Lactoferrin (LF): a natural antimicrobial protein

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ABSTRACT

Improved awareness regarding diet and health linkages has motivated researchers to give more attention to the health perspectives of individual foods. During the previous two decades, the global dairy industry diverted its interest from simple processing of commodities to the value addition. A significant income is being earned in the dairy industry from specialty proteins. Pakistan ranked 3rd in milk production worldwide and different milking animals e.g. cow, buffalo, goat and camel should be studied for their potential for generating new health-promoting diets and nutraceuticals. Bioactive proteins and peptides from having abundant applications including an antimicrobial food additive, dietary supplements, functional foods and nutraceuticals. Proteins from milk have a balanced composition of amino acids promising novel functional products. These also enhance the consistency and sensorial characteristics of various dairy products. Amongst, lactoferrin reveal bacteriostatic as well as bactericidal bustle against a variety of microorganisms. It binds iron thus depriving it required for growth like L. monocytogenes, Salmonella spp, Escherichia coli, Bacillus stearothermophilus, Shigella dysenteriae and Bacillus subtilis. Lactoferrin executes as an alternative to antibiotics. It acts as a natural antimicrobial for bio preservation ranging from dairy, meat, seafood, beverages, bakery products, acne care, infant formulas, extending shelf life, ensuring safety and improving health by acting against life-threatening diseases like cancer, hepatitis, respiratory infections, and foodborne diseases in infants, children and adults etc. In the nutshell, the current review discusses the importance and safety perspectives of lactoferrin in the food industry as well as the health sector.

Introduction

Use of chemical preservatives in processed foods is a distressing situation for consumers and their demand is increasing day by day for natural foods. So, there is a great interest for researchers to discover natural antimicrobials. [1] Currently, the scarcity of new antimicrobials available into the market has directed to the problem of antibiotic resistance, rising into a worldwide health calamity. Overuse or misuse of antibiotics has exerted the major issue in the development of resistance by bacteria to these antimicrobials. The principal driving force behind the assortment and extent of antimicrobial resistance throughout the food chain is due to stress employed by consumption of antibiotics and biocides. In addition, prevention and control of the growth, selection, and spread of...
antibacterial resistance in the food industry have now been considered.\textsuperscript{[2,3]} Fresh produce cut is susceptible to the risk of pathogen contamination and growth which is one of the main safety concerns. A cumulative number of produce-linked foodborne epidemics in recent years have been reported. Use of natural additives are gaining attractiveness among the people due to their consciousness about their diet and health.\textsuperscript{[4]} Food antimicrobials are the compounds or substances that are assorted as preservatives that decelerate microbial decay of foods. They may inhibit the growth of microbes in foods by inactivation process. Examples of implementations of antimicrobials in foods are the use of lysozyme, nisin and nitrite in cured meats and certain types of cheese for the inhibition of \textit{Clostridium botulinum}, by spraying organic acids, diacetate and lactate for the inactivation of \textit{Listeria monocytogenes} in the processed meat products, and the application of benzoates and benzoic acid in beverages to suppress the growth of mold and yeast.\textsuperscript{[5]} Antimicrobials are frequently used as preservatives in many food products for the prolonged shelf life, free from the contamination of end product, and sustain food quality.\textsuperscript{[6]} However, the potency of antimicrobials is reduced when it interacts with the macronutrients, certain enzymes, surfactants and ions present in foods.\textsuperscript{[7]} Aziz and Karboune\textsuperscript{[8]} reviewed natural antimicrobial compounds separately or in combination with other hurdles in food. Quality and shelf life of meat products, fruits and vegetables could be improved by using naturally occurring anti-microbial compounds as food preservatives.

\textbf{Anti-microbial proteins}

Milk protein contains approximately 20\% whey proteins and 80\% caseins. Biological roles are primarily owing to the peptides and milk proteins. Major proteins such as alpha-lactalalbumin, beta-lactoglobulin, bovine serum albumin, immunoglobulins, glycomacropeptides, and minor proteins i.e. lysozyme, lactoperoxidase, and lactoferrin are included in whey proteins. The antimicrobial perspective of milk is mainly endorsed to nonimmune like lactoperoxidase, lysozyme, lactoferrin, and to immunoglobulin. These proteins acquire imperative dietetic and nutraceutical prospectives with special reference to the promotion of health and impediment of diseases.\textsuperscript{[9]} Processing of whey proteins remunerates numerous bioactive peptides that can activate physiological effects in the human body mainly on the nervous system via their opiate and ileum contracting activities; on the cardiovascular system via their anti-thrombotic and anti-hypertensive activities; on the immune system via their antimicrobial and antiviral activities; and on the nutrition system via their digestibility and hypocholesterolemic effects.\textsuperscript{[9]} Significant milk proteins contain lactoferrin, transferrin, ferritin, immunoglobulins, calmodulin protease, prolactin and peptone.\textsuperscript{[10]}

Lactoperoxidase possesses antibacterial activity together with thiocyanate and hydrogen peroxide. It makes an intoxicating natural anti-bacterial system, so-called lactoperoxidase system. Lysozyme kills bacteria through dispensing the formation of a glycosidic bond between two bioactive constituents of peptide-glycan, an element of the bacterial cell wall. It generally acts speedily in connection with immunoglobulin-A or lactoferrin. Lysozyme functions as an anti-inflammatory agent, active against \textit{E.coli} and limits the movement of neutrophils into damaged tissue, also the synergistic effect of lactoperoxidase and cinnamon essential oil effectively inhibited the growth of total flora and \textit{Salmonella} for 5 days in raw milk stored at 4\textdegree{}C for 5 days\textsuperscript{[11,12]}

Lactoferrin (LF) possesses numerous health benefits regarding anti-fungal, anti-bacterial, anti-parasitic, anti-viral, and anti-tumor properties. It endorses bone growth, protects the intestinal epithelium, and stimulates the retrieval of immune system functions in animals. LF is involved in the treatment of hepatitis-C. It also improves the health status of Alzheimer’s disease patients.\textsuperscript{[13–15]}

Glyco-macropeptide, a peptide of kappa-casein, exhibits antithrombotic and anti-bacterial potential. \textit{a}-lactalbumin also reveals antitumoral, antiviral, and antistress perspectives. It also drops blood pressure in rats, and cause a better weight gain in malnourished children. Lysozyme is utilized in the treatment of periodontitis, in infant formulas, and in the impairment of tooth decay. Milk enriched is used in feeding premature infants that are suffering from concomitant maladies. Interestingly, anti-bacterial characteristics exhibit through lactoperoxidase. Both lactoperoxidase and lysozyme
require joint action with LF in combating the bacterial growth. Conclusively, preparations derived from colostrum and milk is effective, safe, easily bio-accessible, and broad applications in the therapy of adults and newborns.\textsuperscript{[16]}

**Lactoferrin**

Lactoferrin, belonging to transferrin family of proteins is multifunctional participates in the regulation of free iron level in the body fluids making the protein bacteriostatic and beneficial to health.\textsuperscript{[17]} It is the most important iron-binding protein in milk.\textsuperscript{[18]} Lactoferrin is present in higher quantities in saliva, tears, seminal fluid, white blood cells and milk of mammals. It is naturally found in cow’s milk at an average level of about 0.2 g/L.\textsuperscript{[19]} The maximum lactoferrin content is about 50 to 100 g/L in cow mammary secretions. In human milk, 2–4 g/L and colostrum, 6 to 8 g/L of LF has been found. Lactoferrin possesses health benefits including anti-inflammatory and antibacterial activities. It has a protective role against gastrointestinal infections and contributes to secretory immune function systems.\textsuperscript{[20–22]} It is revealed that lactoferrin has bacteriostatic properties against gram-negative bacteria requiring high iron like coliforms. It also acts against bacteria like *Staphylococcus aureus, Listeria monocytogenes* and Bacillus species. Lipo-polysaccharides from the external membrane of bacteria are released leading to changes in the permeability due to lactoferrin interaction. It holds iron at a lower pH and has basic nature because of 300 times higher affinity constant than transferrin.\textsuperscript{[23]} Lactoferrins are polypeptides having a single chain with a molecular weight of 80 kDa. It comprises of 1–4 glycans but it depends upon the species.\textsuperscript{[24,25]} Lactoferrin of Bovine and human origin have the same sequence identity.\textsuperscript{[26]} Bovine Lactoferrin contains sugars including N-acetylglucosamine, acetyllactosamine, galactose, fucose, mannose and neuraminic acid. It has been reported that antibacterial activity is due to N-terminus of bovine Lactoferrin which is cationic.\textsuperscript{[27]} It also has antiviral activity including hepatitis-C, HIV, herpes, influenza and rotavirus.\textsuperscript{[28]} Lactoferrin functions as anti-oxidant by binding the free ferric iron with high affinity. It is helpful for the regulation of iron transport and transcriptional regulation. It also acts as anti-inflammatory, antitumoral and immuno-modulatory. It possesses proteolytic and enzymatic activities.\textsuperscript{[29]} This protein has amino acids with the highest contents including alanine, leucine, glycine, tryptophan, histidine and methionine with 10%, 9%, 7%, 1.5%, 1.3% and 0.6% respectively.\textsuperscript{[30–32]} Current uses of bovine lactoferrin include dietary iron supplements, infant formulas, beverages, chewing gums, fermented milk and milk products, cosmetic formulas, immune-enhancing nutraceuticals, feed and pet care supplements. It inhibits bacterial adhesion on abiotic surfaces. LF is an important bar in the mucosal wall preventing both viral as well as microbial attacks. LF has also been the discovered to be effective against the formation of biofilms. Respiratory and oral infections have also been cured by using oral administration of Lactoferrin Food and Drug Administration certified this protein as GRAS prestige. Biomedical use of lactoferrin needs not only evidence of effectiveness at the clinical level but also guaranteed safety, reliable quality of supply and suitable delivery tools.

**Sources**

In 1971, for the first time, lactoferrin in alpaca milk has been reported by Masson and Heremans. Lactoferrin from the milk of camel, sheep, goat, human, and the elephant has been isolated.\textsuperscript{[33]} Lactoferrin is present in the milk of many species\textsuperscript{[34]} including pig, cow, buffalo, horse, human, sheep, camel, goat, mouse and elephant.\textsuperscript{[35]} 2.3 g/L lactoferrin in camel milk is quantified by ion exchange chromatography.\textsuperscript{[36]} Lactoferrin from buffalo, cow, goat and sheep have 90% amino acids sequence identity.\textsuperscript{[35]} Goat milk is advisable to supplement infant formulas due to noteworthy levels of folate binding protein. Lactoferrin has differences in the thermograms indicating diversity in their structure from the milk of diverse species.\textsuperscript{[35]} Camel lactoferrin exerts the highest antibacterial. MIC values quantified for LF are 0.5 milligrams/milliliter at 24 hours and 1 milligram/milliliter at 48 hours.\textsuperscript{[37]} Goat and sheep milk Lactoferrin are detected to be the next most vigorous proteins against *E. coli O157: H7*. Lactoferrin isolated
from a Korean goat against *Escherichia coli* showed a 0.5 milligrams/mL minimum inhibitory concentration value in 1% peptone broth.[38] HLF exhibits much lower antimicrobial activity than BLF.[39] Studies reflected that antibacterial activity is due to some slight differences in the structure of Lactoferrin. hLF has been exposed to be more impervious to proteolysis than bLF due to the changed glycans.[40]

**Production**

Stanic, Radosavljevic, Stojadinovic and Velickovic[41] studied different ion exchangers for segregation of whey proteins. In this study, different biochemical methods have been discussed to isolate lactoferrin from milk of various species. Lactoferrin isolation has endeavored from the milk of goat, elephant, sheep, alpaca, camel, gray seal and human. Cation exchange chromatography is used for purification of Lactoferrin using an SP-Sepharose. SDS-PAGE with 10% polyacrylamide gel is used for identification of lactoferrin found in the chromatographic isolation. Then it is freeze-dried and after that stored at −20°C. Ion exchange chromatography with use of monolithic column attached with UV-VIS detector can be used with optimized wavelength i.e. 280 nm. The ionic strength of the elution solution is 1.5 M NaCl while the flow rate of the retention and elution solutions are 0.25 ml/min and 0.75 ml/min respectively. The detection limit measured was 0.1 μg/ml of lactoferrin under optimum conditions.[42]

Bylund[43] suggested that lactoferrin can be produced without extensive pre-treatment of milk i.e. removal of caseins and fat through precipitation, centrifugation, filtration and Ca\(^{2+}\) chelation. The processing temperature is retained ostensibly about milking temperature (35 to 37°C). The vigorous capacity for capturing lactoferrin under these circumstances was almost 48.6 mg/mL of resin. Doultani, Turhan and Etzel[44] passed the whey through filter paper and then applied it to the column to harvest numeral protein products using mozzarella cheese whey. Hahn, Schulz, Schaupp and Jungbauer[45] studied commercially available cation exchangers for protein extraction from acid whey.

Presently, dairy proteins owing to high nutritional value are observed as a by-product and earning a major income of the industry which comes from milk powder, cheese and butter etc. Milk is cooled to 4°C but in practice often to the only 10°C. It is held in containers on the farm for up to two days and then it is transported to a dairy processing plant. After a series of unit operations like separation of cream, pasteurization (65-70°C), homogenization and mixing for standardization, it is further processed into discrete yields. After a number of such processes, LF is removed from skim milk or from whey which is manufactured as infiltrating as a by-product of cheese production.[46] Ye, Yoshida and Ng[47] used rennet whey produced from skim milk defatted by centrifugation. Incubation was done for one hour with rennet. The caseins were detached by filtration. Centrifugation was done at 10,000 g for 25 minutes before subjecting it to the column. Anion, as well as cation exchange chromatography, were used to isolate whey proteins especially lactoferrin. The subsequent loss in yield can be noteworthy. 4–8 times more lactoferrin can be captured in acid precipitation of casein[48,49].

Lactoferrin (LF) can be isolated and purified by ultrafiltration and with a fast flow robust cation exchange chromatography system, respectively. The molecular weight of LF was found to be 80,400 Da displaying a single band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purity was 94.20% and the recovery was 82.46%.[50] In another study, purification of lactoferrin by ion-exchange chromatography from the milk of a camel (Camelus bactrianus), human (Homo sapiens), goat (Capra hircus), sheep (Ovis aries), alpaca (Lama pacos), elephant (Elephas maximus) as well as from gray seal (Halichoerus Grypus) was tried. Lactoferrin was recognized in all the milk except the gray seal. Differential scanning calorimetry (DSC) is used for studying the thermal stability of purified lactoferrin, hLF was observed to be the most heat-resistant. Lactoferrins from other species offered varying degrees of thermostability. LF of the elephant was found to be the least resistant.[51]
It has been stated that paramagnetic particles connected with heparin can be used for lactoferrin isolation showing yield 164 mg of lactoferrin per gram of the particles. The purity of Lactoferrin achieved by this method is 94% and recovery is 82%. In overall chromatographic techniques, relatively low cost and quick separation of lactoferrin by most of all the ion exchange chromatography is signified. Cationic surfactant i.e. cetyltrimethylammonium bromide can be used for lactoferrin isolation. Recombinant hLF is produced by goats which offered a yield of 2 g/l. In a study conducted by the production of rhLF in transgenic goat milk was more than 30 mg/ml and formulation was constant during the entire lactation cycle. The rhLF purification coherence milk was approximately 70% and 98% pure. Compared with natural hLF, the rhLF from transgenic goats has similar biological characteristics including N-terminal sequence, immunoreactivity, molecular mass, isoelectric point and digestive stability. More importantly, the purified rhLF showed a particular anti-tumor activity in the mouse model of melanoma experimental metastasis. Consequently, the study shows that the large-scale production of functional rhLF in transgenic goat milk could be an economical and prognosticating source of human therapeutic use in the future. The industrial scale revolutionary manufacture of bLF was initiated by Oleofina Company in 1985. Morinaga Milk Industry Co., Ltd.

Food applications

Bio preservation

Investigations are being carried out on the incorporation of lactoferrin into the cheese at different levels (10, 15 & 20 ppm). It was observed that, as the lactoferrin level in the product increased, the bacterial growth decreased dramatically compared to the control, thereby increasing the shelf life of cheese. Cheese treated with lactoferrin up to 20 ppm showed an increase in shelf life of up to 7 days at room temperature (30°C) and 15 days at refrigerated temperature (4°C) compared to the control, respectively. Similarly, as the lactoferrin level increases, the cheese has a much higher hardness, resiliency, springiness and chewiness. The sensory assessment of the samples treated with 20 ppm lactoferrin was acceptable up to 7 days at ambient temperature and up to 15 days at the cooling temperature.

Chantaysakorn and Richter determined the antimicrobial activity of lactoferrin which was added to carrot juice. Lactoferrin was isolated from raw skim milk and digestion was done by pepsin for 4 hours. Peptone-yeast-glucose broth was used for determination of antimicrobial activity of the digests in carrot juice against Escherichia-coli. The inhibitory effect of the digested Lf and growth of E. coli were better at pH 7 using peptone-yeast-glucose broth than at pH 4. Incubation was done at 23°C for 24 hours. Lactoferrin and casein peptide treated samples showed a lower TPC compared to control sample. Meat samples incorporated with LF had lower TPC than the sample with added casein peptide. HPLC was also used to identify meat glucose, which is a freshness index of meat and the results exhibited that glucose content increased during storage from day 1 to day 3 and slightly decreased at day 5 with the incorporation of LF and casein peptide. However, it showed a decreasing trend for the control sample throughout the storage period. The addition of lactoferrin and casein peptide significantly decreased the bacterial counts for 5 days and enhanced the glucose content during storage of hot-boned pork meat at 4°C. Al-Nabulsi and Holley suggested that LF prohibited the growth of some E. coli O157: H7 strains but other strains proliferated. It was observed that during manufacture of sausage, the decline in growth of E. coli O157: H7 was deliberately boosted by all LF doses. Unencapsulated LF dropped 4.2 log units i.e. highest reduction. Lactoferrin has limited effect against pathogens in fermented meats due to a constricted range of activity and initiation of injury of pathogens. Japan and other countries are using LF as a functional ingredient as well as for biopreservation of yoghurt and other foods.

Anti-microbial food packaging

Research on antimicrobial materials for packaging is anticipated to disseminate in the succeeding decade with the beginning of new polymer materials and antimicrobials. Keeping in view
increased demand for microbiologically safer foods by consumers as well as the prolonged shelf life of the product, new packaging techniques are essential to develop. So it is a challenge for the industry to develop novel food packaging policies. Food quality, safety, and shelf life can be enhanced through research on the utilization of edible films as potent packaging ingredients. Several antimicrobial edible films have already been industrialized to diminish spoilage as well as pathogenic microorganism’s growth contaminating the surface of ready-to-eat foods. Antimicrobial agents can potentially be incorporated in proteins, polysaccharides and lipid-based edible films during manufacture. Lactoferrin can be used in edible chitosan film checking the growth of *E-coli* and *L. monocytogenes*. In a study, the combined use of Lactoferrin and Lysozyme incorporated in chitosan film noticeably reduced the growth of *L. monocytogenes* and *E. coli O157: H7* resulting in an estimated 3-log decrease.\[65\] The application of naturally-occurring antimicrobials such as lactoferrin to food preservation is gaining great attention due to consumers’ trend. Films containing natural antimicrobials work as a physical hurdle and an antimicrobial mediator. The combination of natural antimicrobials and edible films may be a superlative approach to food safety and consumer’s concerns. In another study, the antimicrobial activity of lactoferrin in the chitosan-based edible film and the synergistic effects of lactoferrin with lysozyme against foodborne pathogenic bacteria were studied. Chitosan and glycerol were dissolved in 1% acetic acid solution to prepare chitosan-based films. Three concentrations of lactoferrin, lysozyme or nisin were added into the film to possess final concentrations of 0.176, 0.352, or 0.703 mg in a round disc of 10 mm-diameters. Three levels of lactoferrin i.e. 0.1, 0.2, or 0.4 milligrams/disc were also added to the film combined with lysozyme. Both zones of inhibition assay and cell count assay were performed. Antimicrobial activity against *L. monocytogenes* and *E.coli O157: H7* was examined. Although the film incorporated with lysozyme exhibited substantial antimicrobial activity against both *Listeria monocytogenes* and *E.coli O157: H7*. Lactoferrin did not exhibit any significant antimicrobial activity (*p* < .05). However, a combination of lactoferrin with lysozyme exhibited significant synergistic effects against both bacteria. The addition of lactoferrin to the film with lysozyme revealed greater antimicrobial action than the addition of EDTA at the concentration of 0.4 mg/disc against *Listeria monocytogenes*. The results suggest that lactoferrin can be used to replace synthetic chelator such as EDTA with greater synergistic effects.\[66\] In a study, the effectiveness of lactoferrin and lysozyme integrated into casein or zein films against *E.coli* was measured. Three levels of lactoferrin i.e. 7, 14, or 28 mg and lysozyme i.e. 6, 12, or 24 mg were applied to the surface of the films and were checked against *E. coli*. Films incorporated with lactoferrin or lysozyme expressively stopped the growth of *E.coli* depending on levels of antimicrobials.\[67\] Rollini, Nielsen, Musatti, Limbo, Piergiovanni, Hernandez Munoz and Gavara\[68\] invented a film coated with lysozyme and lactoferrin which was found to be very effective against hydrogen sulfide producing bacteria in salmon fillets.

Encapsulated Lactoferrin was studied in two types of the emulsion to protect interference of divalent cations with antimicrobial activity of LF. LF (20% w/v) in distilled water was used to prepare microcapsules. LF (20% w/v) in sodium lactate (3% w/v) or in sodium bicarbonate (20 mM) was also studied. An oil mixture of 22% butterfat, 78% corn oil and 0.1% polyglycerol polyricinoleate was used for emulsification. Microcapsules were combined in edible Whey Protein Isolate packaging film to investigate the antimicrobial activity of LF against *Carnobacterium viridans*, a meat spoilage organism. The progression of *C. viridans* was hindered at 4 & 10°C. Microencapsulated Lactoferrin had better antimicrobial activity and unencapsulated LF showed lower.\[62\]

Antimicrobial drugs reduced its efficacy in complex food systems than in microbiological media. Nisin activity, however, is improved dramatically when applied in liquid media or foods instead of in solid or heterogeneous products, as it is more uniformly distributed in liquid systems.\[63\] LF is an antimicrobial protein that naturally occurs in bovine and human milk and is highly susceptible to calcium or phosphate levels endorsed in food. LF is a naturally occurring antimicrobial protein present in bovine and human milk, is prone to promoted levels of calcium or phosphates in foods. Environmental factors and processing techniques may affect the activity of antimicrobials. For instance, the nisin activity reduced by 20% during the melting process for the production of
pasteurized process cheese. An ultra-heat treatment for processed cheese may reduce the nisin activity up to 40%. Refrigerated storage may stabilize the nisin activity but after refrigerated storage degradation is increased at ambient temperature.\[64\]

**Antimicrobial delivery systems**

Delivery systems are used for the controlled targeted delivery of drugs to the preferred site. A drug is encapsulated in the carrier material in a designed structure in order that the drug is gradually released from the carrier material by mass transfer\[69\], or released rapidly due to the destruction of a carrier material triggered by environmental changes.\[69\] In addition, supply systems protect antimicrobials against interaction with bioactive components present in food, processing and environmental factors. Nisin encapsulated liposomes showed a 2-log larger eradication of *L. monocytogenes* than free nisin.\[70\] Lysozyme embodiment in poly (lactide-co-glycolide) by hot-extrusion improved enzyme consistency, reduced burst discharge and increased discharge culmination.\[71\] Sustained release of lysozyme from spray- dried zein capsules over 49 days was observed. The emulsification- solvent evaporation method of lactoferrin-loaded chitosan microcapsules showed a high loading level and a gradual release of lactoferrin over 7 hours.\[72\] Antimicrobials coated on films should be used as bioactive packaging in order to prevent processing on food surfaces after processing. For example, sodium caseinate films containing nisin on cheese showed 1.1 log *L. monocytogenes* inhibition after storage at 4°C for 07 days.\[73\] Nisin attenuated on poly (ethylene-co-vinyl acetate) films suppressed biofilm generation from *Staphylococcus epidermidis* ATCC 35984\[74,75\] Soy, zein and wheat gluten proteins are also found to be effective as film carriers for nisin against *L. monocytogenes* in turkey bologna.\[75-77\] Food systems are an important vehicle for delivery of bioactive because of their ability to remain active during processing, manufacturing and storage life of the produce. Lactoferrin when enriched in stirred yoghurt stored at 4°C showed its osteogenic activity up to 21 days.\[78\] To enhance the delivery system and continual release of LF at the mandatory location has been done by the use of biomaterials tools as well as biomedical instruments.\[79\]

**Therapeutic applications**

Lactoferrin (Lf) has been claimed to improve and strengthen the immune system through its antioxidant, antibacterial, and anticarcinogenic properties.\[80\] Bovine lactoferrin (bLF) isolated from cheese whey and skimmed milk is added to foods for specific nutritional applications, i.e. infant formula, dietetic foods, dairy products, yoghurts, yoghurt drinks, and chewing gums. 1.1 g bLF per day is prescribed level for the safety of bLF as an innovative food ingredient. Infant formulas enriched with 1 mg/ml promoted beneficial microflora comprising *bifidobacteria* and reduced *clostridium* in the gut in low weight as a well normal newborn.\[81\] Wisgrill, Wessely, Spittler, Förster-Waldl, Berger and Sadeghi\[82\] opined that LF killed *E-coli* in the gut of adolescents. Bovine Lf is also exhibited to clear an E-coli infection in cattle.

**Immunomodulatory effect of Lf**

This protein is observed to have a number of biotic functions including anti-cancer, anti-microbial, immuno-modulatory and antioxidant effects. It has antiphlogistic, bacteriostatic and bactericidal properties.\[83\] It was determined that lactoferrin level has indicative implication in particular in the cases of colitis; inflammations at colon area thus offering an inexpensive technique to detect intestine inflammation alternative to endoscopy. It is an excellent indicator of inflammation in the colon area and significantly increased in the case of Crohn’s disease.\[84,85\] The enhanced lactoferrin level is also an indicator of malignant tumors of kidneys observed in immuno-histochemical patients.\[86\] Lactoferrin moderates the immune system as well as intestinal epidermal cells by the optimistic outcome of *bifidobacteria* growth.\[87,88\] Lactoferrin was also explored for the treatment of *Helicobacter pylori* infection suppressing the growth of this pathogen.\[89,90\] Its secretion in mucosal
has a therapeutic effect against *Mycobacterium tuberculosis*. [91,92] Moreover, Lf has tested against *Streptococcus mutans*, *Salmonella enterica sv Typhimurium* and *Aggregatibacter actinomycetemcomitans* and found to be effective against these strains of pathogens (Velusamy et al., 2016; Wu et al., 2016; Velusamy et al., 2014).

**Anti-viral properties**

Antiviral effect of LF was detected in the event of papillomaviruses that cause cervical cancer. [93] Camel milk lactoferrin has also been found possessing therapeutic properties in curing hepatitis C and is used in traditional medicines. [94] Wang, Ye and Ng [95] reported LF recorded the highest inhibition potential for HIV-1 reverse transcriptase, particularly when compared to other milk proteins. Bovine lactoferrin suppresses monkey embryo kidney cell poliovirus infection by attaching susceptible cell surfaces and preventing viral adsorption. Thus, LF is therefore effective against enteric viruses. [96] Bovine lactoferrin and lactoferricin block viral entry into host cells and CXCR4 or CCR5 attachment and blocks the further division of HIV. Human cytomegalovirus. [97,98] Florian, Macovei, Lazar, Milac, Sokolowska, Darie, Evans, Roseanu and Branza-Nichita [99] suggested that LF and its derivatives neutralize the viral particles and inhibit the viral entry and interfere with the viral attachments of HBV. Human, cow, sheep and camel LF interact with the virus and suppress viral invasion and amplification in the host cells. [100–102] Bovine lactoferrin prevents adenovirus replication by targeting viral III and IIIa structural polypeptides during the entire replicative cycle, neutralizes infection by binding to virus particles. [103,104] Bovine lactoferrin inhibits hantavirus adsorption to host cells and viral shedding. [105] Human lactoferrin inhibits sindbis virus and semliki forest virus infection by interfering with virus-receptor interaction. [106] An (H5N1) β-lactoglobulin-lactoferrin, esterified β-lactoglobulin, and esterified lactoferrin inhibit avian influenza interaction with viral nuclear proteins (PB1, PB2, PA and NP), which disturbs the overall replication pathways by catalyzing the transcription of viral RNA. [107] An (H1N1) Lactoferrin suppresses Influenza virus infiltration of inflammatory cells in the lungs. [108] H1N1Bovine lactoferrin suppresses cytopathic effects, binds to the HA(2) region of viral hemagglutinin and suppresses virus-induced hemagglutination and infection. [109,110] Lactoferrin binds to the F1 protein subunit of the Respiratory syncytial virus. [111] Bovine lactoferrin and lactoferricin inhibits human echovirus binding, exhibits cytopathic effect and antigen synthesis, inhibits viral replication prior to, during and subsequent to the viral adsorption step. [112,113] Enterovirus Porcine lactoferrin blocks the adsorption or receptor-mediated binding of the virus to the target cell membrane. [114] Poliovirus Bovine lactoferrin targets viral adsorption binds to the surfaces of host cells and prevents attachment of virus particles, competes for viral receptor interaction. [96,115] Human rotavirus Apolactoferrin, Fe3+-lactoferrin and β-lactoglobulin binds to viral particles, impedes viral attachment to cell receptors suppress both rotavirus hemagglutination and binding to a cellular receptor. [116]

Human Lactoferrin derived peptide fragments were used to the destruction of yeast originated infections. [117,118] Lactoferrin prevents the tumor in the small intestine. [119,120] Combination of lactoferrin with the polyphenols enhanced cell death of cancer cells representing anti-proliferative effects. [121] Tomita, Wakabayashi, Shin, Yamauchi, Yaeshima and Iwatsuki [122] suggested 3 g/day of oral LF for one year in cancer prevention without inimical effects. The protective effect was confirmed as a greater increase in weight and lesser prevalence of illness. [123] Duran, Deschler, Precigou and Goulas [124] expounded that the dietary supplements of bovine lactoferrin has antibacterial, antiviral and antioxidant properties and enhance the immune system response. One capsule of control was given to eight healthy males of 30 to 55 years for 7 days. Then 100 and 200 mg of LF for 7 days was given every day for 21 days. Statistically noteworthy increases were observed in T-cell between pre-supplementation levels and with 200 mg of supplementation. These results propose that T-cell activation, as well as antioxidant status, can be achieved by oral supplements of bovine lactoferrin. Diarra, Petitclerc and Lacasse [125] evaluated the therapeutic potential of the combined role of bovine lactoferrin and penicillin G against *Staphylococcus spp*. It enhanced the activity of penicillin by 2 to 4 fold and minimized the growth rate of *S. aureus* strains tested while the
inhibitory activity of Lactoferrin by penicillin was increased 16 to 64 fold. Changes in the protein profile of the bacteria occur due to the presence of a combination of both.\textsuperscript{126} evaluated the nutraceutical potential of LF in relation to estrogen deficiency and bone loss and observed positive results.

Lactoferrin can be potentially used for defense against bone damage. Dietary effect of LF supplementation in rodents has been observed for the treatment of bone problems in post-menopausal condition. In another study in women, it was explored that lactoferrin added in drinking water inhibited resorption of bones. Liposomal-lactoferrin might be a powerful therapeutic agent for diminishing alveolar abolition of bone in patients having periodontitis. Fischer, Debbabi, Blais, Dubarry, Rautureau, Boyaka and Tome\textsuperscript{127} provided strong evidence of bone health promotion by using Lactoferrin; a food supplement.

Lactoferrin (LF) also improves bone metabolism. However, the contribution of LF to the bone strengthening activity of MBP is indistinct. Studies suggest that the effects of milk basic protein (MBP) on bone metabolism are not solely due to LF rather multiple components in MBP may act together to improve bone metabolism.\textsuperscript{128} Wakabayashi, Yamauchi, Kobayashi, Yaeshima, Iwatsuki and Yoshibe\textsuperscript{129} evaluated the effect of LF on the growth of Porphyromonas gingivalis and Prevotella intermedia in the subgingival plaque. Major therapeutic uses of LF also include prevention and treatment of cancer so can be used as a chemopreventive mediator.\textsuperscript{130} Lactoferrin has a significant role in healthy metabolism. In another study, it was evaluated that lactoferrin levels have a relationship with lipid concentrations and obesity. Forty-five obese patients including 15 men and 30 women with Body Mass Index (BMI); 53.4 ± 7.2 kg/m were checked. Concentrations of circulating lactoferrin, at baseline as well as fat-stimulated were inversely linked with postprandial lipemia, oxidative stress and fat-induced inflammation in sternly obese patients.\textsuperscript{131} Lactoferrin has great potential for curing skin infections as well as its maintenance. In another study, lactoferrin was fortified in fermented milk for treatment against acne vulgaris. 200 mg LF was given on daily basis to 18 subjects of age ranging from 18 to 30 years for 12 weeks. The improvement was observed in the lactoferrin group by substantial reductions in triglycerides of lipids in skin surface lipids. Inflammatory lesion count was declined up to 38.6% while 23.1% in total lesion counts, and 20.3% in acne grade as compared with the control group.\textsuperscript{132} Lactoferrin was tested for the prevention of biofilm growth. LF should be continuously exposed to biofilms.\textsuperscript{133} LF has great anti-biofilm effect when used in adjunction with for the healing of infections related to diabetic foot ulcers. Iron chelating characteristic of Lactoferrin is exploited for the destabilization of bacterial membrane\textsuperscript{134,135}, Ammons et al., 2011b). Tang, Cui, Wu, Liu-Mares, Huang and Li\textsuperscript{136} studied the effect of LF in the fibroblastic phase in certain in vitro cultures of human skin fibroblasts and concluded that it has a huge impact on the healing of wounds. Only two studies have been conducted on the wound healing property of Lactoferrin; conducted an experiment on diabetic mice and by Lyons and others\textsuperscript{137} who studied on clinical patients. LF can be used for inhibition of Enterohemorrhagic Escherichia coli serotype O157:H7 strains responsible for haemorrhagic colitis as well as hemolytic uremic syndrome in humans from cattle.\textsuperscript{138} LTF delivered to the ICH-affected brain by infiltrating PMNs may assist in hematoma detoxification and represent a powerful potential target for the treatment of ICH. Cerven, DeGeorge and Bethell\textsuperscript{139} determined safety measures of orally administered lactoferrin of any origin by toxicological tests and claimed that it is safe for use in human beings.

\textbf{Conclusion}

Innovative technologies are needed to resolve issues of food preservation in developing as well as developed countries. Regarding food safety situation, thousands of people die due to lack of hygiene in the food chain from farm to fork. Emerging foodborne, as well as zoonotic pathogens, causes several outbreaks of foodborne diseases in human beings. Antibiotic-resistant bacteria are major issue and challenge for health sector scientists. Lactoferrin; a multifunctional ingredient found in

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milk has numerous applications as a natural antimicrobial food additive as well as pharmaceutical. This minor but mighty bioactive component of milk has amazing properties including anti-microbial, antioxidant, anti-infective, anti-cancer, weight management and can be used as a natural alternative to chemical antibiotics. There is also a need to do work on antimicrobial food packaging incorporated with Lactoferrin, biofilm control in food processing using lactoferrin. Similarly, as an iron-containing protein, it can be used for curing anemia; a major issue in pregnant women and newborn babies. Keeping in view sufficient milk production in developing countries, there is dire need to develop suitable and cost-effective on-farm as well as industrial based technology to isolate high value protein; lactroferrin from milk of different indigenous species as a specialty ingredient for dairy industry and for use in biopreservation of milk, meat, fresh cut fruits and vegetables and their products to increase shelf life, control diseases and enhance public health.

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