

Lactoferrin: The Bioactive Protein in Milk

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Milk is called "complete food" because it has proteins and nutrients that help humans to grow. There are two main types of proteins in milk, casein proteins and whey proteins. Casein makes up about 80% of milk proteins while whey proteins account for nearly 20% and are considered more beneficial due to their higher nutritional value. Some of these whey proteins including lactoperoxidase, immunoglobulins and lactoferrin are found in very small amounts, but they play a very important biological role. They are also called as minor whey proteins. Lactoferrin has gained a lot of attention in recent years because of its health benefits and commercial applications. Lactoferrin is a reddish-pink glycoprotein that binds iron. It was first found in milk in 1939 and was later separated from cow milk and human milk. It is a transferrin family protein that can strongly bind iron. Because of this iron-binding ability, lactoferrin can perform many important biological functions like killing microbes, helps in immune system and improves iron absorption.

Uniqueness of lactoferrin

Lactoferrin is naturally present in milk, colostrum, saliva, tears and other biological fluids. However, its concentration is the highest in colostrum, which is the milk secreted within 3 to 5 days post-parturition (Superti, 2020). Human colostrum contains higher amount of lactoferrin than bovine milk (Table 1). Lactoferrin is considered an important component of the innate immune system because of its protective role. It protects infants and adults from harmful microorganisms by depriving bacteria of iron which is essential for their growth. The structure of lactoferrin has two lobes called the N-lobe and C-lobe and each lobe can bind one ferric iron (Fe^{3+}) ion (Rastogi et al., 2016). Lactoferrin forms a stable complex with iron and carbonate ions. This iron-binding mechanism is extremely strong and allows lactoferrin to hold iron even in acidic conditions such as the stomach. Therefore, lactoferrin helps in better iron transport and its absorption in the body. It also prevents excess free iron from participating in harmful oxidation reactions that can damage cells.

Different forms of lactoferrin

Lactoferrin exists in three major forms namely, apo-, holo- and native-lactoferrin depending on

iron saturation level (Table 2). Apo-lactoferrin has stronger antimicrobial activity because it can bind iron and destroys microbes while the holo-lactoferrin has greater thermal stability. Studies have shown that the biological activity of lactoferrin is strongly affected by heat treatment.

Table 1: Average lactoferrin content in different dairy fluids

Dairy fluids	Concentration (mg/100 mL)
Human colostrum	500-700
Bovine colostrum	50-150
Camel colostrum	200-500
Goat colostrum	100-300
Human milk	100-300
Bovine milk	5-20
Camel milk	20-200
Goat milk	10-300
Human tears	100-220

Mild pasteurization temperatures such as 72°C for 16 seconds preserve most of its activity while heating above 72°C can denature and reduce its beneficial effects. Therefore, proper temperature conditions are maintained during the manufacture of lactoferrin-rich products.

Table 2: Different forms of lactoferrin

Parameters	Apo-LF (Iron-depleted)	Holo-LF (Iron-saturated)	Native-LF (Natural mixture)
Iron saturation	< 5% iron	76–100% saturated with Fe^{3+}	15–20% iron saturation
Color	White to light pink	Pink to deep red	Pale pink
Structure	Less folded, more flexible	More compact, tightly folded structure	Intermediate structural compactness
Thermal stability	Lower heat stability (denatures faster)	High thermal stability due to iron binding	Moderately stable (between apo and holo)
Iron-binding activity	Highest iron-binding capacity	Already saturated, low additional binding	Moderate iron-binding activity

Antimicrobial activity	Higher antibacterial potential (because it scavenges iron)	Moderate antimicrobial effect	Balanced antimicrobial activity
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Extraction of lactoferrin from milk

Lactoferrin is highly valuable protein found in milk but its concentration is very low. Therefore, efficient isolation and purification methods are required for its commercial production. Among all available methods cation exchange chromatography is the most widely used industrial technique. Figure 1 represents the extraction process flow diagram of lactoferrin. This method works on the principle of charge difference between lactoferrin and other milk proteins. Most milk proteins carry a negative charge at normal milk pH while lactoferrin remains positively charged because of its high isoelectric point. In cation exchange chromatography milk or whey is passed through negatively charged resins such as SP-Sepharose or CM-Sephadex. The positively charged lactoferrin binds to the resin whereas the most other proteins pass through the column. Later, high salt solutions such as sodium chloride are used to release lactoferrin from the resin (Plate et al., 2006). Stepwise salt elution is commonly used to separate lactoferrin from another positively charged milk protein such as lactoperoxidase. This method can produce lactoferrin with purity above 90% and is widely adopted by dairy industries worldwide.

Apart from chromatography, researchers are also exploring alternative extraction methods such as ammonium sulfate precipitation which uses the "salting out" phenomenon to precipitate proteins selectively. Another advanced method is reverse micellar extraction where surfactant molecules form tiny structures called reverse micelles in organic solvents. These micelles selectively trap lactoferrin and later release it under controlled conditions. Such methods reduce operational costs and improve industrial efficiency in the future.

The future of lactoferrin production is moving beyond conventional dairy extraction. Due to rising global demand and limited concentration in milk, researchers are focusing on recombinant production technologies. Precision fermentation using microorganisms such as yeast and fungi is emerging as a promising method for producing animal-free lactoferrin. Molecular farming through genetically engineered plants is another innovative approach under development. These technologies may help produce large quantities of highly pure lactoferrin at lower cost in the future.

Methods used for lactoferrin detection

After purification lactoferrin must be accurately detected and quantified. Different analytical techniques are used for this purpose like Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), and Enzyme-Linked Immunosorbent Assay (ELISA). RP-HPLC separates proteins based on their interaction with the stationary phase and identifies lactoferrin through UV detection. SDS-PAGE is another commonly used technique in which proteins are separated according to molecular weight. Lactoferrin generally appears near 80 kDa in electrophoretic analysis. ELISA is considered one of the most sensitive methods because it uses antibodies that selectively bind lactoferrin.

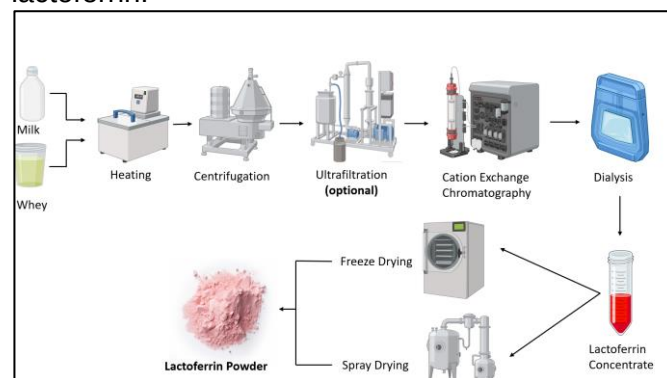


Figure 1: Process flow diagram for isolating and production lactoferrin powder from milk and whey

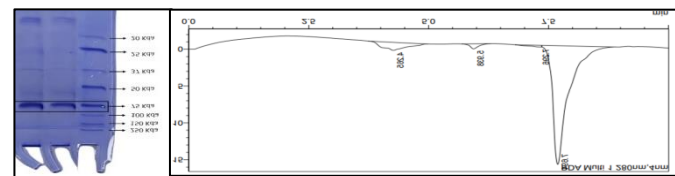


Figure 2: (a) SDS-PAGE profile of lactoferrin band near 80 kDa with a molecular weight marker (20-250 kDa) and (b) RP-HPLC chromatogram of LF standard sample showing the lactoferrin peak at a retention time of 7.69 min.

Commercial applications of lactoferrin

The commercial importance of lactoferrin has increased rapidly over the past decade due to growing consumer demand for natural bioactive ingredients and health-promoting products. Today, lactoferrin is widely incorporated into infant formulas, functional foods, dietary supplements, fermented milk products, cosmetics, oral care products and pharmaceuticals (Demir et al., 2025). The global lactoferrin market has expanded significantly because of its high nutritional and therapeutic value, particularly in premium infant

nutrition and immunity-boosting products. Dairy industries now recover lactoferrin from whey using advanced membrane filtration and chromatographic technologies, making it a high-value dairy ingredient with strong commercial potential. In infant nutrition, lactoferrin helps in strengthening immunity and improving gut health. Its antibacterial, antiviral, anti-inflammatory and anticancer properties have been utilised in numerous medicinal treatments from wound repair to different chemotherapy applications. Researchers have also investigated its role in improving iron deficiency anemia because iron from lactoferrin is better absorbed than from traditional iron salts. Lactoferrin is also finding applications in food preservation and packaging. Because of its antimicrobial activity, it extends the shelf-life of foods and reduces food spoilage. Scientists are developing biodegradable packaging films containing lactoferrin to inhibit microbial growth on food surfaces. Nanotechnology-based delivery systems such as liposomes and nanoparticles are also being explored to improve the stability and absorption of lactoferrin inside the human body.

Conclusion

Lactoferrin is one of the most important bioactive proteins present in milk. Its strong iron-binding ability, antimicrobial action, immune-supporting properties and nutritional significance make it a highly valuable molecule for the dairy, food and pharmaceutical industries. Although its isolation from milk remains challenging because of low concentration and complex purification processes, continuous advancements in biotechnology and dairy processing are opening new opportunities for its commercial production and application. Lactoferrin truly represents the transformation of milk from a traditional food into a source of advanced functional and therapeutic ingredients.

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