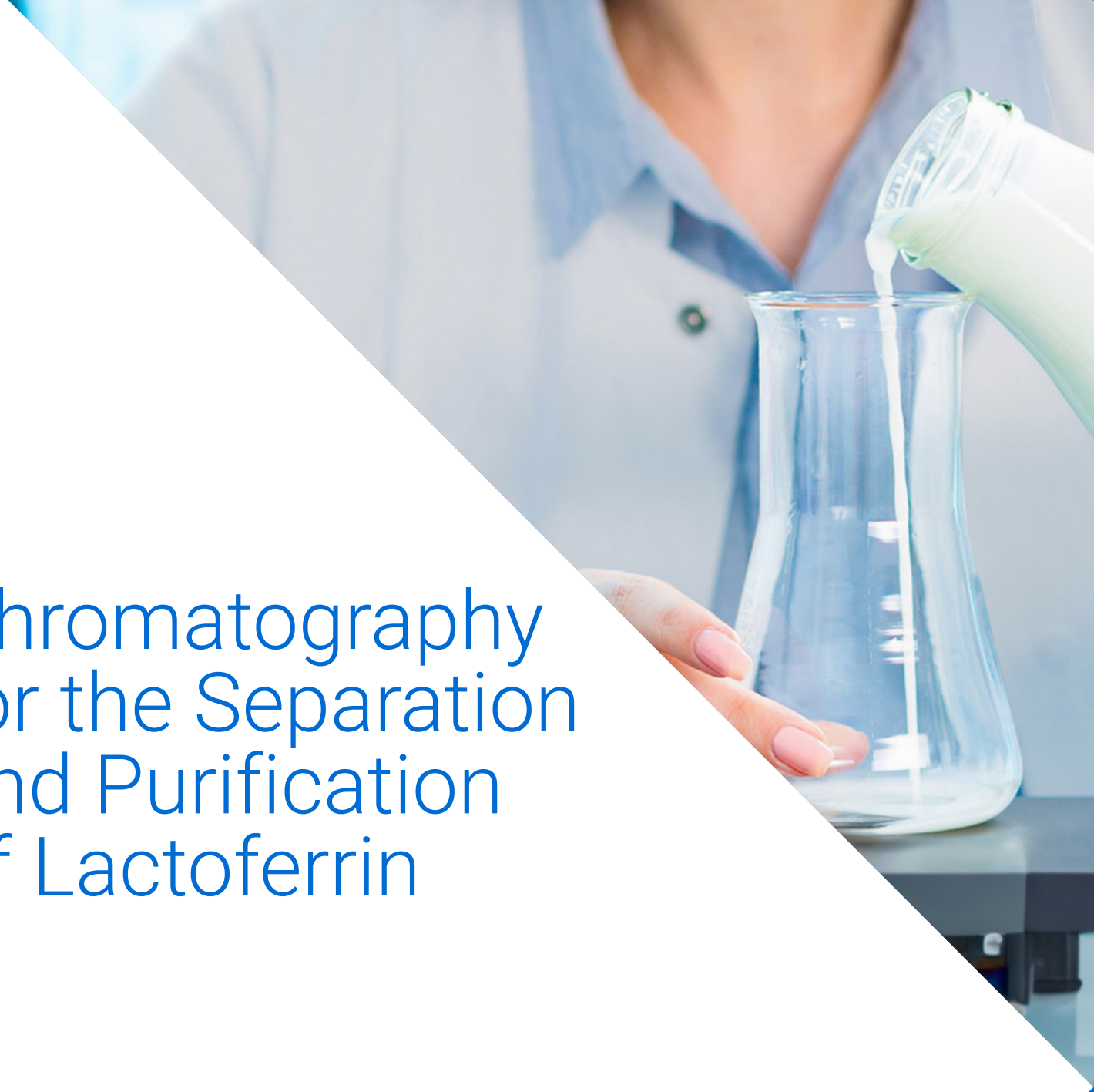


Chromatography for the Separation and Purification of Lactoferrin

Purify high-value proteins to meet the dairy supplement demand

ECOLAB®

Chromatography for the Separation and Purification of Lactoferrin

Praesto SP200 provides optimal purification of milk proteins such as lactoferrin. With the increasing demand for pure and cost-effective dairy proteins like lactoferrin, chromatographic methods must provide efficient and scalable solutions to purify these high-value proteins. This complex mixture of proteins is challenging to separate, but lactoferrin can be readily separated by cation exchange chromatography.

Contents

What is Lactoferrin?

Lactoferrin (LF) is a globular glycoprotein of approximately 700 amino acid residues with a molecular weight of about 78 kDa. The protein also has a relatively high isoelectric point (pI≈8.7), indicating that it is positively charged over a wide pH range, including neutral pH. The capture and purification of LF can be challenging as it is found in deficient concentrations in the whey by-product.

Transform dairy byproducts into high-value products by extracting lactoferrin. Chromatography is a technique used to purify proteins from complex mixtures, such as whey, to help producers turn a waste stream into valuable raw material for high-margin products.

Applications of Lactoferrin

Lactoferrin is an essential protein that has several biological activities, such as:

- Antimicrobial
- Antiviral
- Anti-inflammatory
- Antioxidant
- Anticarcinogenic
- Cosmetics
- Immunomodulation
- Infant formula
- Sports nutrition
- Support in treatment of the Alzheimer's disease

Lately, lactoferrin has also been established in vitro antiviral efficacy against a wide range of viruses, including SARS-CoV, a closely related coronavirus to SARS-CoV-2. Furthermore, lactoferrin possesses unique immunomodulatory and anti-inflammatory effects that may be especially relevant to the pathophysiology of severe COVID-19 cases[1].

FIGURE 1

Lactoferrin Purification Process Overview

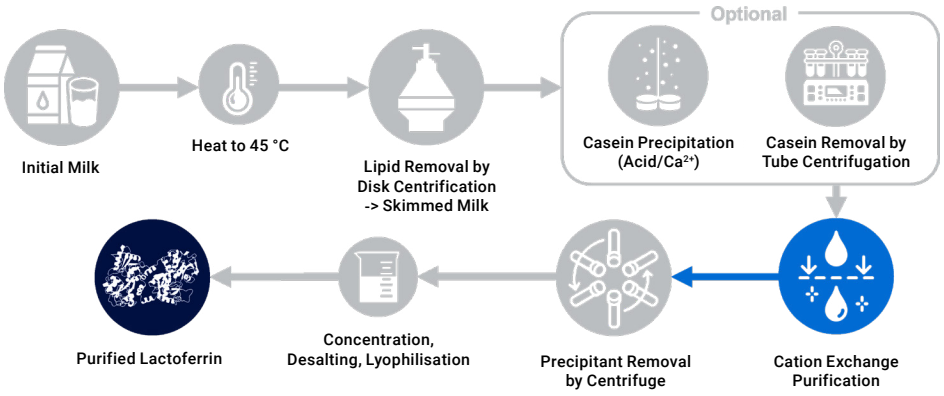


TABLE 1 Product Code: PR02002

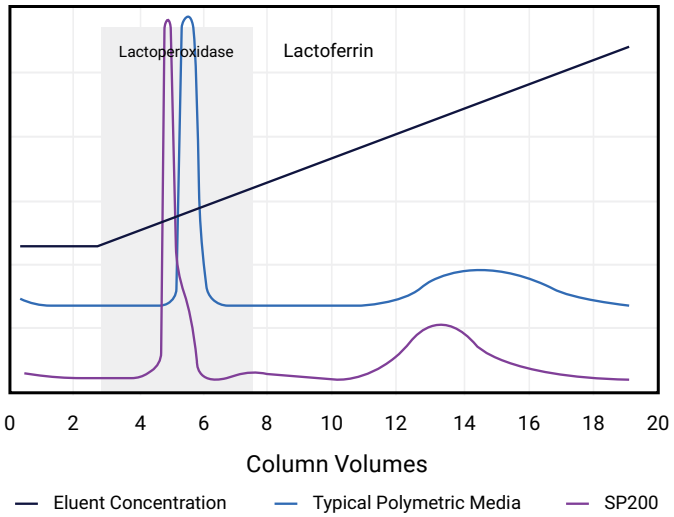
Test/Characteristic	Analysis Method Code	Limits
Ion Exhcange Capacity; mmol H+/mL packed medium	AMQ-14	0.10–0.16
Average Particle Size; d50v/µm	AMQ-13	150–250
Pressure Flow; Back Pressure at 800 cm/h; bar	AMQ-15	< 3.25
Particle Size Distribution	AMQ-13	> 75% within range 100–300 µm
Microbial Contamination; Colony Forming Units/mL suspension	N/A	≤ 20

TABLE 2

Chromatographic Resin	Matrix	Functionality	Particle Size Range (µm)	Mean Diameter (µm)	Volume Capacity (eq/L)	Swelling (Water → 0.5 M NaOH)	Static Binding Capacity (mg/mL)[a]
Praesto SP200	Agarose	Strong Acid Cation	100–300	200-+50	0.10–0.16	No Change	40

FIGURE 2

Profiles of Separation of Lactoferrin and Lactoperoxidase Using Different Cation Exchange Resins



0.5 g of resin was shaken in 20 mL pf phosphate buffer at pH 5 containing 5 mg/mL lactoferrin and the change in absorbance at 280 nm measured over 24 hours and used to calculate the bound lactoferrin.

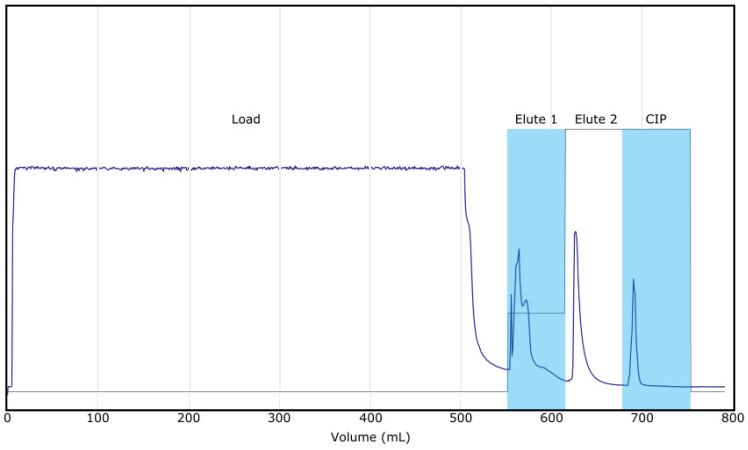
When binding lactoferrin from milk, the other very similar protein to separate is likely to be lactoperoxidase, as this protein has a similar mass and isoelectric point to lactoferrin. However, separation is possible based on the necessary salt concentration to elute the two. A sample buffer containing 1 mg/mL lactoperoxidase and 1 mg/mL lactoferrin was applied to the resin and the proteins eluted by increasing salt concentration. (Figure 2)

In the next set of experiments the purification/separation of lactoferrin and lactoperoxidase was performed on skimmed milk. Each resin was equilibrated with buffer at pH 6.7, then 500 mL of skimmed milk applied.

Lactoperoxidase was eluted by using 300 mM NaCl, which elutes all remaining species bound by ionic interactions. The process was followed by a CIP using 0.5 M NaOH. (Figure 3)

FIGURE 3

Chromatogram Obtained from Lactoferrin Adsorption



Summary

Praesto SP200 performed better in testing than other media types, achieving an LF binding capacity of 13.7 mg/mL, significantly higher than typical polymetric media.

This is an issue when it comes to efficiently treating milk in industrial processes, as the above indicates that the required contact time for typical polymetric media to reach the same level of binding is more extended than can be achieved with the 1 BV/min flow rates. Praesto SP200 chromatography product ranges.

To learn more about how Praesto SP200 can help you reach optimal lactoferrin purification, visit www.puroliteresins.com/lactoferrin

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Praesto SP200 is compliant with FDA 21 CFR 177.1520 as a polymeric component that is safe for food contact. It conforms to the GMP requirements of Regulation (EC) No 2023/2006 and is in compliance with Article 3 of Regulation (EC) 1935/2004 and covered by specific measures listed in Annex. 1.

Praesto SP200 meets the Total Organic Carbon (TOC) release requirements set forth in the European Resolution ResAP(2004)3 "Ion Exchange and Adsorbant Resins in the Processing of Foodstuffs," when measured according to the specified AFNOR test T 90-601 after conducting the recommended pretreatment.

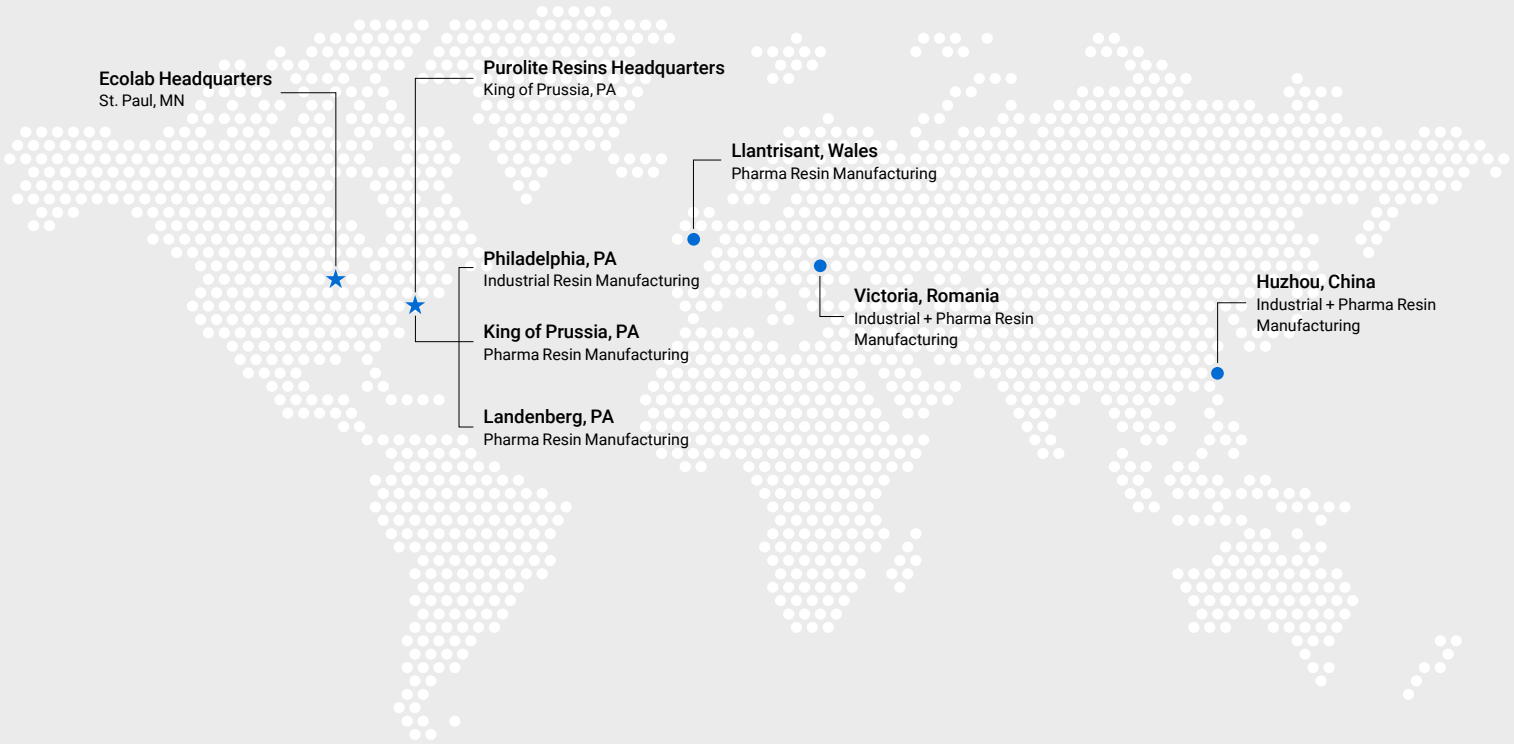
Pre-use treatment recommendations for Purolite Resins are available on our web site at:
Pre-use Conditioning of Ion Exchange Resins.

References

1. Raymond Chang, Tzi Bun Ng, Wei-Zen Sun, Lactoferrin as potential preventative and adjunct treatment for COVID-19, International Journal of Antimicrobial Agents, Volume 56, Issue 3, 2020

Ecolab is a global developer, manufacturer, and supplier of Purolite™ Resins including ion exchange, catalyst adsorbent and advanced polymers that make the world cleaner and healthier.

www.puroliteresins.com



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Americas
americas@ecolab.com

Asia Pacific
asiapacific@ecolab.com

EMEA
emea@ecolab.com

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