Removal of lactate from acid whey using nanofiltration

Jayani Chandrapala^{1*}, George Q. Chen^{2*}, Kezia Kezia², Emily G. Bowman², Todor Vasiljevic¹, Sandra E. Kentish^{2**}

¹Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, Werribee 3030, Australia

²ARC Dairy Innovation Hub, Department of Chemical and Biomolecular Engineering, University of Melbourne, Victoria 3010, Australia.

Running Title: Removal of lactic acid from acid whey using nanofiltration

**Corresponding Author: Prof Sandra Kentish

Tel: + 61 3 8344 6682

Email: sandraek@unimelb.edu.au

*These authors contributed equally and thus regarded as joint first authors.

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ABSTRACT

The utilisation of acid whey by the dairy industry is limited by the high concentrations of lactate in the solution. In the present study, three commercially available nanofiltration membranes: HL, XN45 and DK were used in to evaluate the separation of lactose and lactate as a function of pH. Raw acid whey was used after microfiltration, while two laboratory prepared solutions comprising lactic acid and lactose; and sodium lactate and lactose were also tested for comparison. The rejection of lactic acid was found to increase with the degree of acid dissociation. About 50% of the lactate could be removed at pH ~3 due to the lower degree of dissociation. For all pH values tested, over 90% of the lactose was retained. The molecular weight cutoff and permeability of the membranes had little influence on the separation performance.

1 Introduction

The production of fermented dairy products involves the generation of waste streams termed “whey”. Whey is the serum phase of milk and contains about 20% of the protein and most of the lactose from the original input milk (Blaschek et al., 2007; Fox et al., 2015b). It can be further classified based on processing conditions into sweet whey and acid whey. Sweet whey is a by-product of rennet cheese manufacture and is commonly spray dried to form high value dairy powders for use in infant nutrition and body building applications. Conversely, acid whey comes from the production of acid coagulated dairy products including fresh and cream cheeses and strained (Greek style) yoghurts (de Wit, 2001; Prazeres et al., 2012). A shift in market trends towards Greek style yoghurts has resulted in the production of very large quantities of this acid whey (Prazeres et al., 2012). Despite the profits generated from yoghurt production, the industry has been struggling to find solutions for such increasing volumes. In general, the disposal of waste whey is complicated due to its high biological oxygen demand that leads to the need for costly treatment facilities. Some small scale facilities use acid whey in manure as an organic fertiliser, as a supplement of cattle feed, or for conversion into biogas (Prazeres et al., 2012). However, these are all low value applications.

The presence of high concentrations of lactate, either as lactic acid or dissociated into lactate ions, reduces the potential for the use of acid whey in spray dried powders. Specifically, the presence of high concentrations of lactate has been shown to cause increased powder stickiness, which in turn causes operational problems in the dryer (Modler and Emmons, 1978; Prazeres et al., 2012; Shrestha et al., 2006). It has been shown that the temperature at which sticking occurs is directly related to the mass ratio of lactate to lactose (Knipschildt and Andersen, 1994); if there is more lactate present this occurs at a lower temperature. Therefore, a successful reduction in this ratio would allow acid whey to be

converted to higher value powders using the conventional spray drying units that are widely available in the dairy industry.

Nanofiltration(NF) membrane technology is already extensively used in the dairy industry to demineralise and concentrate sweet whey streams prior to spray drying (Kentish and Rice, 2015; Okawa et al., 2015; Rice et al., 2009). NF has also been considered at laboratory scale for demineralisation of acid whey (Kelly and Kelly, 1995; Roman et al., 2010; Roman et al., 2009; Vasiljevic and Jelen, 2000). NF is also used for the recovery of lactic acid from fermentation broths where the lactate concentration is 10-20 times that in acid whey (Bouchoux et al., 2006; Li, 2006; Sikder et al., 2012; Wang et al., 2013).

However, to the best of our knowledge, this approach has not yet been used to remove the lactate content from acid whey. Nanofiltration is an ideal candidate for this operation as separation is achieved without the use of additional chemicals; and as the whey solution would be simultaneously demineralised and concentrated. NF membranes reject molecules based on size exclusion or charge repulsion (Kentish and Rice, 2015). Under uncharged conditions, lactose (LT, 342.3 Da) can be retained preferentially over lactic acid (LA, 90.1 Da) due to its greater molecular weight. However, while lactose molecules are uncharged, lactic acid is a weak acid that can dissociate into its conjugate base (a lactate ion, $\text{CH}_3\text{CH}(\text{OH})\text{COO}^-$) and protons. The dissociation of lactic acid is given by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[\text{CH}_3\text{CHOHCOO}^-]}{[\text{CH}_3\text{CHOHCOH}]} \quad \text{Equation 1}$$

The dissociation constant (pK_a) of lactic acid at 25°C is reported to be 3.86 (Budavari, 1989). The association constant at a different temperature can be estimated by the following correlation (Perrin, 1964):

88 $-d(pK_a)/dT = (pK_a - 0.9)/T \pm 0.004$ *Equation 2*

89 where T is the temperature of the system in Kelvin.

90   Ideally, a NF membrane possessing a molecular weight cut-off (MWCO) between 90
91 and 340 and a positive surface charge could be used to retain lactose while removing lactate
92 ions from acid whey (Bellona et al., 2004; Teixeira et al., 2005). However, almost all
93 commercially available NF membranes are negatively charged at neutral conditions. This
94 means that separation in the present case is likely to rely upon the differences in molecular
95 size, with the larger lactose retained while lactate species are transmitted. However, for this
96 to be effective, it would be necessary to ensure that the lactic acid is substantially
97 undissociated, as the negatively charged lactate ions would not readily pass through the
98 membrane.

99 Therefore, it was the purpose of this work to evaluate the potential for nanofiltration to
100 separate undissociated lactose from charged lactate anions within an acid whey system, to
101 increase the potential for spray drying of the product. Three commercial NF membranes were
102 selected for this purpose, based on their differences in MWCO, isoelectric point and water
103 permeability; so that the effect of these parameters on lactate/lactose separation could be
104 evaluated. Experiments were conducted using both real acid whey and solutions prepared in
105 the laboratory, across a range of pH values and temperature, which in turn varies the
106 membrane permeability, the strength of the membrane surface charge and the degree of
107 lactate dissociation. The separation performance of the different membranes was assessed and
108 related to the underlying membrane characteristics and the operational conditions.

109

2 Materials & methods

2.1 Materials

Raw acid whey samples were obtained from a dairy processing company in Victoria, Australia. The two acid whey batches were microfiltered (MF) commercially in a pilot plant at Dairy Innovation Australia Ltd (Werribee, Victoria, Australia) using 1.4 μm Isoflex ceramic filters (Tami industries, Z.A. Les Laurons, NYONS CEDEX, France) with a transmembrane pressure of 1 bar. The approximate composition of acid whey was found to be consistent with that reported in the literature (Rodriguez et al., 2012; Waldron, 2007) (Table 1).

Artificial solutions were prepared in the laboratory by mixing lactic acid (85%, Ajax Finechem) or sodium lactate (70%, Ajax Finechem) with lactose ($\geq 99.8\%$, Ajax Finechem) to mimic their concentrations in acid whey. The pH of the lactic acid-lactose (LA + LT) and the sodium lactate-lactose (Na-LA + LT) solutions were ~ 2.6 and ~ 7 , respectively. The pH of these laboratory solutions was adjusted using an appropriate amount of 10 M NaOH or 2 M HCl. pH adjustment was only performed in one direction, in order to prevent unnecessarily high increases in ionic strength due to addition of both NaOH and HCl.

2.2 Nanofiltration

A cross flow GE Osmonics membrane filtration module was used for the NF experiments. The module has a maximum operating pressure of 69 bar and an effective membrane area of 0.014 m^2 . Three commercially available flat sheet NF membranes with different MWCO, divalent rejection, isoelectric point and water permeability were supplied from Sterlitech (Kent, WA) (Table 2). Experiments were conducted with a feed flow rate of 2.4 L/min (cross flow velocity = 0.5 m/s) and a transmembrane pressure of 20 bar. The retentate and permeate

were re-circulated to the feed vessel to maintain a stable feed composition (Figure 1). The feed temperature was maintained at 5 °C or 35 °C by passing the feed solution through a temperature controlled water bath. All experimental runs were repeated at least twice.

After each run, the membrane was cleaned-in-place by firstly rinsing the unit with deionised water for 30 minutes; followed by circulation of 0.05 M NaOH for 30 minutes, 0.05 M HCL for 30 minutes and another 30 minutes with 0.05 M NaOH. The system was then further flushed with deionised water for 30 minutes. The water permeate flux was checked to ensure at least 95% of the flux could be restored prior to further experiments.

Lactic acid and lactose concentrations in the permeate samples were determined using high-performance liquid chromatography (HPLC). An Aminex HPX-87H ion exclusion column (BIO-RAD) was used with a mobile phase of 0.009N H₂SO₄ at 0.6 ml/min and 60°C. Lactic acid and lactose were detected by a UV-Vis detector (at 220 nm and 285 nm wavelengths) and a refractive index detector (RID), respectively. The percentage rejection (%R) of each species was calculated by the following equation:

$$\%R = \left(1 - \frac{C_p}{C_f}\right) \times 100 \quad \text{Equation 3}$$

where C_f is the concentration of the feed (g/L), and C_p the concentration of the permeate sample (g/L).

3 Results and discussion

3.1 Flux

Figure 2 shows the permeate flux measured up to 75 minutes for NF of raw acid whey using the XN45, HL and DK membranes at 20 bar, 5°C and 35°C. The flux increases with

temperature due to increases in penetrant diffusivity which increases membrane permeability; and due to lower solution viscosity which increases the mass transfer coefficient in the boundary layer and thus reduces concentration polarisation. The permeate flux decreases with time due to membrane fouling caused by the solids and fats in the raw acid whey. The decline is more pronounced at 5°C, where the fat is in the solid phase (Fox et al., 2015a). After removing the solids and fats using microfiltration, the permeation flux of the microfiltered acid whey (at 5°C) is more stable, with a steady value of about 23 L/m²h (LMH) after the first 15 minutes for both the DK and XN45 membranes (see Figure 2). The similarity in flux for these two membranes at all temperatures suggests that the flux is not controlled by the underlying properties of the membrane, as these had both different MWCO and different MgCl₂ rejection. In contrast, the HL membrane started with a higher flux (~50 LMH) and this gradually decreased within the first 50 min where it started to plateau (~28 LMH). The higher initial flux reflects the greater water permeability of the membrane as indicated in Table 2. However, this higher flux also leads to more rapid fouling, so that after 50 minutes there is less difference between the three membranes (Tang and Leckie, 2007; Widjaya et al., 2012).

The flux achieved after one hour, over a range of pH values, for the four types of feed solutions is presented in Figure 3. The simplest laboratory prepared solution (LA+LT) yields the highest flux, followed by the sodium lactate/lactose solution. This is due to the increase in osmotic pressure as HCl is added to reduce the pH of the latter solution. The permeate flux of the real MF acid whey sample is below half of the flux obtained for the laboratory prepared solutions, due to the higher ionic strength and the presence of other organic species (Braghetta et al., 1997). Again, the laboratory prepared solutions exhibit similar fluxes for all three membranes, with the HL membrane showing the highest flux with the microfiltered acid whey.

While the variation in flux at different pH values is not substantial, slight changes in flux is obtained around the reported isoelectric point of the membrane for the LA+LT solution and the acid whey after MF for all three membranes. It is very common to see such changes in flux at this point, reflecting the loss of charged groups within the membrane structure. This changes the conformation of the pores and reduces electroviscous effects (Childress and Elimelech, 2000; Rice et al., 2011).

3.2 Lactic Acid/Lactate Ions Rejection

The rejection of lactic acid decreases with decreasing pH (Figure 4). The trend closely matches that of the dissociation of lactic acid which also declining with decreasing pH (Figure 4). This suggests that it is the charge repulsion of the dissociated lactate anions that dominate the separation. At pH values greater than the isoelectric point, the membrane surface is increasingly negative and hence these lactate anions are readily repelled (Bellona et al., 2004). As a result, lactic acid rejection is greater than 70% for all pH greater than 4.5, regardless of the ionic strength of the feed. A slight decline in rejection is observed for the XN45 membrane at pH 4.5 when working with acid whey after MF, which reflects the fact that the membrane is uncharged at this point and so ions can pass more readily. Below this pH, the membrane is positively charged and rejection of the remaining lactic acid relies on both repulsion of cations and size exclusion.

The rejections of the lactate ions observed from the acid whey after MF are lower than that from the laboratory prepared solutions. This reflects the higher ionic strength which acts to screen the charge of the membrane surface reducing electrostatic repulsion. Further, the flux of water is lowest for this system. A lower water flux leads to a higher permeate concentration, which in turn results in reduced rejection (Equation 3). For similar reasons, the rejection for the NA+LA+LT is lower than for the LA+LT system.

Similarly, the XN45 membrane rejects ~45% of the lactic acid at pH 2.4 for the Na-LA+LT solution, while the HL membrane rejects ~55% at pH 3. This is again because the bulk flow of liquid through the membrane is lower for the XN45 membrane (Figure 3), leading to a higher concentration for all solutes in the permeate. In a high ionic strength solution consisting of high multivalent ions, it is possible that the concentration of the solutes in the membrane surface boundary layer may completely shield the charged surface from the bulk solution. In this case, the role of electrostatic interactions may be weakened hence other separation mechanisms dominate.

It should be noted that the lactic acid rejection of raw acid whey at 5°C is $71 \pm 1\%$ for the XN45 membrane (Figure 4(c)), similar to the acid whey after MF at the same pH. At 35°C, a rejection of $67 \pm 1\%$ was observed (data not shown). A higher temperature generally relates to greater permeability of all species due to an expansion in the polymer structure (Atra et al., 2005) and lower solution viscosity. This lead to less selective partitioning and thus lower rejection (Freger and Freger, 2000).

3.3 Lactose and Conductivity Rejection

In contrast to lactate rejection, lactose rejection is high and independent of pH (Figure 5). For such uncharged molecules, sieving or size exclusion is responsible for separation. Within experimental error, more than 90% of lactose was rejected over the range of pH values tested regardless of the type of feed used. This was expected as the MWCOs of the three NF membranes are similar to the molecular weight of lactose molecules. NF membranes with a MWCO of 400Da were used by Li et al (Li et al., 2008) to achieve a lactose rejection of as low as 69.8% at 2.1 MPa, 37°C and a feed pH of 5.5. However, the feed solution contained 76.8% lactose, much higher than the concentration of lactose in acid whey. Conversely,

Cuartis-Urbe et al. (2006) used a membrane with a MWCO between 150 and 300Da and achieved a rejection of 98% at 13.1 bar and 22⁰C with a feed lactose concentration of 50 g/L.

The rejection of the NaCl within the microfiltered acid whey can be estimated using the solution conductivity as an indication of ionic strength (Figure 4(b)). On this basis, NaCl rejection for the XN45 membrane was observed to be between 30% - 50% for the various pH values tested (Figure 5(c)). Monovalent ion rejection for NF membranes is typically within the same range. The manufacturer states a NaCl rejection of 10-30% for this XN45 membrane, which is at the lower end of this observed range. The rejection is a minimum at the isoelectric point, reflecting the lower charge on the membrane surface at this point.

4 Concluding remarks

Nanofiltration has been used to successfully separate lactate acid from lactose in acid whey streams. While the NF membranes varied in MWCO, divalent ion rejection, water permeability and isoelectric point, these properties had little impact upon the separation efficiency. Rather, the transmission of lactate was primarily affected by the extent of lactic acid dissociation. In particular, lactate transmission increased at low pH due to reduced dissociation of the acid. Thus, a reduction in pH presents a good method for improving the separation from lactose. Around 50% of the lactate ions could pass through the membrane if the pH of the acid whey was reduced to around 3, whereas only 30% of the lactate ions were removed at the natural pH of the acid whey. Over 90% of the lactose was retained for all types of feed solutions and pH values tested. These results suggest that NF may be used to reduce the lactate content in acid whey, thus potentially allowing the retentate stream to be processed in spray dryers with greater efficiency.

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366

Table Captions

367 **Table 1.** General composition of acid whey reported in the literature (Benitez and Ortero,
368 2012; Bylund, 1995; Durham and Hourigan, 2009; Nguyen et al., 2003; Peri and Dunkley,
369 1971; Rodriguez et al., 2012; Saffari and Langrish, 2014; Spreer, 1998; Waldron, 2007) and
370 the acid whey (raw and microfiltered) used in the current study.

371 **Table 2.** Characteristics of NF membranes used in this work.

372

373 Table 1
374

Properties	Literature	Present Work	
		Raw	After MF
pH	4.0-4.6	4.3-4.6	
Total solids (%)	5-6.4	6.1	4.5–5.5
Fat (%)	0.003-0.38	-	0.02
Lactose (%)	3.9-4.9	3.5-4.5	3.7-4.4
Lactic acid (%)	<0.8	0.63	0.55-0.58
Ash (%)	0.5-0.7	0.76	0.61-0.64

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Table 2. Characteristics of NF membranes used in this work.

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Membrane	Polymer ¹	Minimum Salt Rejection ²	MWCO ¹	Water permeability ³	Isoelectric point ³	References
	-	%	Da	L/hm ² bar	-	-
Trisep XN45	Thin film composite - Polypiperazineamide	92% MgSO ₄	500	4.9	~4.5	(Kappel et al., 2014; Mandale and Jones, 2010)
GE HL	Thin film composite - Polypiperazinamide	95% MgSO ₄	150-300	3.2	~3.8	(Al-Amoudi et al., 2007; Ecker et al., 2012)
GE DK	Thin film composite - Polypiperazinamide	98% MgSO ₄	150-300	2.8	~4.0	(Al-Amoudi et al., 2007; Ecker et al., 2012)

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1. Manufacturer supplied information

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2. Testing conditions: 2,000ppm MgSO₄ solution at 760kPa operating pressure, 25°C, 15 % recovery

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3. Cited from the references shown.

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Figure Caption

Figure 1. Schematic diagram of the nanofiltration filtration system used within the study.

Figure 2. Flux achieved for XN45, DK and HL membranes at 20 bar using raw (5°C and 35°C) and microfiltered (5°C) acid whey.

Figure 3. Comparison of flux obtained after one hour of operation for XN45, DK and HL membranes using different feed solutions at 5°C, 20 bar, and various pH values.

Figure 4. Rejection of lactic acid for (a) HL, (b) DK & (c) XN45 membranes, and the degree of dissociation of lactic acid over a pH range of 2-7 at 5°C, 20 bar, using various feed solutions.

Figure 5. Rejection of lactose, and conductivity for (a) HL, (b) DK & (c) XN45 membranes, over a pH range of 2-7 at 5°C, 20 bar, using various feed solutions.

Figure 1
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Figure 2
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Figure 3
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Figure 4a
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Figure 4b
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Figure 4c
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Author/s:

Chandrapala, J;Chen, GQ;Kezia, K;Bowman, EG;Vasiljevic, T;Kentish, SE

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