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Isolation of lactoferrin from bovine colostrum by ultrafiltration coupled with strong cation exchange chromatography on a production scale

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Abstract

Lactoferrin (LF) was isolated from bovine colostrum by ultrafiltration and then purified with a fast flow strong cation exchange chromatography system in a production scale. A two-step ultrafiltration process was performed with membranes of nominal molecular weight cut-offs of 100 kDa for ultrafiltration (UF) step 1 (UF-1) and 10 kDa in the UF step 2 (UF-2). The UF-1 process was performed at fixed transmembrane pressure (TMP), tangential flow velocity and temperature equivalent to 200 kPa, 5 m/s, and 25 °C, respectively. The optimum operating parameters for the UF-2 were a tangential flow velocity of 4 m/s, limiting TMP of 150 kPa, and an operating temperature of 50 °C. The predicted permeate flux based on a resistance mathematical model was not significantly ($p > 0.05$) different from those of the actual experiment. The LF concentrated in the UF-2 retentate reached a purity of 30.88% (w/w) and a recovery of 94.04%. A stepwise procedure for purification of the crude LF was conducted using a preparative-scale strong cation exchange chromatography. The LF eluted with 1.0 M NaCl aqueous buffer showed a single band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a molecular weight of 80,400 Da. The purity and the recovery of the final LF product were 94.20% and 82.46%, respectively. The process developed in this work is a significant improvement over the commercial practice for the fractionation of LF from bovine colostrum.

Keywords: Lactoferrin; Bovine colostrum; Ultrafiltration; Flux; Cation exchange chromatography; Tangential flow filter membrane

1. Introduction

Some biologically active components of colostrum and milk such as immunoglobulin (Ig), lactoferrin (LF), lactoperoxidase (LP), and lysozyme play important roles in the protection of the neonate. LF is a glycoprotein in external secretions such as milk, especially bovine colostrum. The concentration of LF in the colostrum is 20-fold higher than that in bovine raw milk [1]. LF has various bioactive functions [2], such as broad-spectrum antimicrobial activity [3–8], promotion of iron transfer and absorption [6,9], inhibition of lipid peroxidation [10–12], cancer prevention [13] and antiviral activity [14]. LF has great potential for wide application in improved infant formula, "therapeutic" cosmetics, and mouthwash solutions [15–17].

Previously, LF was separated by diethylamino ethanol (DEAE) anion exchange chromatography [18]. Nowadays, high-purity LF is obtainable on a laboratory scale using gel filtration chromatography [19], immobilized monoclonal antibodies [20], chelating chromatography [21], hydrophobic interaction chromatography, Cibacron Blue affinity chromatography [22,23], carboxymethyl cation exchange chromatography [24,25], cation exchange membranes [26], adsorptive membrane chromatography [27], semi-batch foaming process [28] and microfiltration affinity purification [29]. However, none of these techniques has been used at commercial scales because of the high processing costs and the difficulties associated with the disposal of large quantities of undesirable effluents [15,30,31].

Since the heat stability of bovine colostrum is poor, it is an under utilized product in the dairy industry. Membrane separation process is a non-thermal process that involves no phase change or chemical agents. The introduction of this technology in industry represents one of the technological answers to the problem of isolation of bioactive proteins. Advanced membrane processes also allow for the recovery and purification of valuable milk constituents [30,32–34]. Tangential flow filter membranes are based on cross-flow hydrodynamics, which require less floor

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area per membrane area installed, reduce the pressure-driven declines in permeate fluxes apart from requiring lower pressures to operate [35,36]. Their use in LF fractionation has not been encountered in the literature.

Recently, an approach of industrial production and application of bovine LF from cheese whey or skim milk was reported [37]. However, after that, information is scarce and has not been encountered in the literature reviewed. The purpose of this work was to combine the membrane process with the preparative-scale ion-exchange chromatography to effectively separate and purify LF from bovine colostrum on a production/pilot scale with cost effectiveness. A novel procedure consists of two stages is described in this study, namely: separation and concentration of LF by ultrafiltration (UF), followed by purification of the crude LF product with a fast flow cation exchange chromatography on a production scale.

2. Materials and methods

2.1. Preparation of bovine skim colostrum whey

Fresh bovine colostrum was obtained from a local dairy farm (Tianzi Dairy Company, Wuxi, Jiangsu, PR China). The composition of the bovine colostrum was protein (total Kjeldahl nitrogen × 6.38) 11.90%, fat 4.85%, lactose 4.13%, and ash 0.75%. The bovine colostrum was defatted by centrifugation at 4000 × *g* for 20 min at 4 °C. The skim bovine colostrum was adjusted to pH 4.2 with 0.1 M hydrochloric acid at 37 °C and kept for 30 min. The precipitated casein was removed by centrifugation at 2000 × *g* for 20 min at room temperature (∼25 °C), followed by Optical XLT30 Durapore 0.22 μm Capsule Filters (Polypropylene, Catalogue No. KVGLA3TTH1, Millipore Corp., Bedford, MA, USA). The suspension was the bovine skim colostrum whey.

2.2. Ultrafiltration system

A Pellicon® 2 tangential flow filtration (TFF) model system (Millipore Corp., Bedford, MA, USA) was used for the ultrafiltration (UF) of bovine skim colostrum whey. It consisted of Pellicon® two cassette UF modules with Ultracel™ composite regenerated cellulose membranes, Pellicon® two Cassette acrylic holders and assemblies (Catalogue No. XX42P0060) and Millipore pump (Catalogue No. XX80EL230). A two-step UF process was performed with PLC membranes of nominal molecular weight cut-offs (NMWCO) 100 kDa (Filter Code: PLCHK, catalogue No. P2C100C05) for the first UF stage (UF-1), and 10 kDa (Filter Code: PLCGC, Catalogue No. P2C010C05) for the second-stage UF (UF-2) (Fig. 1).

The UF was operated in either the concentration or the total recycle mode. In the concentration mode, the material retained by the membrane (retentate) was recirculated back to the feed tank. Meanwhile the material that was not retained by the membrane passed out of the permeate outlet port and was collected as the permeate in a suitable collection vessel. In the total recycle mode, both the retentate and permeate were recirculated to the feed container to maintain constant volume and product concentration. This mode enabled the filtration flux to be measured as a function of the TMP, the tangential flow velocity and the operating time.

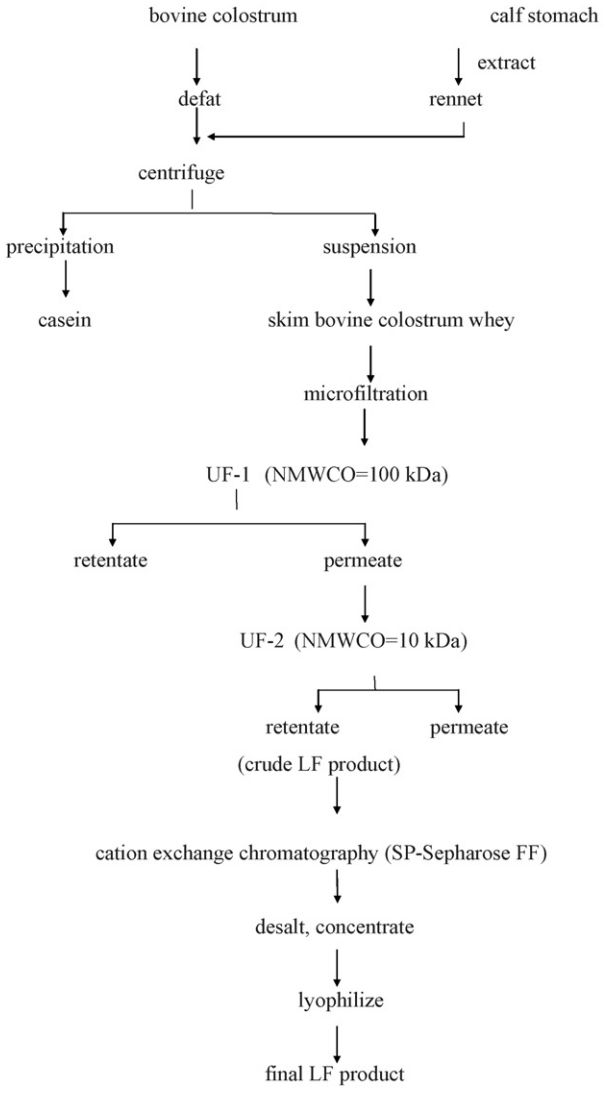

Fig. 1. A flow diagram for the ultrafiltration isolation of lactoferrin from bovine colostrum.

Transmembrane pressure (TMP) was calculated as the following equation:

$$\mathrm{TMP} = \frac{P_{\mathrm{in}} + P_{\mathrm{out}}}{2} \quad (1)$$

where P_{in} and P_{out} are the inlet and outlet pressures, respectively.

Permeate flux (J) is expressed in liters per square meter of membrane area per hour by the following equation:

$$J = \frac{V}{TA} \quad (2)$$

where J is the permeate flux (L/(m^2 h)), V the permeate volume (L), T the time of determination (h), and A is the area of the membrane (m^2, in this study 0.465).