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Lactoferrin as A Natural Preservative: Sources, Mechanisms, and Applications in Food and Pharmaceutical Industries

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Abstract: To stop microbiological deterioration and increase shelf life, preservatives are crucial for food and medicinal items. Naturally produced preservatives have drawn more attention as consumers' preferences for natural and clean-label ingredients expand. The categorization of preservatives is covered in this review, with a special emphasis on lactoferrin and natural preservatives derived from plant, animal, and microbial sources. The iron-binding glycoprotein lactoferrin, which is widely found in milk and colostrum, has antibacterial, antiviral, antioxidant, and anti-inflammatory qualities. The review focuses on lactoferrin's physicochemical properties, sources, and structure that support its biological roles. Iron sequestration, microbial membrane rupture, and immunological regulation are some of its modes of action. Lactoferrin's uses in the food business are covered, including dairy products, baby formula, bio preservation, and antimicrobial food packaging. Lactoferrin is shown to be a promising natural preservative and functional food ingredient by safety assessments and regulatory considerations.

Keywords: Lactoferrin , Natural Preservatives, Antimicrobial Activity, Food Preservation, Biopreservation and Food Safety.

INTRODUCTION

The world is rich in greenery, with a multitude of trees that yield important natural resources for our daily lives. Among these resources are preservatives, which are substances commonly added to food and pharmaceutical products to extend their shelf life. The use of preservatives is particularly crucial for products with higher moisture content, as it helps prevent changes and spoilage caused by microorganisms during storage. Prior to the advent of preservatives, ancient civilizations such as the Egyptians, Greeks, Romans, Sumerians, and Chinese used containers like clay jars to shield food from air and moisture, thus slowing the spoiling process. Drying was also a widespread preservation technique since most bacteria thrive in moist environments; fruits, vegetables, and meats were often dried for this purpose. In the Middle Ages, the earliest chemical preservatives, namely salt and sugar, became commonplace. Salt not only draws moisture out of food (similar to drying) but also creates an environment that is unwelcoming to microbes. Pickling is another method that utilizes salt. Eastern civilizations, particularly in India and China, also employed spices for food preservation. The early 19th century saw significant advancements in food

preservation with the invention of canning, alongside pasteurization, introduced by Louis Pasteur in 1862. Pasteurization involves heating food to high temperatures for extended periods to eliminate nearly all microorganisms. This innovation, when combined with canning, made it possible to store food for many years without spoilage. Preservatives are defined as natural or synthetic chemical agents that prevent decomposition by microbial growth or other undesirable chemical changes in finished products[1]. In the pharmaceutical industry, preservatives inhibit the growth of bacteria, mold, fungi, and other microbes, and are utilized in the production of drugs and cosmetics for their antibacterial, antifungal, and antioxidant properties[2].

CLASSIFICATION OF PRESERVATIVES

Preservatives fall into two primary categories: artificial preservatives and natural preservatives[3]. Figure 1

Artificial Preservatives

These are synthetic chemical compounds utilized to prevent the spoilage and contamination of finished products by microorganisms, including examples like sodium benzoate, propyl gallate, and potassium sorbate.

Natural Preservatives

These are chemical substances derived from natural sources that inherently protect products from microbial growth. They comprise essential oil constituents, flavonoids, phenolic compounds, etc. Natural preservatives can be categorized into four types:

- Plant-derived preservatives
- Animal-derived preservatives
- Certain microbes and/or their metabolites

Natural preservatives are also grouped based on their mode of action into two categories:[4]

Antimicrobial preservatives and Antioxidants. Antimicrobial preservatives are added to formulations to kill or inhibit the growth of microorganisms during production or use. They are further divided into two main subclasses: antifungal preservatives, like benzoic and ascorbic acids and their salts, and antibacterial preservatives, such as quaternary ammonium salts, alcohols, and phenols. Antioxidants are included in pharmaceutical products to prevent degradation from oxidation. They are classified into three sub-groups: true antioxidants, which potentially inhibit oxidation by reacting with free radicals; reducing agents, which have lower redox potentials than the drugs or adjuvants they protect and may also react with free radicals; and antioxidant synergists, which typically have minimal antioxidant effects themselves but may enhance the effectiveness of the first group by reacting with heavy metal ions that catalyze oxidation.

Preservatives from Plant Sources

Herbs have long been used as preservatives due to their antimicrobial properties against certain pathogens and antioxidant capabilities. These herbs and spices contain volatile chemicals that are utilized for preservative production through distillation and enzymatic processes. The aromatic compounds in plants serve as precursors that are broken down by enzymes during tissue damage, resulting in an antibacterial scent[5].

Preservatives from Animal Sources

Certain secretions or products from animals, whether produced inside or outside of their bodies, can act as preservatives, either in their raw state or after appropriate processing[6].

Preservatives from Microbial Sources

Microbes can be both detrimental (such as Helicobacter pylori) and beneficial to human health (like Escherichia coli). During their life cycles, microorganisms generate specific metabolites that serve as preservatives in various food and pharmaceutical products. Here are some preservatives derived from microbial sources:[7]

1. Acidophilin is a low molecular weight nitrogen compound produced by the cultures of Streptococcus diacetylactis and Leuconostoc citrovorum, known for its strong antimicrobial properties.
2. Bacteriocins comprise a diverse group of substances initially defined as colicin-type proteins produced by Escherichia coli. Notable examples include Diplococcin and Nisin, which are produced by Lactococcus cremoris and Lactococcus lactis, respectively. Nisin consists of 34 amino acids and contains unique lanthionines. Since 1969, the FDA has approved Nisin for use as a preservative in

processed cheese, cheese spreads, pasteurized milk, and dairy desserts.

3. Natamycin is a tetraene polyene antibiotic sourced from Streptomyces natalensis, effective against various yeasts and filamentous fungi, including Candida, Aspergillus, Cephalosporium, Fusarium, and Penicillium. It works by binding to the sterol component of fungal cell membranes, altering their permeability and depleting essential cellular constituents. While its fungicidal properties are dose-dependent, Natamycin is ineffective against both grampositive and gram-negative bacteria in vitro.
4. Lactic acid bacteria (LAB) excrete a variety of antimicrobial compounds, including organic acids, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, antifungal peptides, and bacteriocins. Reuterin demonstrated a significant synergistic impact on L. monocytogenes and a minor additive effect against S. aureus when combined with nisin, although it did not enhance antimicrobial effects against Gram-negative pathogens.

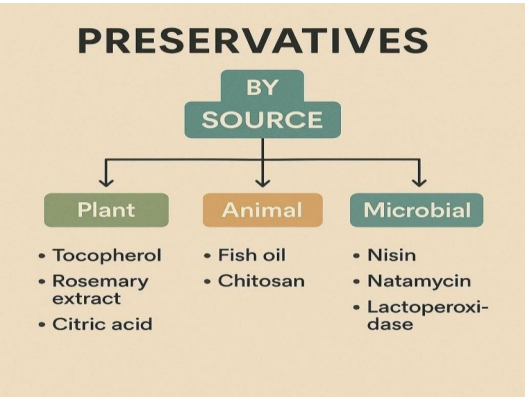

Fig 1: Classification of preservatives.

INTRODUCTION OF LACTOFERRIN

Lactoferrin, also known as lactotransferrin or red milk protein, is a protein found in the milk of various mammalian species[8]. Initially discovered in bovine milk in 1939[9], lactoferrin consists of around 700 amino acids arranged into two globular domains linked by an alpha helix[10]. Its unique structure contributes to its biological activities in both infants and adults. Lactoferrin offers a range of benefits, including antiviral, anti-inflammatory, antimicrobial, and antiparasitic properties, and it supports the growth of beneficial microorganisms while defending against pathogens[11].

Due to its diverse effects, lactoferrin is recognized as a valuable nutraceutical protein that also acts as an immune modulator by adjusting immune system activity as needed. It promotes gut health by strengthening the intestinal lining and fostering the growth of probiotics[12]. Highly present in milk serum, lactoferrin can be found in various bodily fluids of different mammalian species, such as colostrum, the initial milk produced after birth[13].

Colostrum, rich in essential nutrients like oligosaccharides and immune factors, contains higher concentrations of lactoferrin compared to mature milk[11]. Studies have explored the potential of colostrum-derived lactoferrin in combating diseases like COVID-19 and Parkinson's, showcasing its antiviral and neuroprotective properties. Moreover, lactoferrin has been considered a promising therapeutic agent for Parkinson's disease due to its ability to protect nerve cells from damage[14].

Scientists have incorporated bovine lactoferrin into various products, including infant formulas, yogurt, and nutritional supplements. While lactoferrin from bovine sources shows promise in diverse applications, its similarities and differences with human lactoferrin warrant further investigation[15].

This review highlights lactoferrin's characteristics and its health benefits, emphasizing its potential in treating various diseases and microbial infections. Additionally, it discusses lactoferrin's applications in fortifying different food products and assesses its effects and safety for consumption.

Structure and Properties of Lf

Lactoferrin is a cationic protein characterized by an isoelectric pH of 8.7[16]. The level of resistance that lactoferrin (Lf) demonstrates against hydrolytic degradation from proteolytic enzymes such as pepsin, trypsin, and pronase in acidic conditions is contingent upon its iron saturation[17]. The molecular structure and amino acid sequence of human Lf indicate that it is an iron-binding glycoprotein with a molecular weight of 80 kDa, belonging to the transferrin family, and sharing nearly 60% identity with serum transferrin protein[18]. Lf has three isoforms: α, β, and γ. While Lf-β and Lf-γ isoforms have ribonuclease activity, α-Lf binds two Fe3+ ions with high affinity but does not

exhibit ribonuclease activity. Studies by Bagby and Bennet (1982) and Mantel et al. (1994) have shown the presence of polymeric forms of Lf. At elevated calcium concentrations of approximately 1010 mM, Lf molecules aggregate to form oligomers, particularly tetramers. When the protein concentration exceeds 10-5M, Lf forms oligomers with a ratio of monomers to tetramers of 1:4. Lactoferrin is composed of a single polypeptide chain with around 690 amino acids, organized into two symmetrical globular regions (C and N terminal), linked by an α-helix. Each of the C and N terminal regions contains two domains (C1, C2, N1, and N2), each with a binding site for one Fe3+ ion, allowing a single Lf molecule to bind two Fe3+ ions. The Fe3+ binding site in each domain is formed by four amino acid residues (Asp-60, Tyr-92, Tyr-192, and His-253), while Arg-121 is responsible for binding the CO3 2- ion, enabling Lf to bind one CO3 2- ion along with each Fe3+ ion[19]. Glycosylation is a crucial factor for the stability of Lf protein, with the main sugar moieties in bovine Lf being N-acetyllactosamine,

Nacetylglucosamine, mannose, fucose, galactose, and neuraminic acid. In 1997, Siebert and Huang identified a truncated isoform of Lf known as delta Lf (δ-Lf), which is secreted intracellularly, has a molar mass of 73 kDa, and plays a role in regulating cell death. Its expression is induced by the activation of an alternate promoter (exon1-β) of the Lf gene in DNA, and translation occurs without a leader sequence[20]. Based on iron saturation, Lf can exist in three forms: apo-lactoferrin (apoLf), which does not bind Fe3+; monoferric, which binds one Fe3+ ion; and holo-lactoferrin (holoLf), which binds two Fe3+ ions. The C-lobe and N-terminal lobe of apo-Lf have closed and open conformations, respectively, while holo-Lf is a closed molecule that shows increased resistance to proteolytic degradation[21]. Lactoferrin has an iron affinity approximately 300 times greater than that of transferrin, allowing it to bind iron, especially in low pH environments where infections and inflammation are present, and where pH may drop below 4.5, thus sequestering iron released from transferrin and limiting the iron available for bacterial growth[22]. In 2014, Le Parc et al. identified novel glycans in goat milk Lf, revealing that the glycosylation pattern of Lf in goat milk is similar to that of human and bovine Lf. In 2015, Zainab et al. characterized Lf from the colostrum whey of Iraqi goats, finding an iron saturation of 8.7% and an iron content of 123 ppm.

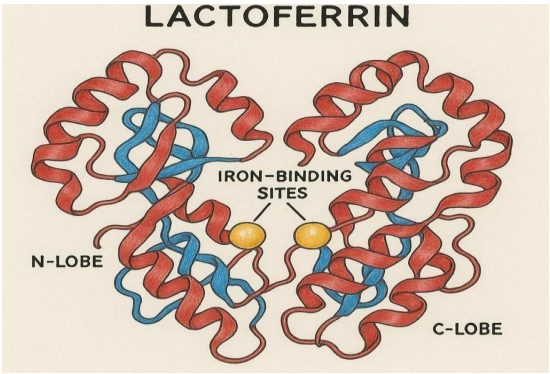

Fig 2: Structure of lactoferrin

Sources of Lf

In adults, lactoferrin (Lf) is found in the majority of mucosal secretions, with the highest concentrations observed in milk and colostrum[23]. Most of the plasma Lf is located in the secondary and tertiary granules of neutrophils[24]. The primary cell types involved in Lf synthesis are secretory epithelial cells and myeloid lineage cells[25]. Research conducted by Ward et al. in 1999 indicated that Lf was first detected in two- and four-cell embryos during early embryonic development, continuing to be present throughout the blastocyst stage and until implantation. Following this period, Lf is undetectable until about halfway through gestation. Subsequently, Lf reappears in the storage granules of neutrophils and in the epithelial cells of the developing digestive and reproductive systems. In 2000, Abrink et al. noted that Lf expression, synthesis, and secretion occurred throughout the collecting tubules, while its reabsorption was restricted to the distal convoluting tubules[26]. Therefore, Lf synthesis also takes place in human kidney

PHYSIOCHEMICAL PROPERTIES OF LACTOFERRIN: Antibacterial, antiviral, and anti-inflammatory

Lactoferrin exhibits a significantly greater affinity for ferric ions—approximately 250 to 300 times higher than that of transferrin (TF). Functioning as a regulator of iron-redox homeostasis (Fe-RH), lactoferrin reduces oxidative stress and enhances host defense by

chelating iron and neutralizing iron-mediated free radicals, thereby optimizing iron metabolism [27]. It primarily facilitates iron ion transport within the intestine, playing a vital role in iron absorption in mammals. Cellular iron uptake is subject to negative feedback regulation; when intracellular iron levels are low, the number of lactoferrin receptors (LfRs) on the cell surface increases, leading to greater uptake of lactoferrin.

Lactoferrin demonstrates antibacterial activity against a broad spectrum of microorganisms, including bacteria, fungi, viruses, and parasites, through three principal mechanisms: (a) It exerts bacteriostatic effects by chelating free iron, which is necessary for bacterial growth and replication—effectively depriving bacteria of essential nutrients [28]. (b) Through its strongly cationic amino-terminal region, lactoferrin increases bacterial membrane permeability, resulting in leakage of lipopolysaccharides and other cellular components, thereby producing direct bactericidal effects. (c) Upon hydrolysis, lactoferrin yields antimicrobial peptides that exhibit enhanced antimicrobial activity (Figure 3) [29]. Bullen et al. initially proposed in 1972 that lactoferrin's antibacterial activity is influenced by iron concentration [30]. Arnold et al., using immunofluorescence, observed that apo-lactoferrin binds to bacterial surfaces and sequesters external nutrients, blocking their entry into bacteria and leading to bacterial death [31]. A prospective randomized trial involving 60 women with bacterial vaginosis (BV) demonstrated that intravaginal administration of 100 mg or 200 mg of lactoferrin for 10 days reduced the incidence of BV, altered the vaginal microbiota, and increased the proportion of Lactobacillus [32].

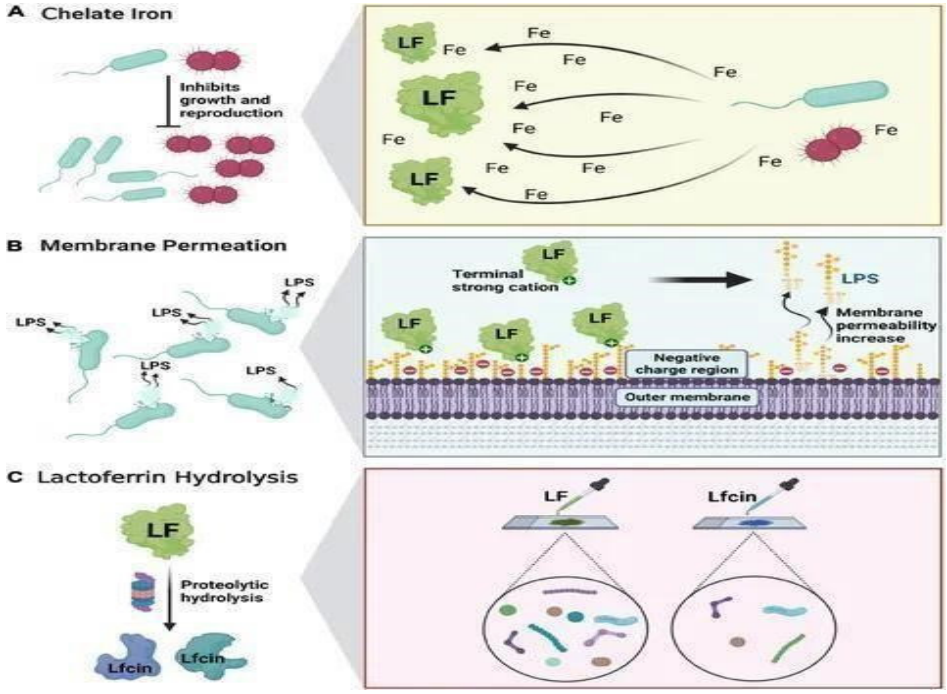

Fig 3: Explanation of how lactoferrin works against bacteria. **(A)** It inhibits bacterial growth by binding to free iron. **(B)** Lactoferrin disrupts bacterial cell membranes, leading to their destruction. **(C)** Lactoferrin breaks down to release antimicrobial peptides for enhanced antimicrobial activity.

Lactoferrin also exhibits considerable antiviral potential. Research to date indicates its efficacy against a range of viruses, including human papillomavirus (HPV), herpes simplex viruses 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV, HCV), respiratory syncytial virus (RSV), hantaan virus (HV), rotavirus (RV), poliovirus (PV), adenovirus (AdV), and SARS-CoV-2, as demonstrated in both in vitro and in vivo studies [33]. In several cell lines, lactoferrin has been shown to block SARS-CoV-2 attachment to cytosolic heparan sulfate and enhance interferon responses [34]. Campione

et al. confirmed in vitro that lactoferrin exerts antiviral activity against SARS-CoV-2 by binding directly to the virus and to components on the surface of host cells[35]. SARS-CoV-2 infects ACE2-expressing cells via direct plasma membrane fusion or endocytosis. Lactoferrin's antiviral mechanisms include: (a) binding to heparan sulfate proteoglycans (HSPGs) on host cells, thereby reducing viral attachment and entry; (b) directly binding viral proteins to inhibit adsorption to target cells; and (c) interfering with intracellular viral transport and preventing delivery of viral genomes to the cytoplasm (Figure 4). The antiviral and antibacterial activities of lactoferrin can vary by source; for example, camel lactoferrin (cLf) has been shown to inhibit hepatitis C virus (HCV) more effectively than human (hLf), bovine (bLf), or sheep lactoferrin (sLf) [36]. This difference may relate to iron saturation, as apo-lactoferrin generally exhibits greater antiviral potency than holo-lactoferrin.

As a modulator of both innate and acquired immunity in mammals, lactoferrin interacts with conserved pathogen structures. It can be converted into an anti-inflammatory molecule that protects the host from detrimental immune responses. Specifically, lactoferrin exerts immunomodulatory effects by binding to specific receptors on immune cells such as lymphocytes, monocytes, dendritic cells, macrophages, and natural killer (NK) cells. For instance, bovine lactoferrin (bLf) alleviates neuropathic pain in mice by inhibiting P38 MAPK activation downstream of TLR4 in microglia, thereby reducing IL-18 production [37]. Oral administration of liposomal bovine lactoferrin (LbLf) effectively hinders the progression of rheumatoid arthritis (RA) in mice by suppressing tumor necrosis factor-alpha (TNF-α) production in the pancreas [38]. Additionally, Fan et al. reported that apo-lactoferrin, more so than holo-lactoferrin, significantly mitigates LPS-induced inflammatory bowel injury by modulating the PPAR-γ/PFKFB3/NF-κB inflammatory pathway [39].

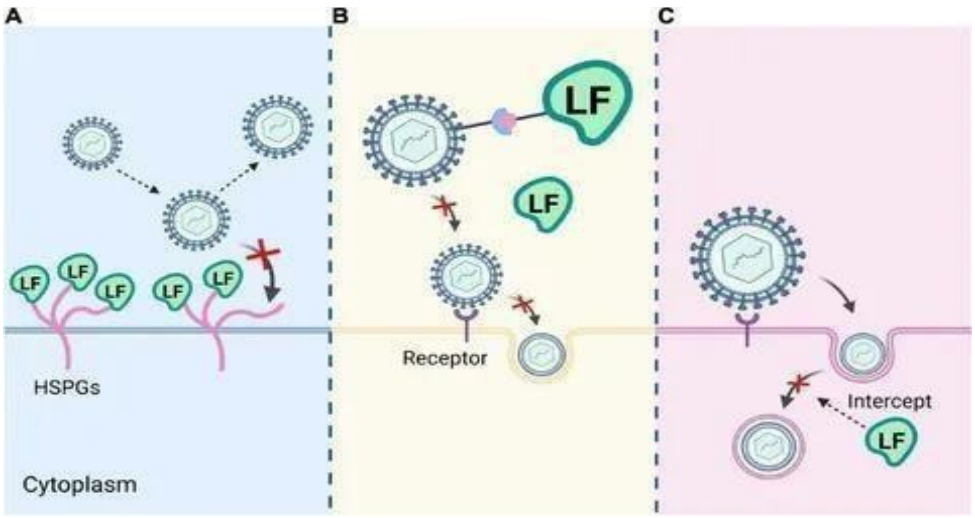

Fig 4: Three ways Lf acts as an antivirus. **(A)** Lf attaches to heparan sulfate proteoglycans (HSPGs) located on the surface of host cells. **(B)** Lf directly interacts with viral proteins, preventing the virus from attaching to target cells. **(C)** Lf disrupts the virus's intracellular transport and hinders the transfer of the viral genome into the cytoplasm.

FOOD INDUSTRY

During the COVID-19 pandemic, the food industry has seen significant transformations, incorporating functional food products like functional chocolate and fermented algae[38]. Lactoferrin (LF) has become a widely used ingredient in numerous dietary supplements, infant formulas, skincare products, and food additives such as yogurt and beverages[39]. Bovine lactoferrin (BLF) is the most frequently utilized LF in the food sector. The Moringa Milk Company introduced BLF into

infant formula under the name "BF-L" in 1986[40]. LF is primarily used as a nutritional additive in dairy products, especially yogurt, where it enhances microbial activity, sensory qualities, nutritional value, and benefits for bone health[41]. The potential of LF-fortified yogurt is also being studied in Drosophila models for its role in body weight regulation and inhibiting the growth of food-borne pathogens. Additionally, it has shown positive effects in treating various health conditions, including acute gastroenteritis, iron deficiency anemia (IDA), and microcytic hypochromic anemia[42]. Besides yogurt, the addition of LF to other foods like cheese and cream is being explored to assess its effects on composition and shelf life. Studies have also investigated the use of LF in sausages, where it was combined with carboxymethyl cellulose (CMC) to evaluate its impact on food properties[43]. Initial research into LF fortification in infant formula included the quantitative analysis of bLF, its role in iron metabolism, and its bioavailability. These fortified formulas have been examined for their potential to address various health issues, such as diarrhea, respiratory infections, acute gastrointestinal symptoms, and anemia in low-birth-weight infants[44]. Moreover, beyond its current applications in food products, LF is being considered as a potential material for food packaging due to its antioxidant and antimicrobial properties[45].

Applications of LF in the Food Industry

The table indicates that a variety of studies have looked into LF fortification. One study from last year concentrated on inhibiting the growth of foodborne pathogens [46]. The research involved Bacillus cereus (B. cereus), Enterococcus faecalis (Ent. faecalis), and Candida albicans (C. albicans) to test the antimicrobial effects of LF. The results showed significantly enhanced effects against these pathogens, particularly under refrigeration. Among the yogurt samples, those with 1.5% LF had the most substantial reduction in B. cereus and C. albicans compared to the 0.5% LF concentration. In 2022, another study assessed the effectiveness of LF-fortified yogurt in reducing obesity-related pancreatic dysfunctions in rats [47]. The results indicated a significant improvement in pancreatic function and some histological changes in the pancreas. The combination of LF (100 mg/kg body weight) with Lactobacillus acidophilus as a probiotic was particularly beneficial for pancreatic health. LF-fortified yogurt has also been evaluated for its health benefits and sensory improvements. One study proposed examining its role in treating IDA and microcytic hypochromic anemia in children [48]. It was found that hemoglobin (Hb) levels and several red blood cell (RBC) parameters improved significantly in children consuming LFfortified yogurt, with effects being much greater than in those receiving only LF. Consistent with previous studies, the applications of LF fortification are being widely distributed to expand its supplementation options [49]. For example, a recent study investigated the addition of LF to cheddar cheese [50]. The research looked into whether LF affected the composition, texture, or sensory properties of cheese. The results showed that LF supplementation did not alter the fatty acid composition of the cheese and was deemed safe for cheddar cheese. Another study focused on sausages, where LF was combined with CMC at concentrations of 5% and 10%, along with a 20% edible coating [51]. This study aimed to evaluate the antimicrobial effects and shelf life. The results were dose-dependent, with the coating showing an overall improvement in shelf life. In 2022, another study examined LF-treated chicken breasts to assess shelf life and microbial efficacy. The findings indicated that LF enhanced shelf life, with varying effects based on the concentration used [52]. In another investigation, LF was added to pasteurized cream made from cow's milk. The study found that LF improved the cream's shelf life and exhibited a dose dependent antibacterial effect against Pseudomonas aeruginosa, Staphylococcus aureus, E. coli, and S. typhimurium. A recent study also explored the use of LF in nanoparticle form, which showed an increased shelf-life effect on fresh apples [53]. Despite these findings, there is still a lack of precise data on the safe maximum dosage for these effects. Therefore, further research is needed.

LF has also been studied for its infant nutrition due to its significant presence in human milk and colostrum . This has led to studies investigating the supplementation of LF in infant formulas. Among other studies, one study investigated the effect of LF-fortified infant

formula on the antibody response [54]. However, the investigation reported an unexpected finding that low-iron formula-fed infants had higher levels of Haemophiles influenzae (Hib) IgG at 12 months, and LF supplementation exhibited no effect on the overall vaccine IgG response. It was also reported that infants who were breastfed had lower levels of vaccine IgG than those who received infant formula. A further study looked at the impact of bLF probiotic infant formula on the prevalence of respiratory and diarrhea-related infections in infants [55]. The results exhibited a significant reduction in morbidity from diarrhea and respiratory illnesses in infants with anemia. Additionally, a study on LF-fortified formula for acute gastroenteritis symptoms in children aged 12 to 32 months indicated that LF supplementation decreased the prevalence of symptoms [56].As mentioned in previous sections, LF has multifunctional priorities, including anti-microbial activity, immune modulation, and iron binding capacity, positioning it as a valuable ingredient in functional groups for supporting gut health and immunity. Given the increasing consumer demands for natural and clean-label products, LF's natural origin and potential for inclusion in minimally processed food formulations ensure this growing demand. To extend the studies, the effects of LF with prebiotics and probiotics that could lead to the development of novel products to improve gut microbiome balance can be investigated [57]. Additionally, rising animal protein demand and scarce resources increased the necessity for feed additives. There are several studies that investigate the potential of LF to improve these effects along with bird health. However, investigations into novel feed additives to improve feed efficiency and growth performance remain limited [58].Similarly, plant-based dairy products are on the rise due to potential health benefits and nutrient preferences. Despite their nutritional value, their nutritional profile can be enhanced when it is fortified with additives, like LF. Therefore, future research should investigate the incorporation of LF in plant-based dairy alternatives to enhance their nutritional value and appeal to more consumers, including health conscious and vegan people [59].In relation to plant-based diary, polyphenol-rich food groups are highly increasing the interest in consumer demands [60]. Therefore, evaluating their nutritional profile with additional studies is required. Furthermore, developing cost-effective and sustainable methods, such as combining various natural antimicrobials with food preservation methods, could significantly reduce production costs and increase accessibility [61].

Ensuring Food Safety in the Use of LF

Food safety while supplementing additional components may lead to a variety of irreversible consequences. The safety has been incorporated in a variety of aspects, including such techniques as freeze-drying to enhance food safety by preserving bioactive compounds [62]. To mitigate potential risks, tests for genotoxicity, animal toxicity, chronic toxicity, acute toxicity, and allergenicity must be performed under toxicology studies. LF has been applied in several food industries, and, during these analyses, researchers must consider potential toxicities. A paper published in 2012 by the EFSA (European Food Safety Authority) reported that the highest dose of bLF tested in sub-chronic toxicity studies was 2000 mg/kg body weight per day, and no toxicity was observed at this level [63]. In recent years, various studies have focused on bLF-peptide toxicity analysis. One such study, conducted in 2021, examined the dual mechanism of LF-derived peptides with angiotensin 1-converting enzyme inhibitory (ACE) and anticoagulant activities [64]. The results suggested that bLF-derived peptides could be potential food ingredients with antihy

The peptides have been assessed for their hypertensive and anticoagulant properties. Through the use of the Toxin red tool, it was determined that these peptides are likely non-toxic. Nonetheless, further toxicity testing is essential before considering any potential applications. A study from 2018 examined the anti amoebic effects of synthetic peptides derived from bLF, such as LF ampin and LFcin [65]. The study revealed that LFampin effectively eliminated amoebae without causing significant harm. However, it was noted that LFampin primarily induced necrosis rather than apoptosis in trophozoites. While these LF-derived peptides show promise as safer alternatives, it is crucial to conduct additional cytotoxicity assessments before widespread use. Although studies indicate no significant toxicity, long-term research on the

safety of dietary LF remains limited. Therefore, investigations into potential benefits like enhanced immune response and gut health require further scrutiny through extended studies. Moreover, potential cumulative effects, such as alterations in gut microbiota or immune responses, necessitate additional exploration to address safety concerns regarding immunogenicity and immunotoxicity potential[66]. Currently, there is no established maximum safe dosage of dietary LF, especially for prolonged consumption by children and the elderly. Hence, it is imperative to expand research on the long-term safety of LF, supported by evidence and addressing existing gaps through extended studies. Furthermore, further inquiries are necessary to meet regulatory standards for incorporating lactoferrin into fortified foods in various countries, particularly those with stringent food safety regulations[67].

Bio preservation

Research is currently underway to investigate the effects of adding lactoferrin to cheese at varying concentrations (10, 15 & 20 ppm). It has been noted that increasing the level of lactoferrin in the cheese leads to a significant decrease in bacterial growth compared to the control, thereby extending the cheese's shelf life. Cheese treated with lactoferrin up to 20 ppm exhibited a shelf-life extension of up to 7 days at room temperature (30°C) and 15 days when refrigerated at 4°C, respectively. Additionally, higher levels of lactoferrin in the cheese result in increased hardness, resiliency, springiness, and chewiness. Sensory evaluations of samples treated with 20 ppm of lactoferrin indicated acceptability for 7 days at room temperature and 15 days when refrigerated[68].

Chantaysakorn and Richter[61] conducted a study on the antimicrobial properties of lactoferrin added to carrot juice. Lactoferrin, extracted from raw skim milk, underwent pepsin digestion for 4 hours. The antimicrobial activity of the digests in carrot juice against Escherichia coli was assessed using peptone-yeast-glucose broth. The inhibitory effect on E. coli and the growth of digested lactoferrin were more effective at pH 7 compared to pH 4 using peptone-yeast-glucose broth, with incubation at 23°C for 24 hours. Samples treated with lactoferrin and casein peptides showed lower total plate counts than the control. Incorporating lactoferrin into meat also reduced total plate counts compared to casein peptide addition. High-Performance Liquid Chromatography (HPLC) was utilized to examine meat glucose levels as an indicator of freshness. The results revealed an increase in glucose content from day 1 to day 3, followed by a slight decrease on day 5 with the addition of lactoferrin and casein peptides. Conversely, the control sample showed a continual decrease in glucose content throughout the storage period. The inclusion of lactoferrin and casein peptides notably reduced bacterial counts over 5 days and enhanced glucose content during the storage of hot-boned pork meat at 4°C.

Al-Nabulsi and Holley[70] found that lactoferrin inhibited the growth of certain strains of E. coli O157: H7 but promoted the growth of other strains. Notably, during sausage production, all doses of lactoferrin deliberately boosted the reduction in E. coli O157: H7 growth, with unencapsulated lactoferrin leading to the highest reduction of 4.2 log units. Lactoferrin's effectiveness against pathogens in fermented meats is limited due to its narrow range of activity and initiation of pathogen injury. Countries like Japan are utilizing lactoferrin as a functional ingredient and for bio preservation in yogurt and various other foods[71].

Anti-microbial food packaging

Research into antimicrobial materials for packaging is expected to grow in the coming decade due to the development of new polymer materials and antimicrobials[72[. To meet the rising consumer demand for microbiologically safer foods with longer shelf lives, the food industry faces the challenge of innovating food packaging technologies. Research into edible films as effective packaging materials could enhance food quality, safety, and durability. Several antimicrobial edible films have already been commercialized to reduce spoilage and inhibit the growth of harmful microorganisms on ready-to-eat foods. Antimicrobial substances can be integrated into protein, polysaccharide, and lipid-based edible films during production. For example, lactoferrin used in chitosan films has shown effectiveness against E. coli and L. monocytogenes. Research indicates that combining lactoferrin with lysozyme in chitosan films significantly decreases the growth of both L.

monocytogenes and E. coli O157: H7, achieving approximately a 3-log reduction[73]. The use of naturally occurring antimicrobials, such as lactoferrin, is increasingly popular with consumers concerned about food preservation. These films not only act as physical barriers but also as antimicrobial agents. The incorporation of natural antimicrobials into edible films may be an excellent strategy to address food safety and consumer concerns. One study examined the antimicrobial effects of lactoferrin in chitosan-based films, investigating its synergy with lysozyme against foodborne pathogens. Chitosan and glycerol were dissolved in 1% acetic acid to create the films, into which different concentrations of lactoferrin, lysozyme, or nisin were added. Antimicrobial activity was evaluated through inhibition zones and cell count assays against L. monocytogenes and E. coli O157: H7. While films containing only lysozyme showed considerable antimicrobial effects against both pathogens, lactoferrin alone did not show any significant activity. However, the combination of lactoferrin and lysozyme demonstrated significant synergistic effects. Lactoferrin combined with lysozyme provided greater antimicrobial action than EDTA at 0.4 mg/disc against L. monocytogenes, suggesting that lactoferrin could be a viable alternative to synthetic chelators[74]. Another study focused on the effectiveness of lactoferrin and lysozyme in casein or zein films against E. coli, finding that these films effectively inhibited E. coli growth depending on the concentration of the antimicrobials used[75]. Rollini and colleagues developed a film coated with lysozyme and lactoferrin that was particularly effective against bacteria producing hydrogen sulfide in salmon fillets. Additionally, encapsulated lactoferrin was tested in two types of emulsions to prevent divalent cation interference with its antimicrobial properties, showing better efficacy than unencapsulated forms against the meat spoilage organism Carno bacterium viridans at varying temperatures. Moreover, the efficacy of antimicrobial agents is often lower in complex food matrices compared to microbiological environments, while nisin shows significantly enhanced activity when employed in liquid systems rather than solid food products. As a naturally occurring antimicrobial protein found in bovine and human milk, lactoferrin's efficacy can be affected by the presence of calcium and phosphates. Additionally, environmental conditions and food processing methods can influence antimicrobial performance, as demonstrated by a reduction in nisin activity during the production of pasteurized cheese due to melting and ultra-heat treatments, although refrigerated storage may stabilize its activity temporarily[72].

PHARMACOLOGICAL POTENTIALS OF LACTOFERRIN AGAINST VARIOUS PATHOLOGICAL CONDITIONS

LF is a mediator secreted by cells that connects innate and adaptive immunity in mammals [76]. The binding of LF influences target cells through a cellular signaling pathway and triggers the activation of specific genes [77]. LF has pharmacological potential in treating various pathological issues, including oxidative stress, inflammation, fibrosis, endoplasmic reticulum (ER) stress, autophagy dysfunction, and mitochondrial Figure 5

Inflammation

Inflammation serves as an adaptive response involving various physiological and pathological processes triggered by harmful stimuli and conditions[78]. This intricate interaction occurs between soluble components and cells in tissues in response to traumatic, infectious, postischemic, toxic, acute kidney injury, or autoimmune damage. Lactoferrin (LF) is notably increased in various inflammatory diseases such as inflammatory bowel disease, allergic skin and lung disorders, neurodegenerative diseases, and arthritis[79].

LF functions as a natural regulator of the body's defense system with important anti-inflammatory properties, primarily through its ability to scavenge excess iron that can accumulate in inflamed tissues and generate harmful hydroxyl radicals. This makes LF a promising candidate for treating common inflammatory disorders. It is one of the first factors released by neutrophils after they encounter pathogens, aiding in the innate activation of adaptive immune responses by promoting active neutrophils to the site of inflammation[80]. While LF levels in blood are normally low (0.2– 0.6 g/ml), they can rise

significantly (over 200 g/ml) at inflammation sites due to neutrophil exposure[81]. Research indicates that LF can inhibit inflammation triggered by microbial challenges and binds to bacterial endotoxin lipopolysaccharides (LPS), thereby disrupting LPS's interaction with receptors and reducing the upregulation of inflammatory cytokines[82].

In laboratory studies, LF has been shown to protect gut mucosal integrity under LPS challenge and alleviate gastritis caused by Helicobacter pylori, as well as endotoxemia and mortality from systemic E. coli or LPS challenges. On monocytes, LF's anti-inflammatory properties in LPS challenges may inhibit pro-inflammatory cytokine production by entering the nucleus and suppressing NF-kB activation[77]. LF may also play a role in sequestering free iron in inflammatory areas, such as in rheumatoid arthritis, where it can prevent harmful free radicals from causing tissue damage. It has been administered intra-articularly in animal models, demonstrating anti-inflammatory effects. Consequently, the localized increase of LF in the synovium is believed to be beneficial in treating rheumatoid arthritis.

Interestingly, in neurodegenerative diseases associated with iron accumulation and oxidative stress, heightened expression of LF in certain brain regions has been observed, which might help mitigate oxidative stress through the transport of plasma LF across the blood-brain barrier during inflammation[83]. Additionally, in vivo studies highlight LF's protective effects against skin and lung allergies, with elevated LF levels noted in allergic individuals. This action likely involves inhibiting mast cell activation and interactions with other immune cells that release proinflammatory cytokines. In skin allergies, LF appears to bind to keratinocytes, preventing TNFα release, and reducing skin inflammation by inhibiting Langerhans cell migration. In both humans and mice, LF has been shown to protect against IL-1β-induced skin inflammation and chemically induced inflammatory bowel disease, often correlating with increased levels of beneficial antiin-flammatory cytokines and decreased pro-inflammatory ones[84].

LF's interaction with specific receptors on various immune cells, including monocytes, macrophages, neutrophils, and epithelial cells, suggests its anti-inflammatory effects may stem from directly influencing cytokine production through receptor-mediated signaling pathways. As a first-line defense glycoprotein, LF is vital in managing systemic inflammatory response syndrome (SIRS) and sepsis. Clinical studies in neonates have shown that dietary LF supplementation reduces the risk of late-onset sepsis, establishing LF as a key innate immune modulator crucial for controlling acute septic inflammation. During infections, systemic monocytes or macrophages generate inflammatory mediators, prompting bone marrow to produce new immune cells and activating mature neutrophils. This sequence results in substantial LF release from neutrophil secondary granules to combat infection, illustrating LF's anti-inflammatory role in the dynamic process of acute inflammation as a manifestation of neutrophil involvement[85].

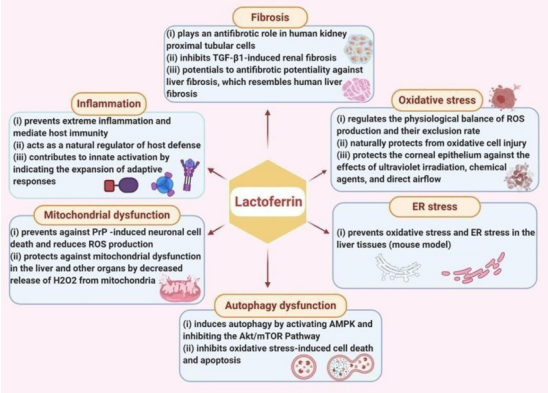

Fig 5: Pharmacological potentials of lactoferrin against pathological conditions.

The diagram illustrates the complex pharmacological potentials of LF in relation to pathological disorders, including fibrosis, oxidative stress, endoplasmic reticulum (ER) stress, autophagy dysfunction, mitochondrial dysfunction, and inflammation. In renal fibrosis and liver fibrosis, LF has been shown to play an antifibrotic role. It maintains the physiological balance of ROS activity and protects cells from oxidative damage in a variety of pathological conditions. In the liver tissues of ob/ob mice, lactoferrin lessens ER stress. It has also been documented that it stimulates autophagy by activating several pathways in order to avoid autophagy dysfunction and apoptosis. Lactoferrin regulates the mitochondrial synthesis of H2O2 to protect the liver and other organs from

mitochondrial dysfunctions. Its anti-inflammatory properties help to mediate host immunity and prevent inflammatory tissue inflammation.

Fibrosis

Fibrosis is characterized by the excessive growth, rigidity, or scarring of various tissues, resulting from an overabundance of extracellular matrix components, particularly collagen. It is the ultimate outcome of chronic inflammatory responses triggered by various factors, such as persistent infections, autoimmune disorders, allergic reactions, chemical damage, radiation, and tissue injuries[86]. Lactoferrin (LF) has been identified as having an antifibrotic effect in human kidney proximal tubular cells. The main pathological features of progressive chronic kidney disease (CKD) include the accumulation of excess matrix proteins, an increase in fibroblasts, and damage to nephron function, all of which contribute to renal fibrosis[87]. Transforming growth factor-β1 (TGF-β1) is a key mediator in the process of renal fibrosis. Connective tissue growth factor (CTGF) and plasminogen activator inhibitor-1 (PAI-1) are known to be effective inducers of tissue fibrosis. Studies have shown that TGF-β1 enhances the levels of CTGF, PAI-1, and collagen I in a concentration-dependent manner. To investigate LF's antifibrotic capabilities, cultured renal epithelial cells (HK-2) were treated with TGF-β1 in the presence or absence of LF to see if LF could inhibit the fibrosis signaling pathway activated by TGF-β1. The findings indicated that LF reduced the expression of profibrogenic target genes PAI-1, CTGF, and collagen I, suggesting that LF inhibits TGF-β1-induced renal fibrosis. Liver fibrosis is a major pathological concern in chronic liver diseases, with various drugs, autoimmune disorders, and genetic conditions being significant contributors. Antifibrotic therapies can help restore normal liver function. LF has demonstrated antiviral effects against a variety of viruses, including hepatitis C, and has been shown to prevent hepatocellular necrosis while providing direct cytoprotective effects in the liver. A recent study involving rats treated with thioacetamide (TAA) revealed LF's antifibrotic potential against liver fibrosis, which resembles human liver fibrosis. Furthermore, aerosolized bovine LF has been shown to reduce infection, inflammation, and iron imbalance in a cystic fibrosis mouse model infected with Pseudomonas aeruginosa. Chronic airway infections are often sustained by Pseudomonas aeruginosa, which is linked to decreased lung function and increased morbidity and mortality[88]. In cystic fibrosis airways, there is a notable presence of infection, inflammation, and disruption of iron homeostasis, with high levels of iron observed in airway secretions. Additionally, increased expression of ferroprotein (Fpn), ferritin (Ftn), and transferrin (Tf) has been noted in the lung tissue of cystic fibrosis patients, along with excessive iron accumulation in the lower respiratory tract. This excess iron in cystic fibrosis airways promotes the growth and biofilm formation of Pseudomonas aeruginosa, exacerbating inflammatory conditions and host injury. LF can chelate two Fe3+ ions per molecule with high affinity and is produced by exocrine glands and neutrophils at sites of infection and inflammation. Bovine LF (bLF), derived from milk, shares a high degree of sequence homology with human proteins and exhibits similar characteristics. It prevents the invasion of host cells by specific intracellular attachments or obligate bacterial pathogens and has strong anti-inflammatory activity that helps protect mucus from inflammatory damage. Treatment with aerosolized bLF or saline after infection demonstrated that aerosolized bLF effectively reduced bacterial lung load and the infiltration of leukocytes in infected cystic fibrosis mice. By reducing pulmonary iron overload in both wild-type and cystic fibrosis mice, bLF acts as a powerful multi-targeting agent capable of disrupting the cycle induced by Pseudomonas aeruginosa, inflammation, and iron imbalance, thereby alleviating the severity of cystic fibrosis-related pathology[87].

Conclusion

Food and pharmaceutical products depend on preservatives to maintain their quality, safety, and shelf life. Because of their safety and many uses, natural preservatives have become increasingly important as worries about synthetic compounds have grown. Because of its potent antibacterial, antiviral, antioxidant, and anti-inflammatory qualities, lactoferrin is highlighted in this review as a promising natural preservative. Lactoferrin is very efficient against a variety of

infections because it can chelate iron, damage microbial membranes, and alter immune responses. Its versatility and promise to improve food safety and quality are demonstrated by its uses in the food sector, which include dairy products, infant formulae, animal products, bio preservation, and antimicrobial food packaging.

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