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Lactoferrin and its application in food technology

Masoud, A.S.; Ebtisam, M.A. Mesalam and Asmaa, E. Hassan

Reference Lab for Safety Analysis of Food of Animal Origin, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt.

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Abstract

Lactoferrin has gained interest in the food industry for its potential as an additive to fortify products such as yogurt, infant formula, and meat derivatives while also improving the shelf life of foods and providing antimicrobial and antioxidant activity.
For many years, researchers have examined the fundamental qualities of LF, including as its antibacterial, antiviral, anticancer, antiinflammatory, **antioxidant**, and probiotic benefits.
LF's antibacterial, antiinflammatory, and antioxidant qualities are strongl y linked to its iron chelation action.
The characteristics of lactoferrin and its use in food technology are cover ed in this review.

History and structure of lactoferrin

Lactoferrin is a naturally occurring defense protein that has the capacity to bind iron. Formerly known as lactotransferrin and belongs to the proteins that can bind and transfer Fe3+ ions since it is a member of the transferrin family. It is found in exocrine secretions and bodily fluids such as milk, tears, nasal discharge, saliva, bronchial mucus, and gut surfaces that are frequently exposed to regular flora and serve as microbial barriers **(Kruzel *et al.*, 2006).**

Sorensen and Sorensen isolated lactoferrin from bovine milk for the first time in 1939. In 1946, Schade and Caroline separated "serotransferrin," a protein that binds iron, from human serum. This protein was subsequently dubbed transferrin. In 1960, three separate labs simultaneously identified it as the primary iron-binding protein in human milk **(Montreuil *et al.*, 1960).**

According to **Arnold *et al.* (1980),** lactoferrin has bactericidal properties**. Birgens (1985)** observed that lactoferrin levels rose in response to inflammation. As a result, lactoferrin serves a wide range of biological purposes, some of which seem unrelated to its capacity to bind iron **(Brock, 2002).** Due to these factors, the food industry has been more interested in em-

Corresponding author: Masoud, A.S., Reference Lab for Safety Analysis of Food of Animal Origin, Animal Health Research Institute (AHRI), Nadi El-Seid Street, Dokki P.O., Giza 12618, Agricultural Research Center (ARC), Giza, Egypt.
Email address: dr.ahmed.masoud2025@gmail.com

ploying LF as a preservative in dairy products. In 1986, LF was first included in infant formula **(Tomita *et al*., 2002).**

Lactoferrin is a glycoprotein with a molecular weight of about 80 kDa, which shows high affinity for iron. Lactoferrin was categorized within the transferrin family, due to its 60% sequence identity with serum transferrin **(Metz -Boutique *et al.*, 1984**). Its carbohydrate content is 11.2%, with a maximal iron content 1.4 mg/ g, iso-electric point "pl" 8 and have absorbance at 465 nm of 1% solution (iron saturation) 0.58 **(Moor *et al.*, 2002).**

Three distinct lactoferrin isoforms have been identified. The iron-binding form, lactoferrin-α, lacks ribonuclease activity. However, lactoferrin-β and lactoferrin-γ cannot bind iron, while they show ribonuclease activity **(Furmanski *et al*., 1989).** A single polypeptide chain with 703 amino acids wrapped into two globular lobes makes up lactoferrin. A α-helix connects these lobes, which are also known as C- (carboxy) and N- (amino) terminal regions. The two domains that make up each lobe are called C1, C2, N1, and N2. Mannose is the most prevalent sacharide, followed by hexoses at about 3% and hexosamines at 1% **(Metz-Boutique *et al*., 1984).**

The ability of lactoferrin to bind iron is double that of transferrin, which in some situations can act as a donor of Fe3+ ions for lactoferrin. One lactoferrin molecule has the ability to bind two ferric ions. Lactoferrin always binds one carbonate ion simultaneously with every ferric ion **(Baker, 1994).**

Depending on its iron saturation, lactoferrin comes in three different forms. While an arginine chain is in charge of binding the carbonate ion, four amino acid residues—histidine, twice tyrosine, and aspartic acid—are crucial for iron **binding (Ward *et al*., 1996).**

2.3.2 Character and stability of lactoferrin:

Bovine LF partly saturated with iron 15 – 20 % and has a salamon pink colour. While, it colored blue, white and red corresponding to net positive, neutral and negative charge respectively **(Koradi *et al.*, 1996).**

It is crucial to maintain iron binding even at low pH levels, particularly in infection and inflammatory sites where bacterial metabolism may cause the pH to drop below 4.5. In this case, lactoferrin freed from transferrin and absorbed iron. Bacterial growth and replication cannot be reliably rendered **(Valenti and Antonini, 2005).**

Besides iron lactoferrin is capable of binding a large amount of other compounds and substances such as lipopolysacharides, heparin, glycosaminoglycans, DNA sequences which possibly affect green expression within cells, or other metal ions like Al3+ , Ga3+, Mn3+, Co3+, Cu2+, Zn2+ etc. However, its affinity for these other ions is much lower. Apart from CO32–, lactoferrin can chelate diverse anions such as oxalates and carboxylates, allowing to affect the metabolism and distribution of various substances **(Baker, 1994).**

The main source of LF is milk. It is widely eliminated in human milk colostrums with concentrations of up to 7 gm/l, and during lactation, the amount of LF in mature milk decreases by around 7 times to 1 gm/l **(Montagne *et al*., 1998).**

Lactoferrin has proven to be very resistant to trypsin and trypsin-like enzymes' proteolytic destruction. The degree of iron saturation determines the resistance level **(Lonnerdal and Lyer 1995).**

Milk heated for 30 minutes at 65°C had no discernible impact on LF. However, in all types of milk, the full activity of LF was lost at 85 oC for 30 minutes. However, due to a higher concentration of LF, camel milk's antimicrobial properties were noticeably more heat resistant than those of cow and buffalo milk proteins **(Elagamy, 2000).**

Paulsson *et al*. (1994) discovered that whereas pasteurization had no effect on milk LF, UHT treatment fully denaturated it and reduced its ability to interact with bacteria. However, **Luf and Rosner (1997)** discovered that while high temperature short-term treatment of milk had no discernible effect on Lf denaturation, heat treatment at 63 0C for 30 minutes decreased the native LF content by 40%.

Its degree of iron saturation and the properties of the treatment media both affect LF stability. Additionally, LF encapsulation shields the structure and activity of LF before it enters the intestines if it is consumed by the user **(Franco *et al*., 2018).**

Furthermore, LF resisted heating at 80 °C for 5 minutes without significantly losing its ability

to bind iron, **according to Abe *et al*. (1991).** Approximately 85% of the iron bound by the unheated LF could still be bound by the heated LF after 5 minutes at 100°C. Its activity changed as the temperature and growth rate changed. Both the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) decreased in proportion to a drop in temperature **(Vorland 1999).**
Lactoferrin retained their functional activities at PH ranged from 2 to 7.4 (**Suzuki *et al., 2003)***. LF is very stable at pH 4 and high temperature **(Abe *et al.,* 1991).**
In the combination between pH and temperature, **Murdock and Mathewes (2002)** reported that under low pH and refrigeration conditions, LF can limit the growth or reduce the population of pathogenic bacteria in dairy products. Moreover, **Troost *et al. (*2001)** concluded that LF was found to withstands stomach pH and successfully surviving following oral administration.
In the heated samples at 80 to 120 degrees Celsius, LF remained soluble and the solution was clear at an acidic pH (2:5). On the other hand, turbidity and gel formation happened at neutral and alkaline pH values of 6 to 10, and both phenomena significantly increased with higher temperatures. The LF in the heated samples was still soluble at pH 11, but the samples' color darkened **(Abe *et al*., 1991).**

2.3.3 Therapeutic effect and Biological functions of lactoferrin:
Lactoferrin and certain dietary proteins possess specific biological activities offering numerous therapeutic benefits **(Berkhout *et al.,* 2002).**
The innate immune system is thought to include lactoferrin. Further, it indirectly participates in certain immunological responses **(Legrand *et al*., 2005).** Lactoferrin is one of the earliest defense mechanisms against microbial invasion, mainly through the mucosal barrier, because of its advantageous location on the mucosal surface. Lactoferrin affects the growth and proliferation of a variety of infectious agents including both Gram positive and negative bacteria, viruses, protozoa and fungi **(Kirkpatrick *et al.,* 1971).**
However, lactoferrin's similarity to transferring suggests that it has an impact on the distribution of iron in an organism. This might be because, in typical circumstances, lactoferrin plasma concentrations are extremely low. However, when inflammation happens, the amount of lactoferrin rises. Because of the reduced pH in such an environment, lactoferrin facilitates iron exchange from transferrin, which may lead to local iron buildup in inflammatory sites **(Brock, 2002).**
Depending on the organism's iron requirements, lactoferrin from human milk may have an impact on newborns' intestinal iron absorption. Lactoferrin is bound by specific receptors (SI-LfR) found on enterocytes. 90% of the lactoferrin that has been attached to the enterocyte is broken down, releasing Fe3+ ions. The cell membrane carries the 10% that is still intact. Lack of intracellular iron may cause certain enterocyte surface receptors to be expressed more often, which would boost the absorption of lactoferrin-bound iron **(Suzuki *et al*., 2005).**
The bacteriostatic activity of lactoferrin results from its capacity to bind free iron, one of the ingredients necessary for bacterial development **(Arnold *et al*., 1980).** *E. coli* and other iron-dependent bacteria cannot develop when there is insufficient iron present **(Brock, 1980).** Conversely, lactoferrin may act as an iron donor, so promoting the growth of certain bacteria with lower iron requirements, including Bifidobacterium sp. or Lactobacillus sp., which are typically seen as advantageous **(Sherman *et al*., 2004).** Other bacterial species, such as those in the Neisseriaceae family, adjust to novel environments by producing certain receptors that can bind lactoferrin and alter the molecule's tertiary structure, which results in iron dissociation **(Ekins *et al*., 2004).**
Lactoferrin's bactericidal action may be mediated by many pathways and is not just dependent on iron. Some microbes have been shown to contain receptors for the N-terminal region of LF on their surface. Gram-negative bacteria experience cell death when LF binds to these receptors because the cell wall is disrupted. A increased susceptibility to lysozyme and other antimicrobial drugs results from the subsequent production of lipopolysacharide (LPS), which also impairs permeability **(Leitch and Willcox, 1998).**
Lactoferrin can stop *Pseudomonas aeruginosa* from forming biofilms in vitro. Bacteria are forced to move when the environment lacks

iron. As a result, they are unable to stick to surfaces **(Singh *et al*., 2002**). By binding both bacterial invasions and glycoaminoglycans in target cell membranes, LF may help defend against facultative bacterial invasion by preventing pathogen attachment to target cells. This capability was initially documented against enteroinvasive *E. coli* HB 101, and subsequently against *Listeria monocytogenes* and *Yersinia enterocolica* **(Valenti and Antonini, 2005).**

By breaking down proteins required for colonization, lactoferrin's proteolytic action is thought to prevent the growth of certain bacteria, including enteropathogenic *E. coli* and *Shigella flexneri.* However, serine protease inhibitors can turn this off **(Orsi, 2004).**

Some DNA and RNA viruses can be bound by lactoferrin **(Yi *et al*., 1997).** However, its primary function in antiviral defense is to attach to glycosaminoglycans in cell membranes. Lactoferrin stops viruses from entering cells in this way, preventing infection at an early stage **(Ward *et al*., 2005).** Such a mechanism has been demonstrated as being effective against the Herpes simplex virus **(Marchetti *et al.*, 1996),** cytomegaloviruses **(Andersen *et al.*, 2001),** and the human immunodeficiency virus **(Harmsen *et al.*, 1995)**.

There are several ways that lactoferrin combats parasites. For instance, after being incubated with lactoferricin B, the sporozoites of *Toxoplasma gondii* and *Eimeria stiedai* exhibit decreased infectivity. Lactoferricin is believed to compromise the integrity of the parasite's membrane, which alters the host-parasite relationship **(Omata *et al*., 2001).** Its antiparasitic action against *Pneumocystis carinii* is based on the parasite and lactoferrin's fight for iron **(Cirioni *et al*., 2000).**

Lactoferrin can affect immune system cells and cells engaged in the inflammatory response in both positive and negative ways because of its iron binding capabilities and interactions with target cells and molecules. In one sense, LF might boost the immune response by promoting the growth, development, and activation of immune system cells. Conversely, LF has anti-inflammatory properties. LF may stop inflammation and consequent tissue damage brought on by the release of pro-inflammatory cytokines and reactive oxygen species because of its antibacterial action and ability to bind components of bacterial cell walls (LPS) or their receptors **(Legrand *et al*., 2005).**

The growth of human mammary gland cancer cells can be inhibited by lactoferrin. The altered expression or activity of regulatory proteins may be the cause of this detrimental effect on cell growth **(Damiens *et al*., 1999).**

Low dosages of LF (10 μg/ml) induce tumor cell cytolysis, but at larger concentrations (100 μg/ml), cytolysis seems to rely on the cell phenotype. As of yet, the precise mechanism behind this function remains unknown **(Fujita *et al*., 2004).**

One strong anabolic agent that has been shown to influence osteocytes is lactoferrin. It increases thymidine incorporation into osteocytes, promotes osteoblast proliferation, and lowers osteoblast apoptosis by 50–70%. Additionally, chondrocytes showed a similar effect **(Cornish *et al*., 2004).**

In a concentration-dependent manner, LF inhibits or even decreases osteoclastogenesis. On the other hand, it shows no influence on the bone resorption performed by mature osteoclasts **(Lorget *et al*., 2002).** Besides direct influence, LF may affect bone cells through the inhibition of osteolytic cytokines such as TNFα or IL-1β, whose levels rise during inflammation **(Cornish *et al*., 2004).**

Bovine LF suppresses psychological stress, especially when faced with difficult circumstances. Depending on the particular stressor, several routes are used by the mechanisms controlling the stress reactions. There is evidence that bovine LF stimulates one of the response-related pathways. It influences serotonin or dopamine activity as well as other neuronal systems, which in turn influences the activity of the opiodergical system **(Kamemori *et al*., 2004).**

As a prebiotic growth factor, lactoferrin promotes the development of bifidobacteria, which defend against infections that cause gastrointestinal symptoms. Antimicrobial and bifidobacterial development together stimulate milk proteins to produce more highly specialized chemicals that can control the large intestine's microbial makeup **(Lipeke *et al*., 2002).**

In addition to its bacterial activity, LF has antifungal effect on some candida spp *especially Candida albicans* (**Samaranayake *et al.*,**

2001).
When it comes to *Listeria monocytogeness*, lactoferrin has an antimicrobial impact. Its antimicrobial peptides, which are produced when hydrolyzed by proteases, are what give it its activity; pepsin, chymosin, and microbial rennet were used to hydrolyze its hydrolysates **(Ripolles *et al*., 2015).**

2.3.4 Application of lactoferrin in dairy products:
Lactoferrin has antimicrobial properties against certain pathogenic and spoiling bacteria, so it has been used to preserve raw, pasteurized, and UHT milk, butter, and cheese **(Champagne *et al*., 1994).**
Lactoferrin can also be used in infant formulas and similar food products due to its antioxidant and its antimicrobial properties (**Satue- Gracia *et al.,* 2000)**
Lactoferrin not used only in dairy food industries, but also it used in a wide range in food industries. In meat industries, LF can be sprayed onto carcasses at concentration 3.26 ml spray/ kg beef or 62.5 mg / kg beef to help preventing bacterial contamination during processing or can be applied to finished beef surface prior to final packaging to extend the shelf life (**FDA, 2001).** Lactoferrin has been used in the preservation of poultry, pork, fish and other sea foods **(Naidu, 2002).**
The antimicrobial assay of lactoferrin on spores of penicillum spp in industrial bottle water was noticed They found that LF at concentration of 60 – 300 micron / ml peptone yeast glucose medium inhibit the spore germination and mycelial growth for up to 9 and 21 days at 30 0C **(Gesualdo *et al.,* 2001).**
According to **Suzuki *et al.* (2003),** lactoferrin at a concentration of 1 mg/ml prevents 48% of the creation of hydroperoxides, but an application of 11 mg/ml totally prevents the formation of hydroperoxides as well as a number of bacteria.
Murdock *et al.* (2007) investigated how nisin and LF worked together to suppress *Listeria monocytogenes* and *E. coli*. The findings showed that 250 mg/ml lactoferrin and 10 IU/ml nisin inhibited *L. monocytogenes*, while 500 mg/ml lactoferrin and 250 IU/ml nisin inhibited *E. coli.*
In certain nations, lactoferrin derived from cow's milk has been utilized in the food sector for over two decades. Yogurt, milk, dietary supplements, and pet supplements all contain lactoferrin **(Rahman *et al.,* 2008, Tomita *et al.,* 2009).**
For growth, bifidobacteria need iron, which they get from LF. Bifidobacteria use the iron released by lactoferrin, which may release iron at lower pH levels, to thrive. However, strain-dependent iron requirements can result in variable growth promotion yields when lactoferrin is added. Another theory is that different bacterial strains may activate lactoferrin-binding protein based on their needs **(Rahman *et al*., 2009).**
The physical characteristics of the yogurt were not significantly impacted by the addition of bovine lactoferrin to milk at concentrations of 0.5, 1, and 2 mg/mL in the holo (iron saturated) and apo (without iron) forms; however, apo -lactoferrin somewhat postponed the pH drop. This might be explained by the partial suppression of *Streptococcus thermophilus* growth that was noted. On the other hand, the synergy of lactoferrin with other mucosal proteins such as lysozyme, having apotential advantages. In addition had been reported to promote the growth and development of *bifidobacteria* **(Franco *et al.,* 2010).**
Da Silva *et al.* (2012) assessed how adding lactoferrin to Minas frescal cheese affected *Staphylococcus aureus*. Lactoferrin (0.2 g/100 g curd + S. aureus), control (S. aureus), and lactoferrin (0.4 g/100 g curd lactoferrin + S. aureus) were the three cheeses that were produced. S. aureus populations were assessed on days 1, 8, 15, 22, and 29 after the cheeses were packaged and stored at 6°C. Three iterations of the experiment were conducted. During storage, the S. aureus population in the control cheese rose by 1.3 logarithmic cycles, while in the lactoferrin-containing cheeses, it fell to levels below the detection limit. Additionally, it was shown that the antibacterial action was dose-dependent
Lactoferrin affect on the multiplication of some probiotic bacteria and thus improve the quality of some dairy products. Lactoferrin when added at concentration of 2mg/g or 4mg/g on Minas fresh soft cheese inoculated with *lactobacillus casei, the LF* stimulated the multiplication of these bacteria along the shelf life

of the cheese. Moreever they noticed that lac*t*oferrin did not posses a harm to *E.coli* in cheese samples. Furthermore, The lactoferrin activity found to be affected by ph and population of microorganisms ***(Inay et al., 2012).***
Bovine lactoferrin and its pepsin-digested hydrolysate were tested for their ability to combat spoilage bacteria that contaminated the high-moisture mozzarella cheese's governing liquid while it was being kept cold. It has the ability to be efficient against spoilage bacteria as *E. coli* and *pseudomonas* **(Quintieri *et al*., 2012).** Accordingly, the blue discoloration of Italian mozzarella cheese can be resolved by adding pepsin-digested lactoferrin, which has the capacity to inhibit *Pseudomonas fluorescens* bacteria **(Anadel-Olmo *et al*., 2018).**
Some spoilage bacteria are susceptible to the antibacterial effects of lactoferrin. According to **Leonardo *et al*. (2015).** It delays the growth of *Pseudomonas fluorescence*, preventing the blue staining of mozzarella cheese.
Masoud *et al. (2018)* *revealed* that LF had a significant antimicrobial effect on yeast, mould and coliforms with no effect on either the acidity indices or organoleptic properties of the cheese. Moreover, addition of LF with B had better effect on cheese quality than C cheese samples.

Another study looked into how well LF-fortified yogurt worked to treat children's IDA and microcytic hypochromic anemia. It was shown that children who were on LF-fortified yogurt had significantly higher levels of hemoglobin (Hb) and several red blood cell (RBC) characteristics. Compared to the youngsters getting LF alone, the effect was noticeably larger. **(Tsukahara *et al*., 2020 and Hassan *et al*., 2022)ز**

2.3.4 Application of lactoferrin in meat products:
Lactoferrin reduces lactic acid bacterial counts and thiobarbituric acid reactive compounds (TBARS) at concentrations up to 80 mg/kg. According to **Chiu and Kuo (2007),** the addition of lactoferrin had no effect on the pH values of hot-boned ground pork, but they did slightly rise with storage time.

Soyer *et al*., 2020 address the synergistic antibacterial activities of activated lactoferrin and rosemary extract in vitro as well as their possible use in meat storage. They found that lactoferrin and rosemary extract suppress the development of *Salmonella Enterditis, E. coli 0157:H7*, and *Listeria monocytogenes.*
Atteya *et al*., 2023 evaluated the effect of carboxy-methyl-cellulose (CMC) fortified with lactoferrin (LF) (5%, 10% and 20%) edible coating in the sensory and microbial quality of home-made sausage.. Results revealed significant enhancement in the overall acceptability of the treated samples with elongation in the shelf-life up to 12th, 15th and 18th days of storage in the treated samples with LF 5%, 10% and 20%, respectively; Furthermore, the antimicrobial effect of LF appeared to be dose-time dependent, where higher concentrations gave more antimicrobial effects. So, usage of LF edible coating technique is recommended to be applied in the meat production for more safe meat products with longer shelf-life, as a low price and easily applied preservative technique.
Lactoferrin incorporated with alginate film on minced meat has a significant antimicrobial effect on aerobic mesophilic count, with a discernible decline in the APC count between the treatment and control groups with no a significant effect on sensory character **(Masoud *et al*., 2024).**

2.3.5 Lactoferrin and food packaging:
Given the longer shelf life of the product and consumers' increasing need for foods that are microbiologically safer, new packaging techniques must be created. As a result, the industry has challenges in developing novel food packaging rules. Food safety, shelf life, and quality can all be enhanced by research into the use of edible films as effective packaging components. To prevent the growth of hazardous germs and deterioration on the surface of ready -to-eat foods, a variety of antimicrobial edible coatings have already been developed. Lactoferrin can be used to track the growth of *E. coli* and *L. monocytogenes* in edible chitosan film. Furthermore, the growth of *L. monocytogenes* and *E. coli* was significantly inhibited by the combined application of lactoferrin and

lysozyme integrated in chitosan film (**Brown *et al*., 2008).**

Moreover, lactoferrin, added to the lysozyme-containing film, showed greater antibacterial action against *Listeria monocytogenes* than EDTA, which was added at a dose of 0.4 mg/ disc. The results suggest that lactoferrin might work more synergistically than synthetic chelators like EDTA. The potential of lactoferrin and lysozyme in combination with casein or zein films to inhibit *E. coli* was evaluated. Three distinct lactoferrin (7, 14, or 28 mg) and lysozyme (6, 12, or 24 mg) concentrations were applied to the surface of the films. The *E. coli* inhibition level varied according to the antimicrobial concentration, and films containing either lactoferrin or lysozyme successfully stopped the growth of *E. coli* **(Brown *et al*., 2004).**

Encapsulated lactoferrin was examined in two distinct emulsion forms to avoid divalent cations interfering with Lf's antibacterial activity. To create microcapsules, combine distilled water with LF (20% w/v) in either sodium lactate (3% w/v) or sodium bicarbonate (20 mM). An oil mixture of 22% butterfat, 78% maize oil, and 0.1% polyglycerol polyricinoleate was used for emulsification. By integrating microcapsules with edible Whey Protein Isolate packaging film, the antibacterial activity of LF against the meat-rotting organism *Carnobacterium viridans* was investigated. The growth of *C. viridans* was hindered between 4°C and 10° C. Microencapsulated lactoferrin exhibited more antibacterial activity than unencapsulated LF **(Al-Nabulsi and Holley 2007).**

LF incorporated with alginate film on minced meat has a significant antimicrobial effect on aerobic mesophilic count, with a discernible decline in the APC percentage between treated and control groups with without possess a significant effect on sensory character **(Masoud *et al*., 2024).**

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