J. Rakuno Gakuen Univ., 22 (1) : 9~16 (1997)

ULTRAFILTRATION OF YAK RENNET CHEESE WHEY: IMPROVEMENT OF PERMEATION RATES BY PREVENTING FOULING

Neupaney DHANAPATI, Makoto ISHIOROSHI and Kunihiko SAMEJIMA

(Apr. 1997)

ABSTRACT

The partial removal of calcium, phosphorus and lactose from yak rennet cheese whey prior to ultrafiltration (UF) significantly increased flux rate. The fresh yak cheese whey contained 4.8-5.0% lactose, 0.034% phosphorus and 0.031% calcium. The calcium, phosphorus and lactose content in the whey was reduced using low temperature crystalization (0 to −40℃) followed by separation of crystal residues. In comparison with initial calcium, phosphorus and lactose content, the level of calcium, phosphorus and lactose was reduced by freezing by 77.5, 79.4 and 16%, respectively. The flux rate of pretreated whey was increased by UF, and the time for whey protein concentration and membrane cleaning was reduced by about 30%. The protein content in the dry matter of UF concentrate was 75-79%. The UF filtrate of yak cheese whey contained 0.003% calcium.

Introduction

Whey is a by-product of cheese making which contains more than half of the non-fat solids present in the original milk, including about 20% of the protein and most of the lactose and minerals. Whey proteins, β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin, as well as immunoglobulins, are components of very high value to the dairy and food industry. The protein efficiency ratio (PER) of whey proteins (3.2) is very high compared to standard caseins (2.5), and that PER value remains constant during normal heating[19] and is also independent of type of whey.

Since 1971, it has been possible to obtain whey proteins in their native form by the application of ultrafiltration (UF), reverse osmosis (RO) and electrodialysis techniques. UF, RO and microfiltration (MF) are membrane processes based on the selective permeability of one or more components of a liquid mixture through a membrane barrier. The retentate of whey UF contains fat and colloidal minerals in a higher proportion than is present in untreated whey, and permeate consisting of water, soluble minerals, lactose, non protein nitrogen compounds and water-soluble vitamins. UF is commonly applied in the dairy industry to the manufacture of a variety fresh cheeses such as quarg, ricotta and camembert, or brine cheeses, and offers technological as well as economical advantages[10,12,13,14,15]. UF is used to standardize fresh milk in order to achieve the desired ratio of protein and total solids for cheese making. Currently, the application of these membrane processes is aimed at enhancing the manifestation of certain desirable functional properties in whey proteins, fractionating caseins and whey proteins, upgrading the quality of low-quality whey and standardizing milk. Whey, as a by-product of cheese making, is also processed by UF, RO and MF for the production of whey protein concentrate (WPC), whey protein isolate (WPI) and lactose.

The main problem in processing whey by UF or RO is mineral fouling of the membrane, principally by calcium and phosphorus complexes.

酪農学園大学食品科学科，肉製品製造学研究室 Neupaney Dhanapati, 石下真人, 鮫島邦彦
Department of Food Science, Meat Science and Technology, Rakuno Gakuen University, Ebetsu, Hokkaido, 069 Japan

During processing, proteins and calcium phosphates play a significant part in membrane fouling, and consequently membrane permeability[1,7]. Another membrane fouling component of whey is lipid. Der-Chan and Srinivasan[3] reported that the lipid content of whey prior to UF was complexed with chitosan and removed by centrifugation, which helped to prevent membrane fouling. Several researchers[4,5,10,19] have studied techniques for removing calcium from whey by complexing it with iron, magnesium, zinc and/or aluminium salts and precipitating the WP at high temperatures. Many studies have been carried out to investigate the effect of high temperature complexing of calcium and phosphates in various types of whey, but there appears to be no extant literature on low temperature complexing of calcium and phosphate in yak rennet cheese whey. Therefore, the present work aims at reducing fouling by calcium and phosphorus complexes in UF membrane surfaces by altering the pretreatment condition of the whey. pH was maintained at a constant during calcium and phosphorus aggregation so as to render precipitation as complete as possible. Lipid content in pretreated whey was removed by centrifugation.

Materials and Methods

Yak rennet cheese whey was procured from the Langtang and Chandanbari yak cheese production centres in the Rasuwa district of Nepal. For comparison, gouda cheese whey was also obtained from the experimental farm of Rakuno Gakuen University, Hokkaido Japan. The yak cheese whey was pasteurized at 62℃ for 30min and preserved using 0.04% H_2O_2. All the whey was centrifugally clarified at 10,000 xg. A portion of the yak rennet cheese whey was adjusted at pH4.5 using 0.1N HCl and slowly cooled down from 0 to −40℃. The frozen whey was then slowly defrosted and the sediments removed. This freezing and defrosting process was repeated three times.

UF procedure

A model UF SM 16525 (Sartorius, West Germany) was used to prepare the whey protein concentrate. This unit contained 15 sheets of cellulose triacetat membrane, each 225cm^2 in surface area. The pore size of the membranes was selected to cut off somewhere in the range of 10,000kDa. Operation was by batch procedure utilizing 3L lots. A temperature of 20℃ and inlet-outlet pressures of 1.5 bar were used. The whey was continuously circulated through the membranes and back to the holding tank until the permeate was removed to the desired protein concentration. The time taken to obtain the desired concentration was 2 and 3 hr for pretreated and fresh whey, respectively. After completion the WPC processing by UF, the membranes were washed by the surface active chemicals and detergents (Sartorius Co. Germany).

Analytical methods

Ash

Five g samples of yak milk and whey were placed in silica crucibles and evaporated at 100℃ for 2 to 3 hr. The crucibles were then carefully capped and transferred to a muffle furnace at 650℃ for 24 hr. The incinerated samples were removed from the furnace and cooled in a desiccator. Ash content was determined using the following formula:

% ash = weight of ash x 100/weight of sample.

Calcium, magnesium, sodium and potassium

Twenty five ml samples of yak milk and whey were placed in silica crucibles and dried in a furnace at 650℃ for 24hr. The ash was dissolved in 3ml of 3M HCl and made up to 250ml with deionised water. Aliquots of ash solution were used for determining the concentration of Ca, Mg, Na and K by the Murthy and Rhea[9] method using an A-6400F atomic absorption spectrophotometer (Shimadzu Co. Kyoto, Japan). Stock solutions and working solutions were prepared using standards from Cica Merck (Kanto Chemicals Co. Japan).

Protein

Casein from yak milk was isolated by isoelectric precipitation (pH4.6) at 25℃ using 1N hydrochloric acid. The proteins remaining in the

supernatant [whey protein (WP)] were then isolated by adding 12% (w/v) trichloroacetic acid, as described by Rowland[17]. The concentration of nitrogen in the precipitated casein, WP and trichloroacetic acid filtrate was determined by micro Kjeldahl method. The concentrations of casein and WP were determined as follows:
Casein=6.38 (TN-NCN); WP=6.38 (NCN-NPN)
where TN is total nitrogen, NCN is nitrogen in the pH4.6 filtrate, and NPN is nitrogen in the tricholoroacetic acid filtrate.

Fat

Fat content in yak milk and cheese whey was determined by the Gerber method using different type of butyrometers.

Lactose

Lactose content in the yak milk and yak cheese whey was determined according to the method described by Leonard et al.[5]. Twenty five g samples of yak milk and whey were put into 500ml volumetric flasks and diluted with 400ml deionised water. Fehling's solution A (10ml) and 0.1N NaOH (44ml) were then added and the volume made up to 500ml. The contents were thoroughly mixed, allowed to precipitate for 30min., and then filtered. This filtrate was used to determine lactose content. Twenty five ml of each Fehling's A and B solutions were placed in a 400ml beaker and mixed with a 50ml aliquot of the filtrate. The contents were covered with a watch glass and heated on an asbestos gauze over a Bunsen burner which had been previously adjusted so that the boiling point of the solution would be reached in exactly 4min. The contents were boiled for 2min and filtered immediately through a tared porcelain filtering crucible. The precipitated Cu_2O was thoroughly washed with 200ml of hot water using a suction flask. After removing the hot water from the suction flask, the crucible was further washed with 95% ethanol (10ml) first, and then with ethyl ether (10ml), later using suction. The crucible and the precipitate were then dried for 45min at 100℃, cooled for 20min., and weighed. The weight of lactose corresponding to the weight of Cu_2O was read according to the Munson and Walker table[5].

Chloride and salt

The salt content of the yak milk and yak cheese whey was determined according to the method described by Robert[18]. Nine g sample of yak milk and whey were taken, diluted with an equal amount of deionised water, and the chloride content determined. After adding 1ml potassium chromate indicator, the contents were titrated with 0.1N $AgNO_3$ till the end point (pale red-brown colour) was reached. Salt and chloride contents were calculated using the following formula:
% Chloride=(ml $AgNO_3$ xN $AgNO_3$ x0.0355 x100)/weight of sample.

Phosphorus

Phosphorus was determined according to the method described by Dieter et al.[2]. Standard phosphate solution was prepared by dissolving 0.3510g of pure dry monopotassium phosphate in water, adding 10ml of 10N H_2SO_4, and diluting to 1 liter. This solution contained 80μgm phosphorus per milliliter. Working standards were made from suitable dilutions of the standard solution. An appropriate amount of sample was digested with 1ml of 7.2N H_2SO_4 using a micro Kjeldahl flask on a micro Kjeldahl digestion rack until the colour changed, becoming colourless. After that, the flask was cooled and the contents transfered to a test tube to which 4.5ml of molybdate solution (0.25% w/v in water) and 0.5ml of ANS solution (1mg/ml in water) were added. The contents of the test tube were then heated in a hot water bath for 10min and cooled down to room temperature. The contents were poured into a 1cm cell and the absorbance read at 830nm using a Ubesto-50 spectrophotometer (Tokyo, Japan).

Results

Whey is the product obtained by using acids, or rennet and/or chemico-physical processes during cheese and casein production. Yak cheese whey is the milk serum obtained by the separation of casein in cheese making, predominantly by rennet, and contains less than 0.5% fat. The

composition of the milk and minerals contained in yak rennet cheese whey may vary, depending on the pasture in the Himalaya highlands from which it came and the breed of yak. Table 1 shows the minerals contained in yak milk, whey and UF filtrate. The calcium and phosphorus in yak cheese whey was significantly decreased with low temperature treatment before ultrafiltration. On the other hand, the magnesium, sodium, potassium and chloride content of milk, whey and UF filtrate were marginally changed in cheese making and UF processing.

Table 2 shows the gross composition of yak milk, yak cheese whey and WPC. Protein content in hot air dried WPC prepared by UF was found to be 75-79%. Generally the WPC prepared from fresh rennet cheese whey contained more than 4.1% ash. The ash content of WPC produced from pretreated whey was 3.9%. This low amount of ash in WPC may have been be caused by the minimization of the principal minerals of the whey e. g. calcium and phosphorus prior to ultrafiltration.

Table 1 Mineral content of yak rennet cheese whey before and after ultrafiltration in comparison with yak milk.

Contents in mg %	yak milk*	yak cheese whey*	whey after pretreatment*	UF filtrate*
Calcium	119	31	7	3
Magnesium	12	9	9	8
Sodium	60	59	59	57
Phosphorus	116	34	7	5
Potassium	150	148	148	148
Chloride	98	95	94	93

*Expressed results are average of five experiments.

Table 2 Gross composition of yak milk, yak cheese whey and yak whey protein concentrate.

Contents(%)	Yak milk*	Yak cheese whey*	Yak whey protein concentrate*(DM)
Water	80.6	93.3	5 - 6
Fat	7 -10	0.06	4.5-5.9
Lactose	4.8-5.0	4.9	6.0
Total protein	5.5	1.25	75-79
β-lactoglobulin	0.8	0.8	-
α-lactalbumin	0.3	0.3	-
Total solids	19.4	6.7	94
Ash	0.9	0.5	3.9

DM: In dry matter. *Expressed results are average or range of five experiments.

Peri and Setti[13] reported that applied pressure and flow velosity in UF did not affect retention of whey proteins. We applied constant pressure (1.5bar) and temperature (20℃) in each UF experiment. The fresh yak cheese whey contained 1.25% total protein. Protein content in the UF concentrate was gradually increased with volume reduction (Fig. 1A). The maximum concentration of protein in the UF concentrate was 14% at the time of final volume reduction (30). Volume reduction is the ratio of the initial volume of the whey divided by the sum of the difference in that initial volume and the UF filtrate.

Variation in ash content in the UF concentrate is shown at different volume reduction times in Fig. 1B. Ash content in the fresh yak cheese whey was 0.5%. Large amounts of calcium and phosphorus were removed from the fresh yak rennet cheese whey by freezing (Table 1). The ash content in the UF concentrate was insignificantly increased at different volume reduction times. The amount of ash in the UF concentrate was 0.43% at the time of the last volume reduction (30).

Increase in total solids (TS) content in the UF concentrate at different volume reductions is shown in Fig. 1C. The TS of the UF concentrate sharply increased with the removal of water. The principal solid component of the UF concentrate was WP. The final concentration of the UF concentrate was 20.2%.

The main solid component in acid and rennet cheese whey is lactose. The yak rennet cheese whey contained 4.8-5% lactose. Some of the lactose had crystalized during the process of low temperature treatment, and was separated together with the calcium-phosphate complex. Lactose acts as a membrane fouling component, along with calcium, phosphorus and lipids[19]. Ultrafiltration permeate mainly contains lactose and minerals. The lactose content at different UF aliquot concentrates and at various levels of volume reduction is shown in Fig. 1D. The result shows that the lactose content in the UF concentrate reached a maximum (4.37%) at the time of final volume reduction (30). This result indicates

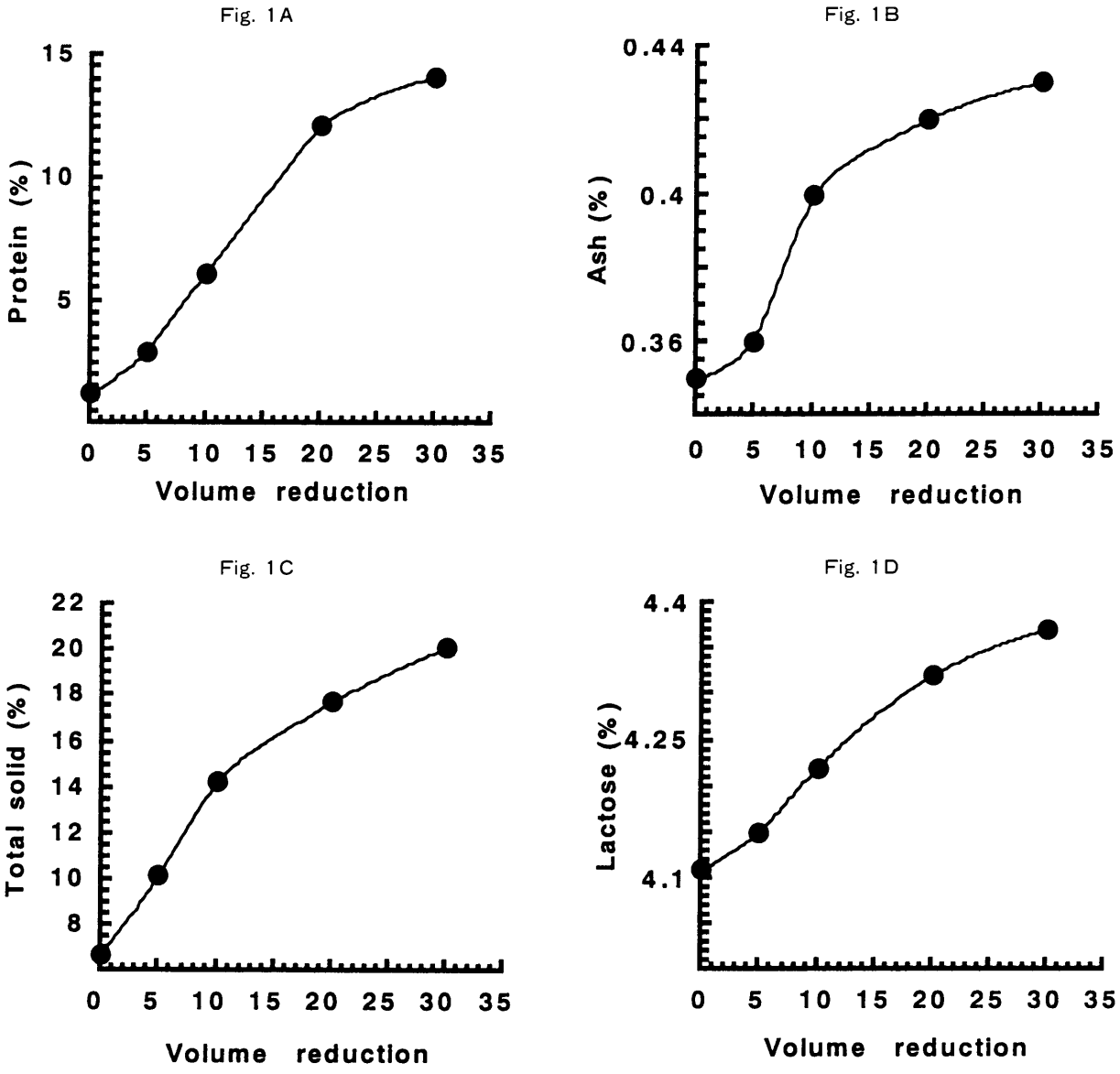

Fig. 1 Proportion of protein (1A), ash (1B), total solids (1C), and lactose (1D) in UF concentrate at different degrees of volume reduction.

that lactose concentration in the UF concentrate was marginally changed at different volume reductions.

UF flux in fresh gouda cheese, yak cheese and pretreated yak cheese whey is shown in Fig. 2. The flux rate of fresh yak rennet cheese whey and that of lactose, calcium and phosphorus level reduced whey was significantly varied. A lower flow rate was observed in the fresh whey in comparison with pretreated whey. The time taken to concentrate the fresh yak rennet cheese whey by UF and to achieve 20.1% TS was 3h. However, in the case of the pretreated whey under identical conditions, only 2h was required to produce the same concentration of TS. We believe that the fresh whey contained more lactose, calcium and phosphorus, which may have caused a fouling of the membrane surface leading to a longer time needed for concentration of the WP. When the lactose, calcium and phosphorus levels in the whey were reduced, the concentration time and cleaning time for the membrane were reduced by 30%. The results show that membrane fouling by calcium and phosphorus complex is more compact and stronger, making it take longer to wash from the surface of the

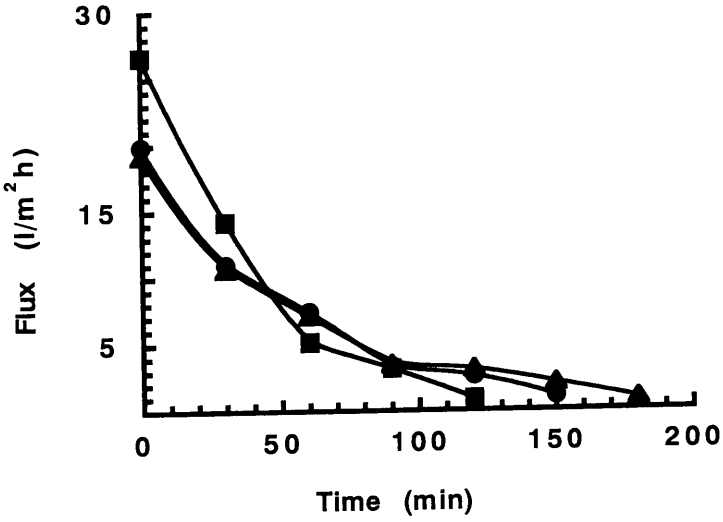

Fig. 2 Change in UF flux rate in relation to membrane fouling time. (●) flux rate of fresh gouda cheese whey; (▲) flux rate of fresh yak rennet cheese whey and (■) flux rate of pretreated yak rennet cheese whey.

membrane. The WP concentration time for fresh gouda cheese whey was lower than that for fresh yak cheese whey, but was higher than for pretreated whey (Fig. 2).

Discussion

A recent estimate of world whey production indicates an amount of approximately 100million tonnes containing about 7,00 000tonnes of whey protein[16]. Whey is processed by a number of manufacturing procedures resulting in a wide range of products, differing mainly in form and composition. Fundamental studies into the physico-chemical properties of individual major whey proteins have indicated that whey products with a high protein content are highly functional and suited to a range of applications requiring solubility, water binding, gelation or surface activity, such as in emulsion and foams. We carried out a comparative study into flux and WP concentration times in UF using fresh gouda cheese, yak rennet cheese and pretreated yak rennet cheese whey. The results showed that pretreated whey, where the content of lactose, calcium and phosphorus had been reduced, increased the flux rate and showed a shorter time for WP concentration and membrane washing. Reduction of such minerals is more important for certain human food requirements such as infant, baby foods and low sodium foods. Low pressure (1.5 bar) applied to the concentration of whey proteins showed a longer time taken to complete the concentration process. Application of low pressure also reduces the incidence of membrane fouling. Georges et al.[4] reported that higher pressure on membrane surfaces developed rapid fouling of the membrane. Our previous study on the structure of β-LG and α-LA[11] showed that the α-helices of these whey proteins were more affected by strong agitation, and it is believed that the application of low pressure helps to maintain the intact structure of whey proteins in the UF processing of whey. Slow and deep freezing of whey, not only formed a complex of calcium and phosphorus, but also formed fine white precipitate and aggrigates of lipoproteins which are easy to remove by either centrifugation or microfiltration. Our results for total solids at the time of 95% water being removed were higher than those reported by McDonough et al.[8]. This discrepancy might be due to the pretreatment of the whey which helped reduce the level of membrane fouling materials and increase the amount of total solids mainly by whey proteins. There-

fore, it is expected that the findings of this study may prove useful for the concentration of WP by UF, so that they may remain highly functional while maintaining a low concentration of calcium and phosphorus.

Conclusion

The results of this study show that the slow and deep freezing of yak rennet cheese whey formed a calcium and phosphorus complex which was easily removed by centrifugation and/or MF. The best results were obtained when the pH of the whey was adjusted 4.5 and centrifugation was performed before ultrafiltration.This pretreated whey increased the UF flux rate by 30%. Dry WPC produced by this method contained up to 79% whey protein.

Acknowledgements

The authors wish to thank Prof. Z. Saito for reading the manuscript and for his valuable comments, and Miss I.Yosida and Mr. J.Kim (doctoral course students of Rakuno Gakuen University, Department of Food Science) for assisting in the chemical analysis and atomic absorption spectrophotometry.

References

1) Dauffin, G., Michel, F., and Merin, U., 1992b. Study of ultrafiltration of deffated whey protein concentrates (WPC) withdrawn from an industrial plant. Lait. **72**: 185-189.

2) Dieter, H., Meun, C., and Kendric, C.M., 1968. A microphosphate method. Analytical biochemistry. **26**: 364-368.

3) Der-Chan, H., and Srinivasan, D., 1995. Selective precipitation and removal of lipids from cheese whey using chitosan. J.Agric. Food Chem. **43**: 33-37.

4) Georges, D., Francoise, M., Jean, P.L., Auguste, Q., and Andre, G., 1993. Ultrafiltration of defatted whey: improving performance by limiting membrane fouling. J. Dairy Res., **60**: 79-88.

5) Leonard, W., Edwin, A.W., and Marion, R.W., 1987. Food composition and analysis. AVI book publisher. New York.

6) Lawrence, R. C., 1985. Bull. Int. Dairy Fed. 240. pp.1-15.

7) Labbe, J. P., Quemerais, A., Michel, F., and Daufin, G., 1990. Fouling of inorganic membranes during whey ultrafiltration: analytical methology. Jurnal of Membrane Science. **51**: 293-307.

8) McDonough, F.E., Margrove, R.E., Mattingly, W.A., Posati, L.P., and Alford J.A., 1974. Composition and properties whey protein concentrates from ultrafiltration. J. Dairy Sci., Vol. **57**: 1438-1443.

9) Murthy, G.K., and Rhea, U., 1967. Determination of major cations in milk by atomic absorption spectroscopy. J. Dairy Sci., **50** (3): 313-317.

10) Mistry, V.V., and Maubois, J.L., 1992. In Advance dairy chemistry vol. 1. (Fox P.F. ed), pp. 493-522.

11) Neupaney, D., Samejima, K., and Ishioroshi, M., 1997. Animal scicnce and technology. (In press).

12) Ottosen, N., 1988. APV Technical information, protein standardization, APV Pacilac, Silkburg, Denmark.

13) Peri, C., and Setti, D., 1976. Whey and skim milk ultrafiltration, 1. Parameters affecting permeation rate in sweet whey ultrafiltration. Milchwissenschaft **31**: 135-138.

14) Puhan, Z., 1992. In IDF special issue No 9201. New application of membrane process. IDF Brucels Belgium. pp.23-32.

15) Pedersen, P.J., and Ottosen, N.,1992. In IDF special issue No.9201. New application of membrane processes. IDF Brucels Belgium. pp. 67-76.

16) Pearce, R. J., 1995. Current utilization of whey proteins in foods. Abstracts of International workshop on functional properties of food hydrocolloids. pp.2.

17) Rowland, S.J.,1938. The determination of nitrogen distribution in milk. J. Dairy Res., **9** : 42-46.

18) Robert, T.M., 1992. Standard methods for the examination of dairy products. Copyright 1993 by the American public Health Association, Washington, DC 2005.

19) Tadeusz S., and Carl L.R., 1990. Whey and whey utilization. Verlag Th. Mann, Gelsenkirchen-Buer Germany.

要　約

ヤクのレンネットチーズホエーのカルシウム，リン，乳糖を部分的に除去すると，限外ろ過の流動速度が明かに速くなった。新鮮なヤクチーズホエーは，4.8～5.0%の乳糖，0.034%のリン，0.031%のカルシウムを含有していた。ホエー中のカルシウム，リン，および乳糖の含有量は低温度処理（0から－40℃）後の遠心操作によって減少した。その減少割合は，カルシウムで77.5%，リンは79.4%，乳糖16%であった。ホエーからあらかじめカルシウム，リンや乳糖を除去することによって，限外ろ過の流動時間と膜の清掃時間は約30%減少した。限外ろ過後の乾燥粉末中にホエータンパク質は75～79%含まれていた。また，限外ろ過したヤクチーズホエーには0.003%のカルシウムが残っていた。