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Separation Science and Technology
Publication details, including instructions for authors and subscription information:
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Microfiltration of Bovine and Ovine Milk for the Reduction of Microbial Content: Effect of Some Operating Conditions on Permeate Flux and Microbial Reduction

Francesca Beolchini[a]; Silvano Cimini[b]; Lia Mosca[b]; Francesco Veglio[b]; Diego Barba[b]
[a] Dipartimento di Scienze del Mare, Universita' Politecnica delle Marche, Ancona, Italy [b] Dipartimento di Chimica, Ingegneria Chimica e Materiali, Universita' dell'Aquila, Italy

To cite this Article Beolchini, Francesca , Cimini, Silvano , Mosca, Lia , Veglio, Francesco and Barba, Diego(2005) 'Microfiltration of Bovine and Ovine Milk for the Reduction of Microbial Content: Effect of Some Operating Conditions on Permeate Flux and Microbial Reduction', Separation Science and Technology, 40: 4, 757 — 772
To link to this Article: DOI: 10.1081/SS-200048160
URL: http://dx.doi.org/10.1081/SS-200048160

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Separation Science and Technology, 40: 757–772, 2005
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ISSN 0149-6395 print/1520-5754 online
DOI: 10.1081/SS-200048160

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Francesca Beolchini
Dipartimento di Scienze del Mare, Universita' Politecnica delle Marche, Ancona, Italy

Silvano Cimini, Lia Mosca, Francesco Veglio, and Diego Barba
Dipartimento di Chimica, Ingegneria Chimica e Materiali, Universita' dell'Aquila, Italy

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Abstract: In the present work a study on bovine and ovine milk microfiltration in a tubular ceramic membrane is reported. The study was aimed at the reduction of milk microbial content through a *cold technology*, with the obvious advantages for the preservation of essential elements. Considering the low availability of ovine milk, which was daily provided by local farmers, most of the tests were performed with bovine milk, which was commercially available. Few tests were then performed also with ovine milk. Firstly, some tests were performed using bovine milk with different fat content. In the presence of fats, a *gradual* start-up procedure (realized by treating a milk diluted with distilled water prior to microfiltration of pure milk) was demonstrated to be fundamental for the achievement of a permeate. Further tests with skim bovine milk evidenced the positive effect of temperature and the negative effect of transmembrane pressure on permeate flux: in fact, average permeate fluxes were 850 and 650 $L\,h^{-1}\,m^{-2}$ when trans-

Received April 12, 2004, Accepted October 10, 2004.

Authors are grateful to Mr. Marcello Centofanti, Dr. Raffaella Marino, and Dr. Laura Patini for their helpful collaboration in the experimental work.

Address correspondence to Francesca Beolchini, Dipartimento di Scienze del Mare, Universita' Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy. E-mail: f.beolchini@univpm.it

membrane pressure was 0.6 and 1.9 bar, respectively, at 40°C, and 650 and 400 L h^{-1} m^{-2}, respectively, at 30°C. The subsequent tests with ovine skim milk, performed at 40°C and 0.6 transmembrane pressure, revealed a significant flux decline from 700 to 200 L h^{-1} m^{-2} in the first 60 min processing and a relatively stable permeate flux around 200 L h^{-1} m^{-2} for further 30 min. In any case, the microbial decimal reduction was about 2–3, in agreement with values found in the literature.

Keywords: Microfiltration, tubular membrane, ovine milk, bovine milk, transmembrane pressure, fats content

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INTRODUCTION

Cross-flow microfiltration has emerged as an industrial separation technology in the dairy industry for many applications, such as the removal of bacteria from skim milk, whey defatting, and micellar casein enrichment of cheese-making (1). In the case of milk, the reduction of microbial content must be achieved in such a way that the functionality of milk proteins is not affected, especially when the milk is to be used for cheese production (2). Consequently microfiltration performed at temperatures lower than 40–50°C represents an interesting alternative to thermal treatments aimed at milk debacterization, which might cause a partial protein denaturation (3). The main problem in the microfiltration of milk is that most fat globules and some of the proteins are as large as bacteria, resulting in a very rapid fouling of the membrane. This fouling is due to the deposition of a layer on the membrane surface and to the constriction of pores that consequently change the microfiltration performances (2). A fundamental help is given by the back-pulse device, which allows a partial cleaning of the membrane, by periodically reversing the transmembrane pressure (2).

Numerous studies have led to the technology and the equipment called Bactocatch® by the Tetra Laval Co. (1) applied on bovine skim milk. No significant application was found in the literature for the treatment of ovine milk.

The aim of the present work was to perform a study on bovine and ovine milk microfiltration. A previous preliminary investigation was already reported, which demonstrated the potential application of microfiltration both for bovine and ovine milk debacterization (4). In this case, a deep study on the effect of the main operating conditions was performed. Considering that ovine milk was daily provided by local farmers and was not always available, most of the tests were performed with bovine milk, which was commercially available. Further tests were then performed with ovine milk. The effect of fats content, temperature, and transmembrane pressure on permeate flux was evidenced, also monitoring the microbial decimal reduction.

MATERIALS AND METHODS

Milk

Three kinds of commercially available bovine milk were tested: a skim milk Punto Weight Watchers, a partially defatted Parmalat, a whole one Parmalat. Considering that it was treated by UHT (ultra high temperature) by the producer, and where specified, a contamination with raw ovine milk bacteria was performed in order to increase the bacterial population to measurable numbers. As concerns ovine milk, it was provided by a local farmer and stored for maximum 1 day at 4°C (L'Aquila, Italy). Table 1 shows the characteristics of all processed fluids. It can be observed that the pH of all kinds of bovine milk was 6.5, instead of the typical value of natural bovine milk which is 6.7–6.75. This slight drop might be attributed to treatments performed to milk prior to commercialization (UHT). Before microfiltration tests, ovine milk was previously centrifugated at 4000 rpm × 5 min (centrifuge *CHERMLE* mod *ZK380*), in order to remove fats, and filtered (pressure filter *MILLIPORE* 142 MM-paper filter).

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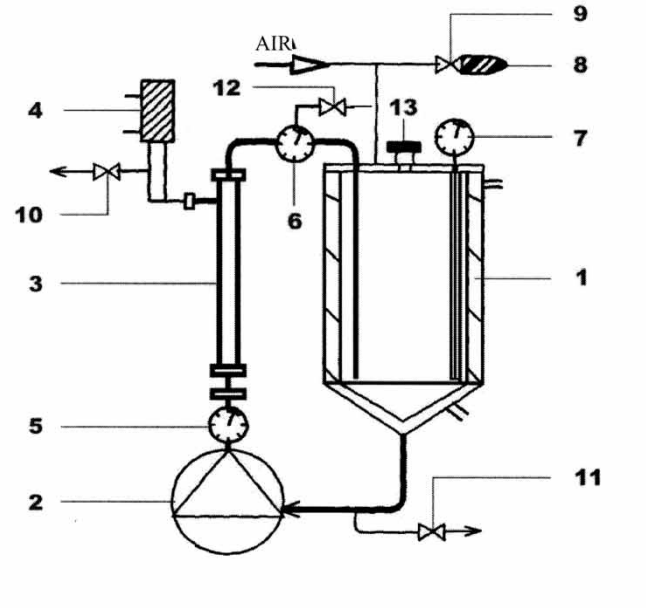
Experimental Apparatus for Microfiltration

Figure 1 shows the experimental apparatus employed. It is a tangential flow laboratory pilot plant *Membralox® XLAB3* (*EXEKIA*, Bazet, France), with a single tube ceramic membrane *Membralox® T1-70* (pore diameter 1.4 μm, membrane surface area 50 cm^2). Recirculation pump gives a tangential velocity of about 7 m/s, nominal flowrate 1 m^3/h. Temperature is controlled by the tank jacket, which is connected to a thermostate *CRIOTERM 10-80*. The plant is equipped with a *Back-pulse BF3*, controlled by an electrovalve (pressure 7 bar; reinjected volume 3 mL, activation for 1 sec every 1 min (4)). The washing procedure is shown in Table 2. During microfiltration tests,

Table 1. Physical properties of the tested milks

Physical property	Punto weight watchers bovine skim milk	Parmalat partialy defatted bovine milk	Parmalat whole bovine milk	Local farmer whole ovine milk
pH	6.5	6.5	6.5	6.6 ÷ 6.7
Proteins (%)	3.2	3.2	3.2	6.1
Lactose (%)	4.8	4.8	4.8	4.2
Calcium (%)	1.2	1.2	1.2	2
Fats (%)	0.05	1.55	3.35	7.6
Microbial content (UFC/mL)	—	—		6 ÷ 7 10^6



LABEL	COMPONENT
1	Jacketed feed tank
2	Pump
3	Membrane module *T1-70*
4	*Back-pulse BF 3*
5-6	Manometer (0÷4 bar)
7	Temperature gauge
8	Muffler
9	Valve
10	Valve
11	Valve
12	Air purge valve
13	Stopper

Figure 1. Experimental apparatus employed for microfiltration tests.

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Table 2. Washing procedure for the pilot unit

Washing solution	Temperature (°C)	Duration (min)
Ultrasil P 25	60	20
Ultrasil P 25	80	60
Distilled water	60	Up to neutral pH
HNO_3 1%	60	30
Distilled water	60	Up to neutral pH

after setting temperature, 30 min were waited in order to re-establish salts-proteins equilibria. Microfiltration tests were operated in concentration mode.

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Analytical Determinations

Lactose concentration was determined through lactose/galactose UV method, Boehringer Mannheim. Calcium content was determined by complexometric titration with EDTA, in the presence of calconcarbonic acid as an indicator (5). Protein concentration was determined by the Kjeldahl's method for protein nitrogen (heating digester Velp Scientifica mod. DK6; automatic steam distilling unit Velp Scientifica mod. UDK 130; automatic titrator Crison mod. microTT 2050); the analyzed values of elemental N were multiplied by 6.38 in order to obtain protein concentration. Fats were determined by Gerber butirrometer (5). Microbial content was determined by direct count on solid medium (6).

Data Analysis

Bacteria removal was expressed in terms of microbial decimal reduction (3), R_D, defined as:

$$R_D = \log N_0/N_P \tag{1}$$

where N_0 and N_P represent the microbial content in the fed milk and in the permeate, respectively.

The following empirical models were used to represent permeate flux (J_p) decline vs. time (t) (7):

$$J_p = J_\infty + D\mathrm{e}^{-t/\tau} \tag{2}$$

$$J_p = a - bt \tag{3}$$

where D ($L\,h^{-1}\,m^{-2}$) is the drop in flux from the start of the experiment to the development of steady state and it is indicated in the following as *stationary flux decline*, J_∞ ($L\,h^{-1}\,m^{-2}$) is the steady-state flux, τ (min) is the flux decline time constant, that is the time where the 63% of the stationary flux decline is achieved, a and b are empirical parameters. All parameters were estimated during fitting of equations (2) and (3) to experimental data by nonlinear regression techniques (8).

Permeate flux vs. fats concentration was mathematically represented by the film model, usually applied for proteins concentration (7):

$$J_P = k \ln \frac{C_G}{C_{fats}} \tag{4}$$

where k ($m\,s^{-1}$) represents fats mass transfer coefficient and C_G (%) a critical fats concentration (in the case of proteins it is proteins gelification concentration (7), but it is not correct here to talk about a fats "gelification concentration").

The milk components' retention coefficient was calculated according to the following relation:

$$\sigma = 1 - \frac{C_P}{C_R} \tag{5}$$

where C_P and C_R were experimentally determined in the permeate and in the retentate, respectively. The considered components were proteins, calcium, and lactose.

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RESULTS AND DISCUSSION

In this section, the experimental results obtained during microfiltration tests of bovine and ovine milk are presented. Taking into account the relatively low availability of ovine milk, which was daily provided by local farmers, most of the tests were performed with bovine milk, which was commercially available (see Table 1). A few tests were also then performed with ovine milk.

Bovine Milk

Effect of Fats Content on Permeate Flux

A first investigation was performed in order to verify the effect of fats content on permeate fluxes. This aspect might be very important: in fact, microfiltration is usually preceded by a centrifugation operation, aimed at the removal of fats. Consequently, a study performed at different fats levels simulates

microfiltration performances after an inefficient centrifugation (or, in the case of whole milk, in the absence of centrifugation). These experiments were realized with the three different types of bovine milk (Table 1): skim, partially defatted, and whole. Considering that in a preliminary test no permeate flux was observed in the case of whole milk, a gradual start-up procedure was performed, by treating pure milk only after previously treating a diluted milk with distilled water. Figure 2 shows permeate fluxes vs. time profiles in the case of pure whole (a), partially defatted (b), and skim (c) milk, both for gradual and direct (treating directly pure milk) start-up procedure. Data are related to pure milk microfiltration: in any case data obtained during treatment of diluted milk have not been reported.

Results in Fig. 2 evidence the significant effect of the start-up procedure, especially in the presence of fats. In fact no permeate flux was obtained with direct start-up on whole milk and a very low one in the case of partially defatted milk (Fig. 2b). On the other hand, no significant effect of the start-up procedure was observed in the case of skim milk, after a preliminary period of about 20 min. Obviously, the initial permeate flux with pure milk in the case of direct start-up was higher than the one obtained with gradual start-up, because the membrane was clean at the beginning of the process; but after a transitory of about 20 min the profiles obtained for the two start-up procedures were similar.

Figure 3 shows the average values (gradual start-up) of permeate flux (calculated during time, not considering values obtained in the first 20 min) represented as a function of fats concentration natural logarithm. It can be observed that a straightline relationship is suitable for data fitting with a very high square regression coefficient ($R^2 = 0.996$) although the low number of data points. Consequently, Eq. (4) parameters, k and C_G, were estimated after linear regression analysis ($k = 2\ 10^{-5}\,\mathrm{m\,s^{-1}}$; $C_G = 53.6\%$). It is known that Eq. (4), which represents the film model (7), is valid for proteins, which form a gel layer on the membrane surface, but an analogy for fats was performed, considering the perfect agreement between experimental and calculated data. Furthermore, this equation can be used to predict permeate fluxes in the case of varying fats concentration in milk, as it happens during the whole year (9).

A very interesting aspect comes out from the profile obtained with whole milk, in the case of gradual start-up (Fig. 2a). In fact, permeate flux does not decline significantly with time: it starts from $280\,\mathrm{L\,h^{-1}\,m^{-2}}$ and it is $210\,\mathrm{L\,h^{-1}\,m^{-2}}$ after 80 min processing. It is obviously lower than fluxes obtained in the case of skim milk (around $500\,\mathrm{L\,h^{-1}\,m^{-2}}$) but it might be interesting to hypothesis a process without centrifugation. In fact, the removal of bacteria from whole milk without cream separation would be a more economical process even if it implies higher membrane areas. Further work should be performed in this direction, both with ovine and bovine milk gradual start-up.

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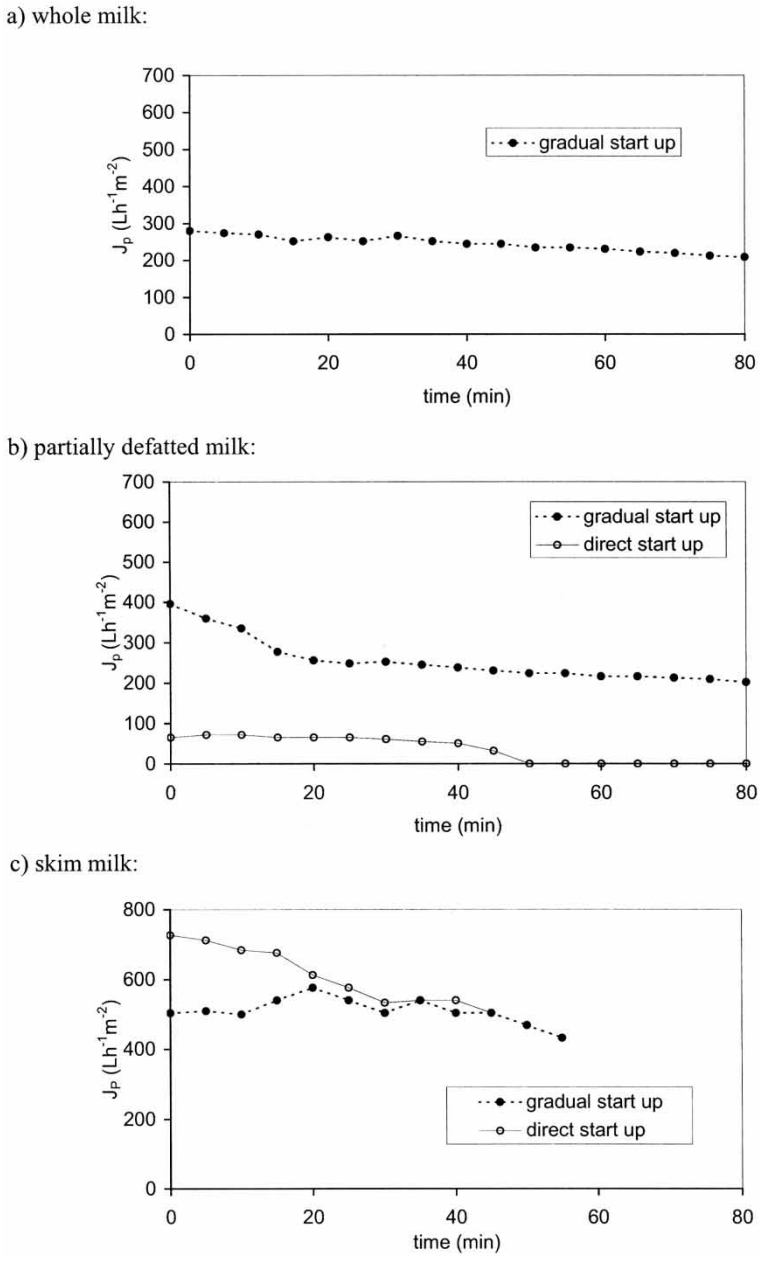

Figure 2. Permeate flux vs. time profiles in the case of whole (a), partially defatted (b), and skim (c) bovine milk: effect of the start up procedure (TMP 0.9 bar, temperature 30°C). No permeate flux was observed with whole milk (a) in the case of direct start-up.

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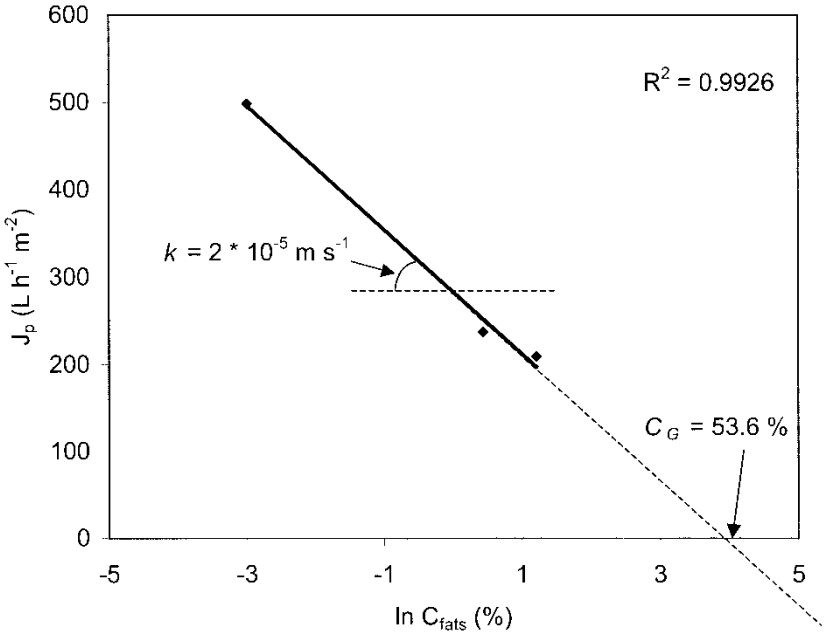

Figure 3. Average permeate flux vs. fats concentration: estimation of mass transfer characteristic parameters (Eq. 4).

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Effect of Transmembrane Pressure and Temperature

Both transmembrane pressure and temperature might significantly influence permeate fluxes. Consequently the present investigation was performed in order to optimize these two operating conditions with respect to permeate flux and microbial reduction. Table 3 shows factors and levels investigated. Temperature levels were 30 and 40°C, since higher temperatures would not give a "raw" milk (1). Transmembrane pressure goes from 0.6 to 1.9 bar, in a range typical of literature. Experimental tests were performed with bovine skim milk (Table 1), where a previous contamination with raw ovine milk bacteria was performed, in order to increase the bacterial population to measurable numbers ($\approx 10^7$).

Table 3. Factors and levels investigated with bovine skim milk

Factors	Levels			
Temperature	30		40	
Transmembrane pressure	0.6	0.9	1.4	1.9

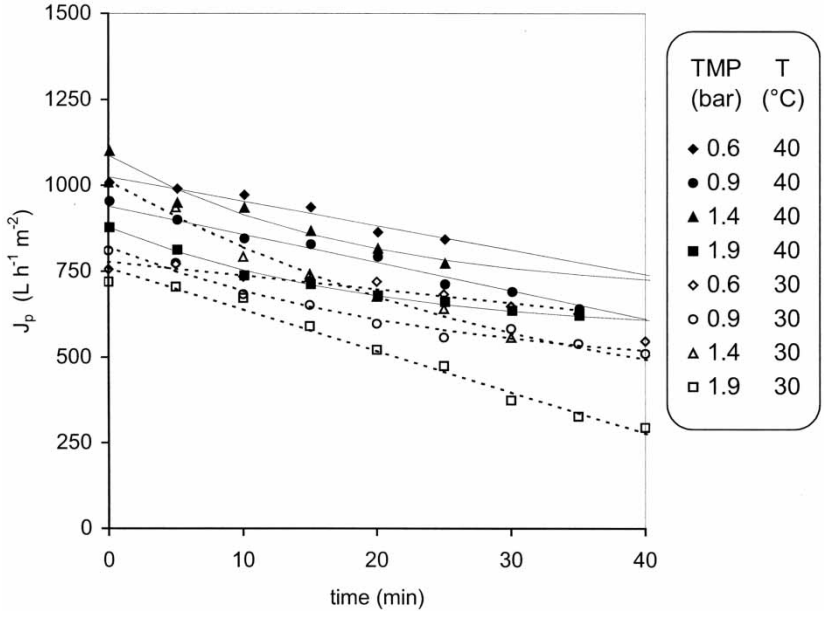

Figure 4. Permeate flux vs. time profiles during microfiltration of contamined bovine milk (continuous lines have been calculated by Eqs. (2) and (3)—see Table 4 for tests at 40°C and dotted lines for tests at 30°C).

Figures 4 to 6 show the obtained results in concentration tests. Figure 4 shows permeate flux vs. time profiles, obtained in all tests. Lines have been calculated either by Eqs. (2) or (3), according to which one has given the best fitting. Table 4 shows also the estimated values for parameters, together with standard errors (95%). A first analysis of data in Fig. 4 and in Table 4 suggests the positive effect of temperature on permeate fluxes, as expected (7). In fact, almost all experimental determinations obtained at 40°C are higher than the ones at 30°C. Figure 5 shows the permeate flux determined after the first 30 min microfiltration as a function of transmembrane pressure and temperature. It is evident that while temperature has the expected effect, on the other hand transmembrane pressure has an unexpected effect on permeate flux. In fact, Fig. 5 shows clearly that permeate flux decreases when transmembrane pressure increases, both at 30°C and 40°C. This aspect will be discussed later, together with the next figures.

Figure 6 shows the microbial decimal reduction, calculated according to Eq. (1), as a function of temperature and transmembrane pressure obtained in the same tests previously reported in Table 4 and Figs. 4 and 5. It can be observed that this parameter is around 2 when transmembrane pressure ranges from 0.5 and 1.5 bar, at any investigated temperature; on the other

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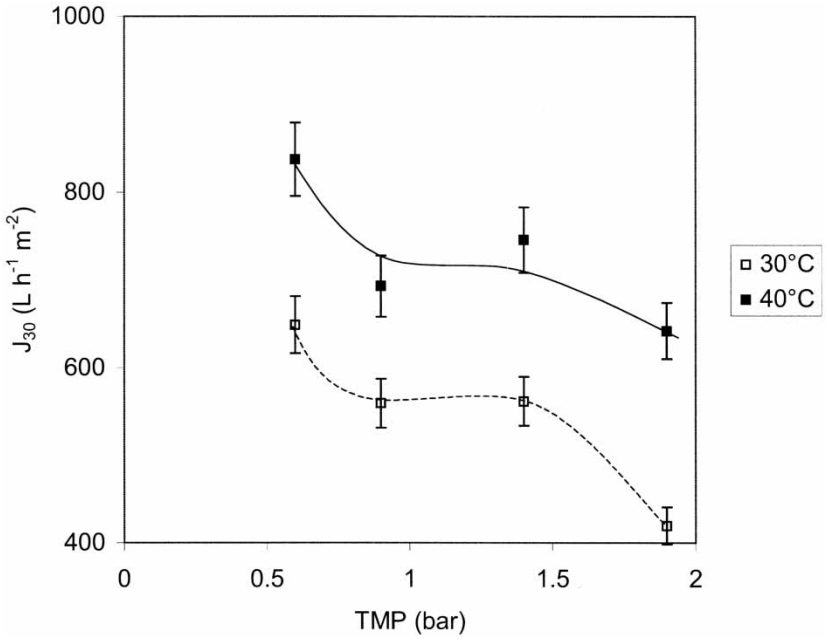

Figure 5. Permeate flux after 30 min microfiltration as a function of transmembrane pressure and temperature, in the case of contamined skim milk microfiltration.

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hand, a significant effect of temperature is evident when transmembrane pressure is 1.9 bar. In fact, the microbial decimal reduction increases as temperature decreases: it is about 3 at 30°C. The increase of cells retention (associated to a relatively high value of the microbial decimal reduction) with transmembrane pressure is probably due to a deposition on the membrane surface of a consolidated layer (consisting of microrganisms and other milk components) which acts as a membrane itself and also causes a relatively low permeate flux (see Fig. 5). The formation of this layer is reasonably strongly influenced not only by pressure but also by temperature: this can explain why the same increase of microbial retention was not observed at 40°C and 2 bar. As concerns lactose, calcium, and proteins, they were not retained by the membrane in the investigated experimental conditions. Consequently the permeated milk contained lactose, calcium, and proteins at the same concentrations as fed milk (Table 1).

Figure 7 gives a confirmation of the layer's deposition on the membrane, which takes place at high pressures. In this figure, the permeate flux is reported as a function of transmembrane pressure, during processing, as before, of bovine skim milk in two different cases: after a 0.6 bar concentration test (test N. 5 in Table 4) and after a 1.4 bar concentration test (test N. 8 in

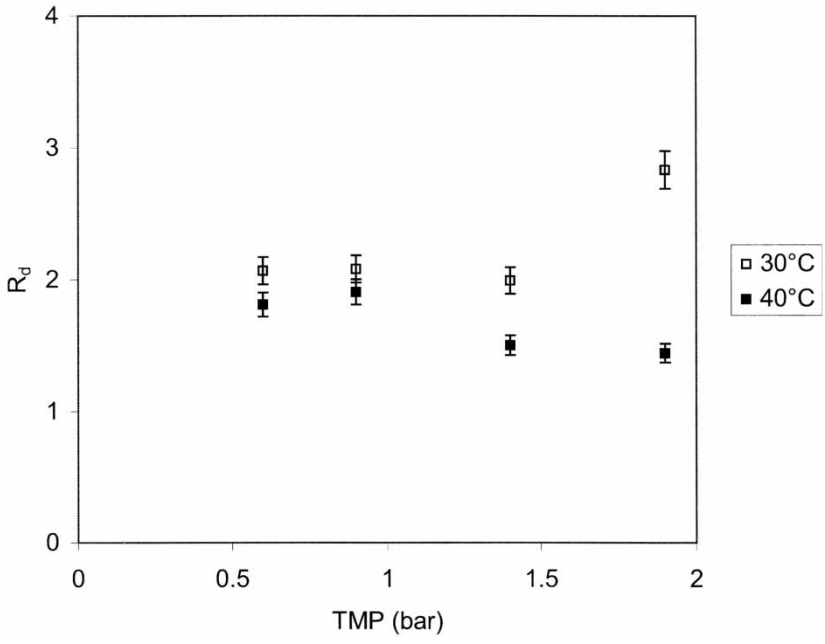

Figure 6. Microbial decimal reduction as a function of transmembrane pressure and temperature, in the case of contamined skim milk microfiltration.

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Table 4). Transmembrane pressure was changed in two directions: firstly, increasing its value from 0.6 to 2.2 bar and then decreasing it, in the same range.

The following remarks come out from the results shown in Fig. 7:

The operating pressure of the preceding concentration test significantly influences permeate fluxes. In fact relatively high permeate flux were determined when transmembrane pressure was increasing, in the case of a prior operating pressure of 0.6 bar. On the other hand significantly lower values for permeate flux were observed with a prior operating pressure of 1.4 bar.

Important hysteresis phenomena are evident (7), especially in the case of a prior operating pressure of 0.6 bar. This was also found in the literature, by Guerra et al. (2).These aspects confirm that an irreversible deposition of a layer takes place on the membrane surface as pressure increases, and suggest that relatively low values for the transmembrane pressure should be chosen in the application of microfiltration for microbial removal. In conclusion, the following operating conditions have been chosen for the subsequent ovine milk microfiltration tests: transmembrane pressure 0.6 bar and temperature 40°C.

Table 4. Experimental treatments and flux decline fitting results (bovine skim milk, Table 3)

Treatment	Temperature (°C)	TMP (bar)	Best equation for flux decline fitting	Parameters
1	30	0.6	(3)	$a = 778 \pm 17$ $b = 4.0 \pm 0.6$
2	30	0.9	(2)	$J_\infty = 452 \pm 99$ $D = 369 \pm 95$ $\tau = 24 \pm 12$
3	30	1.4	(2)	$J_\infty = 279 \pm 114$ $D = 736 \pm 102$ $\tau = 32 \pm 10$
4	30	1.9	(3)	$a = 760 \pm 28$ $b = 12.1 \pm 0.9$
5	40	0.6	(3)	$a = 1025 \pm 16$ $b = 7.1 \pm 0.6$
6	40	0.9	(3)	$a = 939 \pm 19$ $b = 8.2 \pm 0.6$
7	40	1.4	(2)	$J_\infty = 683 \pm 43$ $D = 406 \pm 44$ $\tau = 18 \pm 6$
8	40	1.9	(2)	$J_\infty = 570 \pm 19$ $D = 308 \pm 19$ $\tau = 19 \pm 3$

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Ovine Milk

Figure 8 shows permeate flux (left axis) and microbial decimal reduction (right axis) vs. time profiles during microfiltration tests performed under the previously chosen operating conditions: transmembrane pressure 0.6 bar and temperature 40°C. These conditions were considered as optimum for both permeate flux and microbial retention in the case of skim bovine milk, and, even if it has a different composition, they were chosen also for ovine milk. Prior to microfiltration, ovine milk was centrifuged in order to remove fats and filtered (paper filter) in order to remove any other suspended solids, as had emerged from preliminary investigations reported elsewhere (4).

Results in Fig. 8 evidence a significant flux decline in the first 60 min processing. In fact, permeate flux ranges from about 700 to about 200 $L\,h^{-1}\,m^{-2}$.

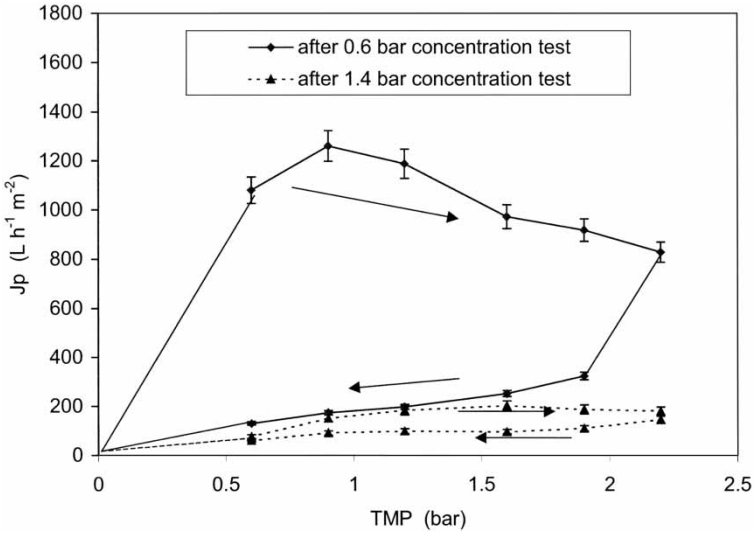

Figure 7. Permeate flux as a function of transmembrane pressure during permeability tests performed after concentration tests at 0.6 and 1.4 bar transmembrane pressure (temperature 40°C).

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On the other hand a relatively stable permeate flux around 200 L h^{-1} m^{-2} was observed for a further 30 min. An increase of the microbial decimal reduction from about 2 (at the beginning) to about 3 (at the end of the process) can also be observed. Both these profiles evidence the very fouling nature of ovine milk: the decrease of permeate flux is probably caused by a partial occlusion of membrane pores which implies an increase in the microbial retention. A comparison with permeate flux vs. time profiles obtained in the test with bovine skim milk under the same experimental conditions (test N. 5 in Table 4) gives a further indication of the very fouling characteristics of ovine milk with respect to the bovine one. In fact permeate flux ranges from about 1000 to 900 L h^{-1} m^{-2} with bovine milk in the first 30 min processing (see Fig. 4), while it ranges from about 700 to 350 L h^{-1} m^{-2} with ovine milk in the same period of time. Nevertheless, the obtained values for microbial decimal reduction are satisfactory and in agreement with values in the literature (Table 5). As concerns proteins, calcium, and lactose, they were not retained by the membrane, like in the case of bovine milk. Further tests are now in progress just with ovine milk, aimed at the optimization of microfiltration's performances considering its differences with respect to bovine milk. Transmembrane pressure will be an important parameter to be considered, considering that the obtained results suggest that its optimum value might be lower than 0.6 bar.

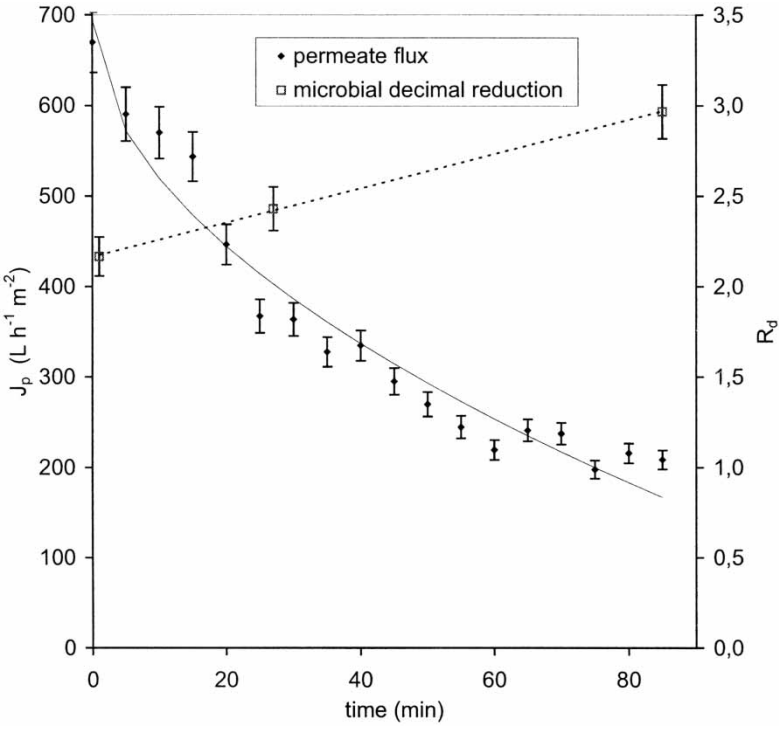

Figure 8. Permeate flux decline and microbial decimal reduction during microfiltration of ovine milk (TMP 0.6 bar, temperature 40°C).

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CONCLUSIONS

The following aspects come out from the performed work:

- Experimental tests with whole and partially defatted milk evidenced that a gradual start-up make possible the removal of bacteria from whole milk

Table 5. Microbial decimal reduction in the literature

Ref.	Milk	Microbial decimal reduction (R_d)
(10)	Bovine	3
(3)	Defatted bovine	2.6
(11)	Defatted goat	2.34
(12)	Defatted bovine	4 ÷ 5
This work	Defatted bovine	2
This work	Defatted ovine	2 ÷ 3

without a prior cream separation. According to this start-up procedure, milk can be microfiltrated just after previously processing a diluted milk in order to prepare the membrane.

- Microfiltration tests with bovine skim milk evidenced optimal operating conditions in the investigated range. In particular, it is very important to have good control of transmembrane pressure, considering that permeate fluxes decrease when transmembrane pressure increases.
- Microfiltration tests with ovine milk demonstrated that the process is effective also with this kind of milk, even if the fouling reduction still needs to be optimized.

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