Mini-review

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The antimicrobial and bioactive properties of lactobionic acid

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Abstract

Lactobionic acid (LBA) is a bioactive molecule that has generated keen interest in different industries. However, its future application in the food area is one of the most promising. Chemically, it is a polyhydroxy acid formed by the union of two molecules (galactose and gluconic acid) linked by an ether-bond, showing many interesting and unusual properties due to its structure and composition, although it is traditionally known in the food industry for its chelating, moisturizing, gelling, and antioxidant properties. There has been much research into the production of LBA, either by microbial fermentation or biocatalytic approaches such as enzymatic synthesis, but its use in foodstuffs, to produce new functional products and to evaluate its antimicrobial activity against food-borne pathogens, is a relatively new topic that has attracted the interest of the international research community recently. Furthermore, in spite of the potential of LBA, it has been approved only by the US Food and Drug Administration, and for its use as the salt form, but the publication of new comprehensive studies, able to agglutinate all the new food-related LBA research results, could disseminate knowledge about this compound and have an influence on its current regulation status. The aim of the present review is to describe the most recent advances and research on its antimicrobial potential, as well as summarizing the significant aspects that make LBA a promising bioactive compound for the food sector.

Keywords: organic acid; bioactive compound; antimicrobial; preservative; prebiotic

INTRODUCTION

Lactobionic acid (LBA) is a natural polyhydroxy acid (Fig. 1) whose systematic name is 4-*O*-*β*-D-galactopyranosyl-D-gluconic acid and which is produced by the oxidation of the glucose component of lactose to gluconic acid. This compound has attracted the attention of the international research community owing to its particular chemical composition and properties. According to several authors, LBA exerts chelating, antioxidant, emulsifying, and antimicrobial properties that have been exploited by different industries.[1-3] In addition, owing to its high biocompatibility it has been widely used as an organ preservative, preventing cell swelling, and as an antioxidant, preventing the oxidation of lipid-based cosmetic products.[1]

LBA is produced mainly by chemical, enzymatic, or microbial synthesis. Although, at present, chemical synthesis is the predominant method employed to produce this organic acid,[4] the method requires expensive, toxic metal catalysts together with the production of undesirable by-products.[5] One alternative to chemical synthesis for the production of LBA is the enzymatic oxidation of lactose, producing a high lactose conversion rate in an eco-friendly process. However, this method suffers from enzyme deactivation problems and the use of additional enzymes and high-cost cofactors.[6] Finally, a third option is the microbial fermentation of whey to oxidize lactose into LBA, which has been developed using different *Pseudomonas* species, in particular *Pseudomonas taetrolens*, although other bacterial species such as *Burkholderia cepacia*, *Zymomonas mobilis*, or *Acetobacter orientalis* have been reported as LBA producers.[1,7] In this case, it is an eco-friendly process that does not generate highly polluting by-products. However, the LBA produced by microbial fermentation subsequently requires expensive purification processes.[8] With respect to large-scale commercial production, bearing in mind that LBA is a growing market, it is no surprise that several international companies have this organic acid in their portfolio, among them Solvay (Germany), FrieslandCampina Domo (New Zealand), Sadoz (Germany), Reliable Biopharmaceutical Corp. (USA), and US Dairy Ingredient Comp. (USA), although there

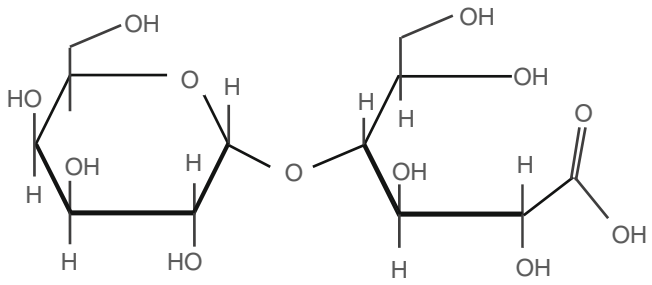

Figure 1. Structural formula of lactobionic acid.

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are also many other chemical-related companies from China involved in LBA production.

At the present time there is only one commercial food product marketed with LBA as a prebiotic compound, namely 'Caspian Sea yoghurt'. This product is sold in Japan and is considered as a 'Food for specified health use'[9,10] and it contains LBA in its salt form (calcium lactobionate, CaLb) at a concentration of 0.45 mg CaLb g^{-1} yoghurt. The US Food and Drug Administration (FDA) has approved LBA for use as an additive, antioxidant, stabilizer, and gelling agent in dessert products. There are also patents in existence referring to its use as an aging inhibitor for bread, as a water retainer for processed meats and a flavor enhancer for foods or beverages, among others.[7]

One of the applications that has attracted attention in recent research is its potential as an antimicrobial. The use of natural bioproducts to control food-borne spoilage and pathogenic bacteria is considered a novel antimicrobial strategy[11,12] and LBA could play a key role. Knowing the mechanism of action of this bionic acid is one of the main points to improving and extending their use in the food field.

Within this context, the objective of this comprehensive study is to provide an overview of the antimicrobial potential of LBA. Recent discoveries about the antimicrobial properties of this organic acid and its incorporation in food matrices for antimicrobial purposes will be analyzed. Thus, the mechanism of action will be summarized and discussed, in order to clarify the potential use of this organic acid as a protective agent against food-borne pathogens. In addition, recent research into food products containing LBA as a bioactive compound will also be highlighted.

ANTIMICROBIAL PROPERTIES IN FOOD

Foodborne pathogens are an important public health problem.[13] In fact, the World Health Organization estimated that in 2015 one out of ten people suffered from a food-borne disease.[14] In this context, the use of natural preservatives with antimicrobial capacity is an important topic in food science, since these preservatives avoid the use of potentially harmful chemicals and can help to reduce food spoilage and contamination. One of these antimicrobial preservatives is LBA, which has recently been studied in depth in order to understand its mechanism of action. In addition, its antimicrobial capacities have been tested in various food matrices in order to estimate its potential range of application.

Mechanism of action of LBA

One of the key requirements for the promotion of the use of LBA in the food field as a natural preservative with antimicrobial capacity is to understand its mechanism of action and to know what its targets are inside the bacterial cells. The mechanism of action of LBA against a Gram-positive bacteria, *Staphylococcus aureus*, has been investigated extensively, as staphylococcal food poisoning is the most prevalent foodborne intoxication worldwide.[15] The mechanism of action is summarized in Fig. 2(A).

Cao *et al.*[16] treated a suspension of *Staphylococcus aureus* with a microbial load of 6 $\log_{10}$ CFU mL^{-1} with 7.5 and 15 mg mL^{-1} LBA and analyzed the growth of the bacteria. As a result, they found that LBA negatively affected the growth of *Staphylococcus aureus* in a dose-dependent manner, damaging the integrity of the cell membrane. In this case, the possible disruptive effect of the LBA on the cell wall membrane was suggested by the presence of alkaline phosphatase and nucleotides in the culture medium and by the transmission electron microscopy (TEM) observations. In a further study, Cao and Zheng[17] provided a deeper insight into this subject by carrying out an iTRAQ-based quantitative proteomic analysis, which suggested that the LBA was affecting the expression of ABC transporters, altering the cellular metabolism, and decreasing the levels of proteins involved in the survival of the bacteria under stress conditions. Likewise, LBA could attenuate the virulence of *Staphylococcus aureus* and reduce its capacity of infection. These authors observed the morphological changes in *Staphylococcus aureus* caused by LBA and visualized them by TEM and scanning electron microscopy (SEM).

Kang *et al.*[18] thoroughly studied the mechanism of action of LBA on methicillin-resistant *Staphylococcus aureus* (MRSA) variants. These variants are resistant to a wide range of antibiotics such as penicillin, oxacillin, and tetracycline and are highly resistant to environmental factors. Therefore, they can contaminate a variety of foodstuffs such as meat and dairy products and so imply a risk to the health of food handlers and consumers.[15] In this case, Kang *et al.*[18] studied the antimicrobial effect of LBA on MRSA N315 using quantitative proteomics, experiments of reactive oxygen species, virulence-associated gene expression, and quantitative real-time polymerase chain reaction (PCR). As a result, they found that LBA's mode of action against MRSA was similar to that against non-MRSA, causing cell wall damage and loss of membrane integrity. Furthermore, there was inhibition of DNA repair and protein synthesis, induction of oxidative stress, and inhibition of metabolic pathways. Regarding cell surface proteins and virulence factors, after a 2-h treatment with LBA, the virulence, biofilm production, and adhesion to the MRSA host were decreased.

Although the mechanism of action has been studied in depth in a Gram-positive bacteria (*Staphylococcus aureus* species), research into the mechanism of action has also been done on Gram-negative bacteria (Fig. 2(B)), such as *Pseudomonas fluorescens*.[19] This bacterium is responsible for the spoilage of foods of animal origin, such as meat, poultry, milk, and fish. It is also the most common psychotrophic bacterium found in milk and dairy products.[20] In this study, *P. taetrolens* ATCC 13525 cells at an exponential growth phase were incubated with 6.25 mg mL^{-1} LBA and a proteomic analysis was performed. As a result, it was found that LBA causes cell damage at different levels. SEM and TEM results showed that LBA may cause cell membrane dysfunction. Besides, folate biosynthesis may be inhibited, blocking DNA and protein synthesis, causing cell death. Regarding the outer membrane, LBA may rapidly increase its permeability in a dose-dependent manner, causing hypoosmotic shock and compromising bacterial survival. In addition, in another study with the same strain, it was also observed that LBA was able to intercalate into bacterial DNA, affecting normal cellular functions.[21] Another Gram-negative microorganism in which the antimicrobial mechanism of action of LBA has been recently studied is *Vibrio parahaemolyticus*,[22] the main pathogenic bacterium associated with seafood-borne illnesses. In this case, the antimicrobial mechanism was studied in two different strains: *V. parahaemolyticus* ATCC 17802 and ATCC 33847 and with a concentration of 4 and 8 mg mL^{-1} of LBA, respectively. The antimicrobial effect of LBA was studied by measuring intracellular adenosine triphosphate (ATP) concentrations, leakage of proteins, changes in bacterial morphology, and membrane integrity. As a result, it was observed that LBA treatment was able to reduce intracellular ATP concentration and to increase protein loss. Regarding changes in the bacterial morphology, it was

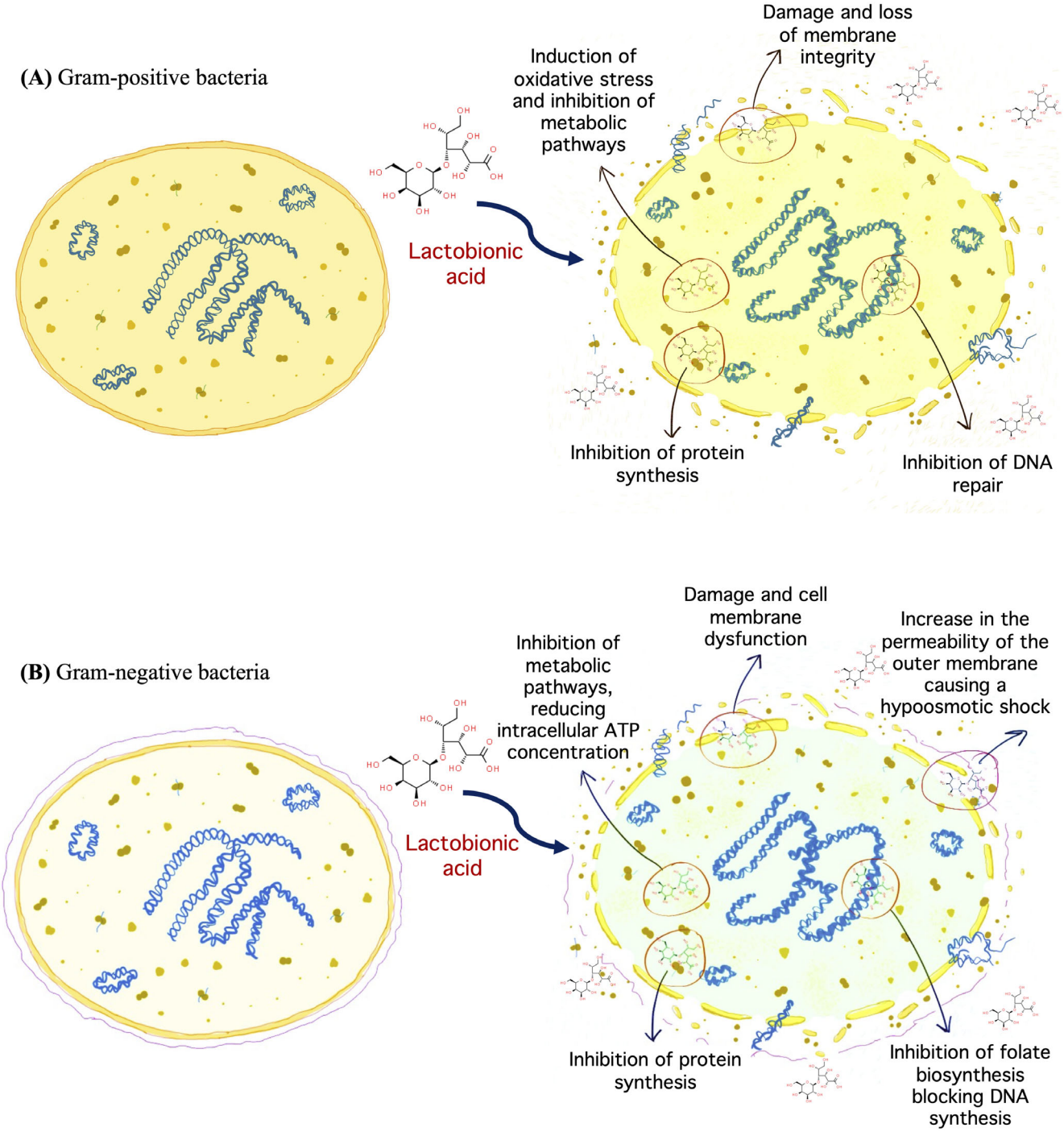

Figure 2. Summary of the mechanism of action of lactobionic acid. (A) On *Staphylococcus aureus* as a Gram-positive bacterium and (B) *Pseudomonas fluorescens* as a Gram-negative bacterium. In both cases, observed effects include induction of oxidative stress, loss of membrane integrity, inhibition of metabolic pathways, protein synthesis, and DNA repair. Besides, on Gram-negative bacteria an increase in the permeability of the outer membrane that causes hypoosmotic shock was observed.

observed that LBA caused alterations in the bacterial shape, affecting the integrity of the membrane.

LBA-spectrum of action against food-borne pathogenic and spoilage bacteria

The antimicrobial capacity of LBA has been studied in several bacterial species (Table 1), including the most important foodborne pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, and *Listeria*.[13] Antimicrobial activity can be measured as minimum inhibitory concentration (MIC) or the minimum bactericidal concentration (MBC). The MIC is the lowest concentration which prevents visible growth of bacteria while MBC is the lowest concentration of an antibacterial agent require to kill a specific bacterium. In the case of LBA, different concentrations have been tested with various spoilage and pathogenic foodborne bacteria in order to determine their MIC or MBC.

Table 1. Summary of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lactobionic acid (LBA) for some spoilage and pathogenic food-borne bacteria

Microorganism strain	MIC/MBC (mg mL^{-1})	Reference
Staphylococcus aureus ATCC 25923	15/50	16
Methicillin-resistant *Staphylococcus aureus* (MRSA) N315	18.75/NA	18
Bacillus cereus ATCC 11778	300–400/NA	23
Salmonella spp. ATCC 13076	300–400/NA	23
Pseudomonas fluorescens ATCC 13525	12.5/NA	21
Listeria monocytogenes Scott A	10.0/20.0	24
Escherichia coli O157:H7 ATCC 43895	10.0/20.0	24
Escherichia coli ATCC 25922	300–400/NA	23
Enterococcus faecalis 29 212	35.8/NA	25
Vibrio parahaemolyticus ATCC 17802	4.0/4.0	22
Vibrio parahaemolyticus ATCC 33847	4.0/4.0	22

NA, not available.

In addition to studying the mechanism of action, Cao *et al.*[16] calculated the MIC and MBC for *Staphylococcus aureus* ATCC 25923. To determine the MIC, the agar well diffusion method was employed, and the inhibition zones were measured. The MBC was calculated on a 96-well plate where the final LBA concentrations tested ranged from 10 to 100 mg mL^{-1}. Kang *et al.*[21] also determined the MIC of MRSA N315 and *P. fluorescens* ATCC 13525 by broth microdilution. Besides that, Chen and Zhong[24] determined the MIC and MBC for *Listeria monocytogenes* and *Escherichia coli* O157:H7 ATCC 43895. In this case, the MIC and MBC were determined by the serial microbroth dilution method using a microtiter plate reader to measure optical density (OD^{630nm}). Fan *et al.*[22] calculated the MIC and MBC of *V. parahaemolyticus* ATCC 17802 and ATCC 33847, using the modified broth dilution method with increasing concentrations of LBA (from 1 to 32 mg mL^{-1}). As a result, the MIC and MBC for these strains was observed to be 4 mg mL^{-1}. Cardoso *et al.*[23] calculated the MIC for *Bacillus cereus* ATCC 11778, *Salmonella* spp. ATCC 13076 and *Escherichia coli* ATCC 25922 by the diffusion disk test. The inhibition of microbial growth was determined by measuring in millimeters the inhibition halos. The same method was used by Wojciechowska *et al.*[25] to determine LBA MIC for *Enterococcus faecalis* 29 212.

As a result, it can be seen that LBA has a wide range of activity, affecting the growth of both Gram-positive (such as *Staphylococcus aureus*, or *Listeria*) and Gram-negative bacteria (*Escherichia coli* or *Salmonella* spp., among others) (Table 2). Regarding the concentration of LBA needed to inhibit microbial growth, as expected, it was highly dependent on the bacterial species. Due to the broad spectrum of action of LBA, it should be considered as one of the promising natural acids in the food area for the prevention of food spoilage and control of food-borne diseases.

Incorporation of LBA in food matrices for antimicrobial purposes

Although LBA has many properties of interest to the food industry, in particular from an antimicrobial point of view, there are very few studies in which these properties have been tested in a real food model (Table 2).

Kang *et al.*[21] analyzed LBA antibacterial activity against *P. fluorescens* ATCC 13525 and MRSA N315 in whole milk for 12 days at 4 °C. For this purpose, a concentration of 3 $\log_{10}$ CFU mL^{-1} of each of the microorganisms was added to the whole milk together with LBA concentrations of 12.5 and 18.75 mg mL^{-1} (MIC concentration) for *P. fluorescens* and MRSA, respectively. As a result, there was a reduction in the growth of *P. fluorescens* and MRSA with regard to control of 2.29 and 2.13 $\log_{10}$ CFU mL^{-1} respectively. Chen and Zhong[24] studied the combined effect of LBA with other antimicrobial agents against *Listeria monocytogenes* Scott A and *Escherichia coli* O157:H7 ATCC 43895 in 2% reduced fat and whole milk for 120 h at 21 °C. The best results were obtained when a ternary combination of 500 IU mL^{-1} of nisin, 2 mg mL^{-1} thymol and 10 mg mL^{-1} of LBA was employed. In 2% reduced fat milk, this combination caused a reduction in both the bacterial populations to a level below the detection limit (1 $\log_{10}$ CFU mL^{-1}). In the case of whole milk, there was a large reduction in the concentration of both bacteria, which was maintained throughout the 120 h of testing. In both cases, the presence of LBA increased the synergistic effect of nisin and thymol against the two microorganisms tested. Furthermore, the importance of the study matrix was also reflected, as in whole milk the antimicrobial effect of the ternary combination was weaker compared to that for the reduced fat milk. Cardoso *et al.*[26] used LBA in the production of *requeijão cremoso* with the aim of studying the effects of LBA as a food additive. They used different concentrations of LBA (0.25, 0.50, 0.75 and 1.00 g LBA per 100 g of food product) and monitored it for 22 days, to investigate the effect on different spoilage and pathogenic bacteria. The best results were obtained for a concentration of 0.75 mg LBA (100 g)$^{-1}$ product. For all concentrations tested, the inhibition halos measured in order from highest to lowest were obtained for *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Salmonella enteritidis* ATCC 13076, and *B. cereus* ATCC 11778. Despite this variation, after 22 days all the samples showed a loss of antimicrobial activity.

In recent years there has been little investigation into the antimicrobial properties of LBA in real food products and the few studies into this topic were carried out using dairy products. However, as was seen in the previous section, LBA possesses notable *in vitro* antimicrobial properties against some of the most worrying food borne pathogens, but it is important to note that this characteristic has not been studied using processed meat, fish or vegetable foodstuffs. In this sense, more research is necessary to assess interactions between LBA and other food compounds that may tend to suppress or enhance its antimicrobial properties, and thus evaluate the real potential of LBA as an antimicrobial agent of practical use in the food industry.

LBA AS A BIOACTIVE COMPOUND IN INNOVATIVE FOOD PRODUCTS

In recent years, several new food products have been developed in which LBA plays a fundamental role, and which have been designed to benefit from other properties it possesses, such as its preservative and prebiotic capacities. Like other bioactive compounds, such as galactosyl derivatives[25] and glucooligosaccharides,[27] LBA can show a prebiotic or antimicrobial effect depending on the bacterial type and dose. A recent study has shown that the utilization of prebiotics by lactic acid bacteria have species and strain specificity.[28] In the case of bifidobacteria and other bacteria of the gastrointestinal tract (GIT)

Table 2. Summary of recent studies on food products to which lactobionic acid (LBA) has been added as an antimicrobial compound

Food product	Concentration of LBA used	Microorganisms tested	Highlighted results	References
Whole milk	12.5 mg mL^{-1} 18.75 mg mL^{-1}	*Pseudomonas fluorescens* ATCC 13525 MRSA N315	There was a reduction in the growth of *P. fluorescens* and MRSA with respect to control of approximately 2.29 and 2.13 log_{10} CFU mL^{-1}, respectively.	21
2% reduced fat milk and whole milk	10 mg LBA mL^{-1} in combination with 500 IU of nisin and 2 mg mL^{-1} thymol.	*Listeria monocytogenes* Scott A *Escherichia coli* O157:H7 ATCC 43895	In 2% reduced fat milk, this combination reduced the bacterial populations to a level below the limit of detection (1 log_{10} CFU mL^{-1}). In the case of whole milk, a large reduction was maintained in both bacterial populations throughout 120 h of testing.	24
Cream cheese (*requeijão cremoso*)	0.75 mg LBA/100 g of food product.	*Listeria monocytogenes* ATCC 7644 *Staphylococcus aureus* ATCC 25923 *Salmonella enteritidis* ATCC 13076 *Bacillus cereus* ATCC 11778	The highest inhibition halos (in order from highest to lowest) were obtained for *Listeria*, *Staphylococcus aureus*, *Salmonella*, and *Bacillus*. Nonetheless, after 22 days all samples showed a loss of antimicrobial activity.	26

MRSA, methicillin-resistant *Staphylococcus aureus* variants.

microflora, LBA acts as a prebiotic, which implies that it is an ingredient that is selectively fermented by this type of bacteria,[29] while it shows antimicrobial activity against several food pathogens. The most recent research work into the incorporation of LBA in food-related products in order to take advantage of its bioactive properties are summarized in Table 3.

Regarding its properties as a preservative, Marques *et al.*[30] prepared yacon juice and tested the effect of several heat treatments and acids, including LBA, on some of its properties. In this case, an LBA concentration of 1% combined with steam blanching was found to be the best option for preserving the pH, antioxidants, color, and total polyphenols content of this juice. When the same yacon juice was tested for a long-term storage (120 days), the appearance of molds and yeasts was delayed from day 60 in the control to day 120 for the juice with LBA added.[31] Goderska[34] added LBA dissolved in glycerol to rapeseed oil to check the preservative properties of this organic acid on a lipid-based product. In this research and as the author expected, the larger the LBA concentration, the higher the antioxidant effect observed. The best results were obtained at the highest LBA concentration tested (1%, *w/v*), with a decrease in the peroxide value of 19.9%. There have also been recent studies on dairy food products. Cardoso *et al.*[26] prepared a kind of acid-set cream cheese (*requeijão cremoso*) using LBA and/or lactic acid, testing the effect of these acids on several cheese properties under refrigerated storage up to 22 days. LBA showed no effect on the water activity, pH and color of the cheese produced and the antioxidant properties of the cheese were higher only during the first day of storage. Kang *et al.*[21] added LBA to whole milk at a concentration of 12.5 mg mL^{-1}, testing the sensory properties of the milk during 12 days of storage at 4°C. In this case, the milk with LBA exhibited lower thickness and sourness, had less precipitate after boiling and its natural milk color was preserved for longer when compared to the control milk. In general, according to all these recent research papers, it is noticeable that the preservative effect of the LBA was most significant when it was incorporated in liquid food products, but when it was added to a more complex, concentrated food matrix, such as the *requeijão cremoso*, these preservative properties were almost unnoticeable.[26] These results suggest some kind of interaction in cheese between the LBA and the milk compounds, resulting to some extent in the suppression of the preservative properties of LBA. In any case, no other study was found in the literature about the preservative effect of LBA in cheese that might have been able to provide a deeper insight into this question.

Regarding its prebiotic properties, several new food products have been developed. García *et al.*[35] prepared a novel dairy product by fermenting milk sequentially with *P. taetrolens* LMG 2336 and then with *Lactobacillus casei* CECT 475 in order to obtain a synbiotic product enriched in LBA. This double fermented milk contained, in its best formulation, 30 g L^{-1} of LBA and an active *Lactobacillus casei* population of 9 log_{10} CFU mL^{-1}. In addition, the textural properties of this dairy product were improved by the gelling capacity of the LBA. Part of the new research has focused on the preparation of biomaterials. In this sense, some authors have prepared microparticles loaded with LBA using casein, gelatine, maltodextrin, and gum arabic.[33] Several studies have shown that LBA is consumed by different probiotic bacteria belonging to the genera *Bifidobacterium* and *Lactobacillus*,[34,37] which are generally present in dairy products. Therefore, LBA was incorporated in the microparticles to prevent its consumption by the lactic acid bacteria that are naturally present in this kind of foodstuff, and thus allow it to reach the low GIT intact. Results showed that the concentration of LBA contained in the microparticles remained almost invariable for 12 days in the cheese matrix. Furthermore, LBA was released abundantly in stomach and intestine, which suggests that the LBA is able to reach the low GIT in a free form, exerting its prebiotic function. If LBA is combined with probiotic bacteria, synbiotic products and bioactive packaging are developed. In this respect, Sáez-Orviz *et al.*[32] prepared an alginate-based synbiotic coating that contained LBA as prebiotic and *Lactobacillus plantarum* CET 9567 as

Table 3. Summary of the most recent research works about the incorporation of lactobionic acid (LBA) in food-related products

Food product	LBA activity	Final product parameters tested	Highlighted results	Reference
Yacon juice	Preservative (up to 48 h).	Juice antioxidant and phenolic content, pH, color, and enzymatic browning protector capacity.	The best preservative tested was a combination of LBA (1%) and steam blanching. All the parameters.	30
Yacon juice	Preservative (up to 120 days). Prebiotic	Juice color, antioxidant content, polyphenols bioavailability, iron(II) ion (Fe^{2+}) and calcium ion (Ca^{2+}) chelating capacity, gastrointestinal digestion, prebiotic assessment.	The best preservative tested was LBA at a concentration of 1%.	31
Cream cheese (*requeijão cremoso*)	Preservative (up to 22 days). Acidulant	Cheese pH, moisture, water activity, color, rheology, antioxidant content, iron chelating capacity, and antimicrobial properties.	When the cheese was produced using LBA instead of lactic acid, better antioxidant and antimicrobial results against *Listeria monocytogenes* were obtained after 1 day of storage.	26
Whole milk	Preservative (up to 12 days).	Sensory properties.	Milk with added LBA showed a better score on the sensory parameters tested over the whole time of storage assessed than the whole milk control.	21
Synbiotic coatings to cover cheese pieces	Prebiotic	*Lactobacillus plantarum* survival under storage conditions and after being subjected to simulated gastrointestinal tract conditions. Textural properties of the pieces of cheese.	The coexistence of LBA and *Lactobacillus plantarum* in the coatings noticeably increased the survival of the bacteria.	32
Microparticles loaded with LBA in order to protect it from its consumption by lactic acid bacteria	NA	LBA encapsulation efficiency, microparticle solubility, microstructure and enriched cottage cheese digestibility and textural analysis.	Microparticles exert a protective effect against consumption of lactobionate by lactic acid bacteria found in the cheese matrix.	33
Rapeseed oil	Antioxidant	Lipid oxidation level.	At the highest concentration of LBA tested (1%, *w/v*) a 19.9% antioxidant effect was observed.	34
Synbiotic dairy product	Prebiotic and gelling agent.	Concentration of LBA in the final product and textural properties.	Final product can be marketed as synbiotic due to an adequate amount of probiotic and prebiotic. The final product had higher viscosity due to the gelling effect of LBA.	35
Bioactive packaging based on delipidated edible egg yolk protein	Prebiotic	*Lactobacillus plantarum* survival under storage conditions and after being subjected to simulated gastrointestinal tract conditions.	The coexistence of LBA and *Lactobacillus plantarum* in the films and coatings increased noticeably the viability and survival of the bacteria after the digestion test, reaching final values above 6 $\log_{10}$ CFU mL^{-1}.	36

NA, not available.

probiotic in order to coat cottage cheese. The presence of LBA in the coating increased the survival of the probiotic during the analysis (15 days). Thus, a simulated *in vitro* digestion of the bioactive coating was made, and only those coatings with both probiotic and prebiotic in their composition met the minimum legal requirements to be considered as probiotic (6.0 $\log_{10}$ CFU g^{-1} cheese). Also, synbiotic films and coatings using delipidated egg yolk protein as the matrix, *Lactobacillus plantarum* CECT 9567 as a probiotic microorganism and LBA (10 g L^{-1}) as a prebiotic compound were developed.[36] The resulting bioactive packaging possessed suitable properties for coating foodstuffs for 15 days at 4 °C. Furthermore, LBA exerted a protective effect on the probiotic bacteria, and when pieces of gelatine were coated by dipping in the film-forming solution containing LBA and probiotic, the LBA also exerted a protective effect on the probiotic population subjected to *in vitro* oral gastrointestinal digestion. It is of interest that LBA can exert a protective effect on the bacterium when encapsulated or trapped in a film or coating, enabling a greater degree of survival in the simulated *in vitro* digestion studies mentioned earlier,[32,36] which is an issue that deserves more attention. LBA, like other prebiotics, would show a protective effect when it is encapsulated or trapped with probiotic bacteria, since it would provide an extra nutritional contribution that improves their survival in the acidic conditions of the digestion process.[38-40] However, the industrial use of these new products is limited since these laboratory concepts have not been scaled-up. In addition, despite the fact that the prebiotic capacity of LBA has been studied by several authors[41,42] and LBA is known to be resistant to digestive enzymes and able to reach the lower

GIT to be fermented by the GIT microflora, more *in vivo* research is necessary.

Current food applications for LBA are scarce and it may be due to a lack of regulation in Europe discouraging research initiatives in the food area. At the moment, LBA has been approved by the FDA for its use as a food preservative only in its salt form (CaLb, E-399),[43] although it is expected that its human consumption will be approved by other food authorities, such the European Food Safety Agency (EFSA), in the near future. However, Japan has approved its consumption in different types of foodstuff, such as 'Caspian Sea yoghurt' and other dairy products.[44] One of the obstacles to the approval of LBA for use in the food sector is the lack of *in vivo* studies involving testing human populations[45] and need for the establishment of concentrations suitable for its consumption. Although very few studies have been conducted, some authors have found that up to 24 g d^{-1} of LBA can be ingested without adverse effects, effects which would be similar to those caused by lactose intolerance.[46]

CONCLUSIONS

In this review, the great potential of LBA as a natural food additive that is of interest for its antimicrobial capacity has been highlighted. In both Gram-positive and Gram-negative bacteria, the antimicrobial mechanism of action involves the loss of cell membrane integrity, inhibition of DNA and protein synthesis and the induction of oxidative stress. LBA could also intercalate into bacterial DNA and is able to decrease virulence factors in some cases. In addition, in Gram-negative bacteria, there is an increase in the permeability of the outer membrane, causing hypoosmotic shock. Therefore, LBA has a spectrum of action against a variety of foodborne bacteria (Gram-positive and Gram-negative) and some studies have already demonstrated this antimicrobial activity in dairy products, but more research is needed in order to assess antimicrobial capacity when LBA is included in other food matrices. Something similar must be said about its preservative properties. There is a general lack of studies in the literature into the possible interactions of LBA with biopolymers such as lipids or proteins from a variety of food sources. The study of these interactions could clarify its antimicrobial and preservative potential as a valuable additive for the food industry. As to the innovative products containing LBA and developed in the last 5 years, it is clear that most of them have been prepared only on laboratory scale and they have not been subjected to any sensory testing. All in all, from the perspective of the food industry, LBA may be considered to remain a promising but understudied compound. Therefore, further in-depth studies into this subject would be valuable for the food research community.

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