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Review

Study of exopolysaccharides from lactic acid bacteria and their industrial applications: a review

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Summary This review describes and discusses the structure, biosynthesis and applications of exopolysaccharides from lactic acid bacteria. These substances are classified as homopolysaccharides, which are synthesised from sucrose through the action of extracellular glycosyltransferases or heteropolysaccharides, which are synthesised from repeating unit precursors formed in the cytoplasm and assembled extracellularly by the sequential addition of nucleotide sugars. The industrial application of exopolysaccharides is linked to enhancing the texture and rheological properties of certain fermented products and their production *in situ* being of particular interest. The chemical characteristics of exopolysaccharides influence interactions with milk proteins in fermented dairy products. These compounds reduce gel syneresis and increase the viscosity, water retention capacity and firmness of the gel, all of which are desirable characteristics for the development of low-fat dairy products. Similarly, they have applications in the production of gluten-free bakery products and fermented meat products.

Keywords Exopolysaccharides biosynthesis, exopolysaccharides, gluten-free bakery products, industrial application, lactic acid bacteria, low-fat dairy products, textural properties.

Introduction

Lactic acid bacteria (LAB) have been used for centuries in the production of fermented dairy products, meats, vegetables and alcoholic beverages, as well as in sourdough breads (Behare *et al*., 2009). LAB present complex proteolytic and lipolytic systems that are responsible for the sensory characteristics of fermented foods (Torino *et al*., 2015; Zannini *et al*., 2016). Furthermore, some LAB strains are also able to produce substances such as exopolysaccharides (EPSs) that can improve food quality (Capek *et al*., 2011). Zhou *et al*. (2019) describes EPSs as biopolymers synthesised extracellularly or secreted in the extracellular medium by microorganisms during their growth. These polymers show variability in monosaccharide composition as well as in branching range degree.

In contrast with that of other polysaccharides that are used as energy sources (Zannini *et al*., 2016), EPSs play an important role in the ecology of LAB controlling cell surface physicochemical characteristics and protecting bacterial cells from abiotic or biotic stress (Nguyen *et al*., 2020). In addition, EPSs play key roles in adhesion and anchorage to surfaces, biofilm formation, cellular recognition and quorum-sensing control (Das *et al*., 2014; Caggianiello *et al*., 2016). This can encourage symbiosis relationships between the microorganisms and the host. In fact, EPSs-producing LAB can easily adhere to the cells of the intestinal epithelium, which allows probiotics to colonise the intestine and inhibit the growth of pathogenic microorganisms. However, the opposite effect could also take place. It means, if the EPSs adhere to the intestinal mucus, it could interfere, by competitiveness, with probiotics cell adhesion. In this case, enteropathogens could bind to EPSs through components on the surface, thereby leading to their adhesion to the intestinal epithelium. Although these possibilities cannot be ignored, *in vitro* studies seem to corroborate that EPSs control pathogen growth and biofilm formation, with a broad antibacterial spectrum (Xu *et al*., 2019).

Food applications of EPSs from LAB have recently been published, especially in fermented products (Korcz & Varga, 2021). Some works have shown the relationship between the structure of EPSs and their application in the food industry. (Bajpai *et al*., 2016; Xu *et al*., 2019; Daba *et al*., 2021). EPSs offer an effective alternative to the use of stabilising additives. In recent years, microbial EPSs have increasingly been

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used to improve texture and rheological properties in food products, mostly dairy products, thanks to their pseudoplastic rheological behaviour and water-binding capacity (Bajpai *et al*., 2016; Nguyen *et al*., 2020).

The literature available for the relationship between the terms 'Food' and 'EPSs' is very wide to date (2762) (Web of Science, 2021). In fact, it constitutes approximately 45% of the total research/review works about EPSs (6278). These data evidence the great interest of the scientific community in this field. In this sense, there has been an increase in the number of papers published about EPSs in foods, from 42 publications in 2000 to 327 in 2020. Regarding the application of EPSs in the food industry, approximately 20% of the articles are related to dairy field. The number of works published about dairy products and EPSs has tripled since 2000, being more significant in the last 5 years. It should be highlighted that fermented milk have been the main area of interest, representing approximately 60% of publications. Evolution in the number of publications since the year 2000 is represented in Fig. 1.

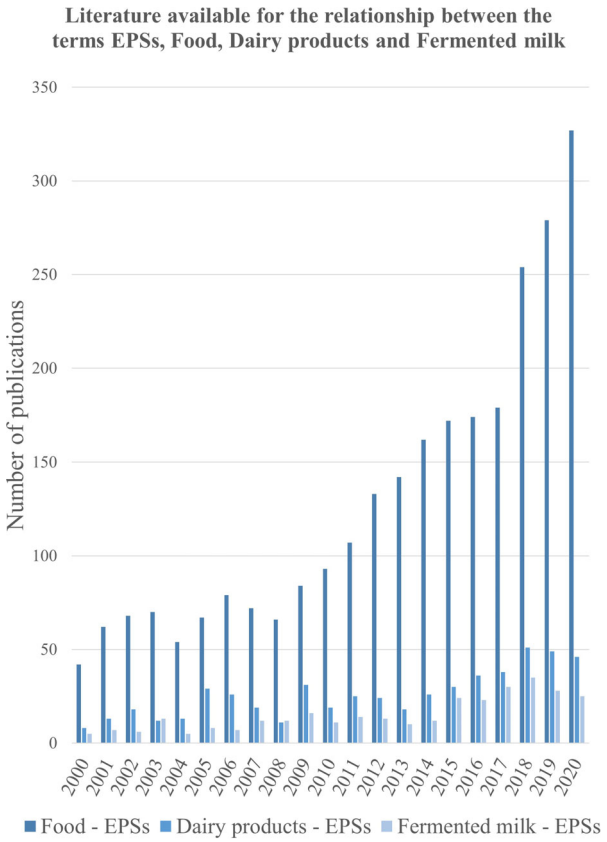

Figure 1 Literature available for the relationship between the terms EPSs, Food, Dairy products and Fermented milk localised in the Web of Science (Clarivate Analytics).

Based on those above described, the aim of this review was to summarise and discuss the knowledge available with regard to EPSs produced by LAB. It will lay most emphasis on the differential aspects of these substances that make them useful for the food industry, especially the dairy industry.

Classification of EPSs synthesised by LAB

Exopolysaccharides are long branched-chain polysaccharides, constituted by repeating units of carbohydrates or carbohydrate derivatives, which can be secreted into the environment in the form of slime or can be closely linked to the cell surface, as capsular polysaccharides (Zannini *et al*., 2016). EPSs produced by LAB are diverse and can be classified using several criteria. On the basis of the monomeric composition of EPSs, these substances are classified as homopolysaccharides or heteropolysaccharides (Abid *et al*., 2018). The main characteristics of each group of EPSs are described below and summarised in Table 1.

Table 1 Main differences between homopolysaccharides and heteropolysaccharides produced by lactic acid bacteria

	Homopolysaccharides	**Heteropolysaccharides**
Types of monosaccharides	Contain only one type	Contain two or more types
Main monosaccharides	Glucose or fructose	Glucose, galactose and rhamnose
Type of link	α or β link present	α and β link present
Structure	Typically linear or branched	Typically branched
Molecular mass	$>10^6$ Da	10^4–10^6 Da
Mainly produced genera	*Lactobacillus*, *Leuconostoc*, *Oenococcus* and *Weissella*	*Lactobacillus*, *Lactococcus*, *Bifidobacterium* and *Streptococcus*
Biosynthesis precursors	Produced extracellularly from sucrose or starch	Produced from intracellular intermediates.
Production level	g L^{-1}	mg L^{-1}
Presence of non-carbohydrate groups	Absence	Presence
Charge	Typically carries no charge	Can contain charged groups
Health benefits	Associated with prebiotic capacity	Associated with immune modulation

Homopolysaccharides

Homopolysaccharides (HoPSs) are the structurally simplest group of EPSs, since they are composed of one type of monosaccharide repeating units, D-fructose in the case of fructans or D-glucose in case of glucans, which are linked to form either linear or ramified structures (Abid *et al.*, 2018; Nguyen *et al.*, 2020). HoPSs are characterised by having high molecular weights, in excess of 10^6 Da. They are produced in large amounts, reaching values of up to 10 g L^{-1} (Lynch *et al.*, 2018a).

Biosynthesis pathway of HoPSs

Lactic acid bacteria use the extracellular synthesis pathway to synthesise HoPSs (Zhou *et al.*, 2019) employing extracellular glycosyl transferases and fructosyl transferases (Angelin & Kavitha, 2020). The biosynthesis of HoPSs consists of two steps, first the hydrolysis of a substrate, such as sucrose, and the binding of the resulting monosaccharide residues to a glycan acceptor chain, followed by the direct release of polymerised chains to the extracellular environment (Zhou *et al.*, 2019).

Beta-glucan synthesis presents differences from the synthesis of other HoPSs. The synthesis of β-glucan is carried out by the synthase-dependent pathway (Zeidan *et al.*, 2017). The synthesis is catalysed by β-glycosyltransferase that uses nucleotide sugars as substrate. The nucleotide sugars are added to growing repeating units in the cytosol and are exported by membrane transport to extracellular medium (Werning *et al.*, 2012).

Types of HoPS

Glucans. Glucans (Fig. 2) are high-molecular-mass polymers, which are classified into α- and β-D-glucans in accordance with the linkages in the main chain (Angelin & Kavitha, 2020).

On the one hand, α-D-glucans are subdivided into dextrans, mutans, reuterans and alternans (Angelin & Kavitha, 2020). Dextran is a polymer of α-D-glucopyranose linked predominantly by α-(1→6) bonds in the main chain and a variable number of α-(1→2), α-(1→3), α-(1→4) branched linkages. Mutan is a water-insoluble glucan composed of α-D-glucopyranose, which has primarily α-(1→3) glycosidic bonds. Alternan is composed of D-glucopyranosyl residues linked by alternate bonds α-(1→3) and α-(1→6). Finally, reuteran is an α-glucan containing α-(1→4) and α-(1→6) glycosidic bonds with α-(1→4)/α-(1→6) branching points (Zannini *et al.*, 2016; Wangpaiboon *et al.*, 2019).

On the other hand, β-D-glucans are composed of glucose residues linked by β-(1→3) bonds and β-(1→2) branching (Lynch *et al.*, 2018b).

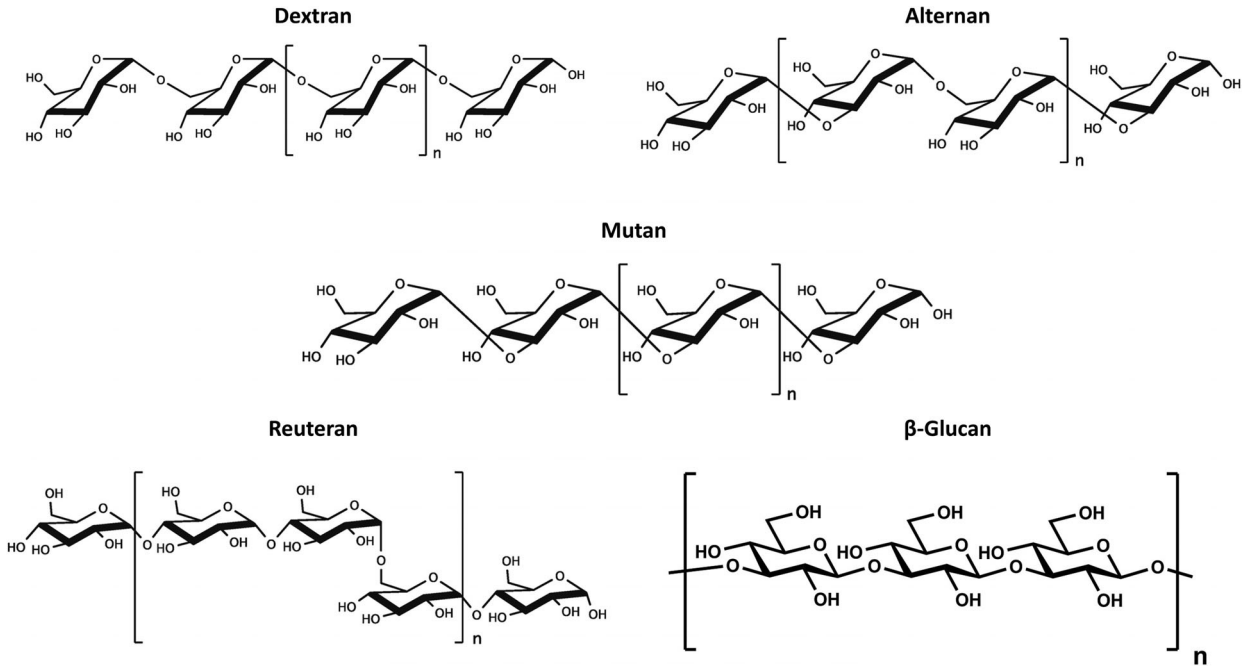

Figure 2 Structures of the glucans produced by lactic acid bacteria. Schematic representation of the repeating units of dextran, alternan, mutan, reuteran and β-glucan.

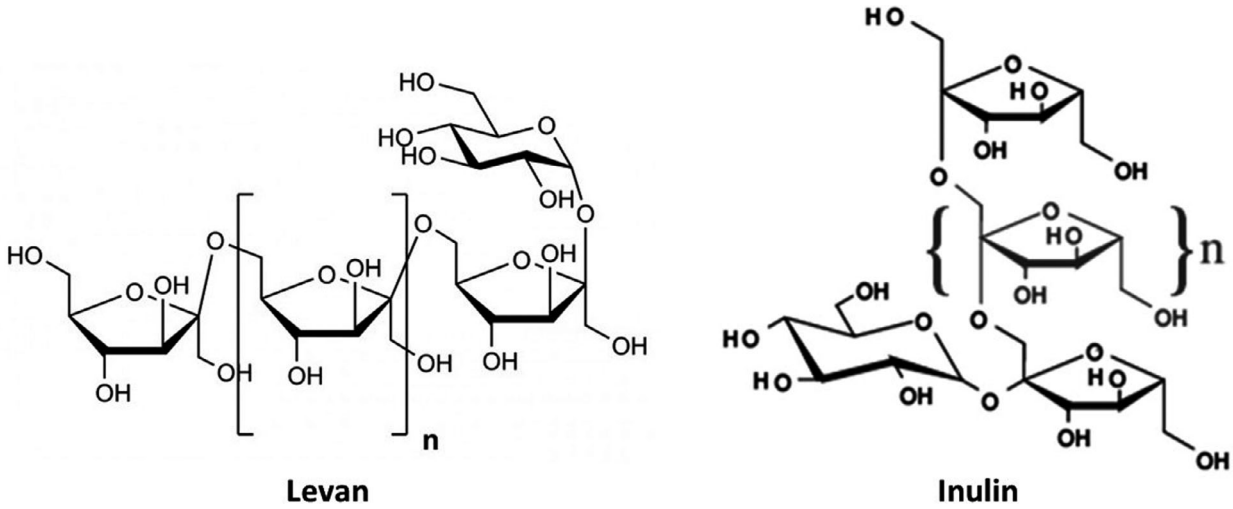

Figure 3 Structures of the fructans produced by lactic acid bacteria. Schematic representation of the repeating units of levan and inulin.

Fructans. Fructans are high-molecular-mass polymers that are classified into levans and inulins (Angelin & Kavitha, 2020) (Fig. 3). Levans are synthesised by the action of levansucrase which catalyses the transfer of D-fructosyl residues from fructose to yield β-(2→6) glycosidic bonds. Inulins are polysaccharides composed of β-D-fructose monosaccharides linked by β-(2→1) glycosidic bonds (Patel *et al*., 2012).

Heteropolysaccharides

Heteropolysaccharides (HePSs) are a structurally more complex group of polysaccharides composed of different monosaccharide repeating units. These may be branched at positions C2, C3, C4 or C6, or unbranched (Zannini *et al*., 2016). The regular repeating units consist of three to eight monosaccharides (D-glucose, D-galactose, L-rhamnose and others), derivatives of monosaccharides (N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and glucuronic acid) and non-carbohydrate substituents (Torino *et al*., 2015; Mende *et al*., 2016). The monosaccharides can be present as the α- or β-anomer in the pyranose or furanose form (Werning *et al*., 2012), and unlike what is found in HoPSs, carbohydrate moieties are synthesised from intracellular sugar nucleotide precursors (Bajpai *et al*., 2016). In general terms, the molecular weight of HePSs ranges from 10^4 to 10^6 Da (Angelin & Kavitha, 2020) and they are usually produced in the range between 25 and 600 mg L^{-1} (Torino *et al*., 2015; Leroy & De Vuyst, 2016).

Biosynthesis pathway of HePSs
Biosynthesis of HePSs in LAB occurs through the Wzx/Wzy-dependent pathway (Schmid *et al*., 2015; Zhou *et al*., 2019) in an energy-demanding process. This pathway takes place in five phases, beginning inside the cell and ending outside (Fig. 4): (i) sugar is transported into the cytoplasm until Glucose-1-P is obtained; (ii) sugar nucleotides are synthesised; (iii) repeating units are synthesised through sequential addition of activated sugar residues by specific glycosyltransferases (GTF) which catalyse the glycosidic bond with attachment to the glycosyl carrier lipid; (iv) repeating units are translocated to the extracellular surface by the Wzx-protein, having flippase activity; and (v) extracellular polymerisation of HePSs through the action of the Wzy-protein, breaking the bond that joins the HePSs to the lipid carrier and releasing into the medium or anchorage on the bacterial wall (Silva *et al*., 2019; Zhou *et al*., 2019).

Types of HePSs
Kefiran (Fig. 5) is a widely studied HePSs produced by microorganisms present in kefir grains. It is a water-soluble branched glucogalactan composed of a branched hexa- or heptasaccharide repeating unit containing approximately equal amounts of D-glucose and D-galactose residues (Patel *et al*., 2012).

Applications of EPSs in foodstuffs

Microbial EPSs have functional effects in food processing due to their interaction with food components, which can be translated to the improvement of rheological and sensory food properties. EPSs can act as texturisers, stabilisers, viscosifiers, bio-thickeners or emulsifiers, depending on the temperature, pH and ionic strength of the medium (Angelin & Kavitha, 2020; Korcz & Varga, 2021). Depending on the

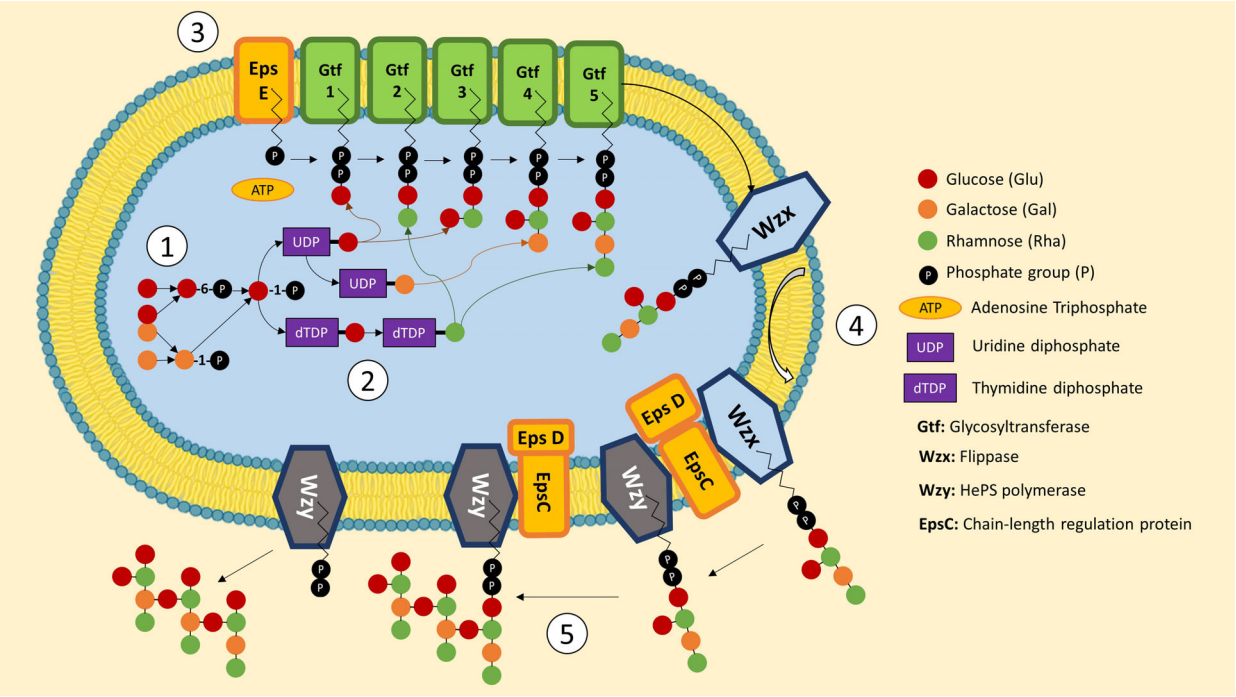

Figure 4 Generic model for heteropolysaccharides biosynthesis via the Wzy-dependent pathway. The main phases are represented: (1) the sugar transport into the cytoplasm, (2) the synthesis of sugar nucleotides, (3) the formation of repeating unit and attached to the glycosyl carrier lipid, (4) the translocation of repeating units to extracellular surface and (5) the extracellularly heteropolysaccharides polymerisation, the rupture of the heteropolysaccharides bond of the lipid carrier and release to the medium.

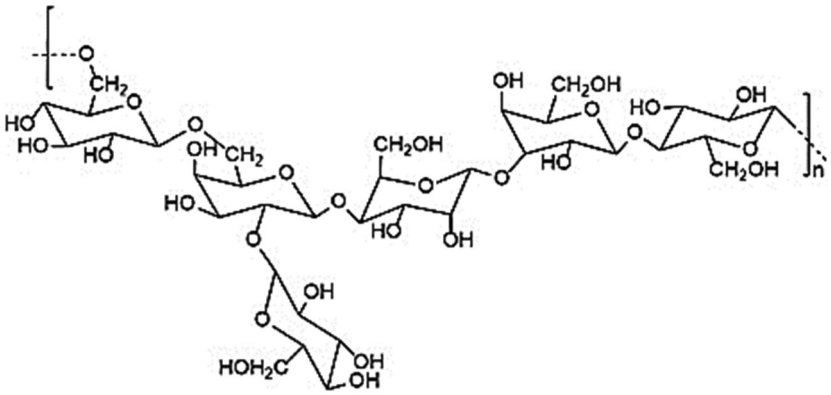

Figure 5 Schematic representation of the repeating unit of kefiran.

monomer composition, type of bonds, number and length of the side chains and molecular weight, EPSs can modify the viscoelastic properties of foods in different ways (Korcz & Varga, 2021).

Exopolysaccharides from LAB have received special attention because of their potential industrial applications, especially those formed *in situ*. They can be used to replace or reduce the use of external hydrocolloids, because they bind water and retain moisture within the product (Lynch *et al*., 2018a).

There are two possible phenotypes of EPSs-producing strains: ropy or non-ropy. These are defined as a function of whether EPSs are released into the medium as free EPSs (fEPSs) or remain attached to the bacterial cell wall as capsular EPSs (cEPSs), respectively. Some findings indicate that both, fEPSs

and cEPSs, can contribute to enhanced viscosity, creaminess, shear resistance or water-holding capacity. However, the apparent viscosity of ropy cultures is greater than that of non-ropy (Mende *et al*., 2016; Zeidan *et al*., 2017). Cirrincione *et al*. (2018) related that ropy strains confer a smoother consistency and higher viscosity than non-ropy strains in fermented dairy products. Moreover, the usefulness of EPSs as viscosity modifiers improve product taste perception by allowing it to persist longer in the mouth (Nampoothiri *et al*., 2017).

Sugar composition, chain length, sugar linkages, frequency of branching and molecular mass affect the technological properties of EPSs (Abid *et al*., 2018; Silva *et al*., 2019). The relationship between the structure and the function of EPSs has not been yet fully understood, due to their complexity and the difficulty of their structural analysis (Riaz Rajoka *et al*., 2020). The contribution of EPSs to the properties of food products depends on the properties of the EPSs and the interactions of EPSs with the food components, especially proteins (Kleerebezem *et al*., 1999). Furthermore, several studies indicate that EPSs influence the rheology and texture of fermented products at extremely low concentrations relative to other thickeners (Zarour *et al*., 2017).

A further aspect to be taken into account is the method of production and use of EPSs. At present, microbial polysaccharides represent only a small fraction of the current biopolymer market, because knowledge of their biosynthesis is still lacking, as is also an appropriate bioprocessing technology (De Vuyst & Degeest, 1999). However, under controlled growing conditions, it is possible to increase the production of EPSs (Cirrincione *et al*., 2018). In fact, it has been reported that *Lactiplantibacillus plantarum* strains can produced between 450 and 515 mg L^{-1} under optimal conditions (Aarti & Khusro, 2019; Mıdık *et al*., 2020).

Applications of EPSs in bakery products

The positive effect of EPSs on bakery products is based on their ability to bind water and form a network with different dough components. Thus, EPSs may improve the rheology, structure and volume of bread, resulting in decreased staling rates and an extended shelf life (Korcz & Varga, 2021).

Incorporation of sourdough when baking bread has repercussions on its texture and sensory properties (Casado *et al*., 2017). Sourdough ecosystems are characterised by stable associations of yeasts and LAB, particularly *Lactobacilli* (De Vuyst *et al*., 2014). One of the main metabolic activities of LAB species from sourdough is the production of EPSs that can affect the viscoelastic properties, texture and technological properties of sourdough as well as the texture and shelf life of bread (İspirli *et al*., 2020; Riaz Rajoka *et al*., 2020). Technologically, the beneficial effects of EPSs on dough and bread include the following: (i) greater water absorption by the dough, (ii) better dough rheology and machinability, (iii) maintenance of bread structure, (iv) larger loaf volume and (v) increased crumb softness and delayed bread staling, leading to a longer shelf life (Tieking & Gänzle, 2005).

Future perspectives focus on the use of EPSs from LAB for the production of gluten-free bread, since EPSs can potentially act as hydrocolloids improving their rheological properties (Torino *et al*., 2015). Gluten is the main structure-forming protein in flour and as a consequence of its major role in determining the viscoelastic properties of dough it makes a definite contribution to the appearance, texture, crumb structure and mouth-feel of the final baked product.

Gluten-free products are characterised by low water absorption, changes in crumb characteristics, decreased bread volume and poor stability. These defects can be eliminated by adding LAB EPSs to gluten-free sourdough (Lynch *et al*., 2018a). The beneficial effect of EPSs on bread is based on their ability to form a network with other components of the dough and improve shelf life due to the water-binding property (Xu *et al*., 2019).

The *in situ* production of EPSs might replace gluten, making it possible to produce gluten-free bread with acceptable sensory properties (Lynch *et al*., 2018a). Recent research has shown the possibility of improving the quality of gluten-free bread through EPSs-producing *Limosilactobacillus reuteri* strains and consequently, some EPSs may reduce the need for using expensive hydrocolloidal polysaccharides in the baking industry (Daba *et al*., 2021).

Applications of EPSs in dairy products

The use of strains of LAB that produce EPSs is no novelty in the manufacture of fermented milk. Indeed, they have traditionally been used in Scandinavian fermented milk products to improve texture and rheological properties (Lynch *et al*., 2018a). EPSs from LAB influence viscosity and the ability to bind water, thus, prevent syneresis and improve firmness and other sensory properties, enhancing mouth-feel (Lynch *et al*., 2018a; Daba *et al*., 2021). Moreover, EPSs interact with other milk constituents, such as proteins and micelles improving casein network firmness (Daba *et al*., 2021; Korcz & Varga, 2021). Thus, research focused on identifying EPSs-producing LAB and Bifidobacteria strains is an aspect of great interest (Xu *et al*., 2019; Ayyash *et al*., 2020; Nachtigall *et al*., 2020).

Furthermore, in the dairy industry there is a demand for low-fat or fat-free fermented milk products (Leroy

& De Vuyst, 2016). In dairy products, as in other foods, fat has a key role in determining physical and technological properties. Removing the fat from dairy products, whether totally or partially, results in a loss of flavour or a down-grading of texture and, consequently, reduces their acceptability (Behare *et al.*, 2009). However, the creaminess and firmness of fat-low dairy products can be enhanced by incorporating EPSs, or by adding strains able to produce them (Nampoothiri *et al.*, 2017).

The successful of the application of EPSs in terms of adjusting the rheological properties of milk products will depend on several factors affecting EPSs. These include their location (capsular or free), structure (molecular mass, charge, degree of branching, and stiffness), concentration and their interactions with other substances (Mende *et al.*, 2016). In fermented dairy products, the interaction of EPSs with proteins plays a key role. Fermented dairy products are complex systems where EPSs are progressively produced while the pH value decreases, causing changes in the casein micelles (Gentès *et al.*, 2013). The drop in the pH of milk down to values of around 4.6 to 4.3 leads the casein to change from a negative charge to neutral or positive by the end of fermentation (Girard & Schaffer-Lequart, 2008). Under these conditions, neutrally and negatively charged EPSs can interact with the protein network and interfere with protein coagulation. This interaction forms a continuous branched protein network which improves viscoelastic properties and leads to a greater water retention capacity, resulting in less syneresis and a high viscosity (Zeidan *et al.*, 2017). However, the role that EPSs play on the rigidity and elastic modulus of yoghurt and other fermented milk has not been well stablished yet, due to the complexity of milk matrix (Xu *et al.*, 2019; Nachtigall *et al.*, 2020). On the other hand, Angelin & Kavitha (2020) reported that EPSs produced by *Leuconostoc* and *Pediococcus* strains showed high thermal stability with the melting points higher than 224 °C, being this key feature in the dairy industry (Angelin & Kavitha, 2020).

In terms of their applications in foods, the type and structure of EPSs are important characteristics that determine the techno-functional properties and their potential for application in the food industry. EPSs with higher molecular weight, higher degree of branching and stiffer chains provide viscosity, improve water-holding capacity and may find application as thickening agents (Xu *et al.*, 2019). In contrast, EPSs with low molecular weight tend to have better solubility and bioactivity. When EPSs are linear, rigid and negatively charged, they can establish electrostatic interactions with positive charges on caseins at pH 4.6, leading to complexes and positively influencing the structure and rigidity of the gel (elastic modulus) and viscosity (Gentès *et al.*, 2013). The anionic EPSs contribute to increase not only the viscosity but also the elastic modulus (G′) and the hardness of the gel (Doublier *et al.*, 2000). Rigid and neutral EPSs also contribute to viscosity due to their ability to bind water, increasing volume. However, they do not contribute to gel hardness because they do not establish bonds with proteins due to thermodynamic incompatibility (Gentès *et al.*, 2013). Negatively charged, low molecular weight EPSs can enhance the immune effect while high molecular weight neutral EPSs can have anti-inflammatory effect (Wang *et al.*, 2015).

Applications of EPSs in yoghurt

Exopolysaccharides produced by LAB play an important role in the rheology and texture of yoghurts (Zannini *et al.*, 2016). Thus, it is possible to improve the gel sensory characteristics, even at low protein contents, without compromising other properties of this fermented dairy product (Priyashantha *et al.*, 2019; Madhubasani *et al.*, 2020).

The production of EPSs from LAB in general shows generally wide range of variations. Jolly *et al.* (2002) studied the production of EPSs by strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in yoghurt. They found that *Streptococcus thermophilus* yielded from 30 to 890 mg L^{-1}, while *Lactobacillus delbrueckii* subsp. *bulgaricus* from 60 to 150 mg L^{-1}. Although EPSs are produced in a very small amounts, they are able to enhance the yogurt smoothness and creaminess (Bajpai *et al.*, 2016). These authors observed that EPSs contributed to improvements in the viscosity and texture of yogurt without altering its flavour.

In the yoghurt industry, the most important textural characteristics are firmness and the ability to retain water, with syneresis, or the exudation of liquid, being one of the greatest problems to be overcome. The industrial solution most widely used to avoid syneresis in the form of the accumulation of whey on the surface is the addition of skim milk powder or concentrated milk. However, starter cultures producing EPSs may offer a solution for circumventing low viscosity, gel fracture or high syneresis (Patel *et al.*, 2012). In fact, set-type probiotic goat milk yogurts produced using an EPSs-producing starter culture exhibited high apparent viscosity, low syneresis and improved sensory properties (Madhubasani *et al.*, 2020).

Apart from syneresis, the presence of large particles produced by vibrations during fermentation is another defect in yogurt. Körzendörfer *et al.* (2017) studied the impact of sonication during fermentation with starter cultures differing in EPSs synthesis upon the physical properties of set (syneresis, firmness) and stirred yogurt (large particles, laser diffraction, rheology). They concluded that sonicated set-style yogurts

exhibited considerable syneresis that could be counteracted when a culture with EPSs production was used. For stirred yoghurts, sonication significantly increased particle numbers, but it proved feasible to reduce this effect, too, when a starter producing EPSs was used. Other authors reported that EPSs produced by *Lacticaseibacillus paracasei* during milk fermentation could inhibit casein aggregation and improve their stability due to electrostatic and hydrophobic interactions between EPSs and proteins (Li *et al*., 2020).

Application of EPSs in cheese

In cheese-making, the use of cultures producing EPSs can improve rheological and textural characteristics like softness, creaminess and humidity, especially in reduced-fat cheeses (Lynch *et al*., 2014). The application of strains producing EPSs has shown that such strains achieve greater water retention, larger cheese yields and greater viscosity of whey (Dabour *et al*., 2006). For example, several strains of *Lactobacillus helveticus* and *Streptococcus thermophilus* producing EPSs are used to make Mozzarella, contributing to water retention (Duboc & Mollet, 2001).

Fat reduction in cheese is associated with many textural and functional defects, since the removal of fat results in a denser protein network, which affects cheese texture (Mozzi *et al*., 2006). Nevertheless, cheese made with starters producing EPSs has higher moisture levels and a microstructure similar to full-fat cheeses, because binding and water retention are increased (Lynch *et al*., 2018b). Moreover, the excellent water-binding capacity of EPSs could contribute to increase the shelf life of cheeses (Mazhar *et al*., 2020).

A study involving three strains of *Streptococcus thermophilus* (an EPSs-producing moderate ropy strain, a non-EPSs-producing strain and a non-ropy strain), together with a ropy *Lactococcus lactic* subsp. *cremoris* strain, used as starters for Cheddar cheese, led to the conclusion that ropy cultures principally improved the textural, melting and sensory characteristics of reduced-fat Cheddar cheese (Awad *et al*., 2005). In another study, ropy EPSs produced by *Lactococcus lactis* gave rise to lactic gels with higher hardness than those produced with non-ropy EPSs strains of the same species. The fresh lactic cheeses showed less syneresis, higher yield and better texture. These are aspects of great importance for the elaboration of fresh lactic cheeses with low-fat content (Angelin & Kavitha, 2020).

Studies have also been carried out to verify the combined effect of EPSs and hydrocolloids. Low-fat Burrata cheese was made by combining semi-skimmed milk inoculated with EPSs from streptococci and a suspension of xanthan gum. The Burrata cheese obtained was very similar to traditional full-fat cheese (Costantino *et al*., 2020).

To sum up, EPSs offer features enhancing the rheological and textural characteristics of cheese. These improvements are especially relevant for reduced-fat cheeses. The water-binding property of EPSs increases the moisture in the non-fat portion of these cheeses, interferes with protein–protein interactions, reduces the rigidity of the protein network and increases the viscosity of the serum phase (Torino *et al*., 2015).

Applications of EPSs in meat fermented products

Exopolysaccharides produced by LAB on meat products have been generally considered spoilage factors and their potential applications in processed meat remain unexplored (Prechtl *et al*., 2018).

In relation to these aspects, Dertli *et al*. (2016) found EPSs could enhance the textural properties of sausages, providing a harder, less adhesive and tougher product. Loeffler *et al*. (2020) have recently reviewed the use of *in situ* EPSs-forming LAB in meat products, describing their positive role in different meat matrix, such as low-fat fermented sausages. Korcz & Varga (2021) reported that the *in situ* formed EPSs influenced the quality of fermented meat products, which undoubtedly expands the possibilities of their application, particularly in fat-reduced meat products, since their consumption is currently growing. Consequently, several studies have shown that the production of EPSs by LAB can find application to improve the texture of meat products, particularly those that are low in fat. However, to guarantee their quality, the concentration of EPSs in the meat product must be within a well-defined range (Loeffler *et al*., 2020).

Future prospects

The addition of EPSs as a bioingredient implies the *ex situ* production, the isolation and purification of EPSs, and then their use as ingredient. The advantage of this process is that EPSs can be incorporated into foods in higher quantity than EPSs produced *in situ* (Lynch *et al*., 2018b).

In the progress of the knowledge about EPSs, the development of methodologies to identify and analyse EPSs produced by LAB acquires particular importance. Qualitative methods for the detection of EPSs include electron microscopy (EM) and confocal laser scanning microscopy (CLSM) (Loeffler *et al*., 2020). In order to quantify and further analyse the composition and structure of EPSs, colorimetric methods, such as the phenol sulfuric acid method, high-performance liquid chromatography (HPLC), gas chromatography (GC), size-exclusion (SEC) or ion-exclusion chromatography (IEC) and Fourier transform infrared (FTIR) spectroscopy. On the other hand, several

recent studies have used nuclear magnetic resonance (NMR) spectroscopy to analyse the structure of pure EPSs from different bacteria (Zhang *et al*., 2019; Loeffler *et al*., 2020). This technique is an interesting tool to understand the structure of these substances.

Among the limitations, many obstacles that must be overcome before the widespread industrial use of EPSs-synthesising LAB become a reality. For example, uniformly high food quality can only be guaranteed if EPSs production by LAB is maintained within a certain, well-defined concentration range (Loeffler *et al*., 2020).

The ability of LAB strains to produce EPSs varies greatly in terms of quantity and quality, and this constitutes a limitation in the commercial use of EPS-producing lactic cultures. However, selection of LAB strains with adequate EPSs production ability and optimisation of their production could solve this problem (Korcz & Varga, 2021). Currently, EPSs production improvement studies often focus on optimising culture media, using genetic engineering, using cheap fermentation substrates and environmental stress (Nguyen *et al*., 2020).

The first step towards EPSs production engineering technology has shown that the combination of gene expression from different sources can lead to functional production systems and that the production of EPSs having modified repeating subunits might be realised. The polymerisation and export processes are still poorly understood, although they are expected to be like most EPSs synthesis. Therefore, a better understanding of these processes can optimise the production of EPSs (Nguyen *et al*., 2020).

In addition to the technological applications referred in this review work, numerous physiological functions attributed to EPSs have been described, among which are their antimicrobial, immunomodulatory, anti-inflammatory, antioxidant, anti-tumour, antiviral, anti-diabetic, cholesterol-lowering activities, among others (Rahbar Saadat *et al*., 2019; Angelin & Kavitha, 2020; Riaz Rajoka *et al*., 2020).

The influence of EPSs from LAB on the immune system has been studied, and it was shown that EPSs acts as immunomodulators (Vinderola *et al*., 2006). Some EPSs are able to induce the activation of immune cells, including activation dendritic cells (DCs), macrophages and splenocytes; and the production of specific cytokines as well as to exercise an immunostimulatory effect on macrophages and lymphocytes, among other functions (Vinderola *et al*., 2006; Laiño *et al*., 2016; Nampoothiri *et al*., 2017). The exact mechanism of cholesterol lowering by EPSs is not fully understood but is believed that EPSs act in a manner similar to dietary fibres, increasing excretion of bile acids and reducing absorption of cholesterol (Nampoothiri *et al*., 2017). EPSs-producing probiotics allow in the intestine the cholesterol assimilation and conversion and induce co-precipitation and short fatty acids promotion to decrease cholesterol (Lynch *et al*., 2018a; Zhou *et al*., 2019). EPSs also play a role as antioxidants because they are able to neutralise reactive oxygen species (ROS) (Nampoothiri *et al*., 2017). The antioxidant activity of the EPSs may be due to the presence of hydroxyl group and other functional groups in EPSs, which can donate electrons to reduce the radicals to a more stable form, or to react with the free radicals to terminate the radical chain reaction (Silva *et al*., 2019).

Concluding remarks

The study of EPSs is relatively recent, and there are still certain aspects requiring more extensive investigation. As has been recorded in this overview, EPSs produced by LAB can find diverse industrial applications, especially in dairy products, such as low-fat cheeses and fermented milk, where they improve texture and rheological properties. Moreover, the use of sourdough containing EPSs-producing LAB could improve the texture of gluten-free bread. The *in situ* production of EPSs may be able to limit the need to use additives, while improving consumer product perception and reducing financial costs.

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Author contribution

Daniel Abarquero: Conceptualization (equal); Resources (equal); Software (equal); Writing-original draft (equal). **Erica Renes:** Supervision (equal). **Jose Maria Fresno:** Supervision (equal); Validation (equal). **María Eugenia Tornadijo:** Supervision (equal); Writing-review & editing (equal).

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Data availability statement

Data sharing not applicable – no new data generated, or the article describes entirely theoretical research.

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