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Iron transport mechanism of lactoferrin and its application in food processing

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Abstract

Lactoferrin (LF) is a non-heme protein binding Fe^{3+} tightly and plays a role in regulating the absorption and metabolism of iron under physiological condition. The absorption of non-heme iron occurs in the duodenum. LF is incorporated as a functional factor of food products during food processing. In this review, we describe the structural and functional changes of LF due to thermal processing of food, as well as the metabolic processes of LF iron binding and release in *vivo*, which characterizes the value of LF as a food matrix from nutrition and biochemistry and provides a basis for the comprehensive utilization of LF in food.

Keywords: lactoferrin; iron binding and release; lactoferrin digestion; lactoferrin function.

Practical Application: a. Changes in lactoferrin structure and function induced by thermal processing. b. Mechanism of iron-binding release from Fe3+ bound lactoferrin in vivo. c. Comprehensive application of lactoferrin as food functional factor.

1 Introduction

Lactoferrin (LF) is an iron binding glycoprotein with a molecular mass of approximately 80 kDa, due to its ability of binding Fe^{3+} ions and its structural similarity with serum TF (Transferrin), LF belongs to the transferrin family (Iglesias-Figueroa et al., 2019; Yang et al., 2017). It is found in milk, semen, mucous membrane secretions, saliva and tears (Wang et al., 2019). Bovine lactoferrin (bLF) and the human lactoferrin (hLF), having similar 3D structures (Lönnerdal et al., 2011), the similarity of amino acid sequence between which reaches 69% (Zlatina & Galuska, 2021).

In order to sterilize, milk products has to be treated at high temperature, thus the structure and functional properties of LF in changed accordingly (Xiong et al., 2021). Either LF is added to infant milk formula (IMF) prior to thermal processing (required for product safety) or LF is thermally processed alone for subsequent addition to IMF by dry blending, it is important to consider the influence of thermal processing on the LF physicochemical structure, digestibility and bio-functional properties (Goulding et al., 2021b).

Iron is an essential trace element in *vivo*, and the regulation of iron absorption largely controls the maintenance of collective iron homeostasis. LF plays an important role in the processes of iron regulation at the cellular level, preventing the body from being damaged by high levels of free iron ions. LF also has other bio-functional activities such as anti-microbial, anti-viral, anti-oxidant, anti-cancer and anti-inflammatory activities (Moreno-Expósito et al., 2018), many of these functions are closely linked to the iron binding capacity of LF. Although LF has many biological activities, it is difficult to preserve its biological functions in the processed food. Of note, there is a stimulated increased research interest in the multiple health promoting functions of LF, and its wide real-life applications.

2 Structure and source of LF

2.1 Fundamental structure of LF

LF consists of two homologous structural domains, N-lobe and C-lobe, which are connected by a α-helix structure and a β-sheet structure with two structural domains on each lobe (Figure 1). Each lobe can be further divided into two similar-sized domains in the N-lobe (N1 and N2) and the C-lobe (C1 and C2), respectively (Rastogi et al., 2016). One LF molecule can bind two Fe^{3+} ions together with two CO_3^{2-} ions, each lobe has the ability to reversibly bind a single ferric ion. bLF consists of a polypeptide chain composed by two sugar chains, which fold spatially into two similar ginkgo biloba-shaped structural domains with an ordered secondary structure (Dierick et al., 2021).

2.2 Iron bound structure of LF

Despite the different origins of LF, the iron binding sites are all roughly the same. A bLF molecule contains 40 glutamic acid and 36 aspartic acid residues, accounting for 11% of the total number of residues, which may lead to its high metal ion chelating potential (Pomastowski et al., 2016). There is an iron binding site in each lobe and the metal ion is coordinated by four amino acid side chains: a carboxylic acid in one aspartic acid residue, two phenolic oxygen atoms in two tyrosine residues and an imidazole in one histidine residue (Figure 2). The iron ion is stabilized at the protein binding site by two oxygen atoms in a carbonate ion. In the N-lobe of LF, the iron ion is bound to Asp60, Tyr92, Tyr192 and His253. In the C-lobe of LF, the iron ion is bound to Asp395, Tyr433, Tyr526 and His595. When saturated with iron, the structure of LF is more compact; when iron ions are not saturated, only the C-lobe of the LF molecule is bound to iron ions, indicating that LF releases iron ions from the N-lobe first. The two lobes of holo-LF (iron-saturated lactoferrin) has

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a compact structure and those of the apo-LF (iron-free lactoferrin) are more or less broadly open (Voswinkel et al., 2016). LF binds or releases Fe^{3+} by opening or releasing specific iron binding sites in the N- and C- lobes. Crystallographic studies have shown that the binding of iron to LF made the structural conformation of LF more compact (Baker & Baker, 2012; Rastogi et al., 2016).

2.3 The primary source of LF

LF is available from a wide range of sources, table 1 shows the range of LF concentrations in the milk of different mammals. Mammalian colostrum normally contains higher levels of LF than their mature milk, only Murrah buffaloes' mature milk contains higher levels of LF than its colostrum (Abdel-Hamid et al., 2022; Kell et al., 2020).

3 Effect of thermal processing on LF

3.1 Changes of LF structure in heating process

The denaturation of LF depends on the environmental factors such as temperature, pH, ionic strength, and the presence of other proteins and polysaccharides (Li & Zhao, 2017). Thermal processing is a very important step in the processing of dairy products, not only for sterilization but also for changing the organoleptic properties of the product (Goncalves et al., 2022; Tadjine et al., 2021). High temperatures not only alter the physicochemical, organoleptic and nutritional properties of milk, but also damage the biologically active substances in it (Prestes et al., 2022). LF contains 17 intramolecular disulfide bonds and has low thermal stability. The pasteurization in human milk at 62.5 °C for 30 min, LF therefore changes its structural by thiol-disulfide exchange reactions, resulting in the loss or reduction of bioactive components (Liu et al., 2020b; Picaud & Buffin, 2017). The study demonstrated that thermal processing of LF resulted in changes to the native secondary protein structure which contains the reduce of α-helix domains and the increase of intermolecular β-sheet structures, loss in color of LF, the increases in surface hydrophobicity and cationic surface charge, and the formation of disulfide linked protein-protein aggregates (Goulding et al., 2021b). Besides, thermal processing induced LF to show a less compact protein structure in the new exposed regions of the surface, further to trigger the denaturation and aggregation (Goulding et al., 2021a). The binding of bLF to iron promotes changes in tertiary structure which increases its structural stability (Barros et al., 2021).

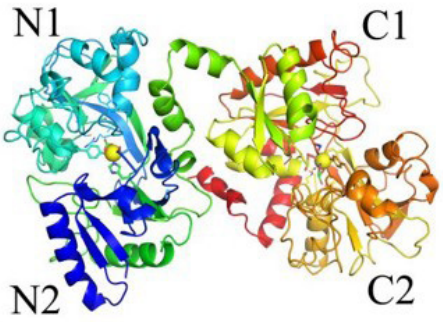

Figure 1. 3D crystal structure of iron saturated bLF at 2.8 A resolution (protein databank code: 1BLF), ferric ions are represented as spheres. N-lobe (N1 and N2) and C-lobe (C1 and C2) are two homologous structural domains of LF.

3.2 Changes in LF thermal stability

It has been reported that apo-LF denatures faster than holo-LF with the increasing temperature and time and with

Table 1. Concentrations of lactoferrin (LF) in mammalian species milk.

Species	LF mg/mL	References
Human	1.0-7.0	(Rai et al., 2014)
Buffalo	0.03-0.81	(Giacinti et al., 2013)
Sheep	0.06-0.72	(Navarro et al., 2018)
Camel	0.02-2.1	(Azhar et al., 2020)
Equine	0.2-2.0	(Pieszka et al., 2016)
Bovine	0.03-0.2	(Liu et al., 2020a)
Goat	0.02-0.2	(Park, 2010)
Donkey	0.10-0.13	(Altomonte et al., 2019)

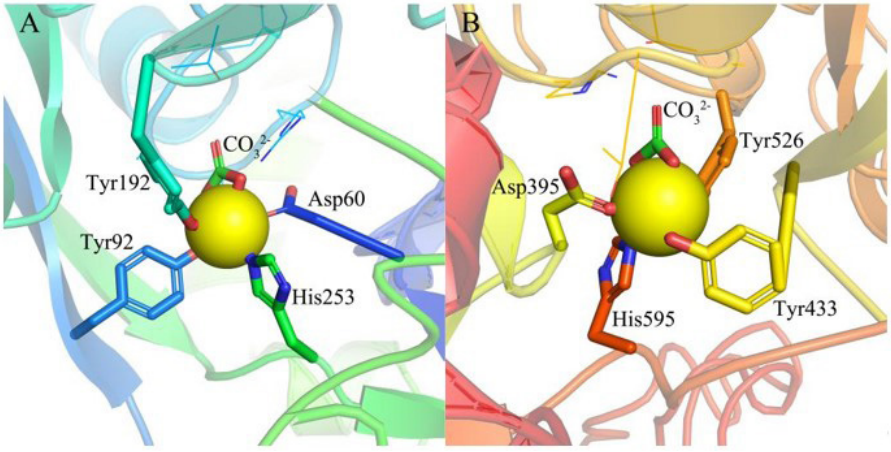

Figure 2. Cartoon representation of the overall structure of bLF (protein databank code: 1BLF), with iron ions represented as spheres. Metal ion coordination sites in the closed form of bLF, including residues Asp60, Tyr92, Tyr192, His253 in N lobe (A) and Asp395, Tyr433, Tyr526 and His595 in C lobe (B).

their deformation temperatures after purification being around 70 °C and 90 °C respectively, holo-LF has a denser structure, thus it is more stable (Franco et al., 2018; Morel et al., 2022). The difference of denaturation temperature between holo-LF and apo-LF is due to the more compact structure of holo-LF formed by iron binding (Rastogi et al., 2016). Therefore, iron saturation increases its resistance to thermally induced denaturation. In IMF, thermal processing promoted the formation of LF-casein complexes by binding LF to casein, this binding readily altered the thermal stability of LF, which explains the more rapid thermal denaturation of LF compared to a pure protein solution of LF (Halabi et al., 2020; Li & Zhao, 2018).

3.3 Effect of iron binding on LF stability

LF was found to be able to maintain its iron binding capacity after being heated at temperatures ranging from 65 °C to 90 °C and ionic strength of about 0.01 or below (Sabra & Agwa, 2020). However, continuous thermal processing can alter the structure of proteins and thus lead to the loss of iron, which reduces the stability of LF. Fernández-Menéndez et al. (2020) synthesized an isotopically labelled iron-lactoferrin complex [$^{57}Fe(III)_2$-LF], which was used to fortify milk samples. After pasteurization, the LF levels increased higher in the iron-added samples than in the non-added ones, suggesting that the binding of LF to iron reduced the effect of the pasteurization thermal processing on it, thus strengthening the LF binding of iron may be a potential way to maintain LF stability under heating condition. Thermal processing forms the so-called Maillard reaction products (MRP) based on the interaction between protein and sugar or lipid. MRP decreased the extent of complex formation of Chrome Azurol S with iron, which reflected the MRP ability to efficiently chelate iron (Bhattacharjee et al., 2020, 2021). Thus, LF glycation may play a greater role in LF function than what has been recognized to date.

3.4 Effects of thermal processing on LF physiological activity

Hot pasteurization can trigger the loss or reduction of LF (Picaud & Buffin, 2017), thus it is important to improve the thermal stability of LF in order to maintain its biological activity. Although light heat treatment (70 °C, 10 min) prior to in *vitro* digestion did not have any significant effect on digestibility compared with the unheated LF samples (Wang et al., 2019), the protein aggregates formed at 75 °C are more resistant to digestion, leading to a reduced release of peptides from LF and thus reducing the bacteriostatic activity of bLF (Xiong et al., 2021). In recent years, non-thermal pasteurization methods have been proposed as potential alternatives to pasteurization, such as optimal conditions of hydro-autoclaving (400 MPa for 5 min at 25 °C) guarantee bioactive components include LF in breast milk samples (Zhang et al., 2022). In addition, the complex of soy soluble polysaccharides and LF at thermal processing is electrostatically generated to prevent aggregation, denaturation and loss of the α-helix of LF, thus preserves the antibacterial capacity of LF during thermal processing (Lin et al., 2022).

Of course, proper thermal denaturation sometimes favors the release of some biological activities in LF. Compared with natural bLF, heat-denatured bLF is susceptible to digestive enzymes, the studies of mice found denatured bLF is hydrolyzed by pepsin and released the neutrophil-binding peptide lactoferricin, which enhanced the production and proliferation of IgM, IgG and IgA (Bielecka et al., 2021; Godínez-Victoria et al., 2017).

4 Iron binding and release of LF in *vivo*

The iron binding and release of the LF is closely linked to its function in *vivo*. As shown in Figure 3, Fe^{3+} in food is converted to Fe^{2+} by duodenal cytochrome b (Dcytb), and Fe^{2+} enters the small intestinal epithelium via recombinant divalent metal transporter 1 (DMT1) or heme carrier protein 1 (HCP1). LF enters the small intestinal epithelium via endocytosis and the Fe^{3+} carried by LF can be converted to Fe^{2+} by hydrochloric acid in the intestine. The absorption of new iron depends on the total previous iron storage. In the Duodenum, there is a sensor transferring carrying iron - the HFE protein, transcriptional expression of the HFE gene can act on Dcytb and hephaestin (Hp) to regulate duodenal absorption of iron. There are two routes for Fe^{2+}, one is to bind to ferritin, which is present in the cytoplasm as mucosal ferritin and subsequently shed outside the cell, where the normal epithelium regenerates; the other is that Fe^{2+} crosses the basement membrane of intestinal epithelial cells by the combined action of ferroportin 1 (FPN1) and Hp and is subsequently converted to Fe^{3+} by ceruloplasmin (CER) in the small intestinal epithelium.

Fe^{3+} enters the vasculature, binds to transferrin, and is transported to the bone and liver. Some Fe^{3+} reach the bone marrow via the blood for hemoglobin synthesis and erythropoiesis, finally iron is used in various tissues and organs. Some Fe^{3+} transported as TF-Fe^{3+}, they reach the liver via the portal system, Fe^{3+} entering the liver stimulates a protein called Hepcidin (Hepc), this is a regulator as well as an inhibitor. When Hepc senses that there is too much Fe^{3+} coming in, Hepc acts on FPN1 to inhibit Fe^{2+} production until FPN1 is not receiving Fe^{2+}, at which point Fe^{2+} in the cytoplasm is shed outside the cell as mucosal ferritin and excreted in the feces or urine. Meanwhile, HFE gene acts on Dcytb and Hp to inhibit their translational uptake of LF. If there is too little Fe^{3+}, Hepc acts on FPN1 to increase Fe^{2+} production, and HFE gene acts on Dcytb and Hp to promote their translational uptake of LF. There is also a way to maintain iron homeostasis in the body, when there is too much Fe^{3+}, Hepc acts on macrophages until macrophages stop releasing Fe^{3+}; when there is too little Fe^{2+}, Hepc does not act on macrophages and macrophages release Fe^{3+} as normal. Most of the body's iron is derived from the recirculation of heme iron after senescent red blood cells are phagocytosed by macrophages, and another part comes from the absorption of iron from food, with the small intestine being the only site for iron absorption.

The release of iron from LF follows the reverse pathway of iron binding, with the structural domain of the closed iron binding site opening, followed by the release of iron. Three factors contribute to the structural changes necessary for iron release: the presence of a specific receptor similar to serum transferrin, the reduction of Fe^{3+} to Fe^{2+} and the lowering of the pH in the environment (Baker et al., 2002). Iron absorption occurs in the proximal duodenum (Cassat & Skaar, 2013). When LF with iron ions reaches the intestine, it binds specifically to receptors

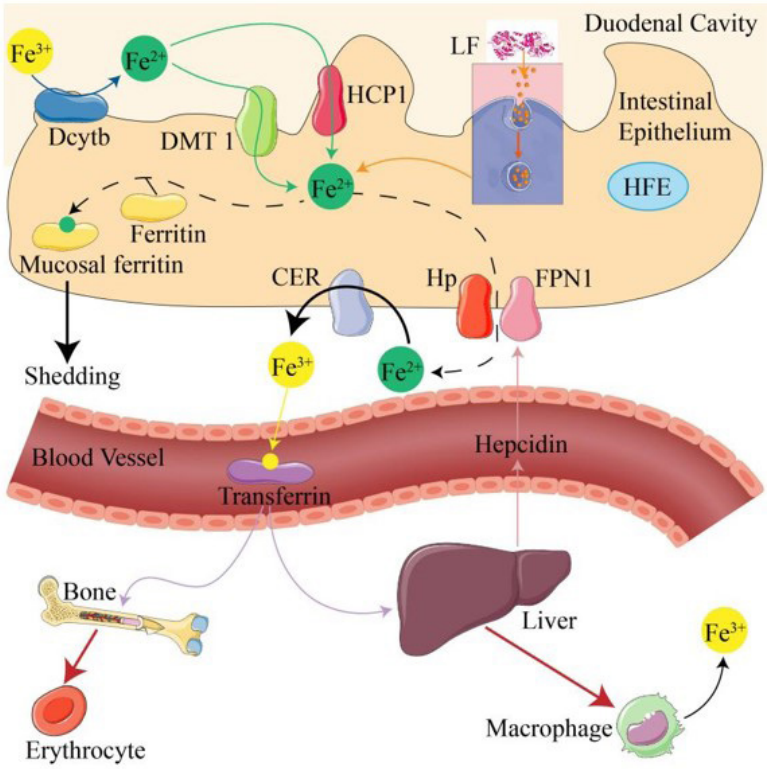

Figure 3. The mechanism of iron binding and release in vivo.

on the cell surface (Figure 3), facilitating the entry of iron and intact LF into the enterocyte and the release of Fe^{3+} ions via an endocytosis-mediated pathway (Jiang et al., 2011; Suzuki et al., 2005). The absorbed LF is then transported by the microsomes and ultimately participates in the redox reactions of the iron cycle (Lönnerdal, 2016). LF enhances intestinal iron absorption by binding to iron and improves hemoglobin and total serum iron levels, thereby maintaining homeostasis of iron in the body and in cells (Mayeur et al., 2016; Sienkiewicz et al., 2022).

5 Digestion of LF

5.1 Digestion of LF in vivo

The digestion of food starts from mouth which is mainly mechanical chawing, and most of the LF is ingested in liquid form, which makes its digestion in the oral stage even less. LF is initially broken down by pepsin in the gastric juice with the participation of gastric acid, and it is at this stage that strong antimicrobial peptides such as lactoferricin and lactoferrampin are produced.

With or without thermal processing, LF is more susceptible to simulated infant intestinal digestive conditions than simulated infant gastric digestive conditions. The effect of thermal processing on LF gastric digestion is negligible, as LF and its aggregates are highly resistant to gastric digestion. In addition, the difference in digestion between bLF and hLF may arise from the different levels of iron saturation in LF (Bokkhim et al., 2013; Sabra & Agwa, 2020). The iron saturation degree of LF interferes with its degradation, apo-LF in bovine milk are more easily digested than holo-LF (Troost et al., 2001). All the same, the gastric digestion may also demonstrate a positive impact on LF activity, because LF can form antimicrobial derivatives in the stomach (Lizzi et al., 2016).

5.2 Simulation digestion studies of LF in vitro

Most of the current LF studies are based on simulation methods in *vitro*. The digestion model in *vitro* facilitates the subsequent extraction and isolation of digestion products by simulating the physiological conditions in *vitro* digestion and then analyses the structure, composition, interactions, and digestibility of the digestion products, with the advantage of being easy to manipulate and reproduce. This allows relatively large numbers of samples to be measured in parallel for screening purposes and is well suited to mechanistic studies and hypothesis construction (Minekus et al., 2014). Thus, the use of adequate digestion tools in *vitro* is a priority for the optimization of IMF (Ménard et al., 2018).

In simulating gastric digestion in infants, compared to the undigested or untreated LF samples, the gastric digest revealed an almost identical molecular weights profile, suggesting that LF undergoes little protein hydrolysis. The heat-treated LF samples showed a lesser resistance to gastric digestion than the unprocessed LF (Goulding et al., 2021b). This result is consistent with those of high temperature short duration pasteurization (HTSDP) on the dynamic digestion of human milk in a premature neonatal model

(Nebbia et al., 2020). LF can act biologically as an intact protein and possibly as a hydrolyzed form, in them LF-derived peptides showed a potent anti-microbial activity (Vogel, 2012). In a static model simulating gastrointestinal digestion in infants, LF resists gastric digestion, instead of intestinal digestion (Halabi et al., 2020; Xiong et al., 2021). Although the amount of LF excreted intact through infant intestinal digestion is low, the protein hydrolysis resistance that allows LF to persist in the infant's gastrointestinal tract may be the key to allowing the protein to affect the infant's intestinal microbiota (Manzoni, 2016). Bokkhim et al. (2016) used an aerosol technique to encapsulate LF and then found that encapsulating apo- and native-LF with alginate microgel particles (composed of a mixture of apo- and holo-LF) protects them from the action of pepsin and allows their release in the intestine.

6 Active functions of LF in *vivo*

There are many medicinal foods in human lives that not only provide the body with nutrients, but also have preventive, palliative, or curative effects. LF is expected to bring some active functions to food. Table 2 summarizes the biofunctions and mechanism of LF that have been studied in recent years.

The anti-cancer activity of LF is reflected in the activation of innate and adaptive immune responses, in addition to stimulating the proliferation and differentiation of T-helper cells and their release of tumor-killing cytokines in the intestine (Zhang et al., 2015). Virus-dependent binding is attributed to LF leaf termini (N- and C-) and is dependent on charge interactions, with LF inhibiting the entry of viral particles into host cells either by direct attachment to viral particles or by blocking their cellular receptors (Redwan et al., 2014). LF increases the cytotoxicity of natural killer cells in *vitro* while inhibiting the release of reactive oxygen species (ROS) from leukocytes at sites of inflammation, however, its antioxidant capacity decreases with decreasing iron saturation (Cutone et al., 2020a). LF inhibited oxidative stress-induced cell death and apoptosis by enhancing autophagy (Hsu et al., 2020). It is worth noting that, compare to holo-LF, apo-LF had a more pronounced stimulatory effect on the proliferation of crypt cells associated with inflammation in colon cancer (Fan et al., 2022). The N-terminal region of LF binds to the bacterial cell wall and destroys the bacterial cell. It inhibits the growth of pathogens (especially Enterobacteriaceae) and stimulates the growth of bifidobacterial intestinal flora, thus protecting the intestinal epithelial cells (Vega-Bautista et al., 2019). In addition to the more representative bio-functional activities mentioned above, LF has also been found to have anti-parasitic, osteogenic, enzymatic activity, and neurological modulation functions.

7 LF application in food

7.1 Food fortification

Good product acceptability is one of the biggest challenges for food development (Santos et al., 2022), and as people become more health conscious, nutritional fortification has become one of the reasons for product acceptance. Nutritional fortification refers to natural or synthetic nutrients or other nutritional ingredients added to foods to increase their nutritional value. bLF has been approved as a generally recognized safe compound by the United States Food and Drug Administration and as a dietary supplement by European Food Safety Authority (Cutone et al., 2020b; Superti, 2020). LF can be used as a nutritional fortification in IMF, milk mixes, flavored fermented milk and dairy drinks. Figure 4 shows different applications of LF in the food industry.

LF has been reported to be a very important addition to IMF due to its antibacterial, anti-inflammatory, anti-cancer, immunomodulatory, enzyme activity and many other bioactive functions (Giansanti et al., 2016; Niaz et al., 2019; Yan et al., 2022). bLF is usually added to IMF to improve its functional properties based on its high homology with hLF and easy availability

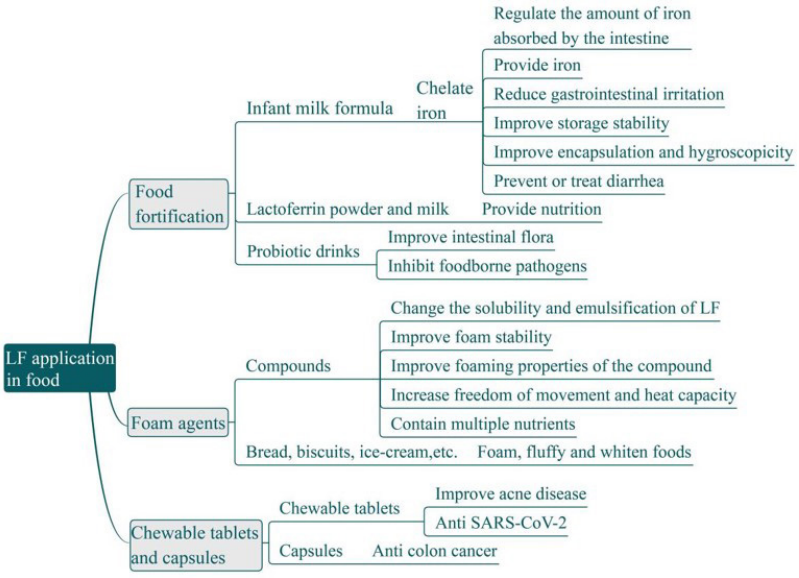

Figure 4. LF application in food.

Table 2. Research on lactoferrin (LF) in *vivo*

Functions	Categories	Mechanism/Results	References
Anti-cancer	Glioblastoma	bLF blocked the migration of human glioblastoma cell lines by reversing epithelial-to-mesenchymal transition-like processes and inhibiting the IL-6/STAT3 axis.	(Cutone et al., 2020a)
	Lung Adenocarcinoma	A novel human recombinant LF inhibited lung adenocarcinoma cell growth and migration with no cytotoxic effect on normal human epithelial cells.	(Olszewska et al., 2021)
	Breast Cancer	Lactoferricin B triggered mitochondrial membrane depolarization and elevated cytoplasmic calcium levels in MCF-7 cells.	(Guerra et al., 2019)
	Prostate Adenocarcinoma Cell	bLF inhibited proliferation, induced apoptosis, intracellular acidification and disrupted lysosomal acidification only in highly metastatic cancer cell lines, whereas BJ-5ta cells were insensitive to bLF.	(Guedes et al., 2018)
	Colon Cancer	bLF played a role in the protective mucus barrier that covers the intestinal epithelium.	(Tanaka et al., 2021)
Anti-viral	COVID-19	The binding of bLF to heparan sulfate proteoglycans blocked the attachment of the virus to the host cell, while HSPG mimetic heparin to antagonize the anti-viral activity of bLF.	(Hu et al., 2021)
	SARS-CoV-2	bLF interacts with pepsin during digestion and releases LF B17-41 with moderate anti-SARS-CoV-2 viral activity	(Wotring et al., 2022)
Anti-inflammatory	Acute Kidney Injury	Camel milk LF protected the kidney from 5-fluorouracil-induced inflammation and oxidative damage, while scavenging ROS, inhibiting MAPKs and NF-κB and activating the PI3K/Akt/eNOS pathway.	(Arab et al., 2018)
	Enteritis	In mouse model, by regulating the expression of PPAR-γ, PFKFB3 and NF-κB genes and proteins, apo-LF suppressed colonic mucosal inflammation and repaired mucosal damage.	(Fan et al., 2022)
	Arthritis and Air Pouch Edema	After internalization of LF into monocytes, LF in camel milk inhibited the activation of NF-κB, thereby inhibiting the production of pro-inflammatory cytokines.	(Arab et al., 2017)
Anti-bacterial	Burkholderia	A construct combining two antimicrobial structural domains of bLF lactoferrampin265-284 and lactoferricin17-30 resulted in disruption of the bacterial plasma membrane and subsequent leakage of intracellular nucleotides leading to cell death.	(Kanthawong et al., 2014)
	Vibrio Cholerae	bLF interacts directly with the negatively charged components of the microbial membrane, inducing changes in their permeability by dispersing them.	(Acosta-Smith et al., 2018)
	Neisseria Meningitidis	The C-lobe of hLF interacts with the bilobed outer membrane of Gram-negative bacteria at two different sites of lipoprotein, where binding of hLF prevents iron uptake or disrupts the protective membrane-bound lipoprotein against the cationic antimicrobial peptide.	(Ostan et al., 2017)
	Aflatoxin M1	LF resulted in a reduction in afm1-induced intestinal permeability, increased expression of claudin-3, ocludin and ZO-1 proteins, and repair of the damaged intestinal barrier.	(Gao et al., 2021)
	Cronobacter and Pseudomonas spp.	bLF inhibited the growth of sepsis-causing microorganisms in recombinant IMF and bacteria.	(Sawale et al., 2022)
	Salmonella enterica and E. coli O157:H7	The growth of E. coli O157:H7 was significantly reduced at LF concentrations greater than 14.05 mg/mL and the growth of S. enterica was reduced at LF concentrations equal to or greater than 112.5 mg/mL.	(Biernbaum et al., 2021)
Anti-parasitic	Amoebiasis	bLF-derived peptides were effective in resolving murine intestinal amoebiasis in *vitro*.	(Díaz-Godínez et al., 2019)
Osteogenesis	Osteogenic Factor	By activating Smad2/3 and p38 MAPK, bLF enhanced osteoblast differentiation from MSCs, resulting in increased transcriptional activity of Runx2. bLF treatment enhanced osteoblast differentiation and mineralized nodule formation, as well as the repair of bone defects in *vitro*.	(Inubushi et al., 2020)
Enzyme activity	DNA Binding	LF has a sequence similar to ribonuclease A and has DNA-binding properties that allow it to act in the transcriptional activation of specific DNA sequences and also as a mediator of signal transduction.	(Brandl et al., 2010; García-Montoya et al., 2012)
Nerve Function Regulation	Neural Development and Cognition	LF improved neurodevelopment, cognition, and memory in piglets through upregulation of brain-derived neurotrophic factor signaling pathways.	(Chen et al., 2015)

(Artym & Zimecki, 2013; Cornish et al., 2004). By participating in the transport of iron in the body, LF can directly or indirectly chelate iron, thus regulating the amount of iron absorbed by the intestine (Hao et al., 2019). Infants are prone to nutritional iron deficiency anaemia and direct iron supplementation can cause irritation to their gastrointestinal tract. The addition of LF to IMF ensures the absorption and utilization of iron and prevents nausea, vomiting and disruption of feeding due to the irritation of the gastrointestinal tract in infants. Moreover, bLF is added to reconstituted IMF and HTSDP retained bLF in IMF with high quality, together with the binding capacity and storage stability of iron, this retention confirmed the possibility of adding bLF to HTSDP products (Wazed et al., 2020). LF combined with whey protein hydrolysate maintains the stability of IMF emulsions and the yield, encapsulation and hygroscopicity of IMF containing LF are improved (Figueiredo Furtado et al., 2021). The iron binding bLF found in colostrum was effective in treating diarrhea in calves, suggesting that bLF may have a potential therapeutic role in infant diarrhea.

As mentioned in above, LF has many beneficial biological activities and therefore direct consumption of LF powder is a good option, but the daily dose per person should be strictly controlled. LF can be powdered and turned into drink, however, due to its instability, storage conditions and limited shelf life, the processing and packaging of beverages still needs to be improved. LF is mainly added to milk as a nutritional fortifier, as milk is pasteurized and sterilized, packaged tightly and mostly in small individual packs, and is not stored for long periods of time like milk powder, thus the antibacterial properties of LF are less represented in milk.

It has been reported that beneficial microorganisms need protein to proliferate, and most proteins do not reach the gut, and there are no protein-containing prebiotics on the market (Peled & Livney, 2021). LF is able to improve intestinal flora, strengthens the body's immunity and has a regulatory effect on iron absorption in mammals, thereby maintaining iron homeostasis in the body (Li et al., 2017). Chen et al. (2013) combined apo-bLF or bLF hydrolysis products with specific supernatants produced by different probiotic bacteria and observed that they inhibited foodborne pathogens. Therefore, LF can be added to yogurt and probiotic drinks. However, the high addition of LF can lead to a decrease in the quality of the probiotic, when developing probiotic products with LF, the amount of probiotic added should be increased to ensure enough live bacteria. Besides, the zinc-bLF complex was reported to be used as a food additive or as a wound healing agent (Pryshchepa et al., 2022).

7.2 Foam agents

Foaming agent is a kind of substance that makes the target substance pore, which can be divided into three categories: chemical foaming agent, physical foaming agent and surfactant, LF belongs to surfactant. Protein is the main foaming agent in food industry (Murray, 2020), but its foam is not easy to produce and control. Due to the foaming and emulsifying properties, the proteins in milk are a good choice for surface active molecules and bLF is a globular protein found in milk whey protein (Liu et al., 2018). Rather than using the foaming properties of LF alone, there is now a preference for using LF in combination with other substances to form complexes, which retain the foaming properties of LF and increase the biological functionality of the complex.

At present, LF is widely used to be foam agents, but this function is not yet perfect. Therefore, it is necessary to further modify the foam agent to make a compound foam agent with better performance. Covalent modification of LF with epigallocatechin gallate, chlorogenic acid and gallic acid resulted in significant changes in the solubility and emulsification of LF, as well as enhanced thermal stability of the LF-polyphenol conjugate (Liu et al., 2015). It is reported that a stable complex was formed by hydrophobic interaction between LF and procyanidin and the foaming properties of the complex were also improved (Li et al., 2021). Dai et al. (2022) used bLF and tannins to form a complex to improve foam performance, although poor foam compared to bLF alone, but with good foam stability. Although rosmarinic acid interacts to a lesser extent with bLF, the complexes formed are more stable than the protein alone and also have more freedom of movement and heat capacity (Ferraro et al., 2015). The conjugation of polyphenols to LF increases the ζ-potential of the complex and decreases the surface hydrophobicity, leading to a reduction in turbidity during thermal processing, and this change in physicochemical properties can also affect the foaming properties of LF (Liu et al., 2016). These complexes are able to fully perform the dual role of the ingredients, suitable for functional food foams, with nutritional and technical benefits. LF offers new resource ideas for purely natural foaming agents and a viable strategy for enhancing the use of functional molecules in food stabilization and industry.

As a pure natural additive, LF can be widely used in food industries such as bread and biscuits. In addition to increasing protein nutrients, it also has the functions of foaming, loosening, and whitening food. Thus, it is widely used in cold drinks such as soft drinks and ice cream.

7.3 Chewable tablets and capsules

Other products of LF included health food products with both nutritional functions and medical efficacy. Subjects took bLF chewable tablets twice daily for 8 weeks and the result showed that bLF was well tolerated in mild and moderate acne vulgaris (Mueller et al., 2011). Wotring et al. (2022) found that custom chewable LF tablets formulated with glucose or sorbitol had anti-SARS-CoV-2 activity. New capsules loaded with LF have been reported using polyelectrolyte complexes, which can be used as anti-colon cancer protein products (Wu et al., 2013)

8 Conclusion

This review illustrates the foodstuffs into which LF may be made, but the processes by which LF can be incorporated into foodstuffs are still to be discovered, and this provides a wider range of ideas for the application of LF in foodstuffs. As LF is easily inactivated during thermal processing, it is particularly important to find out how to obtain highly active bLF in an efficient isolation method. The high extraction cost and high price of bLF set currently in the market have severely limited

the development and application of lactoferrin health products. In order for LF to pass through the stomach in its intact structural form, many protection measures have been proposed one after another and that microencapsulation and PEGylation are the most effective methods used to deliver LF to the site of intestinal absorption, which holds promise for future research. Despite these findings, the kinetics of thermal processing on LF digestion and the biological activity of LF digestion require further study. In addition, the physiological functions of LF need to be studied in depth in order to solve the technical problems of food industry. This is also of great importance for a more rational use of bLF, such as the simulation of breast milk nutrition and the development of breast milk substitute food and IMF. Due to the special status and role of iron in many physiological and pathological processes in the body, further systematic studies will have good application prospects.

Abbreviations

apo-LF: iron-free lactoferrin. bLF: bovine lactoferrin. CER: ceruloplasmin. Dcytb: duodenal cytochrome b. DMT1: divalent metal transporter 1. FPN1: ferroportin 1. HCP1: heme carrier protein 1. hLF: human lactoferrin. holo-LF: iron-saturated lactoferrin. Hp: hephaestin. HTSDP: high temperature short duration pasteurization. IMF: infant milk formula. LF: lactoferrin. MRP: Maillard reaction products. ROS: reactive oxygen species. TF: transferrin

Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Jianing Fu: Conceptualization, Writing- Original draft preparation. Liu Yang: Writing - Review & Editing. Dehong Tan: Supervision. Ling Liu: Project administration. All authors have read and agreed to the published version of the manuscript.

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