Protein fractionation prototype to extract minor proteins on-farm

A. CHAND[1] AND C.J. FEE[2]

[1]Dept of Materials & Process Engineering, The University of Waikato, P.B. 3105, Hamilton, New Zealand

ABSTRACT

The current dairying environment in New Zealand consists of producers (farmers) as suppliers and centralised processors where economies of scale have driven the production of dairy products to large-scale processing facilities. Farm management practices such as breeding or supplementary feeding can produce milk with different fat, proteins and lactose content. These differences, however, are minimised when milk from different suppliers is amalgamated into large silos. An automated milking system (AMS) (Greenfield's project, Dexcel and Sensortec Limited, Hamilton, New Zealand) with a high degree of automation and control can be used to collect individual high-value components from milk. A Protein Fractionation Robot (PFR) prototype was developed and coupled to the AMS to demonstrate the concept of "on-farm fractionation of proteins". Two model proteins, lactoferrin (LF) and lactoperoxidase (LP), were extracted using cation exchange chromatography in a single-stage stirred tank from raw whole milk without any disruption to the milk harvesting process. Trials showed that 30-60% of LF and 80-100% LP can be captured in a single-stage, batch ion exchange of freshly harvested milk at ~32^{o}C.

Keywords: milk proteins; on-farm; extraction, fractionation, automated milking system.

INTRODUCTION

Dairying, via processed dairy products, is the largest contributor to New Zealand's GDP and to export earnings, with at least 95% of the milk produced being exported. The current dairying environment in New Zealand consists of producers (farmers) supplying centralised processors where economies of scale drive production of commodities such as milk powder, casein, cheese and butterfat in large scale processing facilities. Therefore standard practice is to transport milk in bulk to centralised dairy factories, with each plant specialising in its own product line. There are ever present needs to maintain a competitive advantage over competitors, to innovate and deliver better technologies and products to get the best returns for the New Zealand farmer. It is now recognized that large-scale bulk processing has reached its maturity, while meeting specific customer requirements is instead becoming the issue (Marshall & Fenwick, 1998).

The dairy industry in New Zealand has a vertical integration and thus the farmer owns the value chain. However the returns to the farmer have always been indirect, via the dairy companies, who only pay on the basic milk composition i.e. fat, protein, lactose and total solids basis.

Traditionally, high-value dairy proteins have been viewed as by-products, with the major income of the industry coming from commodity dairy foods such as milk powder, cheese and butter. However over the past two decades, the dairy industry globally has moved from being based solely on commodity food production to earning a significant income from specialty proteins. Minor proteins are normally extracted from processed streams such as skim and whey (Hahn, *et al.,* 1998; Pederson, *et al.,* 2003; Zydney, 1998). It is a generally accepted principle of bioseparation process design that proteins should be separated from a source material as fast and in as few steps as possible to avoid loss of activity and yield (Denman, *et al*., 1991; Frey & Kang, 2005; Korhonen *et al*., 1998; Wan, *et al.,* 2005; Yoshida, 1991). Extensive pre-treatments of milk and whey prior to ion exchange capture of proteins is detrimental to their functionality.

An on-farm protein purification system may produce products of superior quality by minimising processing but first must overcome the challenges associated with chromatographic processing of a feed stream (raw whole milk) traditionally perceived as impractical to process by current techniques. A feasible, robust on-farm operation may shift the value back to individual farmers, who can earn directly from innovations to produce premium quality milk and/or enhanced milk components with special functionality.

An Automated Milking System (AMS) at the Greenfields dairy site (Sensortec and Dexcel Limited, Hamilton, New Zealand) offers a high degree of automation and control and, combined with herd management; capabilities for processing

[2]Dept of Chemical & Process Engineering, The University of Canterbury.

of milk from individual animals and batch traceability of resultant product(s).

In this project we sought to identify protein capture techniques and to define processing parameters that would allow minor proteins to be extracted from raw whole milk without changing overall milk characteristics. LF and LP, two multi-functional proteins currently extracted from bulk milk on a commercial basis both in New Zealand and overseas were chosen as model proteins for on-farm capture.

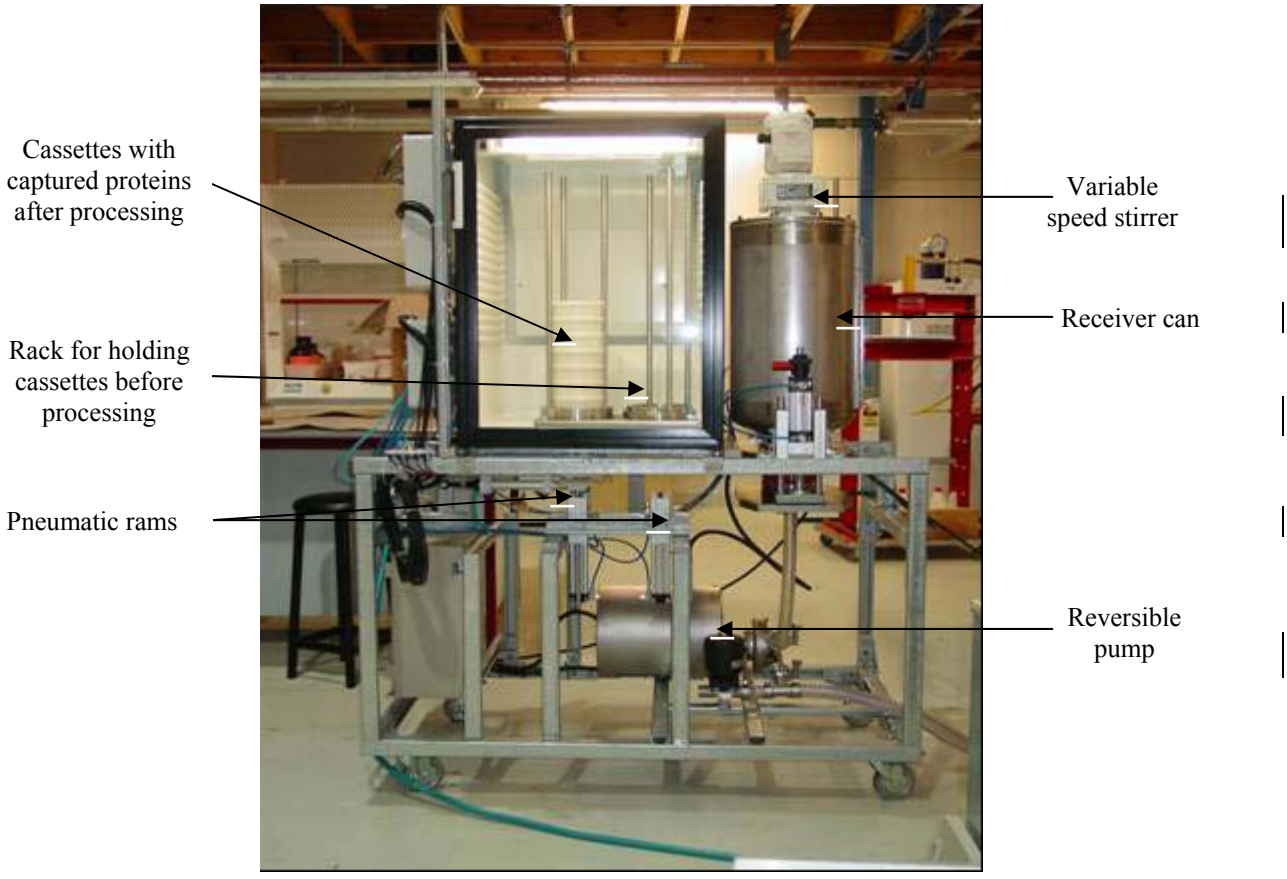
The LF content of bovine milk averages about a few hundred milligrams per litre. However large variations between individual animals exist, along with seasonal variations, with LF present in highest concentrations in colostrums. Other factors such as stress, stage of lactation, frequency of milking, etc. also affect the daily LF and LP concentrations in individual cows milk. Because of these variations and the viable milk volumes expected at each milking, the technology developed would need to be versatile.

MATERIALS AND METHODS

Laboratory scale experiments were undertaken to evaluate three cation exchange resins: BR 70 (Bio Rex 70, Bio Rad Laboratories, Hercules, CA, U.S.A), SP Sepharose Big Beads™ and Fast Flow™ (GE Healthcare Technologies, Uppsala, Sweden) for their suitability to extract LF and LP. Both column chromatography and a single-stage stirred tank system were assessed for their ability to process fresh raw whole milk. The column, milk and stirred tank temperatures were maintained at 37°C during experiments. For both laboratory and on-farm experiments, raw whole milk was obtained from the Greenfield dairy farm site from cows milked using a Merlin AMS milking robot (FV Holdings, UK) integrated with Crystal management software (Fusion Electronics, Netherlands).

For on-farm experiments, a Protein Fractionation Robot (PFR) prototype was designed (Figure 1) (Fee & Chand, 2005a) and fabricated for coupling to the AMS. Trials were undertaken by diverting milk from the AMS to the PFR, where it was processed for LF and LP capture.

FIGURE 1: The Protein Fractionation Robot prototype for on-farm capture of LF and LP.



Cassettes containing 250 mL of swelled drained resin were kept at 4°C (refrigerated). When a signal from the AMS was received, a cassette (with active resin; SP Sepharose Big Beads) was picked up by pneumatic rams and delivered to the load position. Once milking was completed, the AMS sent a signal to the PFR and a reversible pump (Fristam Pumps Inc., Middleton, Wisconsin, U.S.A.) on the PFR pumped the milk into the receiver can (secondary can) with an upward flow which suspended the resin with the milk for adsorption. Stirring was applied for 10 minutes at a constant rate (150 rpm). Once adsorption was complete, the pump drained the milk (in reverse direction from which it was initially pumped), assisted by gravity flow hence straining the resin on the 44 μm sieve of the cassette. The stirrer speed was increased to 450 rpm to assist draining. An impeller was specifically designed to enhance the draining process by keeping the resin suspended during draining and thus preventing blockage of the retention screen. Level sensors, installed within the processing can, signaled the end of the draining process The resin was rinsed twice using warm water (40-50°C) and drained. The cassette was then transferred automatically to the stacking position (4°C storage) to await elution of proteins. Cow identifications, volume produced, pH and conductivity were noted and samples were taken for LF, LP, milk composition and protein analyses. Processing temperature after completion of milking and after 10 minute adsorptions (using the PFR) were noted.

LF and LP were eluted consecutively by washing the resin with 1 litre of 0.4 M NaCl followed by 1.5 litre of 1.0 M NaCl. LP was analysed using an activity assay using 2,2'-azinobis[3-ethyl-benzothiazoline-6-sulphonic] diammonium salt (ABTS) substrate and LF using the surface plasmon resonance (SPR) assay of Indyk & Filzoni (2005) with minor modifications (Fee & Chand, 2005b). Yields of LF and LP were calculated. Milk composition (fat, protein, lactose and total solids content) analyses were carried out by Dexcel (Dexcel Laboratories, Hamilton, New Zealand) using a MilkoScan FT120 Analyser (Foss, Hillerød, Denmark).

RESULTS AND DISCUSSION

BR 70, SP Sepharose Fast Flow and Big Beads were all capable of effectively capturing LF and LP from raw whole milk in a packed column if the temperature was kept above 25°C.

In laboratory processing it took over 1 hour to process 1 L of milk in a 10 mL column at 300 cm/hr. To scale a column chromatography process so 16 L of milk (average yield for an individual cow) is processed within 10 minutes requires high flow rates or impracticably large diameter columns (Fee & Chand, 2005a). Prolonged exposure to elements at high temperatures and repeated loading within a column were also undesirable due to possible microbial growth. Therefore a stirred tank was concluded to be a more practical approach for LF and LP capture from fresh raw whole milk, and this formed the basis of the design of the PFR for the on-farm trials.

The average milking time is 6-7 minutes (Mein, 1998). The PFR was designed so that an individual cows milk could be processed within the average milking time. An adjustable timer was included in the PFR to alter adsorption period if desired.

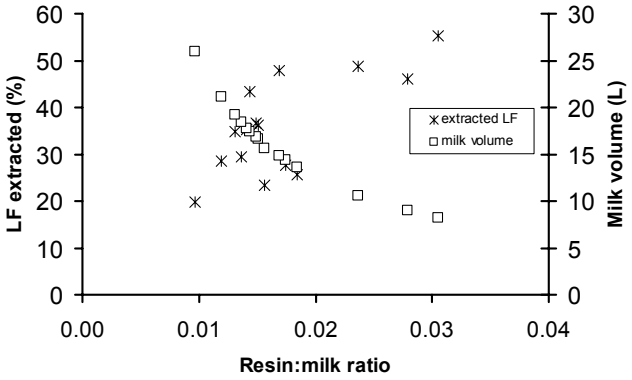
The average temperature after milking was 31.4 ± 1.2 °C and after processing through the PFR it was 30.4 ± 1.4 °C. Warm temperatures minimizes viscosity and increases protein adsorption (Chand & Fee, 2005b). The milk volumes ranged from 8.2-26.0 L (Table 1). A minimum volume of 8.0 L was set for acceptance for processing using the PFR, partially because it is was not possible for the AMS to automatically sub-sample volumes less than 5.0 L.

TABLE 1: Milk volumes, resin: milk ratios, LF and LP recoveries (yields) from on-farm capture with freshly harvested raw whole milk using the PFR.

Cow Id	Milk volume (L)	Resin:milk ratio (v/v)	% LF recovered	% LP recovered
3532	8.2	0.030	55.2	99.5
3022	9.0	0.028	46.0	91.0
9570	10.6	0.024	48.9	82.9
3109	13.6	0.018	25.6	84.7
1922	14.4	0.017	27.6	77.1
1922	14.8	0.017	47.8	92.0
3402	15.6	0.016	23.4	92.9
1607	16.6	0.015	36.1	94.6
2409	16.8	0.015	36.7	90.4
9564	17.4	0.014	43.5	95.9
694	17.8	0.014	35.3	92.4
5710	18.4	0.014	29.5	98.8
480	19.2	0.013	34.9	51.2
1401	21.1	0.012	28.5	81.6
7656	26.0	0.010	19.7	81.9
Mean	15.9	0.017	35.6	87.1
SD	4.6	0.006	10.2	12.0

The average volume of fresh, clean resin before processing and after being regenerated were 250.2 (± 1.8) and 248.2 (± 13.6) mL, respectively, indicating minimal loss of resin. The average LF and LP extracted relative to feed, from 16 individual cows were 35.6 and 87.1% respectively (Table 1). Generally a higher resin:milk (v/v) ratio yielded higher levels of extractions (Figure 2).

FIGURE 2: Effect of resin:milk ratio and milk volume on LF extraction (%) for on-farm capture of LF from raw whole milk of individual cows, using the PFR.



Milk composition of feed (raw whole milk before processing) and outflow (after LF and LP capture using the cation exchanger, SP Sepharose Big Beads) is summarised in Table 2. Differences in milk composition between feed and outflow was statistically significant for all bulk milk composition i.e. fat, protein, lactose and total solids (Student's t-test, $p<0.05$).

Sample 19, corresponding to cow 480 (Jersey cow) had an unusually high fat content (8.09 % compared to 4.50 % fat content on average during this series of experiments). This high fat content may account for the low yield (51 % LP) obtained for this particular cow (compared with 87 % on average) but the effects of other milk components on the LF and LP yield were not investigated during this study.

CONCLUSIONS

This on-farm capture of target milk proteins bypassed many unit operations that are normally undertaken during the centralized processing of minor, high value proteins such as LF and LP in dairy factories. It shows that on-farm fractionation is possible without disruption to the milk supply chain or daily milk harvesting operation. This first capture using the PFR showed that good yields (35-60 % for LF and 81-99 % for LP) are possible. These may be further improved by optimisation of the system and selective processing of animals that produce high levels of LF and LP. If milk is processed on the farm quickly enough, microbial contamination may not be a significant problem. Further work should be undertaken to test our expectation that extracting proteins with minimal processing steps helps retain functionality, in accordance with the central dogma for design of bioseparation processes for highly valuable proteins and peptides.

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TABLE 2: Milk composition (% ^{w}/v) of feed (fresh raw whole milk), and outflow (processed milk) from on-farm capture experiments for LF and LP.

	Feed					Outflow				
Cow Id	**Fat**	**Protein**	**Casein**	**Lactose**	**Total Solids**	**Fat**	**Protein**	**Casein**	**Lactose**	**Total Solids**
480	7.83	3.82	3.01	3.74	15.9	8.09	3.74	2.95	3.71	16.1
694	4.59	3.75	2.93	4.90	13.8	4.67	3.68	2.85	4.74	13.8
1401	5.13	3.09	2.45	4.91	13.7	4.94	3.08	2.39	4.79	13.4
1607	3.91	3.40	2.68	4.72	12.7	3.78	3.27	2.50	4.51	12.2
1922	2.94	2.87	2.22	4.47	11.0	2.97	2.84	2.15	4.32	10.9
1922	5.51	2.92	2.31	4.37	13.3	5.17	2.91	2.21	4.37	13.0
2409	4.07	3.40	2.74	5.18	13.2	4.13	3.34	2.62	5.17	13.1
3022	4.09	3.46	2.59	4.74	13.0	4.35	3.34	2.42	4.48	12.8
3109	1.91	2.78	2.09	4.46	10.0	1.85	2.64	1.88	4.15	9.5
3402	4.34	3.34	2.62	4.94	13.2	4.12	3.25	2.46	4.71	12.7
3532	6.38	3.48	2.68	4.50	15.0	6.08	3.37	2.51	4.35	14.4
5710	5.00	3.41	2.67	4.59	13.7	4.77	3.27	2.50	4.41	13.1
9564	4.63	3.84	3.03	4.79	13.9	4.98	3.74	2.92	4.72	14.0
9570	4.90	3.87	3.00	4.96	14.4	3.93	3.20	2.36	4.31	12.3
Mean	4.66	3.39	2.64	4.66	13.3	4.56	3.26	2.48	4.48	13.0
SD	1.36	0.35	0.29	0.34	1.42	1.38	0.31	0.29	0.33	1.49

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