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Lactoferrin: Structure, biological functions as functional food and maintaining health

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ABSTRACT

Lactoferrin is part of a range of bio-proteins and dairy ingredients specifically designed and developed for nutritional products, particularly in the infant and sports nutrition sectors. Lactoferrin is known as a leader in the technology of milk fractionation and processes. These agents could positively influence the individual's growth and/or their nutritional status and alleviate various health aliments including inflammatory conditions, endotoxin shock, and drug induced damage.

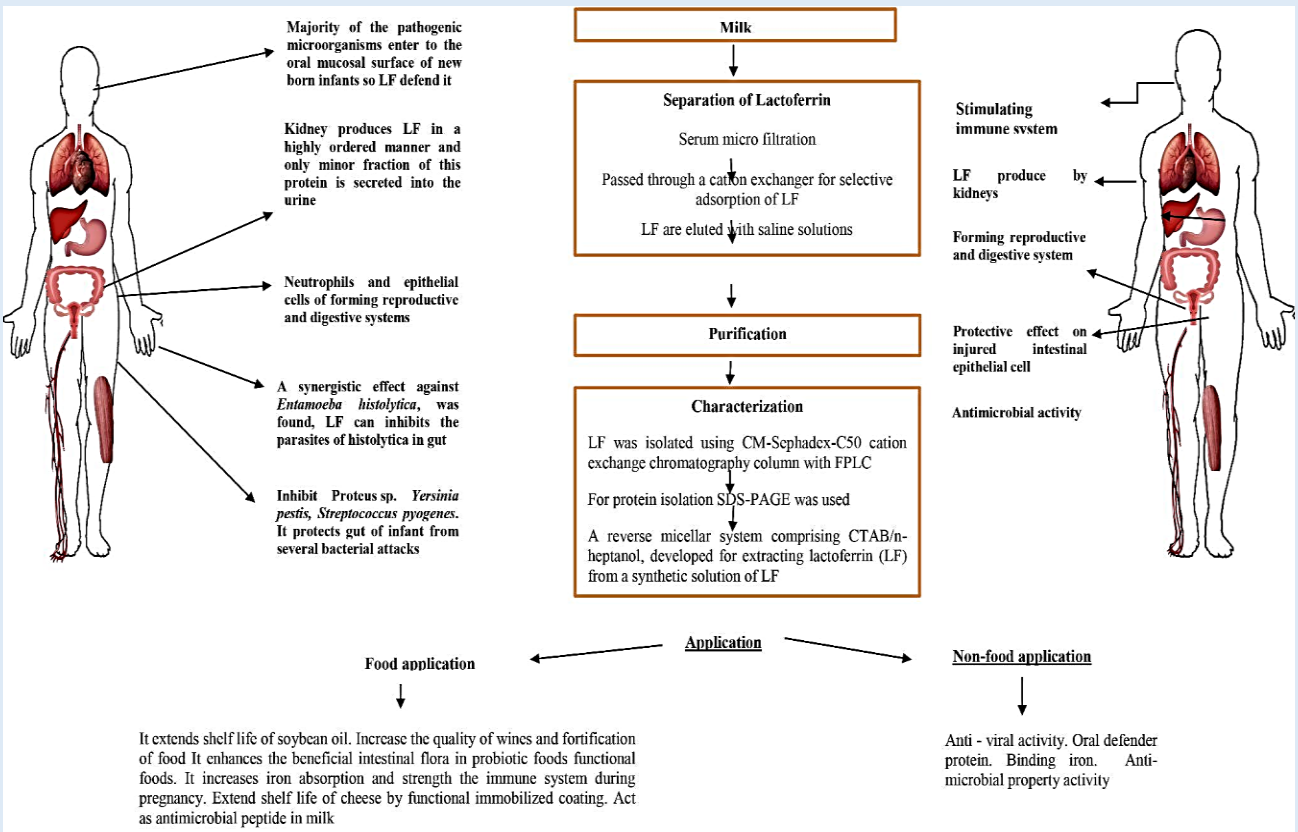
Lactoferrin is generally used as a nutritional supplement. In the last few decades, bovine lactoferrin, an iron-binding glycoprotein and whey protein known for its beneficial effects, has been purified and characterized by scientists from both bovine milk and tears. Due to their various biological properties, they are used to treat diseases, and regular dietary recommendations are being made to address several disorders in the body.

For this review, an extensive web search has been carried out using various scientific databases for lactoferrin related studies. The search keywords used were "lactoferrin", "nutraceuticals", "sources", "chemical structure", "biological functions", "synthesis", "immunity", "disease".

Lactoferrin has several activities such as anti-microbial, antioxidant, antibacterial, anti-inflammatory, antiviral, cytoprotective, antifungal, and iron-binding activity. Research has demonstrated various beneficial effects, especially in inflammatory conditions and in the fight against cancer. Lactoferrin is likewise applied in fish to modulate the intestinal immune system and can constitute a nice supplement in aquaculture. For viral and microbial inhibition, it is recommended to take 64 mg per day for adults.

Lactoferrin can be applicable in both food and non-food system. In the food system, it can be applicable in functional foods, probiotic foods, milk, soybean oil, and edible coating of cheese.

Keywords: Biological functions, health, immunity, lactoferrin, structure.



INTRODUCTION

Lactoferrin is a sugar conjugated protein (glycoprotein) consisting of a sugar molecule and amino acids. The term lactoferrin is not a new one as it was previously termed lacto transferrin. Lactoferrin (LF) protein has a relation with the transferrin family this protein is one of the members of the mentioned family, LF can bind and transfer the ions of Fe^{3+} [1] and its concentration is about 150 mg/L in human colostrum. As the name implies, it possesses affinity towards iron as one molecule of LF reversibly binds with two Fe^{3+} ions [2]. This iron-binding glycoprotein, with a molecular weight of 80 kDa, exhibits various properties such as antimicrobial, antiviral, and antioxidant activities, as well as strengthening the immune system [3]. In 1939, Sorensen isolated LF from bovine milk for the first time. It was concurrently identified in human milk during 1960s [4,5]. LF is also present in saliva, tears, synovial fluids and blood [6]. Chemical composition of humans and bovine LF are nearly similar and composed of 703 amino acids which shares 60% of homology [7]. LF is used in several commercial applications in foods, health drinks, fermented milk, chewing gums, and toothpastes.

LF has been the focus of attention due its wide range of applications. Bovine LF has been widely studied for its pharmaceutical applications like anti-microbial, antioxidant, immunomodulatory, anti-metastatic, and anti-carcinogenic activities [8]. LF are used as additives in increasing the storage life of soybean oils, quality of wines and fortification of foods [9]. They extend the shelf life of cheese by the formation of immobilized coating. These LF were found to promote the growth of beneficial intestinal flora in probiotic foods. In functional foods, LF is incorporated to increase iron absorption and strengthen the immune system during pregnancy. LF reduces the risk of cardiovascular diseases, obesity and diabetes. LF acts as an antimicrobial peptide in milk [9] which inhibits Proteus sp. *Yersinia pestis*, *Streptococcus pyogenesis*, and protects gut of infant from several bacterial attacks [10]. LF can stimulate proliferation of osteoblast complements thymidine incorporation in osteocytes and decreases the apoptosis of osteoblasts (50–70%) [7]. LF decreases or even prevents the osteoclast genesis but does not affect the resorption of bones [11]. With increase in applicability of LF for various biological functions and industrial applications their importance has been gaining across all fields of nutrition and health. This current review describes about the identification of LF having different property, its effect on different body systems and application in food industry.

Methodology: Various literatures have been referenced in the preparation of the manuscript, with greater emphasis on their identification, structural chemistry, biological functions, sources, applications, etc. The common search items used were 'lactoferrin identification', 'lactoferrin sources', 'lactoferrin biological functions', in various databases including google scholar, PubMed, Scopus, Researchgate. The recent publications from relevant topics were selected for this work. The manuscripts discussed the structure, properties, functions and applications, so no statistical analysis or interpretations were used.

Physio-Chemical Properties: This single chain polypeptide has a molecular weight of 80 000 Da with 1→4 glycan and this bond will vary among different species [12]. LF of humans and bovine origin has 680 and 690 amino acids respectively and has a similarity of about 69% [13]. LF comprises of two homologous lobes (C and N) and these corresponds to the carbon and nitrogen-terminal. Each lobe consists of two sublobes/domains, which form a cleft where the ferric ion (Fe3+) binds in synergistic cooperation with a carbonate anion [10]. These domains further labelled as N2, N1, C2, and C1, respectively. Among these, C2 presents the hinge and has conformation of the helix with has three turns and performs the role of closing and opening the domains [14]. The secondary structure of the molecule is partly due to the disulfide bonds between cysteine residues. LF molecules contain different numbers of sites for potential glycosylation with mannose is the most common saccharide. Degree of glycosylation in LF can be changed which determines their rate of resistance to proteases treatment or their activity at low pH [15].

Three different isoforms of LF have been reported with iron-binding form as Lactoferrin α, which does not exhibit ribonuclease activity. Lactoferrin β and lactoferrin γ showed ribonuclease activity but can't bind with iron [16]. During inflammation and bacterial infections, due to their metabolic activity the pH may decrease to 4.5 or below. The capacity to bind with iron even at low pH is crucial for control of infections. LF can bind with iron thereby making them non-available to the bacteria for their proliferation [17]. LF has two times higher iron binding property than the transferrin which could serve as Fe^{3+} ions donor for LF. Each molecule of LF can bind with two ferric ions [18]. LF exhibits resistance to enteric proteolytic degradation by both trypsin and trypsin like enzymes. However, this resistance is proportional to their degree of iron saturation [19]. The concentration of LF was higher in colostrum breast milk (7 mg/ml) which decreases in mature breast milk (1-2 mg/ml). Among the other human body fluids, higher concentration was reported in synovial fluids (10-80) followed by saliva (7-10), tears (2.2) and seminal plasma (0.4-1.9 mg/ml). Similarly, the cow's colostrum whey (1.50 mg/ml) and cow's milk (20-200 mg/ml) had higher concentrations of LF [20].

Digestion of Lactoferrin: Like dietary proteins, lactoferrin (LF) is digested into smaller molecules. Important nutrients, such as small molecular weight peptides and essential amino acids, are absorbed by the intestine and then transported into the bloodstream. The gastrointestinal breakdown of LF causes unwanted loss of its useful properties. It has been reported that strong antibacterial peptides are produced by pepsin hydrolysis of LF [21]. Therefore, understanding of LF digestive pattern is important for their functional properties especially the peptides derived during different digestive stages.

Various value-added food products including infant's formulations and nutritional supplements are fortified with LF for better delivery through oral intake. An *in vivo* assimilation study on 20-year-old volunteer's given oral administration of iron soaked LF showed >60% of preparation passes the gastric digestion with fundamentally unblemished structure [22]. LF administered in fluid forms has quick gastric discharging time than other forms [23]. However, detailed studies are needed to understand the degree of changes LF undergoes during digestion. Identification of these structural changes will provide more insights about the LF assimilation and utilization.

Biological functions: LF exhibits different biological functions which could be exploited for commercial use. They are an important component in the primary line of host's defense and responds to a variety of environmental and physiological changes. LF can be used for improving the shelf life of food products due to its iron scavenging activities during their prolonged processing. LF can bind with anions like carboxylates, oxalates, which can affect their metabolism [18]. Vitamin A-based antioxidant mixes with bovine lactoferrin (LF) are used to protect unsaturated fatty acids from oxidation and rancidity. The addition of LF is due to their ability to bind with oxidation impetuses like copper and iron. A study evaluated the role of LF on oxidation prevention in soy fat powder and its anticancer potential at higher processing temperature (70°C), revealed that LF improved the timeframe of realistic usability of soy fat powder. Even at processing temperature of 20ºC, the presentation of two cell reinforcements was practically identical. This model shows the ability of LF as a cancer prevention agent (Fig. 1).

Iron metabolism: During mid-1960s, research on the transferrin family has gained attention due to its high fixation activity in human breast milk [24]. These particles have been distinguished in various mammalian species, like dairy animals, pig, wild ox, equine, goat, and mouse and studies were conducted to identify their roles [25]. Its usefulness as a solid iron-binding protein has been well-established; however, its non-iron-related roles have also been described. The activity of lactoferrin (LF) in iron binding is particularly fascinating, as it shows low concentration in plasma under normal conditions but exhibits higher affinity during infections. As a defense molecule against gastro-intestinal infections they participate in local secretory immune systems [10]. In synergism with other immunoglobulins (IgG) and other protective proteins, they supply iron-binding antioxidant protein in tissues and possibly promote the growth of cells like lymphocytes and intestine. It is important to note that their ability to bind iron ultimately led to the initiation of biological functions and the inhibition of several bioactive compounds, such as LPS and glucosamine-glycans [26]. First, they assimilate iron from the contamination locales which is the food wellspring of the microorganisms and thereby act as a bacteriostatic. Expulsion of iron is the basic component for the development of pathogenic microorganisms. LF, typically 6-8% immersed with iron in organic liquids, restrains bacterial development by contending with siderophore for restricting free iron availability [27]. Hence, LF is inhibitory to high-iron-requiring microbes than low-iron-requiring ones. During inflammation, the level of LF has been increased and when the pH is low the exchanger of iron from the transferrin is easy [20]. However, the role of LF as a main molecule to transfer the iron to the body under the normal situation still needs to be explored.

LF has responsibility of the hypoferraemia for binding of the iron and giving it back to the macrophages [28]. LF has a function known as a control function, which is activated when iron levels increase and are released from depots [29]. Similar relation was also observed in LF from duodenal secretion and metabolism of the iron [30].

Anti-microbial activity: LF acts as bacteriostatic through sequestering the iron from bacterial pathogens, subsequently denying them of this vital supplement and hindering their development [31]. In addition, LF has been demonstrated to possess bactericidal effect on pathogens that were autonomous of iron restriction. LF associate with the LPS layer of the gram-negative microorganisms causing its discharge. Aside from this, LF also supress the biofilm formation by *Pseudomonas aeruginosa* [32]. Antimicrobial proteins and peptides produced by a large variety of organisms that will be the first line of the defense of peptides. The most crucial antimicrobial proteins are lysozymes, and LF [33]. The antimicrobial activity of these proteins is related to opsonization or bacterial lysis of pathogens. In addition, mannose-binding proteins interact with HIV and influenza A virus, which can be neutralized by surfactant protein A [34]. is a multifunctional protein known for its role in bacterial clearance [35]. The main physiological function of LF is iron binding, which is primarily recognized as a key characteristic of the protein. This function contributes to its antibacterial activity and its ability to inhibit the growth of a broad spectrum of bacteria. In addition to antibacterial and antiviral activity, LF can prevent parasitic and fungal infections. LF is thought to be part of the immune system initiating. LF can take part in specific immune reactions, but in an indirect way [36]. Because of its strategic position, mucosal LF can represent the first line of defense against microbial agents invading via mucosal tissues. LF affects the growth and proliferation of a variety of infectious agents including gram-negative and positive bacteria, protozoa, viruses, or fungi [37]. LF harms the external films of gram-negative microorganisms causing the LPS damage and tie with porins and promote the precariousness of the bacterial layer and penetrability [38].

LF not only restrict the growth of iron-dependent pathogens, but they also facilitate the growth of low-iron dependent microbes like *Bifidobacterium* or *Lactobacillus spp* which are beneficial in nature [39]. Some bacteria can create new conditions and produce siderophores (iron-chelating compounds) that compete with lactoferrin (LF) for Fe3+ ions [40]. It is believed that the antimicrobial activity of LF is most pronounced when it is in its apo form, as studies have shown that only apo-lactoferrin retains full antibacterial activity. This property is reduced when LF is iron-saturated [41]. LF's N-terminal flap exhibits a serine protease-like action, and LF is rich in arginine, with the protease active site orchestrated in the N-terminal projection [42].

Anti-fungal activity: The antifungal effect of LF was associated with their ability to sequester Fe^{3+} ion. Subsequently, *Candida albicans* and *Candida krusei* can be killed by altering the permeability of cell surface. Fungicidal activity of LF significantly decreases under anaerobic conditions, in presence of mitochondria and low extracellular K, Na^{2+}, Ca^{2+}, and Mg^{2+} situations. The antifungal action of LF against *Aspergillus fumigates* can be ascribed to its iron restricting property while they eliminate *Candida albicans* and *Candida krusei* by modifying the penetrability of cell surface. LF is reported to have a lower antifungal activity than commercially available drugs [43].

Anti-viral activity*:* LF has antiviral properties against a wide range of viruses which cause infection in humans and animals. LF can bind with certain DNA and RNA viruses. Its main contribution to antiviral defense is due to its binding ability to the cell membrane glycosaminoglycans. Thus, LF inhibits viruses from entering the cells and infection is stopped at primary stage [44]. has a strong ability to inhibit the tobacco mosaic virus [45]. Studies on antiviral action of LF demonstrated that they can prevent viral disease during the underlying phases of a few infections including hepatitis B infection (HBV), herpes simplex infection (HSV), hepatitis C infection (HCV), human immunodeficiency infection (HIV), rotavirus, adenovirus and poliovirus [46]. *In vitro* studies showed that LF present in human plasma and milk proteins, have potent activity against these viruses. The antiviral activity of LF against viral pathogens - flu, common cold, gastroenteritis, and herpes is by blocking their adherence to target cells [47]. LF influences the infections movement and hinder the virus-host association in HBV, HSV or direct association with viral molecule e.g., cat herpes virus infection (FHV-1), hepatitis C virus infection (HCV), hepatitis G infection (HGV) and human immunodeficiency infection HIV [48].

Infections like rota, polio, adeno, and enterovirus are vulnerable to LF. LF shows its antiviral activity at an early period of the decontamination procedure [49]. *In vitro* investigations showed that LF displays cooperative energy, in blend with zidovudine, against HIV-1. A synergistic antiviral action was likewise watched for HSV-2 and HSV-1 when acyclovir was utilized in blend with LF. In clinical preliminaries on a constrained arrangement of HCV patients, it was exhibited that LF essentially diminishes the HCV RNA titter and adds to the viability of a joined treatment with interferon and ribavirin. Oral administration of LF has prompted promising improvement in the invulnerable reactions of antiretroviral treatment youngsters experiencing HIV [50].

Anti-parasitic activity: LF controls parasites in different ways, as the infection of *Eimeria* spores and *Toxoplasma gondii* was reduced after co-incubation. It is known that LF disturbs parasitic membrane integrity which causes subsequent changes in the interactions between the host and the parasite [51]. LF further exhibits parasitic leisure activity, even though the atomic components of such intrigue are significantly more complex.

Antioxidant activity: The antioxidant and protective effects of LF were assessed on H_2O_2-induced oxidative stress in human endothelial cells and umbilical vein [52]. Pre-treatment of cells with LF at a concentration of 25–100 mg/ml is reducing the H_2O_2 induced cytotoxicity in a concentration dependent manner. Hydrogen peroxide levels can also decrease or increase the FRAP value in both intracellular and extracellular assays [53]. LF has been established, that anti-oxidation properties of LF from dried colostrum and cannot be changed at the duration of 12 months by dry colostrum storage under proper condition [52].

USE OF LACTOFERRIN IN CLINICAL CONDITIONS

Hepatitis C prevention: Studies show that LF may inhibit hepatitis C infection as administration of LF increases the levels of interleukin 18 (key role in fighting off the hepatitis C virus). This year-long study involving 63 subjects and demonstrated the benefits of LF in improvements in humans. Clinical trials have pointed out that the LF inhibit intra-cellular virus replication, in addition to its capacity to prevent virus entry into the targeted cells [54].

Preventing osteoporosis*:* Initial studies have demonstrated that, LF can prevent the development of osteoporosis. LF can work with hydroxyapatite mineral to stimulate bone growth by promoting cell formation through osteoblasts [55]. LF supplementation helps in establishment of new blood vessels in women during post-menopause and people who consume LF supplements have a high bone formation.

Prevention of infections: LF has the ability to protect the body from microorganisms, and its antiviral activities involve preventing the entry of viruses into cells, inhibiting their replication, and boosting the body's immune functions [55]. LF can help to protect organs from *Helicobacter pylori* infection which is known as the type of infection which causes ulcers. Use of fermented milk and the different components of milk including protein and LF can prevent this infection. Bovine LF can destroy *H. pylori* and can decrease the rates of infections. Some other benefits which can be performed by using the LF are boosting the immune system, preventing the damage which has relation to age, developing the beneficial bacteria in the human intestine, regulation of the metabolism of iron, preventing diarrhoea, and cancer [56].

Cancer Prevention: LF down regulate numerous disease cell lines and tumors, provoking scientists to investigate the impact of LF on tumor development. It was later exhibited that LF can hinder the progress from G1 to S stage in cell pattern of threatening cells [57]. Cell expansion was reduced because of LF-prompted adjusted action of a few cellscycle administrative proteins that incorporate Cdk inhibitors p1 and p2. *In vivo* examinations have demonstrated that LF may incite apoptosis in malignancy cells by actuating the FAS flagging pathway [58]. The immune-modulatory movement of LF is additionally basic in forestalling disease development by improving the action of NK cells.

Health Benefits of Lactoferrin: LF released from neutrophils plays an essential role in innate immunity, protecting against infection through its antimicrobial action. LF was known to upregulate T-cell multiplication, help regulate executioner cell numbers and advance lymphocyte development. LF is also involved in the myelopoietic process. LF from humans and bovine has health benefits and can be used as a food additive. About 2% of LF can act on the intestinal bacteria and flora of animals special in mice. Feeding of bovine LF to mice increase the Enterobacteriaceae. LF can prevent the translocation of the aerobic bacteria which is present in the gut, and peptides of LF can also prevent gastric infections [59]. LF can decrease the fungal burden in back and foot. It reduces the human body weight loss caused by HSV 1 [60]. LF can increase RBCs and haemoglobin and reduce the nutritional disorders. LF also decrease the fats in liver and serum with the maintenance of iron in the body by increasing haemoglobin [61].

Application in Food Industry: The initial application of LF was directed in infant formulations. Nowadays, LF is used in foods, oral medications, and different skin treatments. The products in which LF is used are skim milk, pet foods, yogurt, and drinks. LF detoxifies the iron-free radicals in the biological fluids. Presently, deficiency of iron is one of the most nutritional deficiencies throughout the world [62]. LF is considered as an effective and safe ingredient to delivered iron to deficient peoples [63]. It has been found that iron from bovine LF is easily absorbed in the intestine compared to inorganic ferrous salts. Clinical research on pregnant women given oral administration of bovine LF has increased absorption of iron than from the salts of ferrous at the same dosage [64]. The usefulness is identified with solid iron-binding properties of LF. The variation in absorption of iron from LF and other salts may be because of the existence of specific LF receptors on the mucosa cells [65]. Oral administration of LF revealed higher absorption rate of iron in new-borns [66].

The EFSA directed an appraisal for ox-like LF to be utilized as a food fixing in different items including the newborn child formulae. The proposed degrees of LF in certain nourishments would fluctuate from 667 mg/100 g for child food sources, 100 mg/100 g for baby formula, and 4000 mg/100 g for vitality bars for athletes and ladies. Administration (g/day) of newborn children (0-6 months) with 1.2 and 1.9 g for babies (8-10 months) improved overall health status. The Commission of Europe has allowed endorsement for LF as a novel food usually utilized in child nourishments. In United States, LF is used in practical nourishments (100 mg/serving). Nonetheless, concerning the GRAS status, FDA has no inquiries over the ends set forward by DMV International (2001) and Morinaga Milk Industry Co., Ltd (2014) who have pronounced milk LF as GRAS. FDA expresses that the GRAS status to dairy animals' milk-inferred LF [67]. Because of the presence of essential amino acids in milk, it is specified that the protein of milk is a very crucial source of bioactive peptides in vegetable and animal protein sources. These peptides which are derived from milk proteins have different physiological bioactivities such as anti-microbial, anti-oxidative antithrombotic, antihypertension, opioid effects, and mineral binding. Milk proteins have the most crucial role in decreasing and preventing the risk of cardiovascular diseases with type II diabetes and obesity related diseases. Contamination with microorganisms is inevitably the main way to give shelf life to milk by preventing bacterial activity [68]. LF is largely used in the industry of infant dairy formula to provide protection and inhibit pathogens. Functional foods will be rich in bioactive compounds which may be either nutrient or non-nutrient in nature. These bioactive compounds will be in smaller fractions which could provide health benefits [69]. Considering the health benefits of LF through its anti-infective, anti-inflammatory, and anti-cancer activities, LF is a bioactive compound found in functional foods [70]. In functional foods, it is used to increase iron absorption in cosmetics and acts like an antioxidant. In oral protecting products, it is used to improve oral hygiene. Probiotic foods are used to enhance intestinal flora that has more benefits [47]. LF can be used as food additives in many foods for its anti-microbial activity and extending shelf life of unsaturated fatty acids. The functional foods are labelled as class A, B and C based on their experimental, pre-clinical and clinical trials. A functional food that needs to be classified under category A should undergo research, including safety studies, while category B is based on epidemiological results, and category C should have undergone pre-clinical and clinical trials and be recognized as a functional food [71]. Since, the LF are recognized as functional food and LF based products are being in market (infant preparations) and are found as effective in uncontrolled study and safety; it is fair to term LF as a category A functional food product.

Lactoferrin in Different Species: LF was known for the first time in bovine and followed by human milk. LF levels differ among various animal species. The relationships between gender and LF concentrations, age, or inflammatory processes were reported. LF concentration in adult human blood was in the range of 0.02-1.52 mg/ml. Concentration of LF in human biological fluids like plasma of venous, milk, and colostrum concentrations were 0.12, 3.1–6.7 and 1.0-3.2 mg/ml, respectively [72]. The glycosylation process of LF among diverse origins (human, cows, caprine, murine) vary and hence, the biological functions of LF from different sources could vary depending on the altered glycan profiles [73].

Denaturation of Lactoferrin: LF are proteins, and they are prone to denaturation under harsh environmental conditions such as acidic or basic environments, sub-zero temperatures, and high salt concentrations. These conditions can alter the conformation of protein and breakdown the disulfide and hydrogen bonds (these bonds provide stability) [74]. In general, LF denaturation is not desirable as it alters their 3D structure and compromises their functional properties, especially the binding of iron and antibacterial activity. Thus, during the production and extraction processes, the extent of denaturation of LF should be carefully considered and the process should be optimized to preserve its biological property [75].

CONCLUSION AND FUTURE IMPLICATIONS:

Lactoferrin is a multifunction glycoprotein found in bovine, human milk, tears, saliva, mucus, and some other liquids of body. LF has gained attention due to its immune-modulatory, antimicrobial and antineoplastic activities and potential in pragmatic medication. Various research and examinations on its movement, interactions have empowered their safe use. Recent studies implicated the use of LF in pharmaceutical and food industry and boosted by the development of various techniques to deliver them. Investigations have been completed to create procedures for developing and purification of LF but none of them at a large-scale development. Therefore, there is a need for consideration from both the scholarly community and industry to develop a new, productive, cost-effective, and environmentally friendly strategy that produces LF with maximum yield. LF has significance for the different physiological procedures of the body. In the past, the various uses of LF have been transformed by the development of membrane separation strategies, with chromatography being the most widely used method for large-scale production. LF performs several activities in the body which are important for the protection of the immune system. In food systems, LF is used as an edible coating for cheese, an extender for soybean oil, a peptide in milk, and in probiotic-based functional foods. It acts as an antimicrobial, antiviral, antibacterial, iron-binding, and oral defence protein. LF in tears provides antimicrobial efficacy by binding free iron, which reduces microbial growth, survival, and pathogenesis. For oral use, a daily intake of 10-15 mg is recommended.

ABBREVIATIONS: LF: Lactoferrin, HUVECs - Human umbilical vein endothelial cells, H_2O_2 – hydrogen peroxide, MTT - 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide, DNA – Deoxy ribonucleic acid, RNA – Ribonucleic acid, LPS – Lipopolysaccharide, Cdk - Cyclin-dependent kinase, FDA - Food and Drug Administration, GRAS - Generally Recognized as Safe, FRAP - Ferric reducing ability of plasma, EFSA - European Food Safety Authority

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