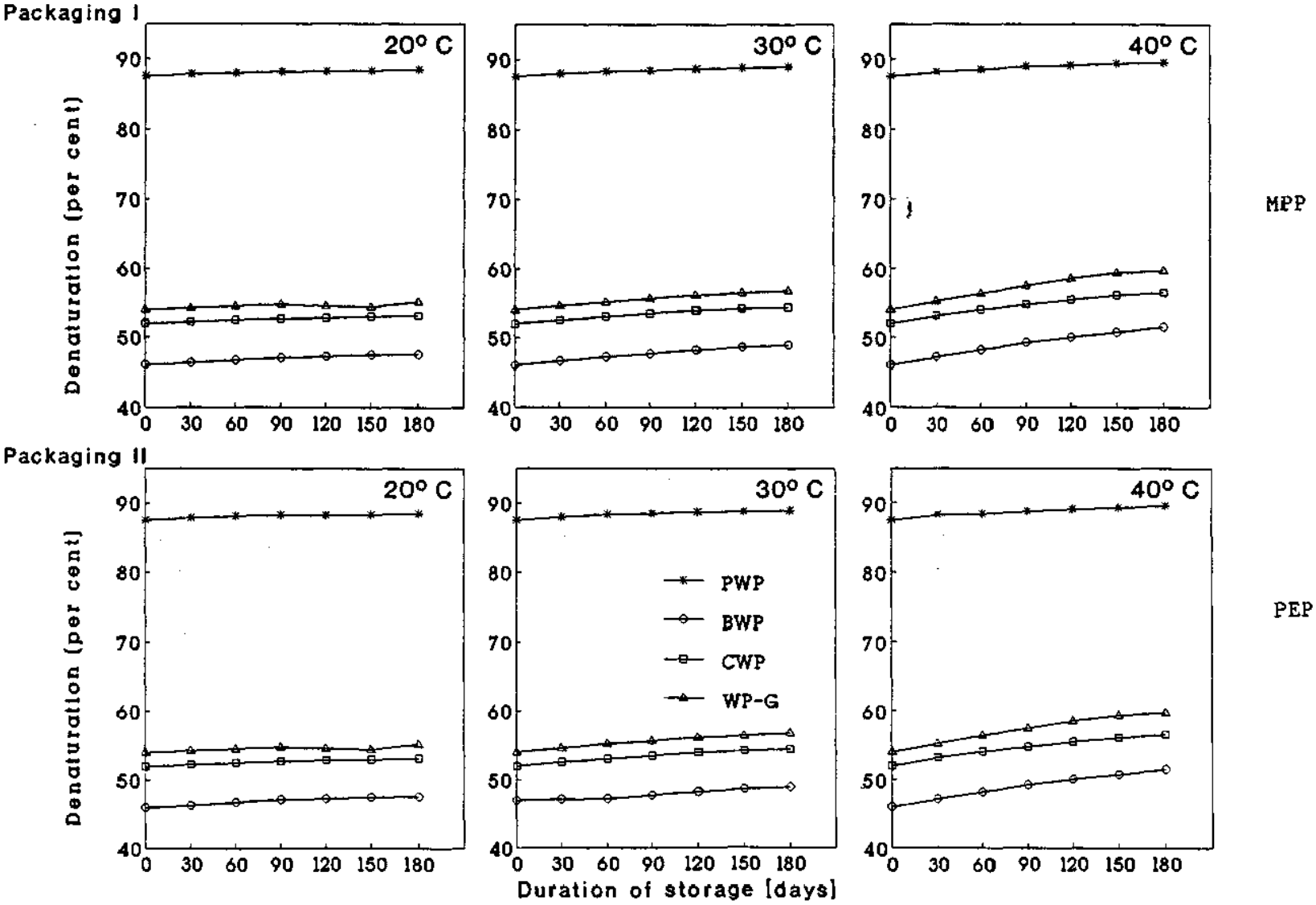
The changes in the extent of denaturation during storage of whey powders are presented in Fig 26. The initial denaturation was observed to be 87.5, 46.0, 52.0 and 54.0 per cent, respectively for PWP, BWP, CWP and commercial whey powder.

It can be observed that there is slight increase in the denaturation during storage. The extent of denaturation appeared to be slightly higher at 40°C than at 20 and 30°C. The change in denaturation was not affected by the type of packaging materials used.

The values for the extent of denaturation for different period of storage are shown in Fig 26. It was observed that after 6 months of storage, the extent of increase in denaturation when whey powders were stored in MPP were estimated to be 1.02, 1.60 and 2.28 per cent for PWP, 2.11, 4.61 and 8.65 per cent for CWP, 3.26, 6.30 and 11.90 per cent for BWP and 2.25, 5.22 and 10.62 per cent for WP-G, respectively when stored at 20, 30 and 40°C. Whereas when stored in PEP the extent of increase in denaturation was 1.08, 1.60 and 2.40 per cent for PWP, 2.19, 4.71 and 8.75 per cent for CWP, 3.36, 6.41 and 11.91 per cent for BWP and 2.22, 5.18 and 10.55 per cent for commercial whey powder.

Regression analysis was carried out to know the dependence of change in denaturation as affected by packaging material and temperature of

FIG 26. CHANGES IN EXTENT OF DENATURATION OF PROTEIN OF WHEY POWDER DURING STORAGE



storage. ANOVA table 12 clearly indicated that there is significant effect of temperature of storage on the extent of increase in denaturation. The effect of packaging material was, however, non-significant ($P \leq 0.01$). Rate of increase in denaturation was found to be 0.4405 and 0.4408 for MPP and PEP, respectively indicating that packaging material has no effect as per the extent of denaturation. The reaction rate constant for various whey powders were 0.2310, 0.5589, 0.4483 and 0.5326, respectively for PWP, BWP, CWP and WP-G. Temperature of storage appeared to have significant effect on denaturation. The rate of increase in denaturation was found to be 0.1842, 0.3997 and 0.7740, respectively for 20, 30 adn 40°C storage temperature.

Results have indicated that there is slight increase in the extent of denaturation of proteins during storage. The extent of increase was more at higher temperatures of storage than at lower temperatures. A maximum of 2.40, 11.91, 8.75 and 10.62 per cent increase in denaturation was observed respectively for PWP, BWP, CWP and WP-G at a storage temperature of 40°C. At other temperatures of storage, the increase in denaturation was found to be negligible. The increase in denaturation specially at higher temperature of storage could be attributed to Maillard reaction. Maillard reaction results in protein and other molecular interactions leading to change in the conformation of the protein structure. Li-chan (1983) observed that during storage of whey protein powders, formation of large aggregates of proteins which could not penetrate the gel of electrophoresis. These results suffice that there will be conformational changes in the protein due to various molecular interactions which probably bring about changes in the extent of denaturation. Packaging materials did not show any significant difference in the extent of denaturation. Though PEP has good permeability to moisture due to high

degree of crystallization of lactose, powders packed in PEP could not absorb moisture and bring about denaturation by accelerating Maillard reaction.

5.2.12.3 Colour

The high contents of lactose and protein (high in lysine) in whey powders are especially conducive to Maillard reactions which may result in undesirable changes of visual characteristics besides change in organoleptic, nutritional and functional characteristics. The change in colour during storage of whey powders was measured as reflectance values. The changes in reflectance values, as affected by storage period, temperature of storage and packaging material are presented in Table 11. Slight variation in the colour was discerned among whey powders with an initial reflectance value of 78, 80, 79 and 78 per cent, respectively for PWP, CWP, BWP and WP-G.

It can be seen from the table that with the passage of storage time, there was progressive increase in the colour development in all the whey powders. However, the intensity varied widely with the storage temperature. When whey powders were stored in MPP, the reflectance values after 6 months of storage period were lowered to 75, 73 and 70 per cent in PWP, 76, 74 and 71 in CWP, 75, 73 and 70 in BWP and 75, 73 and 69 per cent in WP-G, respectively at 20, 30 and 40°C. Similarly, when whey powders stored in PEP, the reflectance values declined to 74.5, 73.0 and 69.5 in PWP, 76.0, 73.5 and 71.0 in CWP, 74.5, 73.0 and 70.0 per cent in BWP and 75.0, 73.0 and 69.0 per cent in WP-G, respectively.

The results of storage studies were subjected to regression analysis and reaction rate constant was computed. The rate of reaction or the reaction rate constant for MPP and PEP was 0.9844 and 0.9807, respectively

Table 11. Changes in reflectance value (%) of whey powders during storage

Packaging material	Type of whey powder	Temperature of storage	Duration of storage (days)						
			0	30	60	90	120	150	180
I	Paneer whey powder	20	78.0	77.5	77.0	76.5	75.5	75.0	75.0
		30	78.0	76.5	75.0	74.0	73.5	73.0	73.0
		40	78.0	75.5	73.0	72.0	71.5	70.5	70.0
	Cow milk cheese whey powder	20	80.0	79.5	78.5	77.0	77.0	76.5	76.0
		30	80.0	78.0	76.5	76.0	74.5	74.0	74.0
		40	80.0	77.5	75.0	73.5	72.0	71.5	71.0
	Buffalo milk cheese whey powder	20	79.0	78.0	77.5	77.0	76.0	75.0	75.0
		30	79.0	77.0	75.5	74.5	73.5	73.5	73.0
		40	79.0	76.5	75.5	73.5	72.0	71.5	70.0
	Commercial whey powder (Germany)	20	78.0	77.0	76.5	76.0	75.5	75.0	75.0
		30	78.0	76.0	74.5	74.0	73.5	73.0	73.0
		40	78.0	75.0	73.5	71.5	70.0	69.5	69.0
II	Paneer whey powder	20	78.0	77.0	77.5	76.0	75.5	75.0	74.5
		30	78.0	76.0	75.0	74.5	73.0	73.5	73.0
		40	78.0	75.0	73.5	72.5	71.5	70.0	69.5
	Cow milk cheese whey powder	20	80.0	79.5	78.5	77.5	77.0	76.0	76.0
		30	80.0	78.0	76.0	75.5	74.5	74.0	73.5
		40	80.0	77.5	75.0	73.5	72.5	71.5	71.0
	Buffalo milk cheese whey powder	20	79.0	78.0	77.5	77.0	76.5	75.0	74.5
		30	79.0	77.0	75.0	74.0	73.5	73.0	73.0
		40	79.0	76.5	74.5	73.0	72.0	71.5	70.0
	Commercial whey powder (Germany)	20	78.0	77.0	76.0	76.0	75.5	75.0	75.0
		30	78.0	76.5	74.5	74.0	73.0	73.0	73.0
		40	78.0	75.0	73.5	71.5	70.0	69.5	69.0

Table 12. ANOVA for the effect of storage on the quality of whey powder

Source of variation	d.f.	'F' values			
		Colour	Solubility	Denaturation	Lysine
Replication	1	0.144^{NS}	0.914^{NS}	1.780^{NS}	0.307^{NS}
Packaging material (a)	1	0.074^{NS}	0.953^{NS}	3.888^{NS}	4.012^{NS}
Temperature of storage (b)	2	1201.407**	152.081**	28684.230**	50444.688**
Type of product (c)	3	39.484**	40.840**	5975.829**	1028.678**
Interaction					
a x b	2	0.207^{NS}	1.106^{NS}	1.313^{NS}	0.090^{NS}
a x c	3	0.233^{NS}	1.071^{NS}	0.920^{NS}	0.526^{NS}
b x c	6	7.847**	10.197**	1759.318**	76.114**
All effect interaction	6	0.200^{NS}	0.960^{NS}	1.285^{NS}	0.329^{NS}
Error	23	-	-	-	-

NS = Non-significant

** Significant at 1 per cent level ($P \leq 0.01$)

indicating that type of packaging material had no significant effect on the intensity of browning. Whereas, these values for 20, 30 and 40°C storage temperature were 0.6161, 0.9161 and 1.4152, respectively, demonstrating an increase in the intensity of browning with an increase in storage temperature. The ANOVA table 12 also revealed that there is significant effect ($P \leq 0.01$) of storage temperature on the rate of change in the intensity of browning whereas the effect of packaging material was observed to be non-significant.

Non-enzymatic browning via the Maillard reaction is one of the important mode of deterioration in whey powders which limit shelf-life (Saltmarch et al., 1981). As observed from the above result, browning was evident in all the four types of whey powders which could be ascribed to the Maillard reaction. The whey powders possessed relatively high amounts of lactose and lysine. In the presence of moisture, these components readily participate in the Maillard reaction. This interaction may result in a decrease in protein quality which is accompanied by undesirable colour changes (Ledl and Schleicher, 1990). In the final stages of the Maillard reaction, condensation and polymerization lead to formation of insoluble melanoidins and a red brown or dark brown colour contributing to the decrease in the reflectance values (Li-chan, 1983) during storage period. However, the intensity of decrease in reflectance values or increase in the degree of browning was higher at 40°C than at 20° and 30°C, which could be attributed to the faster rate of browning reaction at higher temperatures. It was observed by many workers that higher the storage temperature greater is the Maillard reaction and the resultant change in the intensity of colour (Huss, 1971; Holsinger et al., 1973; Labuja and Saltmarch, 1981; Hsu and Fennema , 1989).

It can be inferred from the storage studies of whey powders that it is necessary to store whey powder below 20°C. With regard to packaging material, either MPP or PEP could be used. Taking economic aspects into consideration, polyethylene could be used for storage of powder without affecting the quality of the whey powders.

5.2.13 ENERGY REQUIREMENT FOR CONCENTRATION OF WHEY BY REVERSE OSMOSIS AND CONVENTIONAL EVAPORATOR

Substantial quantities of water have to be removed from whey for production of whey powder. Whey is generally concentrated to 50 per cent TS before spray drying. Removal of water from whey is an energy intensive process due to its low dry matter content. Evaporation of water under vacuum requires energy several orders higher than RO process. Though RO process cannot be a complete replacement for the conventional vacuum evaporator, about 50 to 60 per cent of water can be economically removed by RO leading to considerable savings in energy. The energy requirements in conventional vacuum evaporator, RO and a combination of both were compared and are presented in Table 13. Detailed calculations of energy requirement are given in Annexure IV.

In the present study, energy required for concentrating 1,000 l of PW, CW and BW to 50 per cent TS by RO and conventional evaporator was estimated and compared. To calculate the energy requirement for 1,000 l of whey, following assumptions were made.

Type of whey	Optimum concentration level	Area of membrane (m^2)	Average flux rate (l/m^2h)	Actual permeation (l/h)	Time required (h)
PW	2.0 fold	5.4	35.28	190.05	3.03
CW	2.5 fold	5.4	38.25	260.55	3.30
BW	2.5 fold	5.4	39.80	214.92	3.19

Table 13. Comparative energy consumption for the concentration of whey by reverse osmosis and single effect evaporator (for 1,000 litres of whey)

Type of whey	Energy reqired* for RO process (kJ)	Energy required in single effect evaporator (for 50% TS) (kJ)	Energy required in RO + single effect evaporator (for 50% TS) (kJ)	Net energy saving (%)
Paneer whey (PW) (6.06% TS)	145440.0	3081218.4	1516258.9	50.79
Cow milk cheddar cheese whey (CW) (6.14% TS)	168000.0	3074158.1	1134559.2	63.09
Buffalo milk cheddar cheese whey (BW) (6.15% TS)	153120.0	3049411.7	1099637.3	63.99

* PW, 2-fold concentration by RO (6.06 to 12.12% TS)

CW, 2.5-fold concentration by RO (6.14 to 15.35% TS)

BW, 2.5-fold concentration by RO (6.51 to 16.27% TS)

Energy required for concentrating 1,000 l of PW by RO to 2.0 fold (6.06 to 12.12% TS) was estimated to be 145440.0 kJ. In case of CW (6.14 to 15.35% TS) and BW (6.51 to 16.27% TS) concentrating to 2.5 fold the energy requied was 168000.0 kJ and 153120.0 kJ, respectively. Similarly, the energy requirement for concentrating from their initial TS content to 50 per cent TS by single effect evaporator was observed to be 3081218.4, 3074158.1 and 3049411.7 kJ, respectively for PW, CW and BW. Whereas concentration of whey by removal of 50 to 60 per cent of water by RO as indicated above followed by single effect evaporation to a level of 50 per cent TS, the energy consumption was estimated to be 1516258.9, 1134559.2 and 1099637.3 kJ, respectively. This resulted in considerable savings in energy amounting to 50.79, 63.09 and 63.99 per cent for PW, CW and BW, respectively in comparison to the conventional process alone.

From the results it is clear that substantial savings in energy can be made by employing RO process as a partial substitute for conventional evaporation. The savings are as high as 63.99 per cent as in BW. Wright (1982) reported 45 per cent energy savings in concentrating whey to 50 per cent TS by RO and conventional vacuum evaporation combination. Substantial energy savings by incorporating RO prior to evaporator have also been reported by several workers (Pepper, 1981; Stabile, 1983; Cheryan et al., 1990).

5.3 PRODUCTION OF WHEY PROTEIN CONCENTRATE BY ULTRAFILTRATION

Process optimization was carried out for the production of WPC from BW by employing UF. The flux rate as affected by various processing parameters such as temperature of operation, pH and heat treatments of whey was studied. Retention of various constituents at different stages of UF concentration was estimated. The composition of retentate and the

quality of permeate were determined. Similarly, dried WPC prepared from BW and the commercial WPCs were characterized for their physical, chemical and functional properties and for their storage stability. The results of these experiments are reported hereunder and discussed.

5.3.1 STANDARDIZATION OF PROCESSING PARAMETERS

Ultrafiltration processing of BW was standardized with respect to temperature of operation and pretreatments of whey such as pH adjustments and heating for improving the efficiency of the process.

5.3.1.1 Temperature of operation

Clarified and pasteurized BW was concentrated by UF at 40° and 50°C. The flux obtained at various levels of volume reduction are presented in Table 14. It is evident from the result that there is a significant difference in the flux obtained at 40° and 50°C. When the temperature was raised from 40 to 50°C, there was significant increase in the flux. The initial flux was observed to be 35.07 and 54.80 $l/m^2/h$, respectively at 40 and 50°C. Whereas the average flux values appeared to be 23.79 and 34.00 $l/m^2/h$, respectively at the above temperatures. Significant improvement of flux at higher temperature of operation could be ascribed to decrease in viscosity of the fluid as well as increase in diffusivity of the constituents. It was observed by Cheryan (1986) that the diffusivity of protein increased to an average rate of 3 to 3.5 per cent per °C rise in temperature. Hence, it is beneficial to operate UF at 50°C.

5.3.1.2 Effect of pH and heat treatments

Clarified BW was adjusted to various levels of pH, namely 3.0, 4.5, 5.6, 6.4 and 7.2 and were heated individually at 60°C/30 min and

Table 14. Effect of temperature of buffalo milk cheese whey on ultrafiltration flux*

Volume reduction (%)	Flux ($l/m^2/h$)	
	Temperature of operation	
	40°C	50°C
0	35.07	54.80
10	33.10	49.30
20	31.20	44.80
30	29.40	40.20
40	27.50	36.00
50	25.60	33.70
60	23.50	31.60
70	21.10	29.50
80	16.90	25.80
90	11.80	18.00
95	6.60	10.40

* Average of three trials

80°C/15 sec. At each combination of these pretreatments UF was carried out at 50°C. The flux was measured at a regular interval of volume reduction. The permeate flux as a function of volume reduction is projected in Fig 27.

When the whey was subjected to 60°C/30 min at various pH levels, the flux rate appeared to be better at extreme pH values. At pH 3.0 and 7.2, the flux was significantly higher than at any other pH levels. The initial flux at pH 3.0, 4.5, 5.6, 6.4 and 7.2 was estimated to be 60.60, 49.60, 53.60, 55.50 and 62.30 $1/m^2/h$, respectively and these flux values as it can be observed from the figure declined to 17.10, 5.30, 6.90, 10.90 and 22.30 $1/m^2/h$ after 95 per cent volume reduction. However, the respective average flux values for 95 per cent volume reduction were 44.29, 20.88, 28.75, 34.36 and 46.88 $1/m^2/h$.

Similarly, when the whey was heated to 80°C/15 sec, the flux rate was better compared to 65°C/30 min heating. Higher flux was observed at pH values of 3.0 and 7.2. The initial flux values were found to be 62.40, 51.70, 55.60, 57.40 and 64.20 $1/m^2/h$ and these values reduced to 18.70, 6.50, 8.60, 12.50 and 22.60 $1/m^2/h$ after 95 per cent volume reduction, respectively at pH values of 3.0, 4.5, 5.6, 6.4 and 7.2. The mean flux rates for the above pH values were 48.53, 22.77, 30.50, 36.39 and 51.79 $1/m^2/h$.

The statistical analysis (Table 15) revealed that there is significant effect of pH and heat treatment ($P \leq 0.01$) on the resultant flux at all the stages of volume reduction. The effect of their interaction was also found significant ($P \leq 0.01$).

It is evident from Figs 27a and 27b that as the volume reduction increased, the flux decreased irrespective of the treatment imparted to the

FIG 27. EFFECT OF pH AND PREHEATING OF BUFFALO MILK CHEESE WHEY ON FLUX OF ULTRAFILTRATION

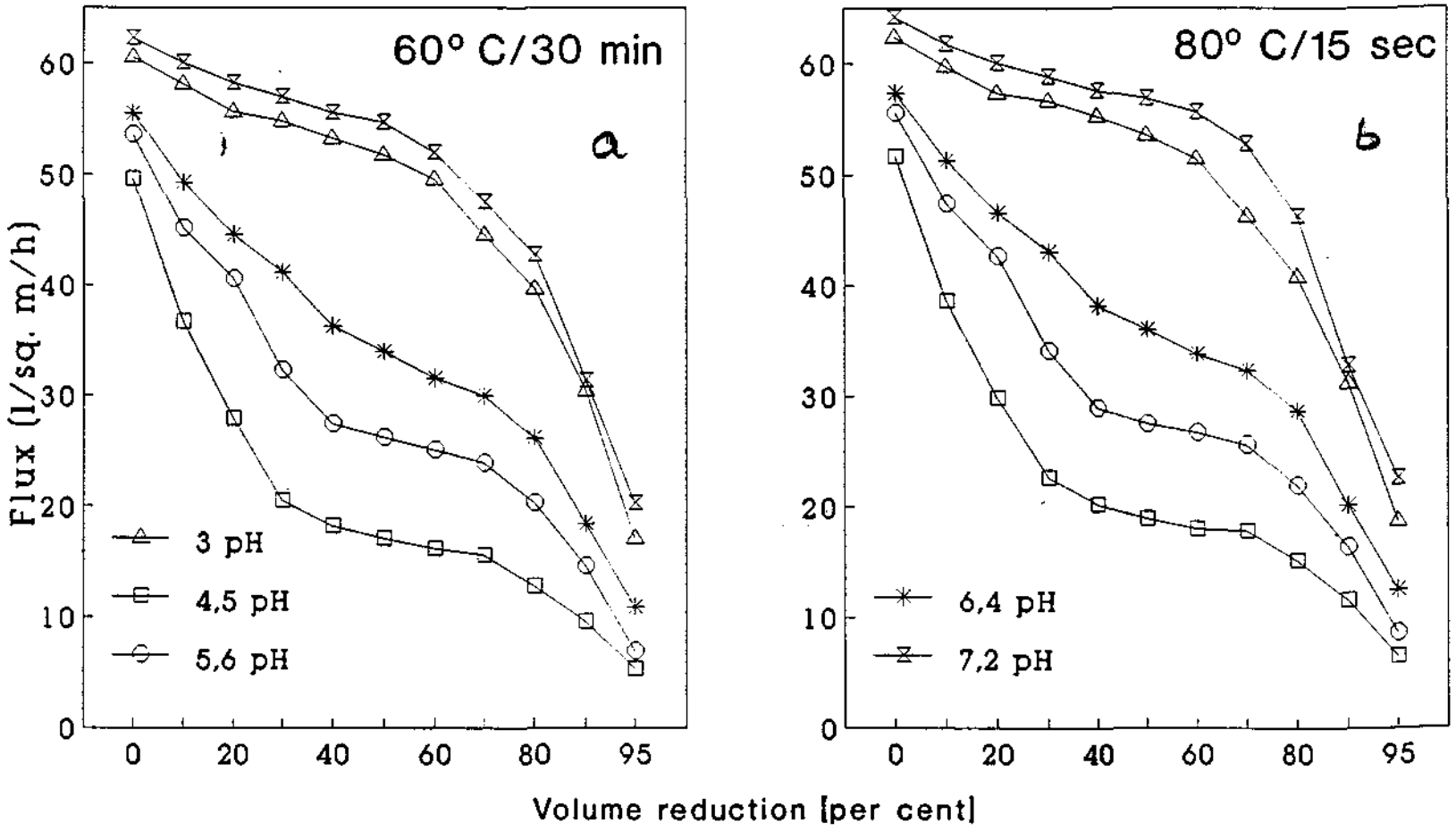


Table 15. ANOVA for the effect of pH and heat treatment of buffalo milk cheese whey on ultrafiltration flux

Source of variation	d.f.	'F' value
Replication	2	2.5162^{NS}
Temperature of heating (a)	1	5058.056**
pH levels (b)	4	153789.047**
Volume reduction (c)	10	76710.180**
Interaction		
a x b	4	28.009**
a x c	10	11.385**
b x c	40	1605.851**
All effect interaction	40	7.172**
Error	218	-

NS = Non-significant

** Significant at 1 per cent level ($P \leq 0.01$)

whey. This decrease in flux can be attributed to the added resistance from fouling and concentration polarization of the membrane surface and its pores as a result of protein and salt deposition (Kaiser and Glatz, 1988; Daufin et al., 1992). However, the rate of decline in flux varied significantly with the pH and heat treatment of whey. This can be ascribed to the fact that UF performance is strongly dependent on physico-chemical characteristics (complexation of calcium ions, pH change) which refers to the solute-solute interaction and solute-membrane interactions (Taddei et al., 1988; Daufin et al., 1992). The intensity of these interactions varies with the kind of pretreatment imparted to whey. Hence, the flux varied widely with the treatments. The change in pH and heat treatment affects the status of calcium salts and the configuration of protein and hence the flux. Several studies reinforce the hypothesis that poorly soluble calcium salts and the adsorption of protein on the surface of the membrane can contribute to fouling or decrease in flux furing UF (Cheryan and Merin, 1981; Taddei et al., 1988).

When BW was processed at pH 7.2 or pH 3.0, the flux rate was observed to be higher than at normal pH. At pH 4.5, the net charge on the proteins is low and hence dispersion of protein is poor. They get adsorbed on the surface of the membrane, forming gel layer which results in the lowest flux rate. As the pH is lowered from 4.5, the dispersion of proteins improves, calcium gets solubilized and pass through the membrane without much fouling (Patocka and Jelen, 1987a) increasing the flux rate significantly. The same is the case when pH is raised from 4.5 to 7.2, but the increase in flux is attributable to other reasons. At pH 5.6 for example, the flux improved but still is at a lower side, probably because of certain deposition of calcium phosphate as amorphous tricalcium phosphate and

adsorption of beta-lactoglobulin as the pH is near to its isoelectric point (Hiddink et al., 1981). It is surprising to note a drastic increase in the flux at pH 7.2. This pronounced increase in the permeate flux may be due to heating of whey at elevated pH. Under such conditions, a considerable precipitation of calcium phosphate is expected in such a form (probably as hydroxy apatitie) that, results in reduced fouling of membrane. Hayes et al. (1974) observed that addition of calcium to whey and adjusting pH above 6.5 and heating, increased the flux. As the calcium content of BW is higher, heating at pH 7.2 probably resulted in calcium induced protein interaction which aided in larger aggregate or apatite formation, which were found to be non-fouling.

Higher temperature of heating 80°C/15 sec had given better flux than 60°C/30 min. Probably higher temperatures are required for the apatite formation or for the calcium induced beta-lactoglobulin self aggregation or for the protein-protein interactions. Muller and Harper (1979) also observed an increase in flux by 50 per cent when cheese whey was heated to 80°C/15 sec instead of pasteurization temperature.

From the results and the statistical analysis, it can be concluded that maximum flux during UF processing of BW could be attained when pH of whey is adjusted to 7.2 followed by 80°C/15 sec heating.

5.3.2 COMPOSITIONAL CHANGES DURING ULTRAFILTRATION OF BUFFALO MILK CHEDDAR CHEESE WHEY

Preliminary trials have shown that it is possible to obtain a maximum of 26.5 per cent solids (97.5% volume reduction) from BW by fractionating through UF. However, due to low flux rate caused by high viscosity, the concentration was restricted to 95 per cent volume reduction (21.60% TS).

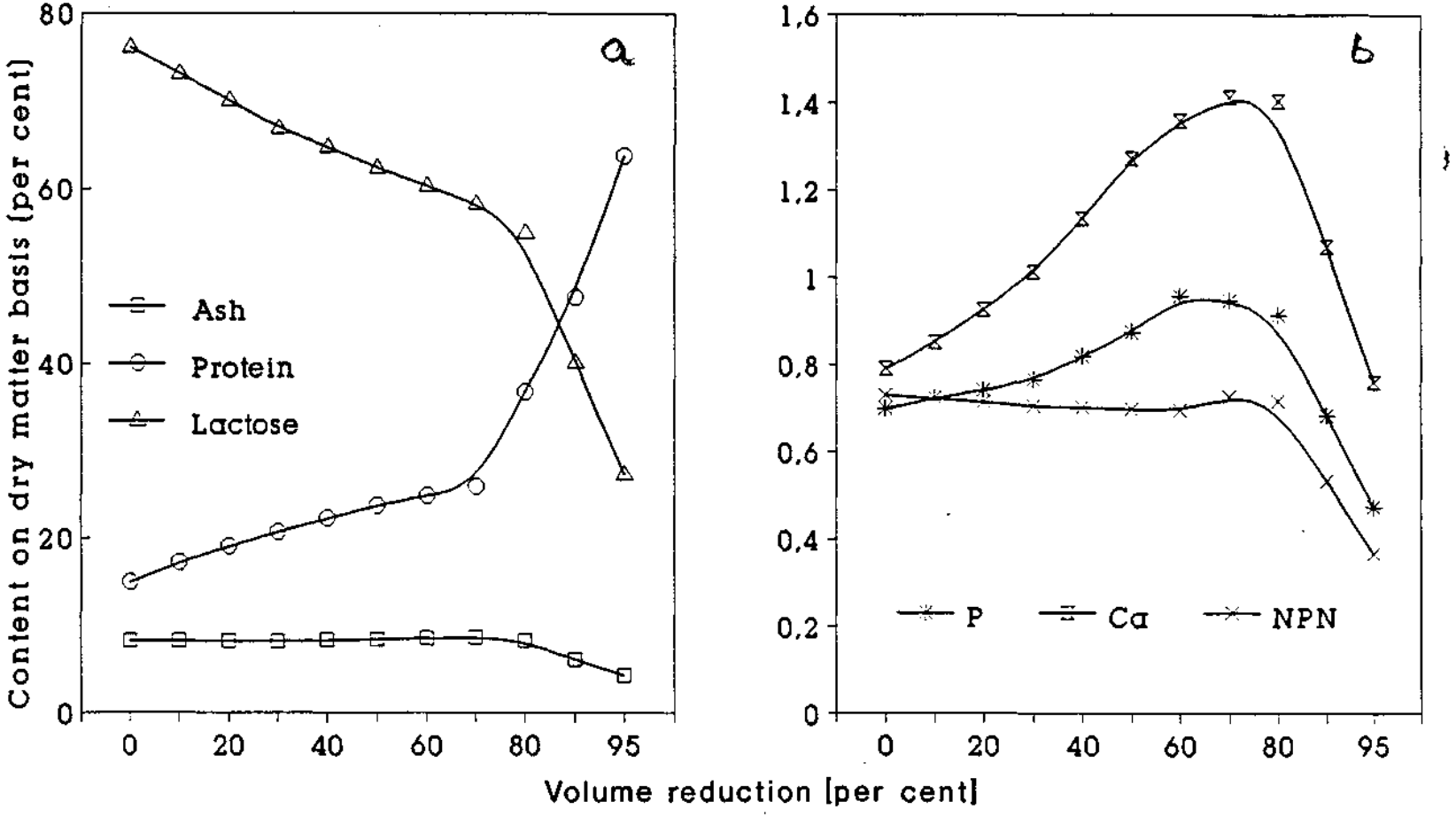
The changes occurring during concentration with respect to total solids, protein, NPN, fat, lactose, ash, calcium and phosphorus were estimated at a regular interval of volume reduction and results expressed on DM basis are displayed in Figs 28a and 28b.

The increase in TS of retentate was found to be gradual till 80 per cent volume reduction. Thereafter, there was sharp increase in the TS content of the retentate. The TS of retentate at 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction was observed to be 6.58, 8.10, 9.04, 9.92, 10.62, 14.66 and 21.60 per cent, respectively.

The changes in protein content as a function of volume reduction during UF of BW is illustrated in Fig 28a. At the initial stages, as the volume reduction increased, the extent of of increase in protein was found to be very small. However, as the concentration progresses, after certain degree of volume reduction, the increase in protein content was found to be rapid. The protein content of retentate on DM basis at 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction was 14.98, 20.61, 23.67, 25.90, 36.72, 47.61 and 63.79 per cent, respectively. Thus, it is possible to obtain desired levels of protein in the end product depending on the degree of volume reduction. For example, by 80 per cent volume reduction, WPC possesses protein content (on DM basis) equivalent to that of skim milk powder.

The changes with respect to lactose were quite opposite to that of the changes in protein. The lactose content of retentate was observed to be almost uniform throughout the processing. The retentate had a lactose content of 5.01, 5.42, 5.64, 5.79, 5.84, 5.88 and 5.95 per cent at 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction. However, it is interesting to note that the lactose content of retentate on DM basis decreased in the order of 76.13, 66.91, 62.38, 58.36, 54.99, 41.10 and

FIG 28. COMPOSITIONAL CHANGES DURING ULTRAFILTRATION OF BUFFALO MILK CHEESE WHEY



27.54 per cent. The lactose content reduced from an initial value of 76.13 per cent to 27.54 per cent after 95 per cent volume reduction. From the results it is clear that varying degree of lactose can be obtained in WPCs by monitoring volume reduction or concentration.

It can be seen from Fig 28a that the changes in ash content during UF of BW have followed a different pattern than those in protein and lactose. Gradual increase in the ash content of the retentate was observed as the concentration progressed. However, on DM basis the increase was found to be uniform until 70 per cent volume reduction. Thereafter, there was decrease in the ash content. At 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction, the ash content of the retentate was 8.20, 8.14, 8.40, 8.66, 8.28, 7.13 and 6.23 per cent (on DM basis), respectively.

The calcium, phosphorus and NPN contents gradually increased upto a level of 70 per cent volume reduction which declined (on DM basis) to a great extent (Fig 28b).

As can be observed from the figure, it is clear that NPN content during UF of BW increased from an initial content of 0.048 to 0.079 per cent after 95 per cent volume reduction. However, at 0, 30, 50, 70, 80, 90 and 95 per cent, the corresponding NPN on DM basis was estimated to be 0.72, 0.70, 0.69, 0.72, 0.71, 0.53 and 0.36 per cent.

The initial calcium content of BW was 0.052 per cent, which had increased to 0.164 per cent during UF after 95 per cent volume reduction of BW. The corresponding calcium content on DM for 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction was observed to be 0.79, 1.01, 1.27, 1.41, 1.40, 1.07 and 0.76 per cent. It appears that there was an increase in retention of calcium upto 70 per cent volume reduction and thereafter a decreasing trend was observed in the calcium content of the retentate.

Changes in phosphorus content followed similar pattern as that of calcium. Phosphorus content increased from an initial value of 0.046 per cent to 0.102 per cent. The retentate had 0.69, 0.77, 0.87, 0.94, 0.91, 0.68 and 0.47 per cent phosphorus (on DM basis), respectively at 0, 30, 50, 70, 80, 90 and 95 per cent volume reduction.

From the results it was observed that at the beginning increase in TS content during UF of BW was slow. However, at later stages the increase was found to be rapid. Similar observations were made by Gupta and Reuter (1987) during processing of cow milk cheese whey by UF. They attained TS content 15, 20 and 25 per cent at about 92.5, 95.35 and 96.80 per cent volume reduction, whereas in our study at 95 per cent volume reduction the TS obtained was 21.60 per cent. On the other hand, Cheryan (1986) observed a TS content of 17-18 per cent for a volume reduction of 95 per cent.

The compositional changes during UF of BW have followed the similar pattern as reported by other workers for cheese whey. Marshall and Harper (1988) have observed increase of protein content from 12 to 66 per cent (on DM basis), decrease in lactose content from 79 to 28 per cent, a smaller reduction in ash content from 9 to 6 per cent during UF of cheese whey. In our experiments with BW, increase of protein content from 14.98 to 63.79, decrease in lactose and ash content from 76.13 to 27.54 and 8.20 to 4.23 per cent were observed, respectively. These changes with respect to protein, lactose and ash are also in agreement with the findings of Cheryan (1986) and Gupta and Reuter (1987). During UF of whey, they have reported that as the concentration or volume production progressed, the protein content increased and lactose and ash content decreased. The increase in protein content was found to be very slow at the beginning stages of volume reduction and later on there was rapid increase in protein

content. Whereas rate of decrease of lactose was slow at the beginning and was rapid at later stages of concentration. Similarly, the ash content slighly increased at the initial stages of volume reduction, whereas it decreased later on.

As the UF membranes are permeable to lactose and minerals, the decrease in ash and lactose contents during UF of whey was evident. At later stages as there will be build up of internal pressure due to higher solids, there will be more permeation of solutes. Hence, at later stages the rate of decrease in lactose and ash content was higher. The rapid increase in protein content at later stages of volume reduction can be mainly ascribed to the continuous removal of water and solutes per unit mass of the retentate.

Zall (1982) observed that during UF of whey, when a level of 10 per cent TS was attained the solids composition on DM basis was 34 per cent protein, 54 per cent lactose and 9 per cent ash, which was approximately equivalent to the composition of skim milk powder. Whereas in our studies at 10.62 per cent TS of retentate, the protein, lactose and ash content on DM basis were observed to be 36.72, 54.99 and 8.28 per cent, respectively. From this, it is evident that concentration of BW to 80 per cent volume reduction results in WPC having similar composition of skim milk powder (on DM basis).

As can be seen from Fig 28, it can be seen that it is possible to obtain a product having varying degree of protein/TS ratio by controlling the volume reduction. Higher the volume reduction, higher is the protein to TS ratio. Marshall and Harper (1988) also reported that quantity of permeate removed controls the protein/TS ratios of the final retentate. According to them, normally the ratios are limited to 0.65:1 because the viscosity in the later stages limits the flux to too low

value. In our experiments, the protein content at 95 per cent volume reduction was found to be 13.65 for a TS content of 21.60. The protein/TS ratio attained was 0.63:1 as against 0.65:1 reported by Marshall and Harper (1988).

Results also indicated that the increase in volume reduction resulted in decrease of NPN compounds as these low molecular weight compounds are easily permeable through UF membrane. The ratio of NPN/true protein decreased as the concentration factor increased. Similar results were obtained by Rommel (1983) who observed that NPN/true protein ratio decreased from 0.46 in whey to 0.19 in UF retentate at a concentration factor of 16 and 0.14 at a concentration factor of 30. It is reported (Renner and Abd El-Salam, 1991) that as the concentration of whey by UF increased, the per cent of NPN to the total nitrogen decreased. They observed that the percentage of NPN in total nitrogen is about 10 per cent in WPC containing 40 per cent protein, which decreased to about 6 per cent when protein content of WPC increased to 70 per cent.

Similarly, calcium and phosphorus content on DM basis increased with the increase in concentration until a certain degree of volume reduction, thereafter a decreasing trend was observed. This can be ascribed to the fact that as the solubility of calcium and phosphorus is low, higher proportion of them would be retained. However, as the UF progresses, there will be build up of internal pressure which aids in driving out more and more of calcium and phosphorus along with the permeate. Hence, at later stages of volume reduction the retention was minimal. These results are in confirmation with the observations of several other workers (Hiddink et al., 1981; Gupta and Reuter, 1987).

5.3.3 COMPOSITIONAL CHANGES OF BUFFALO MILK CHEDDAR CHEESE WHEY DURING ULTRAFILTRATION AND DIAFILTRATION

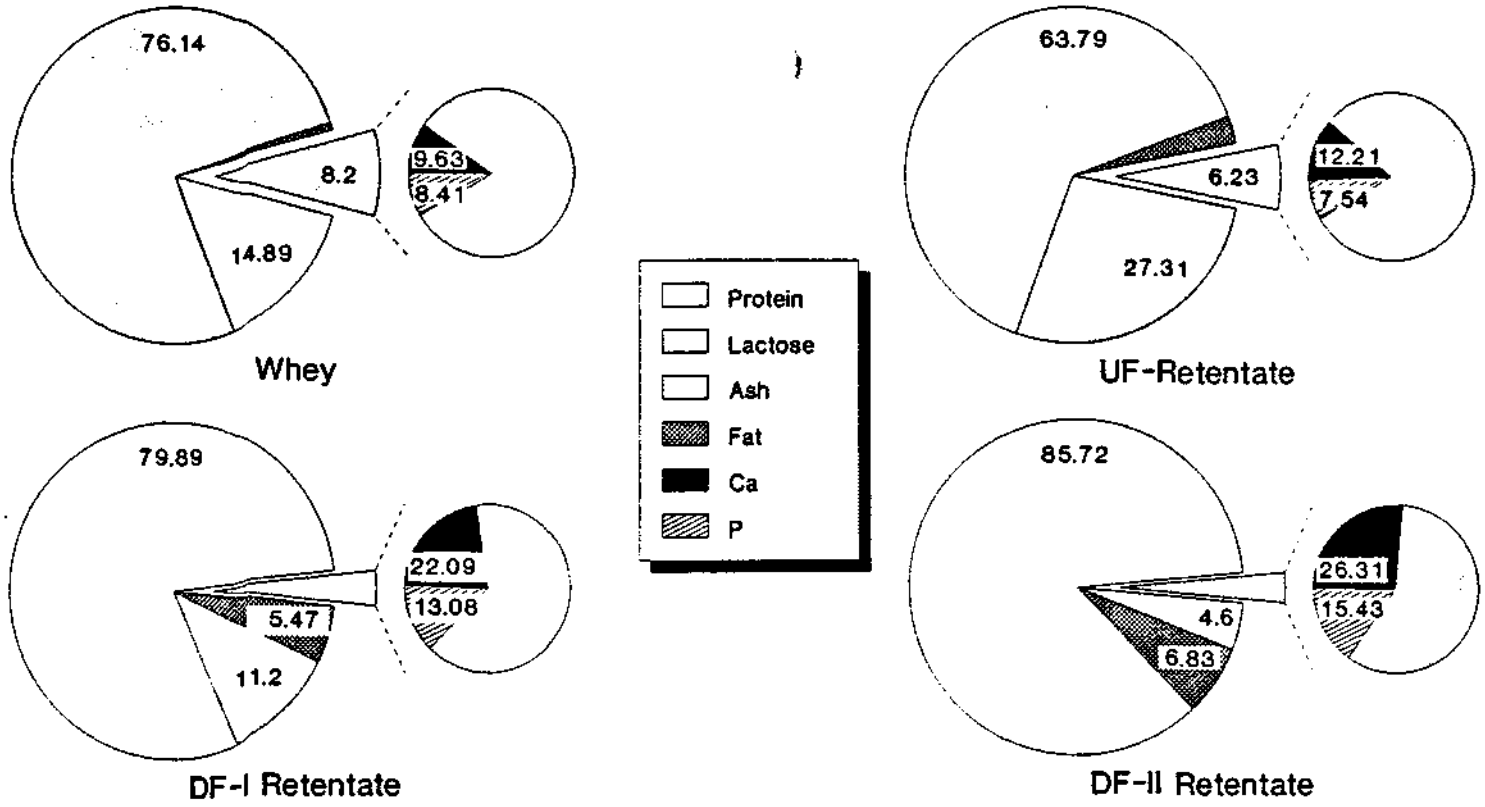
Whey protein concentrates with different protein contents for various end uses are available in the market abroad. The protein contents in them vary from 35 to 80 per cent. By UF process, the maximum protein in WPC obtainable is generally 65 per cent. Higher protein contents can be obtained by diafiltering the UF retentate.

In this study, an attempt was made to check the possibility of getting high protein values in buffalo milk WPC by diafiltration (DF) process. Fractionation of BW by UF was carried out to a level of 95 per cent volume reduction. Further, DF was performed at two stages. The changes occurred with respect to relative composition of protein and other constituents after UF and two stage DF are depicted in Fig 29.

By UF of BW to 95 per cent volume reduction, the protein content of retentate (on DM basis) increased to 63.79 per cent from an initial value of 14.89 per cent. It increased further to 79.89 per cent after first stage DF and to 85.92 per cent after second stage DF. Similarly, the content of other components at the end of UF, first stage DF and second stage DF respectively were 27.31, 11.2 and 4.6 per cent for lactose, 6.23, 3.44 and 2.85 per cent for ash and 2.67, 5.47 and 6.83 per cent for fat. Whereas the corresponding percentages of calcium and phosphorus content in the total ash were 12.21 and 7.54, 22.09 and 13.08, and 26.31 and 15.43.

Buffalo milk cheddar cheese whey had protein, lactose and ash in the ratio of 1:5.11:0.550, which on UF changed to 1:0.43:0.097. This ratio was altered to 1:0.14:0.043 by first DF and subsequently to 1:0.05: 0.033 by second DF. Whereas the protein to NPN ratio was found to be 1:0.048, 1:0.015, 1:0.100 and 1:0.008 in the initial BW, UF retentate, first DF retentate and second DF retentate, respectively.

FIG 29. COMPOSITIONAL CHANGES DURING ULTRAFILTRATION AND DIAFILTRATION OF BUFFALO MILK CHEESE WHEY



These results indicate that it is possible to attain as high as 85.95 per cent protein by UF of BW followed by two stage DF. The lactose content reduced to as low as 4.60 per cent and ash to 2.85 per cent. These observations are in close agreement with the work of Tratnik and Krsev (1991). They have obtained a maximum of 83.20 per cent protein on DM by UF of whey followed by two stage DF. By employing this process, lactose content was reduced to a minimum level of 4.40 per cent on DM basis.

The increase in protein and decrease in lactose, ash and NPN content during UF and DF could be attributed to the addition of water to the UF retentate, which aids in reducing the viscosity and, thereby, more of lactose, minerals and low molecular weight nitrogenous compounds passes alongwith permeate (Zall, 1982; Vuillemard et al., 1989), which helps in increasing the concentration of the protein to the unit mass by reducing the contents of other constituents.

During DF process it was observed that retention of calcium and phosphorus was high, whereas total ash retention decreased to a great extent. This could be ascribed to the fact that repeated DF of whey results in removal of potassium, sodium and magnesium, to a greater extent and phosphorus to a lesser extent (Tratnik and Krsev, 1991). Retention of calcium was high during DF process of BW. Similar results showing the highest retention of calcium during DF and its dependence on the pH of whey (Derham and Chanton, 1986; deWit et al., 1986) have been reported by many workers.

It is evident from the results that a maximum protein content of 63.59 per cent on DM basis could be attained by UF of BW. At this level, protein, lactose and ash ratio was found to be 1:0.43:0.097. At 60 per cent

protein level, several workers have reported a ratio of 1:0.38:0.070. In our study at 79.89 per cent protein level the above ratio changed to 1:0.14:0.043. Similarly, at 85.79 per cent protein level the ratio was observed to be 1:0.05:0.033. These results are in close agreement with the observations of several workers where they have reported 1:0.15:0.040 and 1:0.04:0.040, respectively for 75 and 85 per cent protein (Muir and Banks, 1985; Schmidt et al., 1986; Zadow, 1986; Marshall and Harper, 1988).

From the results it can be concluded that depending on the degree of UF and on the steps of DF applied, WPCs can be produced with enormously varying composition depending on the end use requirements.

5.3.4 COMPOSITION OF ULTRAFILTRATION PERMEATE OF BUFFALO MILK CHEDDAR CHEESE WHEY

The UF permeate contains mainly lactose and ash apart from NPN, water soluble vitamins and traces of protein. The composition of permeate varies with the level of volume reduction and the loss of these components in the permeate depends not only on membrane characteristics but also on pH of the whey. The composition of the pooled permeate of BW after 95 per cent volume reduction was determined and have been reported in Table 16.

The pH was found to have little effect on TS, protein, NPN and lactose contents, but significantly affected calcium and phosphorus contents. The values at pH 3.0, 4.5, 5.6, 6.4 and 7.2 were 5.78, 5.75, 5.70, 5.68 and 5.65 for TS, 0.21, 0.19, 0.18, 0.22 and 0.23 for protein, 0.023, 0.021, 0.020, 0.023 and 0.024 for NPN and 4.98, 5.02, 5.02, 4.98 and 4.99 for lactose. The TS content decreased with decreasing pH of whey but the trend for protein, NPN and lactose was varying.

Wide variation in the total ash content of permeate was observed when BW was processed at different pH values. Ash content of permeate

Table 16. Effect of pH of buffalo milk cheese whey on the composition of ultrafiltration permeate

pH levels	Components (per cent)						
	Total solids	Protein	NPN	Lactose	Ash	Calcium	Phosphorus
3.0	5.78	0.21	0.023	4.98	0.59	0.058	0.045
4.5	5.75	0.19	0.021	5.02	0.54	0.052	0.042
5.6	5.70	0.18	0.020	5.02	0.50	0.048	0.040
6.4	5.68	0.22	0.023	4.98	0.47	0.045	0.038
7.2	5.65	0.23	0.024	4.99	0.45	0.042	0.035

varied from 0.45 to 0.59 per cent. Highest ash content was observed at pH 3.0 (0.59%) and the lowest was at pH 7.2 (0.45%). At other pH values of 4.5, 5.6 and 6.4, the permeate had 0.54, 0.50 and 0.47 per cent ash, respectively.

Calcium content of the permeate followed a pattern similar to that of ash. With decrease in pH from 7.2 to 3.0, there was an increase in the calcium content of the permeate. The permeate had calcium content of 0.058, 0.052, 0.048, 0.045 and 0.042 per cent at pH values of 3.0, 4.5, 5.6, 6.4 and 7.2.

As for changes in phosphorus, its content decreased from 0.045 to 0.035 per cent as the pH was increased from 3.0 to 7.2. The phosphorus content of BW at pH 3.0, 4.5, 5.6, 6.4 and 7.2 was found to be 0.045, 0.042, 0.040, 0.038 and 0.035 per cent, respectively.

The differences in the composition of permeate can be ascribed to the dissimilarities in the solubility of minerals with the change in pH, and the resultant effect on the dispersion or the electrostatic charges on the protein. Lower the pH higher is the solubility of minerals (Patocka and Jelen, 1987a; Renner and Abd El-Salam, 1991) especially calcium phosphate. Hence, these soluble calcium phosphate pass through the permeate, thus, increase the ash, calcium and phosphorus contents of the permeate. These results are in agreement with the results of Hiddink _et al_. (1979) and Taddei _et al_. (1988). For the same reason, an increase in TS content was observed at pH 3.0 than at any other pH. At pH 7.2 when BW was heated, calcium phosphate probably got precipitated and induced calcium-bound protein-protein interaction, thus, the permeation of solute was minimal. Therefore, the permeate was found to contain less TS at this pH than at any other pH. Whereas at pH 4.5 and 5.6 slightly less TS was observed than at pH 3.0. This can be explained by poor dispersion of protein at

these pH values which resulted membrane fouling and formation of secondary membrane. This might have blocked the permeation of protein or reduced the permeation of NPN (Breslau and Kilcullen, 1977). Higher content of protein at pH 6.4 and 7.2 can be reasoned by lesser deposit on the membrane which may aid in the permeation of protein into the permeate. Protein retention is dependent on pH and is the lowest at pH 3.0, a similar effect was observed by Breslau and Kilcullen (1977) and Hiddink et al. (1981).

5.3.5 MANUFACTURE OF SPRAY DRIED WHEY PROTEIN CONCENTRATE

As shown by the results, it is possible to attain differential degree of composition and protein content in WPC depending on the requirement by controlling the degree of concentration or the volume reduction. Manufacture of WPC involves UF of whey to a required degree of concentration followed by vacuum evaporation and drying or directly spray drying after UF concentration of whey.

Six hundred litres of clarified buffalo milk cheddar cheese whey was adjusted to a pH of 7.2 and subjected to a heat treatment of 80°C/15 sec and cooled to 50°C. UF of pretreated BW was carried out at a temperature of 50°C to a level of 85 per cent volume reduction. The retentate so obtained was directly spray dried at 180°C inlet and 80°C outlet temperatures with a atomizer speed of 25,000 rpm by using Anhydro Denmark (35 kg evaporation components) spray drier. Spray dried WPC was cooled to room temperature packed in polyethylene and metallized polyester packaging materials.

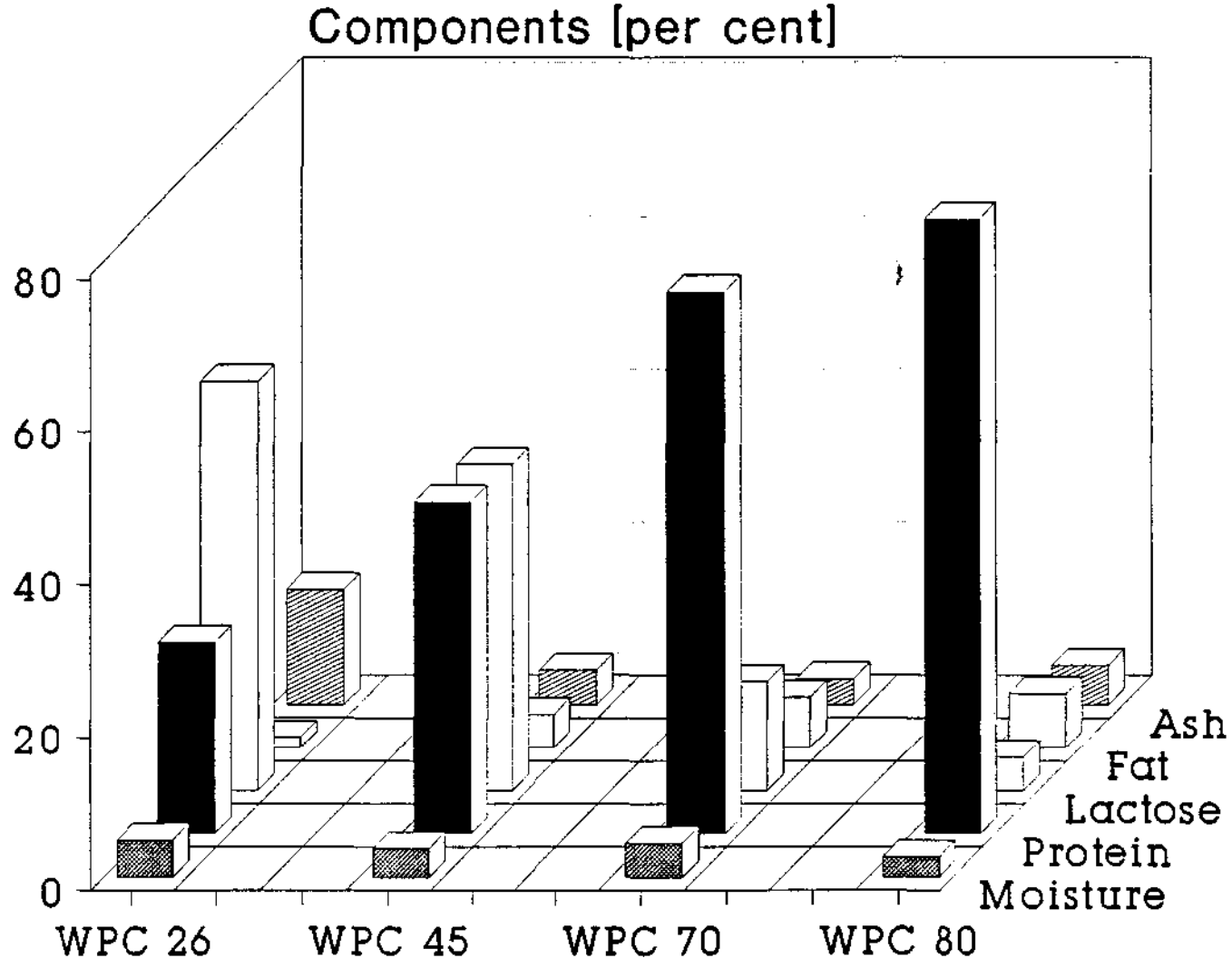
5.3.5.1 Chemical composition of dried whey protein concentrates

Spray dried WPC prepared from BW (WPC 45) and commercial whey protein concentrate (WPC 26, 70 and 80) were analysed for their chemical composition.

The major components such as moisture, protein, fat, lactose and ash contents of the samples are diagramatically projected in Fig 30. It can be seen from the figure that the composition of WPCs with respect to various components varied with the protein content of the samples. The samples WPC 26, WPC 45, WPC 70 and WPC 80 had protein content of 25.11, 43.46, 71.16 and 80.60 per cent. The lactose content of these samples was 53.64, 42.76, 14.37 and 4.45 per cent, respectively. Similarly, in contrast to lactose, the fat contents of these products were found to increase as the protein content increased. The respective fat content of WPC 26, WPC 45, WPC 70 and WPC 80 was 1.42, 4.45, 6.70 and 7.17 per cent. Slight variation in the moisture content of the dried WPCs was observed. The samples WPC 26, WPC 45, WPC 70 and WPC 80 had moisture content of 4.80, 3.74, 4.41 and 3.62 per cent, respectively. Wide variation in the ash content of the samples was observed. The respective ash content of the above samples was found to be 15.03, 5.58, 3.36 and 4.61 per cent.

Slight variation in the moisture content of dried WPCs could be ascribed to the variation in spray drying temperature, type of spray drier employed, variation in the degree of concentration of UF retentate used for spray drying. However, all the samples were observed to contain moisture below 5 per cent. The protein, fat and lactose contents are interdependent. It was observed that higher the protein content, higher was the fat content, since the fat, being impermeable, also gets concentrated during the operation. Similar reports are reported by earlier workers (Morr, 1979; Kinsella, 1985). As the protein content in retentate increased, lactose, which is lost through permeate, decreased. The commercial product (WPC 80) having 80.60 per cent protein contained as low as 4.45 per cent lactose. These results are in confirmation with the results of several other workers (Hugunin, 1987; Marshall and Harper,

FIG 30. CHEMICAL COMPOSITION OF WHEY PROTEIN CONCENTRATE



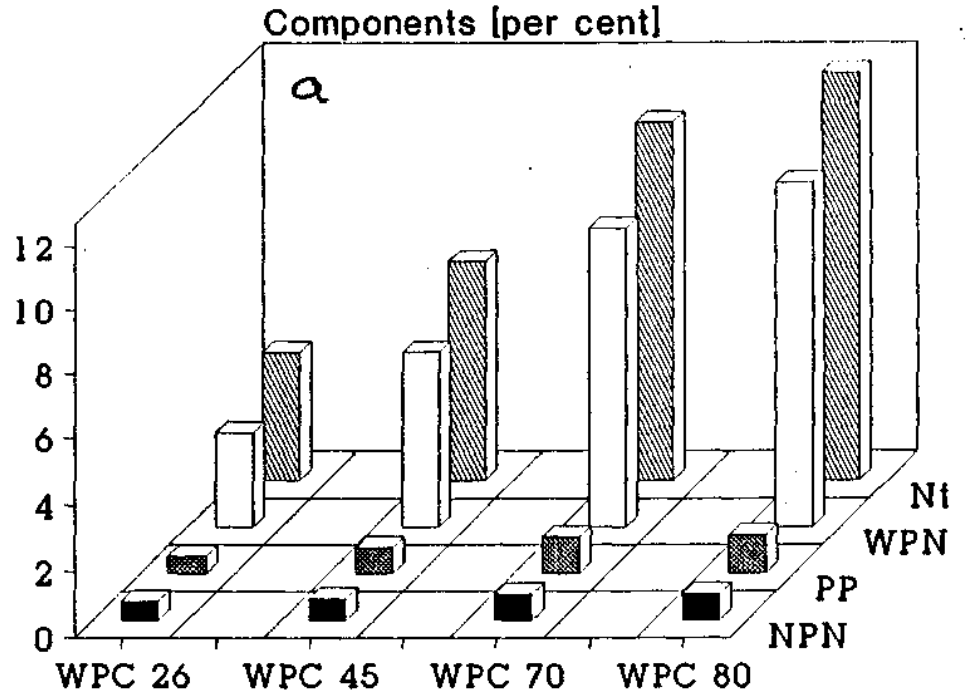
Note : WPC 45 is from Buffalo milk cheddar cheese whey. The rest are from Dairy Industry, Germany.

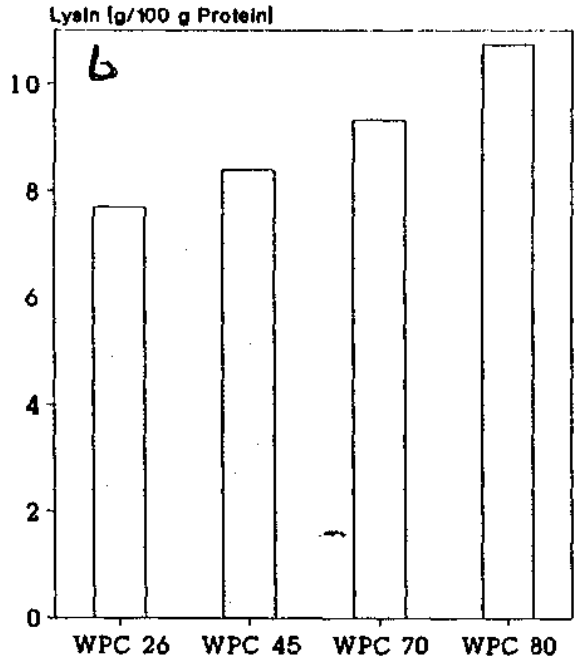
1988; Daufin et al., 1992). Generally, it is presumed that as the protein content increases or the higher the volume reduction lower should be the ash content. However, WPC 70 had shown lower ash content (3.36%) than the WPC 80 (4.16%). Probably during WPC 70 production, the UF might have been carried out at lower pH values as shown by a low pH of 3.20 as against pH 6.30 for WPC powder. It has been observed by many workers that UF at lower pH values results in removal of more minerals through permeate thus considerably decreasing the ash content of WPCs. Ash content of WPCs also depends on the initial load of minerals in whey, preheat treatment imparted, pH, type of whey and the neutralizers used. Hence, wide variations in the ash contents were observed.

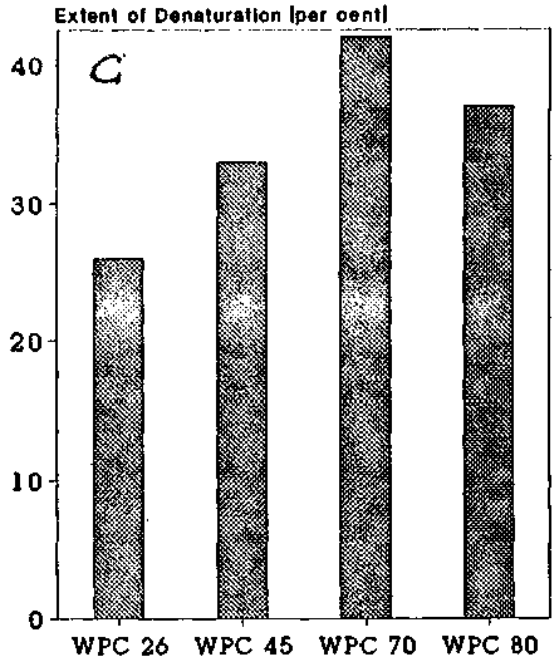
5.3.5.1.1 Protein quality of whey protein concentrate

Total nitrogen NPN, PP and WPN contents of four samples of WPCs are projected in Fig. 31a. It was interesting to observe that as the protein content of samples increased, there was substantial change in the ratio of NPN, PP and WPN. Higher the NT content, lower was the proportion of NPN and PP. It was observed that WPC 26 had 14.73 per cent NPN, 13.40 per cent PP and 71.87 per cent WPN, whereas WPC 45 had 9.70 per cent NPN, 11.60 per cent PP and 78.70 per cent WPN. Similarly, WPC 70 and WPC 80 had 7.10, 9.95 and 82.95 per cent and 6.25, 9.20 and 84.55 per cent NPN, PP and WPN, respectively. These changes can be ascribed to the fact that UF membranes are highly permeable to NPN and PP. Hence, as the concentration increases, more and more of NPN and PP gets permeated and WPN gets concentrated in the retentate. Hence, the nitrogen distribution changes with the degree of UF concentration. These results are in agreement with the results of Matthews et al. (1976).

FIG 31. PROTEIN QUALITY OF WHEY PROTEIN CONCENTRATE







Note : WPC 45 is from Buffalo milk cheddar cheese whey
The rest are from Dairy Industry, Germany.

(a) Lysine content

Lysine content of WPCs are expressed in g/100 g total protein and is delineated in Fig 31b. It was observed that higher the content of protein, higher was the lysine content. The samples were found to have 7.70, 8.40, 9.33 and 10.72 g/100 g protein respectively for WPC 26, WPC 45, WPC 70 and WPC 80 products. The increase in lysine content with the change in total protein content could be attributed to the permeation of NPN compounds during UF. As the UF concentration progresses, more and more of NPN is removed which results in higher proportion of WPN to NT, thus higher lysine content was observed.

(b) Extent of denaturation

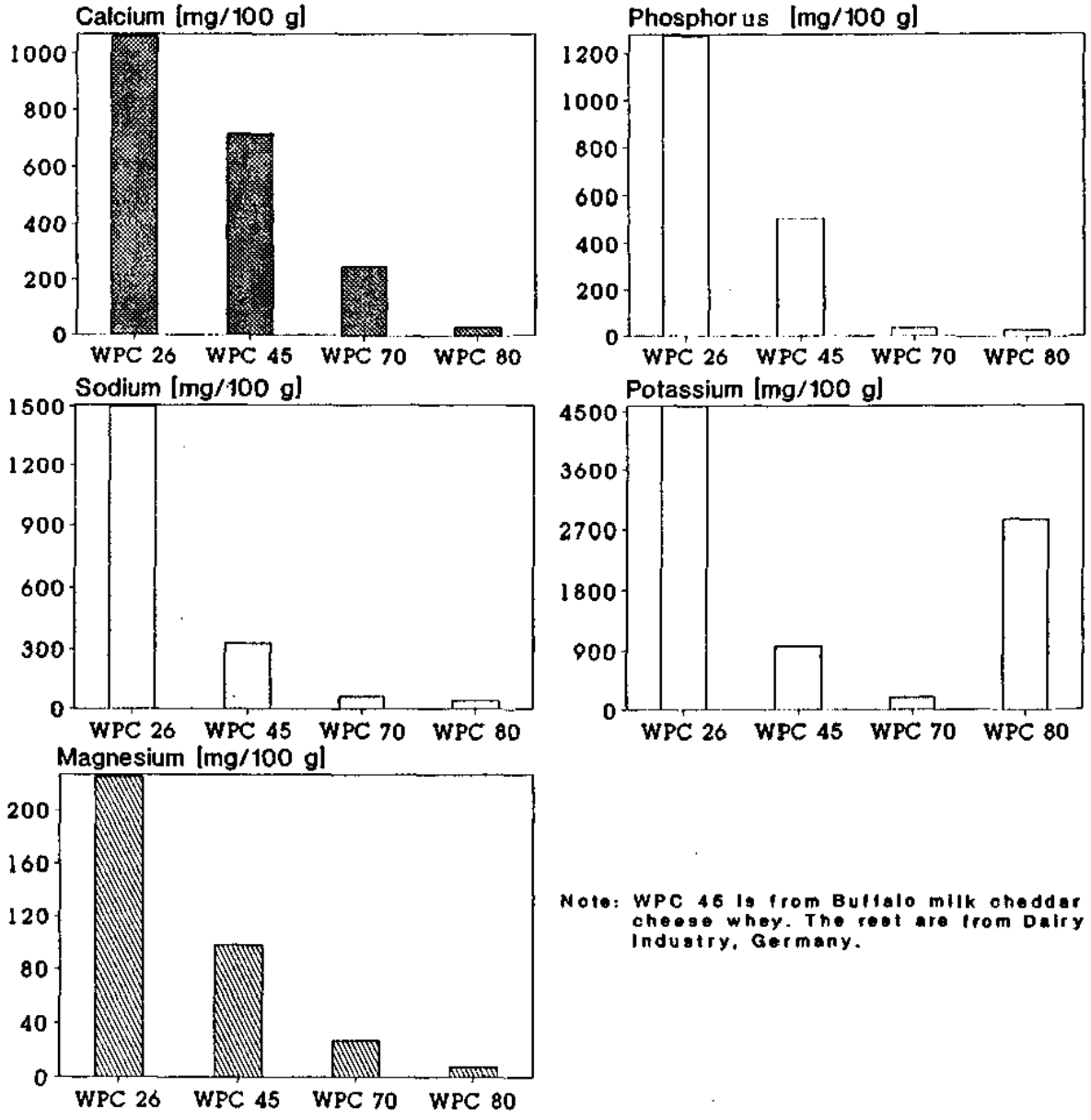
The extent of denaturation in various samples of WPCs are presented in Fig. 31c. It can be seen from the figure that denaturation varied from 26 to 42 per cent. The denaturation was found to be 26, 33, 42 and 37 per cent, respectively for WPC 26, WPC 45, WPC 70 and WPC 80. The denaturation depends on the pH of the concentrate, salt balance and also on the electrostatic charges on the protein. As the sources of these WPCs vary and their relative composition and pH differ widely, variation in the extent of denaturation is also observed.

5.3.5.1.2 Mineral profile of whey protein concentrate

Whey protein concentrates were analysed for their mineral profile. The presence of minerals such as calcium, phosphorus, potassium, magnesium and sodium are summarized in Fig 32.

It was estimated that WPC 26 had 1062, 1273, 4588, 1501 and 226 mg/100 g of calcium, phosphorus, potassium, sodium and magnesium, whereas WPC 45 was found to contain 715, 504, 965, 332 and 98 mg/100 g,WPC 70

FIG 32. MINERAL COMPOSITION OF WHEY PROTEIN CONCENTRATES



Note: WPC 46 is from Buffalo milk cheddar cheese whey. The rest are from Dairy Industry, Germany.

250, 34.74, 190, 63.75 and 27.18 mg/100 g and WPC 8033.43, 27.45, 2858, 41.25 and 7.50 mg/100 g of calcium, phosphorus, potassium, sodium and magnesium respectively.

The WPC 26 had all the minerals in higher proprotion which is also reflected by higher ash content of the sample. This is probably due to addition of neutralizers to whey and also the addition of potassium nitrate during cheese preparation which might have resulted in higher amount of potassium in whey. As the level of protein increased, the content of various minerals decreased, possibly due to permeation of more and more minerals with progressive concentration of whey by UF. The rate or the intensity of permeation of minerals was found to be higher at higher degree of concentration. However, it is surprising to observe that WPC 80 had higher content of potassium than WPC 45 and WPC 70. This may be due to the addition of higher dose of potassium nitrate during production of cheese, which must have been passed on to whey. The amounts of other minerals were found to be very less in WPC 80 as compared to other samples.

5.3.5.2 Physical properties of spray dried whey protein concentrate

The various physical properties of spray dried WPCs such as pH, bulk density, dispersibility, wettability, sinkability and solubility index were determined. The results pertaining to these properties are depicted in Table 17.

It can be seen from the table that there is wide variation in the pH of reconstituted powder. The respective pH of WPC 26, WPC 45, WPC 70 and WPC 80 were 6.40, 6.62, 3.20 and 6.51. The variation in the pH of WPCs could be attributed to the variation in the pH of the whey used for WPC manufacture or it can also be ascribed to the pH at which UF or DF was carried out. WPC 70 had shown lowest pH value of 3.20 as against other

Table 17. Physical properties of spray dried whey protein concentrates*

Properties	Products			
	WPC-26	WPC-45	WPC-70	WPC-80
pH	6.40	6.62	3.20	6.51
Bulk density (g/cm^3)	0.630	0.420	0.340	0.280
Solubility index (ml)	0.48	0.36	0.20	0.15
Dispersibility (%)	94.70	96.50	97.00	98.30
Wettability (seconds)	27	38	68	82
Sinkability (% transmission)	43	47	53	58

Average of five trials

products. Probably UF or DF might have been carried out at lower pH values. Hence, the resultant product had shown low pH value.

Bulk density of WPCs varied from 0.280 to 0.630 g/cm^3. Highest bulk density was observed in case of WPC 26 and lowest in WPC 80. The respective bulk density was found to be 0.630, 0.420, 0.340 and 0.280 g/cm^3 for WPC 26, WPC 45, WPC 70 and WPC 80. During UF process, as the degree of concentration of whey progresses, more and more of minerals and lactose will be removed, hence their content in the retentate gets reduced. Therefore, resultant products exhibit variation in bulk density depending on the degree of concentration or the protein content. As the contents of minerals and lactose were high in WPC 26, it exhibited higher bulk density than the other products.

The dispersibility of WPC 26, WPC 45, WPC 70 and WPC 80 was observed to be 94.70, 96.50, 97.00 and 98.30 per cent, respectively. Whereas the respective solubility index for these four products was 0.48, 0.36, 0.20 and 0.15 ml. The dispersibility and solubility index are interrelated and these properties are dependent on the processing history of the product, especially various heat treatments it has undergone. These also depend on the ionic strength and net charges on the protein surface which vary with the composition of the WPCs. Hence, wide variation in the dispersibility and solubility index was observed. Products with higher proteins have shown good dispersibility and less values for solubility index which can be ascribed to the mineral load of the respective products specially with respect to calcium, which has negative correlation with these properties.

The products WPC 26, WPC 45, WPC 70 and WPC 80 had shown wettability of 27, 38, 68 and 62 sec, respectively. It is observed from the results

that lower the lactose content in the WPCs, lower is the wettability of the products. Wettability is directly related to the lactose content. At higher levels of UF concentration more and more of lactose will be removed through permeate, hence they possess low lactose, which results in poor wettability.

The respective values of sinkability for WPC 26, WPC 45, WPC 70 and WPC 80 were observed to be 43, 47, 53 and 58 per cent (transmission). Higher the transmission, poorer is the sinkability. Higher lactose and mineral contents exhibit better sinkability. Among four WPCs, WPC 80 had shown poor sinkability compared to the other samples. Whereas the sinkability of WPC 26 was higher than the other WPCs which can be attributed to the higher lactose and mineral contents. In general, it is observed from the results that the sinkability decreased with the decrease in lactose content. However, all the products exhibited fairly good sinkability.

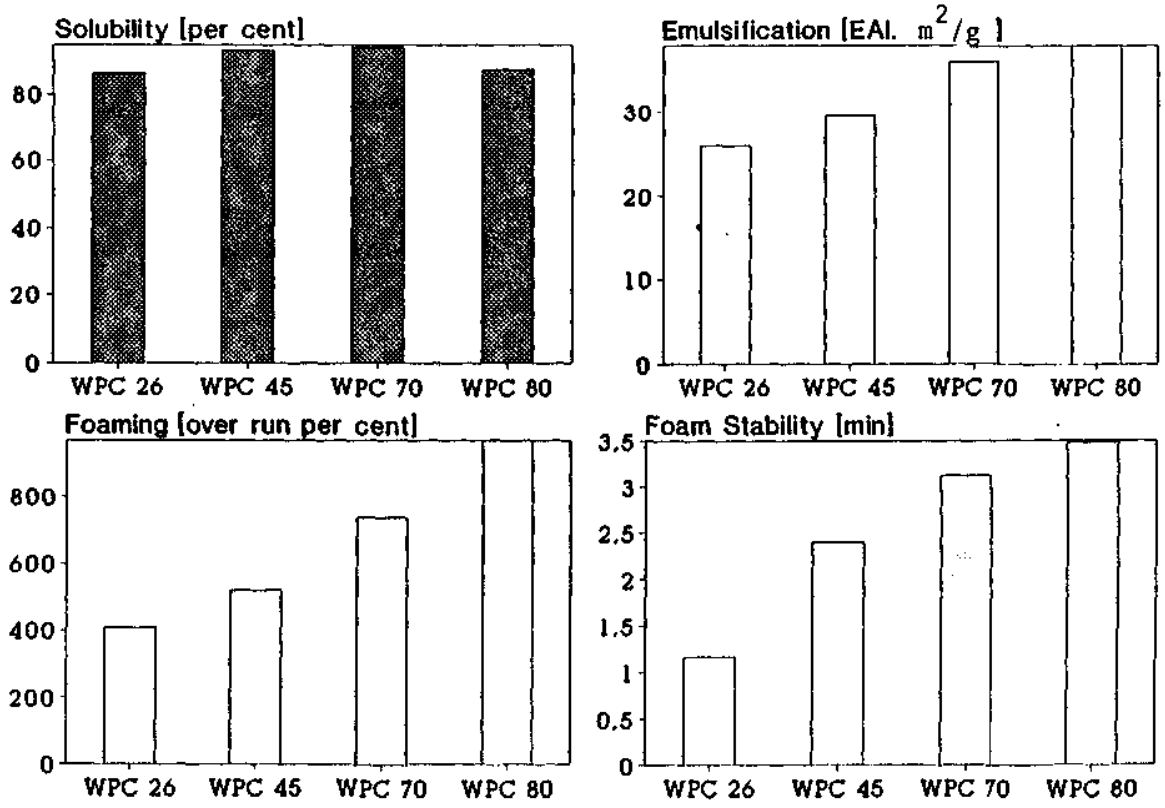
5.3.5.3 Functional properties of spray dried whey protein concentrates

Whey protein concentrates were studied for their various functional properties as these properties have significant role to play in various food applications. The results with respect to the functional properties such as solubility, emulsifying capacity, foaming capacity and foam stability are represented in Fig 33.

5.3.5.3.1 Solubility

The solubilities of WPC 26, WPC 45, WPC 70 and WPC 80 were 86.40, 93.32, 94.15 and 87.64 per cent, respectively. The solubility of WPCs was found to vary with the degree of heat treatment given during processing of whey and it also varied with the mineral profile of the products (Morr, 1979). In particular, the calcium content is negatively correlated with

FIG 33. FUNCTIONAL PROPERTIES OF WHEY PROTEIN CONCENTRATES



Note: WPC 45 is from Buffalo milk cheddar cheese whey
The rest are from Dairy Industry, Germany.

solubility (Zadow, 1986). The variation in the solubility of WPCs may be explained by changes in the electrical, hydrophobic and structural parameters by various treatments imparted during processing (Nakai and Li-chan, 1985), especially the surface hydrophobicity of UF retentate is significantly correlated with the solubility of WPCs.

5.3.5.3.2 Emulsifying capacity

Emulsifying capacity was measured in terms of emulsifying activity index (EAI). The EAI was observed to be 25.88, 29.47, 36.11 and 38.08 m^2/g, respectively for WPC 26, WPC 45, WPC 70 and WPC 80. It can be observed from the results that as the protein content increased the emulsifying capacity was also increased. Higher protein content aids in emulsifying more of fat by reducing interfacial tension between hydrophobic and hydrophilic components in food, and are closely linked to the conformation of protein. Variation in the emulsifying properties can also be attributed to the processing conditions, particularly heat treatment.

5.3.5.3.3 Foaming capacity

Foaming capacity was measured in terms of overrun. The products WPC 26, WPC 45, WPC 70 and WPC 80 had an overrun of 410, 521, 742 and 973 per cent, respectively. It was observed that higher the protein content in the sample, higher was the overrun. This can be explained by the rapid diffusion of proteins to the air water interface to reduce surface tension, followed by partial unfolding of the protein. This will result in encapsulation of air bubbles in association of protein molecules leading to an intermolecular cohesive film with certain degree of elasticity (deWit et al., 1988). Hence, higher level of protein naturally leads to good foaming capacity. It was observed by Patel and Kilara (1990b) that WPCs

having 70 per cent protein were found to exhibit 648 per cent overrun. In our investigation, WPC 70 had shown an overrun of 742 per cent.

5.3.5.3.4 Foam stability

The results pertaining to the foam stability of WPCs are presented in Fig 33. Foam stability varied from 1.16 to 3.48 min. Higher protein content in WPCs resulted in better foam stability. Foaming capacity and foam stability were observed to be interrelated. Higher the foaming capacity, higher was the foam stability. The products WPC 26, WPC 45, WPC 70 and WPC 80 had shown foam stability of 1.16, 2.40, 3.14 and 3.48 min, respectively. Higher the protein content, higher is the stability of foams and they will not drain out easily (Hugunin, 1987). The variation in foaming properties can be attributed to a number of compositional and processing variables that include pH, calcium ions concentration, ash content, redox potential, residual lipid content, sulphydryls, protein stability, heat treatments, fractionation technology and drying conditions (Morr, 1982).

5.3.6 STORAGE STABILITY OF SPRAY DRIED WHEY PROTEIN CONCENTRATES

The versatile use and the increasing demand for WPCs from various sectors of food industries emphasize the need to determine the storage stability. WPCs, owing to their high protein and lactose contents, are conducive to Maillard reaction, which may lead to off-flavour and deterioration of the functional properties and nutritional quality of the product during storage.

Four types of WPCs, viz., WPC 26, WPC 45, WPC 70 and WPC 80 were stored in two types of packaging materials at 20, 30 and 40°C for a period of 6 months. At a regular interval, the changes occurring with respect

to certain parameters were assessed. The results are narrated hereunder and discussed.

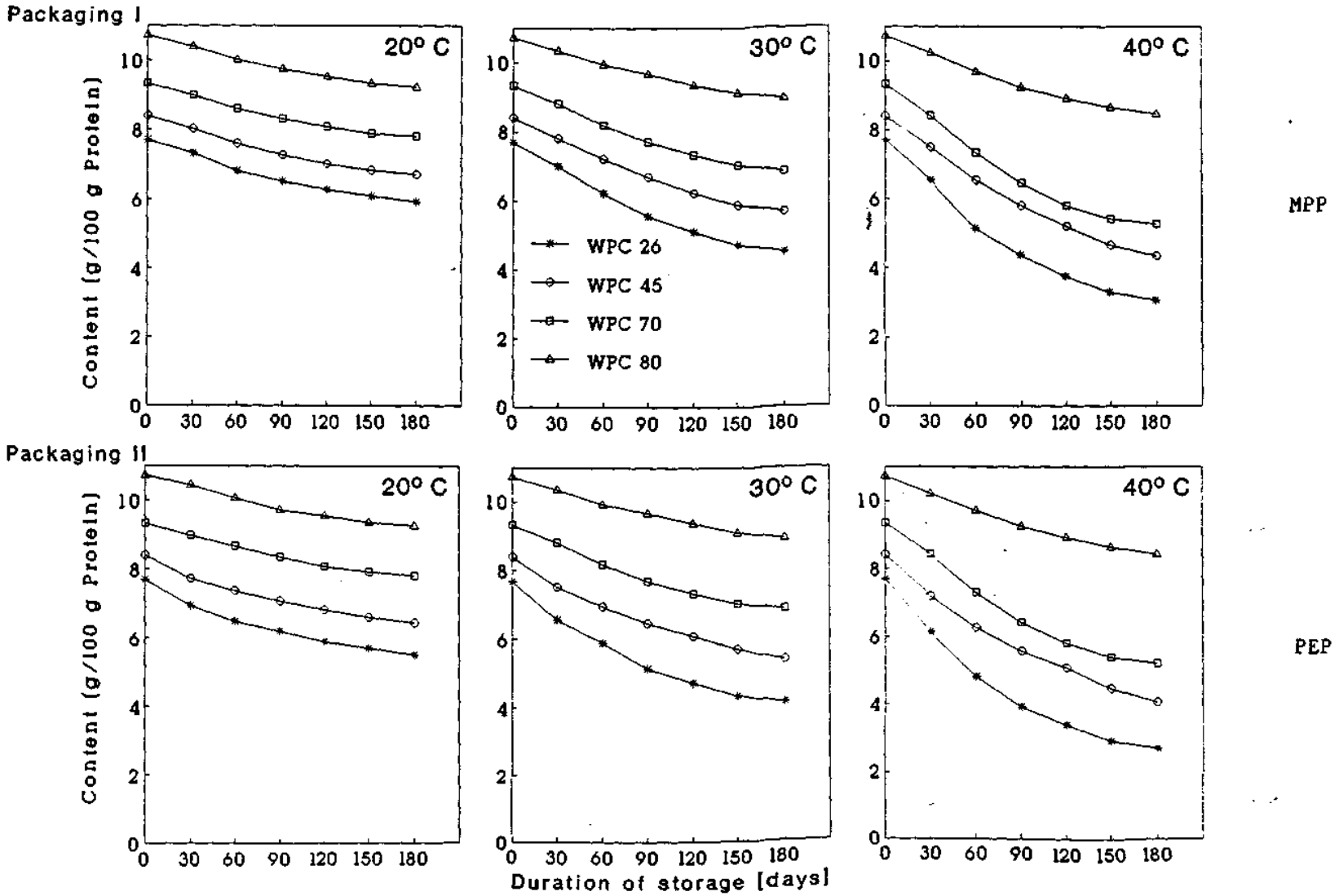
5.3.6.1 Lysine content

Changes occurring with respect to lysine during storage of WPCs are graphically presented in Fig 34. The samples had shown an initial lysine content of 7.70, 8.40, 9.33 and 10.72 g/100 g protein, respectively for WPC 26, WPC 45, WPC 70 and WPC 80.

When WPCs were stored in MPP, the extent of loss in lysine was estimated to be 23.10, 41.35 and 60.13 per cent for WPC 26, 20.10, 32.40 and 48.50 per cent for WPC 45, 16.30, 26.69 and 43.40 per cent for WPC 70 and 13.89, 16.51 and 20.98 per cent for WPC 80, respectively following 6 months of storage at 20, 30 and 40°C. Similarly when stored in PEP, the extent of loss of lysine was recorded to be 28.44, 46.99 and 64.54 per cent for WPC 26, 23.45, 36.07 and 51.19 per cent for WPC 45, 16.40, 26.37 and 43.62 for WPC 70 and 13.62, 16.60 and 21.17 per cent for WPC 80.

The results were subjected to regression analysis and the reaction rate constant were determined for each effect. The reaction rate constant for MPP was 0.4478 and for PEP 0.4632 indicating the significant difference due to the effect of packaging material. The rate of loss of lysine increased with increase in lactose content of the WPCs. The reaction rate constant for WPC 26, WPC 45, WPC 70 and WPC 80 was estimated to be 0.5605, 0.4888, 0.4620 and 0.3108 indicating a significant difference in the extent of loss of lysine among various WPCs. It was also observed from the reaction rate constants that temperature of storage has pronounced effect on the loss of lysine. As the temperature increased, the extent of loss also increased. The respective reaction rate constants for 20, 30 and 40°C were 0.2836, 0.4419 and 0.6420. The interaction effect of packaging

FIG 34. CHANGES IN LYSIN CONTENT OF WHEY PROTEIN CONCENTRATES DURING STORAGE



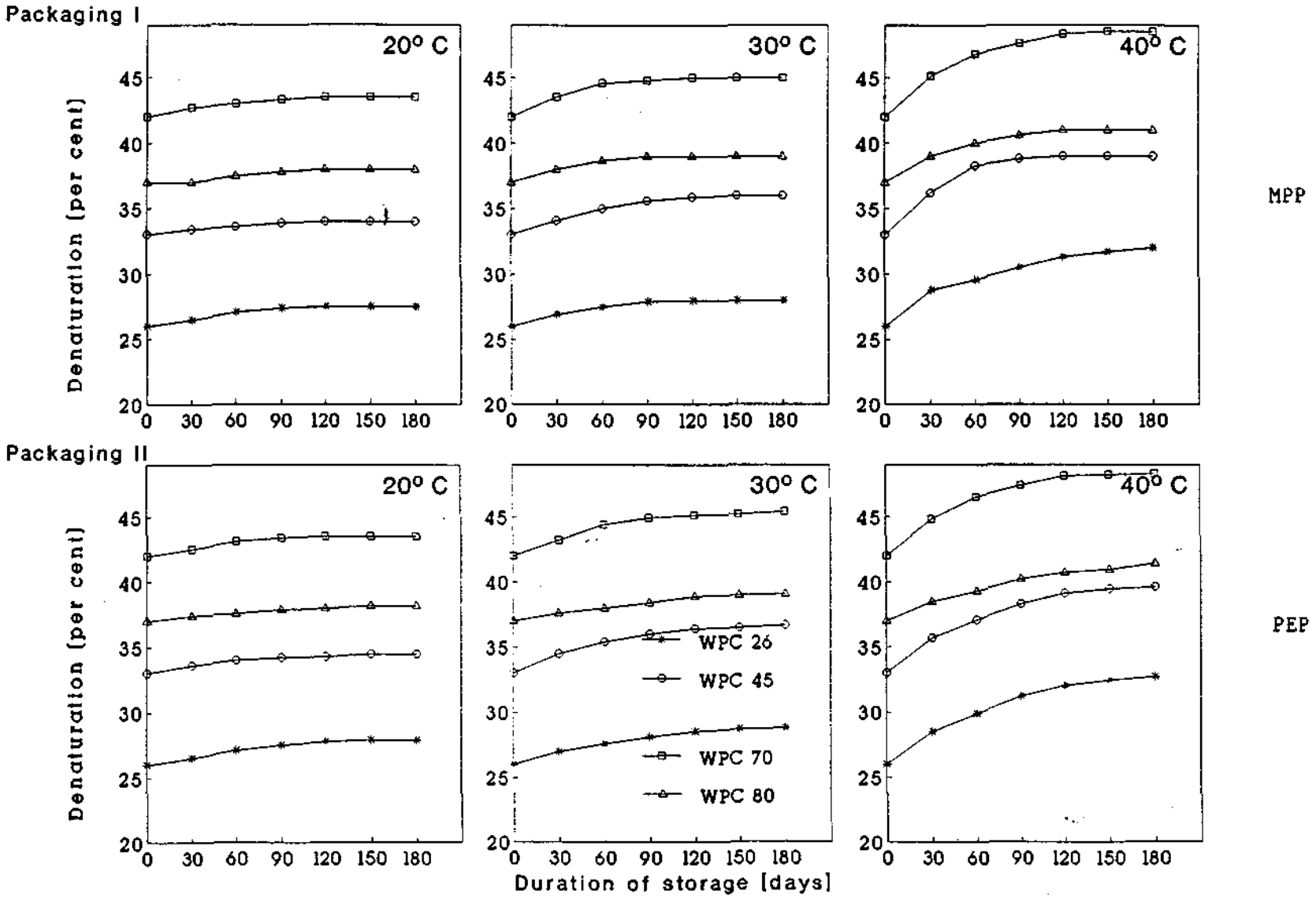
material, temperature of storage and various products also had significant effect on the extent of loss of lysine.

The loss of lysine was higher in WPC 26, which possessed higher lactose than the other WPCs, reverse was true for WPC 80. This can be ascribed to the higher lactose which causes more lysine deterioration because of greater Maillard reaction. Packaging material was found to have significant effect on the loss of lysine. Higher amount of loss of lysine was observed in PEP than MPP. Though there is statistically (Table 18) significant effect ($P \leq 0.01$) of packaging material on the loss of lysine, as observed between WPC 70 and WPC 80 which possess high protein and low lactose, the extent of difference in the loss of lysine was very small. Whereas in WPC 26 and WPC 45, the extent of difference between the packaging materials was high. As the lactose content of these two products is high, which is very hygroscopic, probably absorbed moisture through PEP which has good permeability to moisture (high MVTR). This must have enhanced the Maillard reaction leading to higher loss of lysine. Irrespective of the packaging material, the temperature had significant effect (Table 18) on the loss of lysine. The extent of loss was high at higher temperatures specially with the products possessing high amount of lactose (WPC 26 and WPC 45). As the temperature was raised from 20 to 40°C, the lysine content decreased drastically in all the types of WPCs and for both the packaging materials. The higher the temperature, greater is the Maillard reaction resulting in higher loss of lysine, which has also been confirmed by many workers (Li-chan, 1983; Hsu and Fennema, 1989).

5.3.6.2 Extent of denaturation

Changes in the extent of denaturation during storage of WPCs are projected in Fig 35. The values for initial denaturation were 37, 42, 33

FIG 35. CHANGES IN EXTENT OF DENATURATION OF PROTEIN OF WHEY PROTEIN CONCENTRATES DURING STORAGE



and 26 per cent, respectively for WPC 26, WPC 45, WPC 70 and WPC 80. The figure depicts the monthly changes in denaturation at various storage conditions.

After 6 months of storage, when WPCs were stored in MPP, the increase in the extent of denaturation was 5.77, 7.69 and 23.40 per cent for WPC 26, 3.03, 9.09 and 18.38 per cent for WPC 45, 3.57, 7.14 and 15.48 per cent for WPC 70, and 2.70, 5.40 and 10.81 per cent for WPC 80, respectively at 20, 30 and 40°C. Similarly, in PEP the extent of increase in denaturation was estimated to be 7.31, 10.76 and 25.76 for WPC 26, 4.54, 11.21 and 20 per cent for WPC 45, 3.57, 8.09 and 15.00 per cent for WPC 70 and 3.24, 5.67 and 10.89 per cent for WPC 80.

The effect of packaging material, temperature of storage and their interaction on the rate of change in denaturation was evaluated by regression analysis. The reaction rate constants of 0.4801 and 0.5590 were obtained for MPP and PEP indicating that there is overall significant effect ($P \leq 0.01$) of packaging material on the extent of denaturation. The rate of reaction also varied with the composition of the WPCs. The reaction rate constant values for WPC 26, WPC 45, WPC 70 and WPC 80 were 0.5536, 0.5638, 0.5708 and 0.3911, respectively. Significant variations were observed for the effect of temperature on denaturation. The reaction rate constants for 20, 30 and 40°C were 0.2317, 0.4318 and 0.8952, respectively, depicting that as the temperature of storage increases the extent of denaturation also increases. Rate of reaction was four times higher at 40°C than at 20°C.

From the results, it is evident that during storage of WPCs, there is slight denaturation of protein, specially at higher temperature of storage. However, the extent of denaturation at 20°C was negligible and

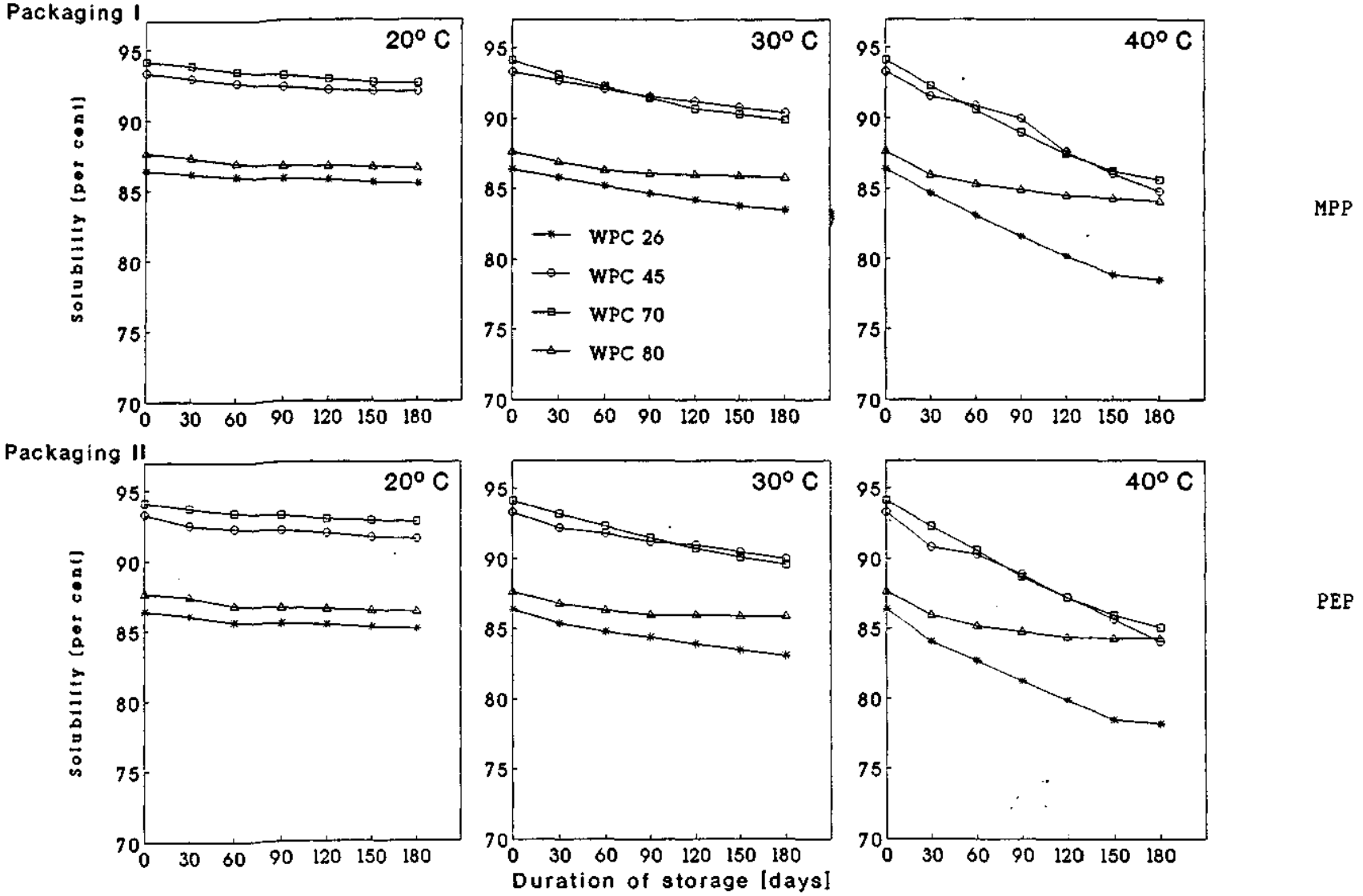
varied from 2.70 to 7.31 per cent, whereas at 30°C the denaturation varied from 5.40 to 11.21 per cent. Highest denaturation was found at 40°C (10.81 to 25.76%). Denaturation of protein during storage probably could be attributed to the Maillard reaction, which may lead to protein-protein interaction resulting in denaturation of protein. The severity of denaturation increased with the increase in temperature of storage which may be due to enhanced rate of Maillard reaction at higher temperature. As can be seen from the results, the extent of denaturation increased with the increase in the lactose content of the product. This could be again attributed to the higher rate of Maillard reaction at higher lactose content. Type of packaging material used had significant effect on the extent of denaturation in case of products having high lactose content. With respect to WPC 26 and WPC 45 as the lactose content was more, the extent of denaturation was higher when stored in PEP than in MPP. This could be ascribed to the absorption of moisture through PEP, as PEP has good permeability, whereas MPP has good barrier for moisture. The other two WPCs, as the lactose content is low, the packaging material used did not show significant effect.

5.3.6.3 Solubility

Changes with respect to solubility of WPCs during 6 months of storage at 20, 30 and 40°C in PEP and MPP are presented in Fig 36.

It is observed that after 6 months of storage, the solubility of WPC 26 decreased by 1.52, 3.36 and 9.14 per cent in MPP and 1.50, 3.82 and 9.49 per cent in PEP, respectively at 20, 30 and 40°C. Whereas the extent of decrease in solubility for WPC 45 was observed to be 1.52, 3.13 and 9.14 per cent in MPP and 1.95, 3.56 and 9.87 per cent in PEP, respectively at the above storage temperatures. The loss of solubility with respect to

FIG 36. CHANGES IN SOLUBILITY OF WHEY PROTEIN CONCENTRATE DURING STORAGE



WPC 70 and WPC 80 was estimated to be 1.74, 4.50 and 9.07 per cent and 1.30, 2.10 and 4.05 per cent in MPP, respectively. These two products exhibited a loss of 1.95, 3.56 and 9.87 per cent and 1.53, 1.99 and 3.77 per cent solubility when stored in PEP, respectively at the above temperatures.

Results with respect to changes in solubility during storage were computed by regression analysis. The reaction rate constants were found to be 0.6358 and 0.6874, respectively for MPP and PEP, indicating that MPP is slightly better than PEP. However, the extent of difference was found to be very small. With respect to storage temperature, the reaction rate constants were observed to be 0.2263, 0.5022 and 1.2083, respectively at 20, 30 and 40°C which revealed that storage temperature had significant effect on the loss of solubility. The values of reaction rate constant also varied from product to product. The ANOVA table 18 has indicated that there is significant effect of packaging material, temperature of storage and their interaction on the extent of loss of solubility.

It is evident from the results that the extent of decrease in solubility was less in all the types of WPCs at 20 and 30°C. A maximum loss of 1.95 and 4.82 per cent was observed at the above temperatures, respectively. However, at 40°C a maximum of 9.87 per cent loss was exhibited. Minimum loss in solubility was noticed in WPC 80 compared to other products. Although packaging material had shown significant effect on the loss of solubility, the difference in the extent of loss was marginal in all the four types of whey protein concentrates.

Our study clearly demonstrated that high solubilities are well maintained during storage. These results are in agreement with the observations of Li-chan (1983) and Hsu and Fennema (1989). Li-chan (1983)

stored WPC, containing 35 per cent protein at 37°C and observed a 14.0 per cent decrease in solubility after 42 days. Whereas Hsu and Fennema (1989) observed a decrease of 7.0 per cent in the extent of solubility when WPC containing 52 per cent protein was stored at 40°C for a period of 6 months. The decrease in solubility during storage could be attributed to the changes in protein structure followed by aggregation. It can also be due to the progressive Maillard reaction during storage which may bring about structural changes of protein leading to denaturation and loss of solubility.

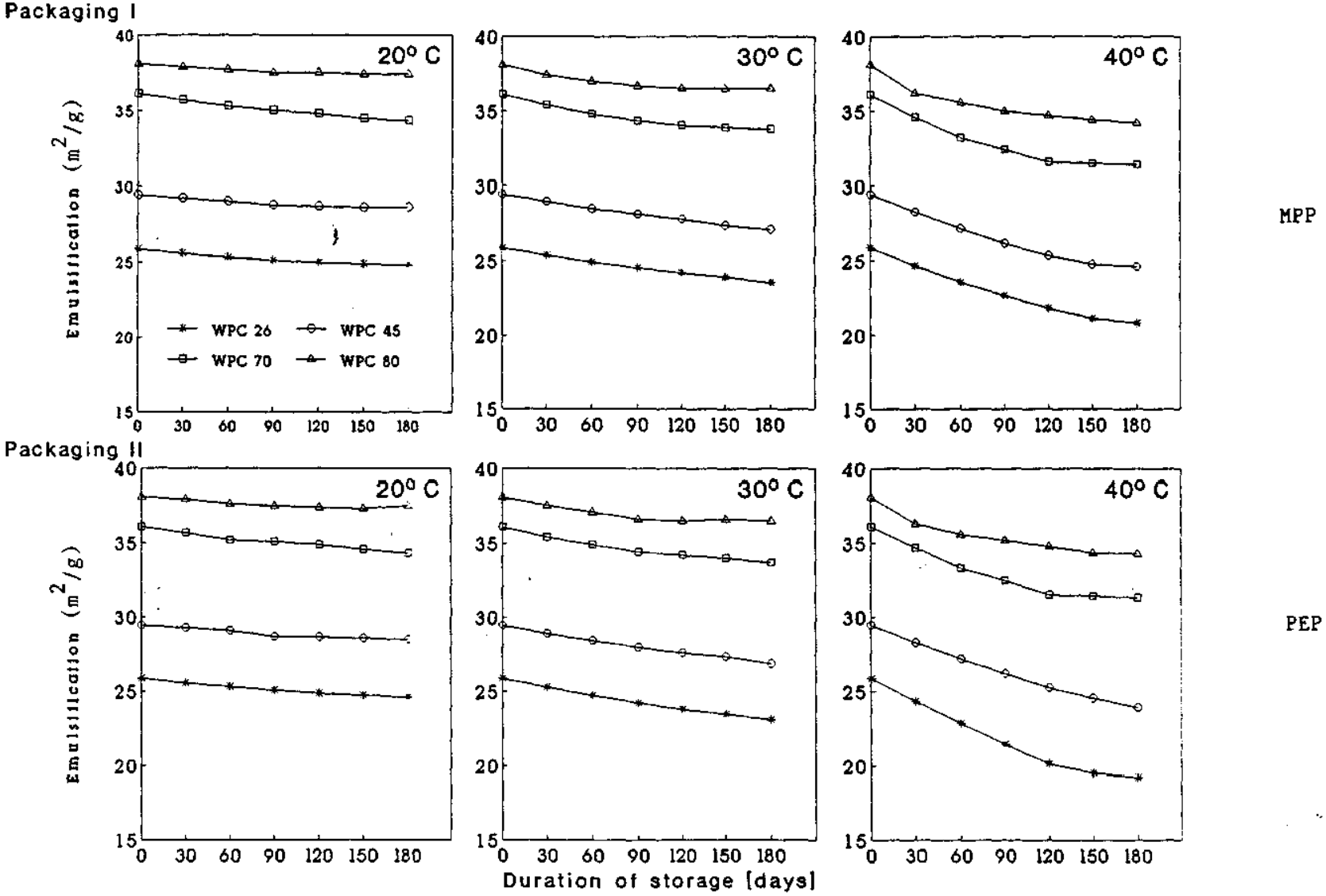
5.3.6.4 Emulsifying capacity

Emulsifying capacity is expressed as emulsifying activity index (EAI). The changes occurring in emulsifying capacity during storage of WPCs are represented in Fig 37. The EAI at the beginning of storage was estimated to be 25.88, 29.47, 36.11 and 38.08 m^2/g, respectively for WPC 26, WPC 45, WPC 70 and WPC 80.

After 6 months of storage, the extent of decrease in emulsifying capacity of WPCs, stored in MPP was observed to be 4.02, 9.00 and 19.51 per cent for WPC 26, 2.82, 8.01 and 16.49 per cent for WPC 45, 4.93, 6.40 and 13.04 per cent for WPC 70 and 1.78, 4.15 and 10.10 per cent for WPC 80, respectively at 20, 30 and 40°C. Similarly, in PEP the extent of loss of emulsifying capacity was recorded to be 4.94, 10.74 and 25.81 per cent for WPC 26, 3.32, 8.85 and 19.00 per cent for WPC 45, 5.01, 6.67 and 13.32 per cent for WPC 70 and 1.52, 4.15 and 9.92 per cent for WPC 80.

Results of storage studies were computed by regression analysis. The reaction rate constants were estimated to be 0.4321 and 0.4821, respectively for MPP and PEP material, indicating that the rate of loss was higher in PEP than in MPP. The reaction rate constant also varied with

FIG 37. CHANGES IN EMULSIFYING CAPACITY OF WHEY PROTEIN CONCENTRATE DURING STORAGE



the type of WPCs and their composition. The temperature of storage was observed to have pronounced effect on the loss of emulsifying capacity. The respective reaction rate constants for 20, 30 and 40°C were observed to be 0.1885, 0.3658 and 0.8167 which represents that higher the temperature of storage, greater is the loss of emulsifying capacity. ANOVA table 18 has also revealed that there is significant effect ($P \leq 0.01$) of packaging material and temperature of storage on the extent of loss of emulsifying capacity. However, the degree of loss varied with the type of WPCs.

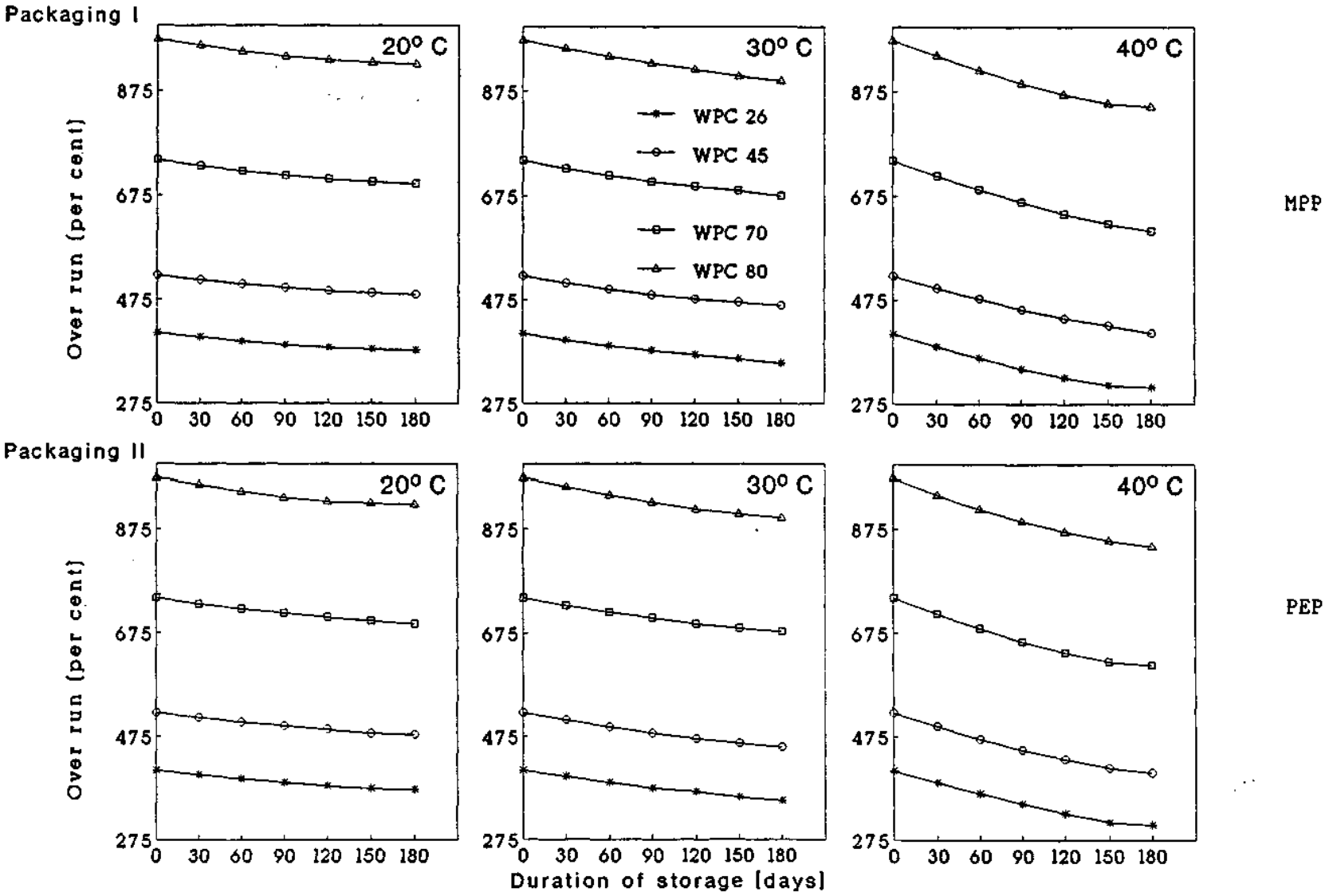
The decrease in EAI during storage could be attributed to the denaturation of protein during storage and this may also be due to molecular interaction occurring during prolonged storage period specially at higher temperatures which may bring about conformational changes in protein leading to an adverse effect on the EAI. The changes in emulsifying capacity are interrelated with the extent of denaturation of protein, conformational changes in protein, probably affected by Maillard reaction. It was observed by Mangino et al. (1987) that denaturation has a negative effect on the emulsifying properties of WPCs. The changes in emulsifying capacity during storage of WPCs were also observed by Hsu and Fennema (1989).

5.3.6.5 Foaming capacity

The effect of storage of WPCs on the foaming capacity is presented in Fig 38. Foaming capacity is expressed as per cent overrun. The figure depicts the monthly changes in foaming capacity as affected by storage temperature and packaging material.

Progressive decrease in the foaming capacity was observed during storage. The intensity of decrease varied with the storage temperature,

FIG 38 CHANGES IN FOAMING CAPACITY OF WHEY PROTEIN CONCENTRATES DURING STORAGE



packaging material and type of WPCs. For instance, after 6 months of storage of WPCs in MPP the extent of loss of foaming capacity was 8.05, 13.90 and 25.12 per cent for WPC 26, 6.91, 10.94 and 20.92 for WPC 45, 6.06, 9.03 and 18.06 per cent for WPC 70 and 4.93, 8.02 and 13.05 per cent for WPC 80, respectively at 20, 30 and 40°C. Similarly, in PEP the products registered a loss of 9.02, 14.39 and 25.85 per cent for WPC 26, 8.06, 12.86 and 22.26 for WPC 45, 6.60, 8.62 and 17.52 per cent for WPC 70 and 5.14, 7.80 and 13.46 per cent for WPC 80.

Reaction rate constants were derived by regression analysis to determine the effect of packaging material, temperature of storage and type of WPCs on the extent of change in foaming capacity during storage. The reaction rate constants were estimated to be 12.52 and 13.08, respectively for MPP and PEP indicating that there is slight variation in the loss of foaming capacity of WPC when these packaging materials were used. Reaction rate constants also indicated that there is variation in the extent of loss from one product to another. Similarly, wide variation in the reaction rate constants was observed with respect to temperature. The values were found to be 7.18, 10.93 and 20.28, respectively for 20, 30 and 40°C, depicting that temperature has significant effect on the extent of loss of foaming capacity during storage. ANOVA table 18 has also revealed a significant effect ($P \leq 0.01$) of packaging material and temperature of storage on the foaming capacity.

The decrease in foaming capacity of WPCs during storage could be justified by denaturation and the conformational changes of protein occurring during storage due to molecular interactions of components. Such protein-protein interaction, ionic interaction and intermolecular interaction may bring about structural changes in protein which, in turn, may effect the foaming capacity of the WPCs. At higher temperature of

Table 18. ANOVA for the effect of storage on the quality of spray dried whey protein concentrates

Source of variation	d.f.	'F' values				
		Lysine	Denaturation	Solubility	Emulsifying capacity	Foaming capacity
Replication	1	0.807^{NS}	1.023^{NS}	3.966^{NS}	1.266^{NS}	2.178^{NS}
Packaging material (a)	1	804.427**	8303.311**	2118.580**	2060.593**	39.779**
Temperature of storage (b)	2	146364.609**	206021.031**	1291184.375**	115698.617**	7709.013**
Type of product (c)	3	37391.551**	9854.271**	180260.250**	8037.651**	277.013**
Interaction a x b	2	8.031**	461.277**	27.205**	706.497**	1.512^{NS}
a x c	3	313.049**	783.187**	203.576**	856.121**	13.785**
b x c	6	7160.872**	3469.653**	68519.125**	2139.228**	29.146**
All effect interaction	6	4.358*	207.541**	630.807**	226.191**	1.306^{NS}
Error	23	-	-	-	-	-

NS = Non-significant
* Significant at 5 per cent level (P 0.05)
** Significant at 1 per cent level (P 0.01)

storage, WPCs have exhibited greater loss of foaming capacity for which pronounced Maillard reaction is certainly the causative factor. It is well known that Maillard reaction proceeds at a higher rate at higher temperatures (Holsinger et al., 1973; Labuja and Saltmarch, 1981) which brings about various conformational and structural changes in protein, thus, affecting the foaming capacity. It is observed that WPC 26 and WPC 45 had shown slightly higher loss of overrun (foaming capacity) in PEP than in MPP. High lactose content of these two products is the main reason for it. Lactose aids in absorbing moisture and may enhance the Maillard reaction which, in turn, lower foaming capacity as PEP has good permeability to moisture as against MPP which has good barrier for the moisture uptake. However, the effect of packaging material on the loss of foaming capacity with respect to WPC 70 and WPC 80 was found to be marginal due to low level of lactose content.

From the results of storage experiments, it can be inferred that it is necessary to store WPCs at or below 20°C. Though there was loss of lysine and functional properties, to some extent, the severity of loss was kept at minimum. Higher storage temperatures affect the nutritional as well as functional properties of WPCs. Between MPP and PEP, it was observed that for higher lactose containing WPCs (WPC 26 and WPC 45),it is advisable to use MPP. Whereas for WPC 70 and WPC 80 (low lactose and high protein containing WPCs), PEP could be used. Hence, for WPCs having high level of lactose, it is necessary to select a packaging material which has a good barrier capacity for moisture. For WPCs with low level of lactose, polyethylene could be used successfully as a packaging material.

CHAPTER 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

With a spectacular increase in milk production, substantial quantities of milk are being diverted for production of paneer, cheese, chhana, casein and shrikhand. This results in enormous quantities of whey as a by-product. In spite of the fact that the whey constitutes nearly 50 per cent of the nutritionally superior milk constituents, almost all the whey produced in the country is being merely wasted. The need for high quality proteins to combat protein-calorie malnutrition and the prevailing stringent environmental Pollution Act emphasize the need to utilize the whey in an effective manner. However, a major hindrance in the recovery of whey solids is the amount of energy involved in the process. A conventional way of evaporation and drying would add significantly to the cost of production, and hence cannot be recommended to our dairy industry which is already ridden with economic problems. Conservation of whey solids in the form of whey powder and whey protein concentrate (WPC) by employing less energy intensive processes such as reverse osmosis (RO) and ultrafiltration (UF) appear to be a viable proposition. Hence, this project was envisaged to develop a cost effective technology for the production of whey powder and WPC to conserve huge quantities of whey solids. The results of the investigation are summarized hereunder.

Three types of whey, viz., paneer whey (PW), cow milk cheddar cheese whey (CW) and buffalo milk cheddar cheese whey (BW) were used in this investigation. Composition of these wheys was determined. Processing parameters for RO, such as clarification of whey, operational temperature, pressure, pH and preheat treatments of whey were optimized. Fouling and deposit formation phenomenon were investigated with respect to cleaning

schedule of the membrane. Variables in the precrystallization process including the level of seeding material, TS level and duration of crystallization were standardized. Comparative energy requirement for concentration of whey by RO and conventional evaporation were computed. Storage studies for experimental whey powder along with commercial whey powder were carried out. Changes in nutritional and functional properties were assessed. A process for the manufacture of whey powder from the PW, CW and BW has been developed.

Similarly, a process for manufacture of WPC from BW using UF was was standardized and storage studies for the WPC along with commercial WPCs were conducted.

6.1 Clarification of whey was found to improve the flux in case of CW and BW. Clarified CW and BW showed respective mean flux of 35.75 and 34.07 $l/m^2/h$ as against 29.90 and 28.27 $l/m^2/h$ for unclarified whey. On the contrary, clarification of PW did not improve the flux.

6.2 RO concentration of whey was carried out at two temperatures, 40° and 50°C. The operation at 50°C yielded higher flux than at 40°C. At 50°C, PW, CW and BW recorded an average flux of 29.80, 35.76 and 34.07 $l/m^2/h$ as against 22.23, 27.35 and 25.14 $l/m^2/h$ at 40°C.

6.3 Among various levels of pressures studied, operation at 35 bar pressure was more advantageous than at 25, 30 or 40 bar. As the pressure increased from 25 to 35 bar, there was substantial improvement in the flux (from 18.5 to 26.40 $l/m^2/h$). However, after 35 bar the increase in flux was non-significant ($P \leq 0.01$).

6.4 The three types of whey were preheated at various pH levels and temperatures prior to RO concentration to select the best treatment

combination which would yield maximum flux. The pH ranged from 3.5 to 7.2 and the heat treatments 80°C/15 sec, 70°C/15 sec and 60°C/30 min. A higher permeation was observed by heating whey at 80°C/15 sec than at 60°C/30 min or 70°C/15 sec regardless of whey type. PW and BW registered the highest flux (32.43 and 36.24 $l/m^2/h$, respectively) when heated to 80°C/15 sec at pH 7.2. However, the other combinations of pretreatment yielded a substantially lower flux. However, in case of CW, adjusting pH to below or above its native pH and heating to different temperatures did not improve the flux. Highest flux (34.09 $l/m^2/h$) was recorded when CW was heated to 80°C/15 sec at its native pH (6.3).

6.5 The fouling phenomenon during processing of whey by RO was studied. Fouling of membrane was found to occur within 3 min of operation. However, the severity varied with the pretreatments imparted to whey. In all the three types of whey, fouling was severe at pH 3.5 and 4.5 with all the heat treatments. In case of PW and BW, fouling was minimized to a great extent when heated to 80°C/15 sec at a pH of 7.2, whereas with respect to CW, fouling was the least when heated to 80°C/15 sec at its native pH(6.3).

6.6 The deposit formation on RO membranes while processing of whey at various combinations of treatments was studied. PW and BW produced lowest deposit when preheated to 80°C/15 sec at pH 7.2 (31.40 and 18.40 g/m^2), while CW produced the lowest deposit at pH 6.3 (20.30 g/m^2). Heat treatment of 80°C/15 sec resulted in a lower deposit compared to 60°C/30 min and 70°C/15 sec. At lower pH values the contribution of ash to the total deposit mass was higher than that of protein, whereas at pH above 5.6, contribution of protein was higher. The calcium and phosphorus contents of deposits were more at lower pH values than at higher ones.

6.7 Duration needed for cleaning RO membrane varied with the type of

pretreatments imparted to whey. When PW preheated to 80°C/15 sec at pH 7.2 was processed, lowest cleaning time was recorded: alkali based (Ultrasil-25) cleaning required 40 min and acid based cleaning (Ultrasil-75) took 35 min to attain the original reference water flux. Similarly, for BW, minimum time for cleaning (35 and 30 min, respectively for Ultrasil-25 and Ultrasil-75) was needed when it was heated to 80°C/15 sec at pH 7.2. In case of CW, time required for cleaning was minimum with preheating temperature of 80°C/15 sec and pH 6.3; the time taken for Ultrasil-25 and Ultrasil-75 was 35 and 30 min, respectively. Whey processing at lower pH values required longer cleaning times.

6.8 Paneer whey, CW and BW could be concentrated to a maximum of 20.50, 22.10 and 23.20 per cent solids by RO respectively. However, from economic point of view, just 2.0-fold concentration (50% volume reduction) of PW and 2.5 fold concentration (60% volume reduction) of CW and BW would be preferable. During RO concentration, slight losses of lower molecular weight compounds such as NPN and ash through permeate were observed. Traces of lactose were also noticed in the permeate. The retention value for true protein, calcium and phosphorus was found to be 100, whereas for TS, lactose, ash and NPN the values were estimated to be 99.65, 99.86, 97.92 and 94.99 per cent for PW, 99.74, 99.88, 97.96 and 95.60 per cent for CW and 99.71, 99.85, 97.94 and 95.57 per cent for BW, respectively.

6.9 RO retentate was further concentrated in a vacuum evaporator to a TS content of 40, 45 and 50 per cent in order to select the optimum level of TS which could give maximum crystallization to produce high quality whey powder free of hygroscopicity and caking problems. Precrystallization was carried out using 0.01, 0.03, 0.05 and 0.07 per cent seeding material for crystallization duration of 1 to 6 h at 30°C in order

to optimize the process. The degree of lactose crystallization increased with the duration but after 4 h, the increase was not significant. Maximum crystallization was attained at 50 per cent TS, at the end of 4 h with 0.03 per cent seeding material in PW concentrate (64.0%) and with 0.05 per cent seeding material in CW (63.0%) and BW (57.0%).

At the end of crystallization at 30°C, the lactose crystal size varied from 5 to 20 μm; about 65 per cent of crystals were smaller than 5 μm. Further cooling of CW and BW concentrates to 10°C (3°C/h) increased the degree of crystallization to 75 and 73 per cent, respectively, whereas in PW concentrate the increase was to the extent of 79 per cent.

6.10 Precrystallized PW, CW and BW concentrates were spray dried and the resultant powders (PWP, CWP and BWP, respectively) were analysed for various physical and chemical attributes along with commercial whey powder (WP-G). The PWP, CWP, BWP and WP-G exhibited, respectively, 0.579, 0.520, 0.510 and 0.508 g/cm^3 bulk density, 90.44, 95.50, 94.90 and 95.80 per cent dispersibility, 8.0, 15.0, 22.0 and 17.0 sec wettability, 67.5, 64.0, 62.0 and 63.0 per cent sinkability, 37°, 32°, 34° and 33° flowability and 1.10, 0.60, 0.70 and 0.75 ml solubility index. Thus, CWP and BWP showed fairly good physical properties and were at par with WP-G. However, PW had a slightly less dispersibility, less solubility and higher bulk density.

6.11 The moisture, protein, fat, lactose and ash contents were estimated to be 3.84, 4.68, 2.06, 80.68 and 8.74 per cent for PWP, 3.86, 12.51, 0.78, 75.49 and 7.34 per cent for CWP, 3.78, 14.33, 0.87, 72.99 and 8.01 per cent for BWP and 3.40, 13.40, 0.58, 74.77 and 7.85 per cent for WP-G, respectively. All the samples except PWP were within the limits of various standards prescribed.

The calcium, phosphorus, potassium, magnesium and sodium contents were 1109, 831, 1887, 180 and 570 mg/100 g for PWP, 549, 454, 1540, 123 and 559 mg/100 g for CWP, 667, 587, 1634, 140 and 551 mg/100 g for BWP and 598, 553, 1563, 115 and 633 mg/100 g for WP-G, respectively. Thus, appreciably higher levels of calcium and phosphorus were observed in PWP than in other three samples. Further, BWP had higher calcium and phosphorus than CWP and WP-G. Sodium content of WP-G was slightly higher than the other whey powders.

The nitrogen distribution of whey powders with respect to total nitrogen (NT), non-protein nitrogen (NPN), proteose peptone (PPN) and whey protein nitrogen (WPN) was estimated to be 0.73, 0.37, 0.164 and 0.20 per cent for PWP, 1.96, 0.53, 0.36 and 1.06 per cent for CWP, 2.25, 0.62, 0.42 and 1.21 per cent for BWP and 2.10, 0.64, 0.39 and 1.06 per cent for WP-G, respectively. The respective lysine contents of PWP, CWP, BWP and WP-G were 7.42, 7.28, 7.70 and 7.52 g/100 g protein, whereas the respective extent of denaturation was 87.5, 52.0, 46.0 and 54.0 per cent.

The PWP, CWP,BWP and WP-G packaged in metallized polyester packaging (MPP) and polyethylene packaging (PEP) were stored at 20, 30 and 40°C for a period of 6 months, and changes in the physico-chemical attributes of the powders assessed.

6.12 During storage, loss of lysine was observed to be higher at higher temperature of storage in all the four types of powder. After 6 months of storage, the respective loss of lysine was estimated to be 8.87, 13.08 and 28.04 per cent for PWP, 8.69, 12.27 and 25.10 per cent for CWP, 9.40, 13.89 and 28.05 per cent for BWP, and 8.77, 12.89 and 26.59 per cent for WP-G when stored in MPP at 20, 30 and 40°C. The extent of lysine loss was

study, it was observed that lysine content did not cross this limit even after 6 months of storage at 20° and 30°C.

6.13 The energy requirements for the concentration of whey by RO and conventional evaporator were compared. 1516258.9, 1134559.2 and 1099637.3 kJ energy was required to concentrate 1,000 l of PW,CW and BW, respectively to 50 per cent TS by conventional evaporator, whereas 3081218.4, 3074158.1 and 3049411.7 kJ was required by employing RO and conventional evaporator together. Thus, a saving of 50.79, 63.09 and 63.99 per cent energy could be effected by RO.

6.14 Whey protein concentrate was produced from BW by employing UF technology. Processing parameters were standardized in order to obtain maximum permeation per unit area of the membrane. Of the two operational temperatures tried, 50°C yielded substantially higher flux compared to 40°C. The mean average flux for 95 per cent volume reduction was 34.00 $l/m^2/h$ at 50°C as against 23.79 $l/m^2/h$ at 40°C. Hence, it is imperative to operate UF at 50°C.

6.15 Various pretreatments of BW were attempted to enhance the flux. BW was adjusted to pH 3.0, 4.5, 5.6, 6.4 and 7.2 and heated to 60°C/30 min or 80°C/15 sec prior to UF concentration. The highest average flux of 51.79 $l/m^2/h$ was observed when BW was heated to 80°C/15 sec at pH 7.2. A slightly lower flux (48.53 $l/m^2/h$) was observed when BW was ultrafiltered at pH 3.0. Other combinations of treatments, however, resulted in significantly lower flux.

6.16 Changes occurring with respect to various constituents of BW during UF were studied. It was observed that with the progress in concentration or the volume reduction, the protein content increased from 14.98 to 63.79

per cent (on DM basis), whereas lactose decreased from 78.20 to 4.23 per cent after 95 per cent volume reduction. It was possible to attain a maximum volume reduction of 97.5 per cent (26.50% TS). However, it is economical to concentrate the whey to a volume reduction of 95.0 per cent (21.60% TS). At this concentration, the retentate possessed 63.79, 27.54, 6.23, 0.36, 0.76 and 0.47 per cent protein, lactose, ash, NPN, calcium and phosphorus on DM basis. At the initial stages of UF concentration, the calcium, phosphorus and NPN (on DM basis) were observed to increase in the retentate. However, at later stages there was drastic decrease in their content. It was evident that by controlling the level of volume reduction, desired level of protein in the end product could be obtained.

6.17 The UF retentate of BW after 95 per cent volume reduction had protein, lactose and ash in the ratio of 1:0.43:0.097. By first stage diafiltration (DF), it was possible to attain protein, lactose and ash to a ratio of 1:0.14:0.043, whereas after second stage DF, the ratio was 1:0.050:0.033. Thus, the protein content increased drastically as a result of DF. Lactose and ash contents reduced to a minimum. However, calcium and phosphorus contents in total ash increased, presumably due to decreases in sodium, potassium and magnesium contents.

6.18 Buffalo milk cheddar cheese whey was ultrafiltered to 85 per cent volume reduction and spray dried. The resultant WPC had 3.74, 43.46, 42.76, 5.58 and 4.45 per cent moisture, protein, lactose, ash and fat, respectively. Commercial WPCs such as WPC 26, WPC 70 and WPC 80 had 4.80, 4.41 and 3.62 per cent moisture, 25.11, 71.16 and 80.60 per cent protein, 53.64, 14.37 and 4.45 per cent lactose, 15.03, 3.36 and 4.61 per cent ash, and 1.42, 6.70 and 7.17 per cent fat, respectively.

6.19 With respect to nitrogen distribution, the WPN:NPN:PP ratio was

71.87:13.40:14.73 in WPC 26. As the protein content increased in the powders to 43.46, 71.16 and 80.60 per cent, the ratio changed to 78.70: 9.70:13.40 (WPC 45), 82.95:9.95:7.10 (WPC 70) and 84.55:9.20:6.25 (WPC 80), respectively, indicating that as the concentration of whey by UF progressed, there was loss of more and more NPN and PP through permeate. The lysine content increased as the protein content increased in the powders. The respective lysine contents of WPC 26, WPC 45, WPC 70 and WPC 80 were 7.70, 8.40, 9.33 and 10.72 g/100 g protein. The per cent denaturation of protein was 26, 33, 42 and 37 for WPC 26, WPC 45, WPC 70 and WPC 80, respectively.

6.20 The contents of calcium, phosphorus, potassium, sodium and magnesium were 1062, 1273, 4588, 1501 and 226 mg/100 g for WPC 26, 715, 504, 965, 322 and 98 mg/100 g for WPC 45, 250, 34.74, 190, 63.75 and 27.18 mg/100 g for WPC 70 and 33.43, 27.45, 2858, 41.25 and 7.50 mg/100 g for WPC 80, respectively.

6.21 The solubility index values of WPC 26, WPC 45, WPC 70 and WPC 80 were 0.48, 0.36, 0.20 and 0.15 ml, respectively. These products exhibited dispersibility of 94.70, 96.50, 97.00 and 98.30 per cent, and wettability values of 27, 38, 68 and 82 sec, respectively. The sinkability was found to be 43, 47, 53 and 58 per cent. The bulk density was 0.630, 0.420, 0.340 and 0.280 g/cm^3, respectively for WPC 26, WPC 70 and WPC 80. Thus, with increase in protein content, solubility and dispersibility slightly improved, whereas sinkability, wettability and bulk density slightly decreased.

6.22 The functional properties varied with the composition of the powders, especially protein content. The solubility expressed as nitrogen solubility index (NSI) was 86.40, 93.22, 94.15 and 87.64 per cent, the emulsifying capability expressed as emulsifying activity index (EAI) was

25.88, 29.47, 36.11 and 38.08 m^2/g, foaming capacity expressed as overrun was 410, 521, 742 and 973 per cent and foam stability was 1.16, 2.40, 3.14 and 3.48 min, respectively for WPC 26, WPC 45, WPC 70 and WPC 80. As the protein content in WPCs increased, emulsifying capacity, foaming capacity and foam stability increased but solubility varied regardless of protein content.

6.23 During storage of WPCs, some changes in nutritional and functional properties of the powders occurred depending on packaging material, type of WPC and storage temperature. The lysine content gradually decreased with progress of storage. The loss was higher at higher storage temperature. The lysine content, emulsifying capacity, foaming capacity and solubility decreased and denaturation increased gradually with advancing storage period. However, the intensity of change was greater at higher storage temperature and also in WPC 26 and WPC 45, which had higher lactose contents. It was observed that these products, when stored in PEP, exhibited greater loss in nutritional and functional properties than when packed in MPP. However, in WPC 70 and WPC 80, the packaging material did not appreciably affect the rate of loss of these properties. The loss of lysine was 23.10, 41.35 and 60.13 per cent for WPC 26, 20.10, 32.40 and 48.50 per cent for WPC 45, 16.30, 26.69 and 43.40 per cent for WPC 70 and 13.89, 16.51 and 20.98 per cent for WPC 80, respectively when these were packed in MPP and stored for 6 months at 20, 30 and 40°C, respectively.

Thus, for WPCs having high lactose content, it is necessary to select a packaging material such as MPP, having good water barrier properties. For WPCs having low lactose, even polyethylene can be used without their quality being affected. It would be better to store WPCs at or below 20°C to prevent the losses of nutritional and functional characteristics.

In conclusion, technology for manufacture of whey powder and WPC employing RO and UF has been successfully developed during this project. The nutritional, physical and functional properties of the powders were fairly comparable with those of commercial powders sold abroad. This simple and energy saving technology can be easily adopted by our dairy industry. This process is first of its kind to be developed in our country which will enable us conserve large quantities of whey solids which are presently drained into the gutter. With the adoption of this process, the dairy industry is certain to be benefited to a great extent. It has to be admitted that whey production in our country exists in pockets. There is a need to channelize the by-product appropriately so as to put it to economic utilization.

Proper emphasis should be laid on further research to explore the possibilities of utilizing whey powder and whey protein concentrate in various Indian food formulations.

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ANNEXURES

ANNEXURE I

CHEMICAL COMPOSITION OF WHEY SYSTEM

Constituents	Paneer whey		Cow milk cheddar cheese whey		Buffalo milk cheddar cheese whey	
	Mean*	S.D.	Mean*	S.D.	Mean*	S.D.
Total solids (%)	6.06	0.051	6.41	0.068	6.87	0.084
Lactose (%)	5.03	0.044	4.83	0.024	5.01	0.033
Protein (%) (Total nitrogen x 6.38)	0.30	0.021	0.78	0.023	0.98	0.037
Fat (%)	0.13	0.017	0.33	0.014	0.34	0.015
Ash (%)	0.60	0.020	0.46	0.015	0.54	0.016
Non-protein nitrogen	35.50	0.512	28.05	0.546	37.55	0.905
Calcium (mg/100 g)	71.05	1.693	43.55	0.807	50.15	0.884
Phosphorus (mg/100 g)	56.05	1.487	35.85	0.647	44.15	0.418

* Mean of ten trials

SD = Standard deviation

ANNEXURE II

REFERENCE WATER FLUX FOR CLEANING SCHEDULE

Pressure (bar)	Temperature of operation (°C)		
	30	40	50
	Flux ($l/m^2/h$)		
20	14.40	22.26	32.93
25	20.40	26.00	37.50
30	22.53	34.26	42.66
35	26.66	40.93	52.40
40	30.00	44.66	59.86

ANNEXURE III

PHYSICAL PROPERTIES OF WHEY CONCENTRATES AT DIFFERENT LEVELS OF TOTAL SOLIDS

Types of whey concentrate	Properties of concentrate	Levels of total solids (%)			
		20	40	45	50
Paneer whey concentrate	Specific gravity	1.1027	1.2092	1.2224	1.2365
	Viscosity (cp)	2.2654	7.2519	8.8749	10.5082
Buffalo milk cheese whey concentrate	Specific gravity	1.1033	1.2093	1.2223	1.2364
	Viscosity (cp)	2.6145	9.5117	17.9732	25.3290
Cow milk cheese whey concentrate	Specific gravity	1.1032	1.2092	1.2220	1.2362
	Viscosity (cp)	2.4850	8.4815	16.5018	22.4540

ANNEXURE IV

CALCULATION OF ENERGY REQUIREMENT FOR CONCENTRATING 1,000 LITRES OF PANEER WHEY

Conventional method (vacuum evaporation alone)

Initial total solids	- 6.06 per cent
Required total solids in the final concentrate	- 50.00 per cent
Water to be evaporated	- 879 l
Steam required to evaporate 879 l of water	- 879 x 1.2 1054.80 kg
Energy required to produce 1054.80 kg of steam	- 2381738.40 kJ
Condenser pump:	
Motor	- 12 HP
Evaporation capacity of the vacuum pan	- 135 l/h
Number of hours to be operated to evaporate 879 l water	- 6 h 50 min
Equivalent energy to run the condenser pump	- 699480.00 kJ
Total energy required to evaporate 879 l water to obtain 50 per cent TS	- (2381738.40 + 699480.00) - 3081218.4 kJ

Combination of reverse osmosis and vacuum evaporation

By reverse osmosis (2-fold concentration) (from 1,000 l to 500 l)

Av. flux ($l/m^2/h$)	Actual permeation (l/h)	Time required (h)	Electrical* energy reqd. (KWH)	Equivalent steam energy (kJ)
35.28	190.05	3.03	12.12 (43632.0 kJ)	145440.00

*(4 KWH motor, presuming the maximum capacity)

contd.....

Vacuum evaporation

Further, 379 l has to be evaporated by vacuum pan to attain 50 per cent total solids.

The steam required	=	379 x 1.2
	=	4848 kg
Steam energy required to evaporate 379 l of water	=	1026938.0 kJ

Condenser pump

Number of hours to be operated	-	3 h 20 min
Equivalent energy requirement	-	343880.59 kJ

Total energy requirement	=	(145440.00 + 1026938.0 + 343880.59)
	=	1516258.9 kJ
Total energy savings	=	(Energy required by vacuum evaporator alone - energy required by the combination of reverse osmosis and vacuum evaporation)
	=	(3081218.4 - 1516258.9)
	=	1564959.5 kJ
Net energy savings	=	50.79 per cent

Similarly, the energy requirement to concentrate 1,000 l of cheese wheys to 50 per cent total solids was calculated.