

## Microbiological stabilization of whey by cross flow microfiltration

G Mucchetti, P Taglietti \*

*Istituto Sperimentale Lattiero Caseario (ILC), Via Lombardo 11, 20075 Lodi, Milan, Italy*

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**Summary** — CFMF of Grana cheese whey, using a 0.8- $\mu\text{m}$  nominal pore size alumina membrane, reduced bacterial population by an average value of 4.6 decimal reductions. The bacterial count of MF whey always ranged between 0–1.9 log CFU ml<sup>-1</sup> and was not proportional to the population of the relevant feed. The 0.8- $\mu\text{m}$  membrane, when utilised under the technological conditions of this study, retained the natural Grana whey microflora almost completely. No significant protein retention was observed. The permeation fluxes of skim whey were > 600 l h<sup>-1</sup> m<sup>-2</sup> even after 4.5 h of filtration. CFMF of raw whey showed a decrease in permeation fluxes to  $\approx$  70% compared to the skim whey. The use of membranes of 0.1 or 0.45  $\mu\text{m}$  nominal pore size, although presenting a similar microbial removal efficiency, resulted in a higher protein retention and lower permeation fluxes. The current interest in the recovery of heat-sensitive whey proteins may increase the use of CFMF technology for the microbiological stabilization of whey as an alternative to the pasteurization process.

### microfiltration / whey / bacterial removal efficiency

**Résumé** — Épuration bactérienne du lactosérum par microfiltration tangentielle. La microfiltration tangentielle du lactosérum du fromage Grana, en utilisant une membrane en alumine avec diamètre moyen des pores de 0,8  $\mu\text{m}$ , a diminué en moyenne la population bactérienne de 4,6 réductions décimales. La population résiduelle du lactosérum microfiltré a montré des valeurs assez constantes, comprises entre 0 et 1,9 log UFC.ml<sup>-1</sup>, et ces valeurs n'étaient pas en relation proportionnelle avec le niveau initial de population bactérienne des fluides entrants. La membrane 0,8  $\mu\text{m}$ , dans les conditions opératoires utilisées, retenait presque complètement la microflore naturellement présente dans le lactosérum du Grana. Aucune rétention significative des protéines n'était observée. Les débits de perméation obtenus étaient supérieurs à 600 l.h<sup>-1</sup>.m<sup>-2</sup> pour le lactosérum écrémé, même après 4,5 h de filtration, par contre ces débits chutaient en moyenne de 70% lors du traitement du lactosérum non écrémé. L'utilisation des membranes ayant un diamètre de pore de 0,1 et 0,45  $\mu\text{m}$  entraînait une importante rétention protéique et des débits plus faibles de perméation, tout en conservant le même niveau d'épuration bactérienne. En raison de l'intérêt porté à la séparation des protéines thermosensibles du lactosérum, la technologie de microfiltration dans le but de réaliser l'assainissement bactérien du lactosérum apparaît comme une alternative au protocole de pasteurisation.

### microfiltration / lactosérum / épuration bactérienne

\* Present address: Caseificio Zani, Cigole, Brescia.

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## INTRODUCTION

The microbiological stabilization of whey, the first step in whey component recovery, is usually carried out by pasteurization; however, since this treatment has a denaturing effect on the structure of whey proteins (de Wit, 1981; de Wit and Klarenbeek, 1984; Morr, 1987), it may be not compatible with production objectives concerning fractionation and recovery of heat sensitive or native whey proteins.

The microbiological stabilization by cross flow microfiltration (CFMF) is one of the alternatives to pasteurization with respect to both microbial reduction and production capacity (Merin *et al*, 1983; Merin, 1986; Merin and Daufin, 1990): the efficiency of bacterial removal seems to be a function of the membrane pore size, the type of CFMF plant and the different bacterial species present in dairy liquids (Piot *et al*, 1987; Olesen and Jensen, 1989; Trouvé *et al*, 1991; Pedersen, 1991). It has also been observed that microfiltered (MF) whey improves permeation fluxes in successive ultrafiltration (UF) steps (Merin *et al*, 1983).

CFMF combined with thermocalcic aggregation has been proposed as a step to remove phospholipoprotein aggregates, as it has been postulated that they are an important part of the polarization layer (Fauquant *et al*, 1985) and one of the factors that contribute to limit UF fluxes (Maubois, 1984). Moreover, phospholipids may be an interesting product (Baumy *et al*, 1990) and their removal from whey would improve the purity of whey protein concentrates (Maubois *et al*, 1987).

The aim of this work was to test some membranes in view of the application of CFMF process to the microbial stabilization of whey.

## MATERIALS AND METHODS

Whey from Grana cheesemaking, produced at the ILC dairy (Lodi, Italy), was used. For some experiments, the whey was skimmed at the dairy with a centrifugal separator (Model CA 40-Frau, Vicenza, Italy) at 50 °C.

Fifteen liters of whey were used for each CFMF experiment, carried out in collaboration with the Hydro Air Research Company (Zerbo di Opera, Milan, Italy) which supplied a laboratory pilot plant with the following characteristics: 20 l batch running by a thermostated feed/product recycling tank, feed/recycling lobe pump with mechanical variator (Johnson Pump, Sweden); temperature and pressure gauges; filtering surface area of 0.0165 m<sup>2</sup>.

Three  $\alpha$  alumina membranes TI 70 (Membralox, SCT, Tarbes, France) of nominal pore size 0.1, 0.45 and 0.8  $\mu$ m were used. The permeation flux was measured using a stop watch and a calibrated flask (100 ml).

The following CFMF conditions were used: tangential flow velocity, 6.5 m.s<sup>-1</sup>; inlet and outlet pressure, 2.8 and 2.2 bar; operating temperature, 40–42 °C for the trials with skim whey and raw whey A, and 48 °C for the experiment with raw whey B. The microfiltered permeate and the retentate were recycled in a constant concentration mode in the feed tank.

Protein content was determined by the Kjeldahl method and by HPLC (Resmini *et al*, 1989). Fat content was measured by the Gerber method using Siegfeld butyrometers (Dr Gerber, Zurich, Switzerland) with 0.01% division.

The outlet line of the permeate was sanitized by recirculating H<sub>2</sub>O<sub>2</sub>, but not steam-sterilized before collecting the MF whey.

The total bacterial count (TBC) and eumycetic flora were tested: the former in plate count agar by incubation at 32 °C for 72 h and the latter in oxytetracycline–glucose agar by incubation at 25 °C for 5 d.

The microbial removal efficiency (*E*) was evaluated as decimal reductions using the following formula (Trouvé *et al*, 1991):

$$\log \text{CFU ml}^{-1} \text{ whey} - \log \text{CFU ml}^{-1} \text{ microfiltered whey} = E.$$

## EXPERIMENTAL RESULTS AND DISCUSSION

### Selecting the membrane pore size

The use of membranes with 0.1 and 0.45  $\mu\text{m}$  pore size with conventional CFMF equipment resulted in a high protein retention rate after 30 min; this was probably due to membrane fouling (Vetier *et al.*, 1986; Merin and Daufin, 1990; Daufin *et al.*, 1991). Daufin *et al.* (1991) showed a decrease in the nitrogen matter in MF whey obtained with a membrane of 0.2  $\mu\text{m}$  pore size, and noted that 30% of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin were retained. Overall, concomitant low permeation flux values were obtained, in the range of 200–120  $\text{l}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$ . However, such values are similar to those described for 0.25- $\mu\text{m}$  pore size membranes by Maubois *et al.* (1987). Tests showed that these pore size membranes were not useful for whey pretreatment with the CFMF equipment utilized, although they had a microbial removal efficiency of  $> 4.5$ .

The 0.8- $\mu\text{m}$  pore size membrane did not show significant protein retention after 4.5 h filtration (table I), as also confirmed by HPLC analysis of individual whey proteins. In figure 1 only 2 plots were reported, the others being similar. These results disagree with the findings of Merin *et al.* (1983), obtained with an organic membrane of the same pore size, which resulted in  $15 \pm 5\%$  protein retention. The suitability of a 0.8- $\mu\text{m}$  membrane for whey CFMF has also been claimed by Pearce *et al.* (1991).

CFMF of raw whey resulted in a decrease of permeation flux values of  $\approx 70\%$  as compared to the skim whey (fig 2). CFMF of raw or centrifuged whey produced MF whey with  $< 0.01\%$  residual fat.

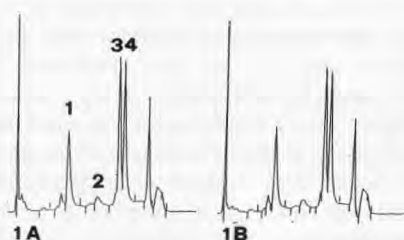
### CFMF efficiency on microbial removal

The microbial removal efficiency  $E$  obtained with the 0.8  $\mu\text{m}$  membrane ranged between 4.0–5.5 for each sample ( $n = 22$ ), with an average value of 4.6 (table II). The bacterial count of MF whey, determined

**Table I.** Protein and fat content of whey (w) and microfiltered whey (M).  
*Teneur en protéines et matière grass de lactosérum (w) et de microfiltrat de lactosérum (M).*

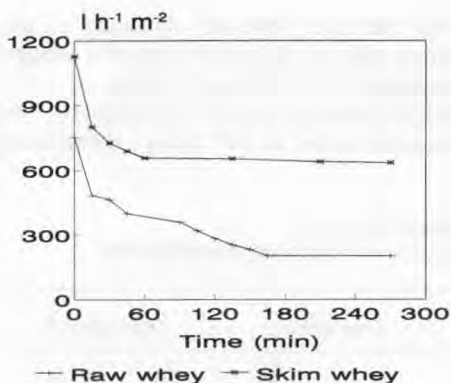
	Skim whey A		Skim whey B		Raw whey A		Raw whey B	
	W	M	W	M	W	M	W	M
Protein (%)	0.84	0.81	0.83	0.82	0.85	0.80	0.86	0.82
SD		0.10		0.02		0.02		0.03
CV (%)		1.19		2.79		2.11		3.77
Min value		0.80		0.79		0.78		0.79
Max value		0.82		0.85		0.83		0.87
<i>n</i>	1	4	1	5	1	7	1	6
Fat (%)	0.01	<0.01	<0.02	<0.01	0.65	<0.01	0.70	<0.01
<i>n</i>	1	4	1	5	1	7	1	6

SD: standard deviation; CV: coefficient of variation.



**Fig 1.** Chromatographic profiles by HPLC of proteins from crude whey (1A) and microfiltered whey collected after 280 min of CFMF (1B). Peaks: 1 =  $\alpha$ -lactalbumin; 2 = bovine serum albumin; 3 =  $\beta$ -lactoglobulin B; 4 =  $\beta$ -lactoglobulin A.

*Profils chromatographiques HPLC des protéines de lactosérum (1A) et de lactosérum microfiltré collecté après 280 min de traitement (1B). Pics : 1-  $\alpha$ -lactalbumine, 2- sérum albumine bovine, 3-  $\beta$ -lactoglobuline B, 4-  $\beta$ -lactoglobuline A.*



**Fig 2.** Permeation fluxes of skim and raw whey by cross flow microfiltration. (---+--- raw whey; ---\*--- skim whey).

*Débits de perméation de lactosérum cru et écrémé par microfiltration tangentielle. (---+--- lactosérum non écrémé; ---\*--- lactosérum écrémé).*

during and at the end of the process, always ranged between 0.0–1.9 log CFU ml<sup>-1</sup>; similar values have been previously reported by Merin *et al* (1983).

The MF whey count was not proportional to the bacterial count of the relevant feed. In fact, during filtration the bacterial count increased significantly in the retentate; the *E* value, calculated as log CFU ml<sup>-1</sup> retentate – log CFU ml<sup>-1</sup> relevant permeate ranged between 4.46–6.19 with an average value of 5.50 (table II): it should, however, be noted that in our experiments both liquids were recycled.

No eumycetic flora was detected in MF whey, while in the whey it varied from 2–3.52 log CFU ml<sup>-1</sup>.

These result showed that the *E* value was affected by the feed count, and that the alumina 0.8- $\mu$ m pore size membrane almost completely retains the natural Grana whey microflora, mostly composed of *Lactobacillus helveticus* and other thermophilic lactic acid rod bacteria (Bottazzi *et al*, 1977); the dimensions of such microorganisms reported in *Bergey's Manual* were 0.5–0.9  $\mu$ m thick by 2–9  $\mu$ m in length.

It should be emphasized that small differences in pore size are fundamental for filtration efficiency. Merin *et al* (1983) using a 1.2- $\mu$ m membrane measured 5–10-fold higher MF whey counts than those determined using a 0.8- $\mu$ m membrane. Trouvé *et al* (1991), using CFMF equipment with a uniform transmembrane pressure system and a 1.4- $\mu$ m membrane, microfiltered selected cultures of *L. helveticus* added to milk microfiltrate, whose biochemical composition is claimed to be similar to that of whey (Fauquant *et al*, 1988). The authors achieved an *E* value of 2.73, independent of the initial level of the bacterial population; the calculated *E* value increased to 3.63 when they microfiltered the same culture added to skim milk. This was in agreement with the data of Olesen and Jensen (1989). The different *E* values obtained by microfiltration of the same microbial cells added to milk microfiltrate and skim milk are probably due to the different fouling of

**Table II.** Microbiological characteristics of whey (W), retentate (R) and microfiltered whey (M): evaluation of bacterial removal efficiency *E* (decimal reductions) of a 0.8- $\mu\text{m}$  pore size MF membrane. *Caractéristiques microbiologiques de lactosérum (W), rétentat (R) et microfiltrat de lactosérum (M) évaluation de l'efficacité E (nombre de réductions décimales) d'une membrane de porosité 0.8  $\mu\text{m}$ .*

		Mean log CFU ml <sup>-1</sup>	SD	CV (%)	Min value	Max value	<i>n</i>
Skim whey A	W	5.90					1
	R	7.08					1
	M	1.53	0.31	20.61	1.14	1.90	4
	E1	4.37	0.31	7.10	4.00	4.75	4
	E2	5.18					1
Skim whey B	W	5.49					1
	R	6.79					1
	M	0.73	0.47	64.41	0.00	1.32	7
	E1	4.76	0.47	9.80	4.17	5.48	7
	E2	6.19					1
Raw whey A	W	6.00					1
	R	7.48					1
	M	1.11	0.35	31.70	0.60	1.64	7
	E1	4.89	0.35	7.20	4.36	5.40	7
	E2	6.19					1
Raw whey A	W	6.00					1
	R	7.48					1
	M	1.11	0.35	31.70	0.60	1.64	7
	E1	4.89	0.35	7.20	4.36	5.40	7
	E2	6.18					1
Raw whey B	W	6.00					1
	R	6.36					1
	M	1.45	0.57	39.61	0.70	1.90	4
	E1	4.55	0.57	12.62	4.10	5.30	4
	E2	4.46					1

E1 = log CFU ml<sup>-1</sup> whey - log CFU ml<sup>-1</sup> microfiltered whey; E2 = log CFU ml<sup>-1</sup> retentate - log CFU ml<sup>-1</sup> microfiltered whey.

membranes issued from the 2 fluids: Madec *et al* (1992) hypothesized that pre-microfiltration of milk with a 1.4- $\mu\text{m}$  pore size membrane reduced the milk components involved in the fouling of the 1.4- $\mu\text{m}$  alumina membrane. Membrane transfer of mi-

crobial cells during subsequent MF would therefore be easier as a consequence of reduced fouling.

Piot *et al* (1987), using a 1.8- $\mu\text{m}$  membrane reduced the total microflora of raw milk by 2 orders of magnitude.

## CONCLUSIONS

A 0.8- $\mu\text{m}$  pore size alumina mineral membrane is suitable for microbial stabilization of whey by CFMF, the MF whey microbial count always being  $< 2.0 \log \text{CFU ml}^{-1}$ . Such microbial reduction without thermal treatment at temperatures  $> 50\text{--}55^\circ\text{C}$  may be the first step in the recovery of heat-sensitive or native proteins from whey.

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