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Review Article

Applications of Microbial Exopolysaccharides in the Food Industry

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Abstract

Exopolysaccharides (EPSs) are high molecular weight polysaccharides secreted by microorganisms in the surrounding environment. In addition to the favorable benefits of these compounds for microorganisms, including microbial cell protection, they are used in various food, pharmaceutical, and cosmetic industries. Investigating the functional and health-promoting characteristics of microbial EPS, identifying the isolation method of these valuable compounds, and their applications in the food industry are the objectives of this study. EPS are used in food industries as thickeners, gelling agents, viscosifiers, and film formers. The antioxidative, anticancer, prebiotic, and cholesterol-lowering effects of some of these compounds make it possible to use them in functional food production.
Keywords: Microbial exopolysaccharide, Functional food, Prebiotic, Health.

Background

Using natural compounds for producing and preserving food has attracted great attention. The exopolysaccharides (EPS) are high molecular weight polysaccharides secreted by plants, seaweeds, and microorganisms to the surrounding environment. EPS generally consist of monosaccharides and other compounds such as acetate, phosphate, pyruvate, and succinate (1). Based on the type of monosaccharide, EPS are divided into two groups of homopolysaccharides and heteropolysaccharides. Homopolysaccharides are composed of one type of monosaccharide, while heteropolysaccharides are made up of two or more types of monosaccharides (2). Glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine, and rhamnose are prominent components of these heteropolymers (2).

Various groups of microorganisms such as bacteria (3,4), cyanobacteria (5), fungi (6), and microalgae (7) can produce EPS. The genes accountable for production are often clustered in the genome of the relevant organisms (8). Biosynthesis of microbial EPS occurs during the growth period and is regulated by various enzymes and proteins. Production of EPS is vital to microorganisms as they play critical biological roles in cell protection, attachment to solid surfaces, cell aggregation, and cell to cell interactions (3,9). Table 1 summarizes the general characteristics of the principal EPS.

EPS can form thick pseudoplastic liquids, and they have been consistently applied in food (emulsifier, stabilizer, viscosifier, and moisture retention), cosmetic (anti-aging activity and reduction of allergic reaction), pharmaceutical (blood flow improving and drug delivery system), and textile (better water holding capacity and flame retardancy) industries (1,10-13). In addition to the technological advantages, some EPS promote human health by different mechanisms such as detoxification of heavy metals, decrease of blood cholesterol levels, provision of a fermentable substrate for intestinal microflora (prebiotic), and modulation of the immune response (4,14). The present review provides the readers with an overview of the characterization and commercial production of some microbial EPSs used in the food industry and their health benefits. Figure 1 highlights the key parts of this review.

Prominent Microbial EPS and their Properties in the Food Industry

The microbial EPS are subdivided into homopolysaccharides and heteropolysaccharides.

Homopolysaccharides

Homopolysaccharides are divided into two general classes of glucan and fructan.

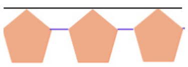
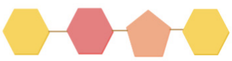
Glucans

Glucans, as described below, are high molecular weight polymers comprised of glucose units linked by different glycosidic bonds.

a. Dextran

Dextran is a high-molecular-weight compound produced from sucrose by the dextransucrase enzyme of bacteria (35,36). Dextran is generally regarded as a safe (GRAS)

Table 1. The Main Characteristics and Structures of Microbial Exopolysaccharides

Microbial EPS	Name	Chemical Structure	Structure	Solubility i Water	Molecular Weight (D)	Producer Organism	References
	Dextran	α (1→6) Glc	Branched	Variable	10^3 - 10^7	*Leu.*, *Strep.*, and *Acetobacter*	15
	Pullulan	α (1,4) Glc α (1,6)	Linear	Soluble	362× 10^3- 480 ×10^3	*Aureobasidium* spp., *Tremella mesenterica*, *Cytaria* spp., *Teloschistes flavicans*, *Rhodototula bacarum* and *Cryphonectria parasitica*	16
 Glucan	Curdlan	β (1,3) Glc	Linear	Insoluble	2× 10^6	*Alcaligenes faecalis* var. *myxogenes*, some rhizobium strains, and *Cellulomonas* spp	17,18
	Alternan	α (1,6) Glc α (1,3)	Branched	Highly soluble	10^6–10^7	*Leu. citreum* and *Leu. mesenteroides*	19
	Reuteran	α (1,4) Glc α (1,6)	Branched	Soluble		*Lb. reuteri*	20
1. Homopolysaccharides	Scleroglucan	β (1,3) Glc β (1,6)	Branched	Soluble	6 × 10^6	*Sclerotium rolfsii*	21
	Cellulose	β (1,4) Glc	Linear	Insoluble	3 × 10^5 - 2 × 10^6	*Komagataeibacter*, *Agrobacterium*, *Rhizobium*, *Salmonella* and *Sarcina*	1, 15, 22
 Fructan	Levan	β (2,6), β (2, 1) Fru	Branched	Soluble	10^4 - 10^8	*Bacillus* sp., *Strep.* spp., *Zymonas mobilis*, *Arthrobacter ureafaciens*, *Halomonas* sp., *P. fluorescens*, *Serratia Levanicum*, *Microbacterium laevaniformans*, *Lb.* spp., *B. stearothermophilus*	15, 25- 28
	Inulin	β (2,1) Fru	Linear	Soluble in hot water	5 × 10^2 - 1.3 × 10^4	*Lb. johnsonii*, *Strep. mutans* strain JC2, *Leu. citreum* CW28 and *Lb. reuteri* 121	15, 29
	Kefiran	(1 ,6)- Glc, (1,3) Gal, (1,4)- Gal, (1,4) Glc, (1,2, 6)- Gal,	Branched	Soluble	534× 10^3	*Lb. kefiranofaciens*, *Lb. kefirgranum*, *Lb. parakefir*, *Lb. kefir* and *Lb. delbrueckii* subsp. *Bulgaricus*	30,31
	Xanthan	(1,4) β- Glc, β- Man-(1,4)-β-Glc-(1,2)-α-Man	Branched	Soluble	3 × 10^6	***Xanthomonas campestris***	20, 32
2. Heteropolysaccharides 	Gellan	1,3-β-D-Glc; 1,4-β-D-Gul; 1,4-β-D-glc; & 1,4-α-L-Rha.	Linear	Insoluble in cold water	5 × 10^5	*Sphingomonas elodea*	33
	Alginate	*β* (1,4)-D-manu.; 1,4 *α*-L-Gul	Linear	Soluble	33× 10^3 - 400× 10^3	***P. aeruginosa*, *Az. vinelandii***	32
	Viilian	Glc β (1,4) Gal β (1,4) Glc; α-L-Rha (1,2) Gal & Gal α- (1,3) phos	Linear			*Lac. lactis* subsp. *cremoris*	34

Glucose: Glc; Fru: Fructose; Gal: Galactose; Man: Manose; Manu: Mannuronic acid; Gul: Guluronic acid; Rha: Rhamnose; ***Leu: Leuconostoc***; Lb: *Lactobacillus*; Lac: *Lactococcus*; ***P: Pseudomonas***; Az: *Azotobacter*; Alc: *Alcaligenes*; ***Sin: Sinorhizobium***. Strep: Streptococcus.

Figure 1. Biosynthesis of Microbial Exopolysaccharides and Their Main Uses in the Food Industry.

compound for animal feeds, medicines, and human foods by Food and Drug Administration (FDA) (37). The European Commission allows using *Leuconostoc mesenteroides* dextran in the bakery to improve the softness, crumb texture, and loaf volume (38). Oil recovery enhancement (39), biodegradable coatings or films (40), and biosensors for the analysis of different biointeractions (41) are some other uses of dextran. Dextran reveals high water solubility and produces low viscosity solutions, so it can be added to foods at high concentrations without excessive viscosity. Adding dextran can raise the glass transition temperature of ice cream mixes and stabilize the final product. It prevents sugar crystallization, increases moisture retention, retards oxidation, and maintains the flavor and appearance of various foodstuffs (42,43). It also has some medical benefits, such as blood coagulation, treatment of hypovolemia, and management of iron deficiency anemia (44).

b. Pullulan

Pullulan is a neutral, non-toxic, non-mutagenic, and non-carcinogenic water-soluble polysaccharide consisting of maltotriose repeating units (45). It is considered a GRAS powder, which can be used as a replacement for starch in pasta or baked products (46,47).

Pullulan is a candidate for packaging film in the food industry due to its high solubility in cold and hot water, mechanical strength, and resistance to pH changes. Pullulan films are colorless, tasteless, biodegradable, oxygen impermeable, high adhesive (48,49), flexible (50), highly impermeable to oxygen and oil (51,52), and heat-sealable (52). Its physical characteristics are dependent on the composition, for instance, adding xanthan and locust bean gums reduces the mechanical properties of the pullulan film (53). However, Gounga et al proposed a whey protein isolate pullulan as a coating to keep the fresh chestnut fruits from moisture loss and color changes (54). Pullulan-based edible films can also serve as a carrier for flavors and antimicrobial substances. The pullulan films incorporated with meadowsweet flower extract (52) and sweet basil extract (55) can retard the growth of *Rhizopus arrhizus* on the apples without changing the color during storage. The number of *Staphylococcus aureus*, *Aspergillus niger*, and *Saccharomyces cerevisiae* in baby carrot was reduced at least by 3 log CFU/g using pullulan films containing caraway essential oil (CEO). The slow release of included antimicrobial agents from the film matrix increases the bacterial lag phase, decreases microbial growth rate in food, and improves its quality (56). Incorporation of pullulan film with sakacin A, essential oils (oregano and rosemary), or nanoparticles (zinc oxide or silver) was useful against pathogenic microorganisms such as *S. aureus*, *L. monocytogenes*, *E. coli* O157: H7, and *S. typhimurium* and improved the safety of refrigerated, fresh, or processed meat and poultry products (57,58).

Pullulan is resistant to mammalian amylases and is considered as a dietary fiber in human nutrition (51). It can be applied as an additive in low-calorie foods. It is predominantly metabolized by bifidobacteria (Ryan, Fitzgerald, and van Sinderen, 2006), and as a prebiotic, it increases the number of bifidobacteria and lactobacilli in feces (51,59). However, Chlebowska-Śmigiel et al did not detect any motivating effect of pullulan on *Bifidobacterium* and *Lactobacillus* growth although confirmed increasing the acidifying activity of these bacteria in the presence of pullulan which reduced the number of *E. coli* (60).

c. Curdlan

Curdlan is a neutral and an acidic linear glucan with a few intra- or inter-chain (1→6)-linkages (13). It is a colorless, odorless, tasteless, and indigestible (61) compound that is used in the medical (drug encapsulation, modulation of immune responses, etc) and food industries (62). Although it is insoluble in water, two types of gel can be produced after heating the aqueous suspension. Curdlan gel strength depends on the heating temperature, time of heat-treatment, and concentration of curdlan. Two types of gel including a low-set gel (thermo-reversible gel formed between 55-80ºC) and a high-set gel (thermo-irreversible gel formed above 80ºC) can be produced. The latter is much more stable during retorting, deep-frying, and cycles of freeze-thawing (13). It is approved as a stabilizer and texturizer in the food industry by the FDA (63). Wu et al suggested the use of thermoreversible curdlan gel as a gel binder and dietary fiber in fish meat gel-based products (64). It increases the chewiness, gumminess, adhesiveness, and viscosity of an emulsified meatball (65) and improves the quality of tofu, noodles, and surimi because of its exclusive resilience and strength through heating and after freezing-thawing. Dense cross-links between curdlan and the fish proteins during heating improve the textural and rheological properties of Alaska pollock surimi gel(66).

Curdlan can reduce fat absorption and moisture loss during deep-frying (67) because it forms a reversible thermal gel that can capture water and makes it a barrier against oil and moisture. There are no digestive enzymes for curdlan in the upper alimentary tract; it can be considered as a fat mimetic by itself or in combination with other hydrocolloids (68,69). Using curdlan in the non-fat sausage as a fat mimetic improves the texture and flavor of sausage, similar to the 20% fat sausage (69).

Curdlan has the potential to use as an edible and biodegradable film for food packaging. Konjac glucomannan/curdlan blend films (70) fish gelatine/curdlan blend films (71), and curdlan/chitosan membranes (72) have been found to show excellent waterproofing properties. The latter case also shows an antimicrobial effect.

d. Alternan

Altrenan is a long-chain homopolysaccharide produced by the alternansucrase enzyme from sucrose (14). Due to its high solubility, low viscosity, and high resistance to enzymatic hydrolysis, it is used as a low viscosity bulking agent in foods. It can also serve as a prebiotic to form symbiotic food (44).

e. Reuteran

Reuteran is a water-soluble α-glucan produced by reuteransucrase. It can improve the quality of gluten-free sourdough and sorghum bread, characterized by a softer crumb, extended shelf life, and prebiotic activity (16,74).

f. Scleroglucan

Scleroglucan is a water-soluble neutral homopolymer, which dissolves in both cold and hot water. Salt concentrations and extreme pH conditions (2.5–12) have no impact on solution viscosity. Its solution is thermostable (stable for 20 hours at 120°C) and shows pseudoplastic behavior with a high yield value. It is a good emulsifier and stabilizer (dressings and ice creams) and can improve the quality of frozen or heat-treated foods. However, it is not approved by food safety legislation in Europe and the USA (75).

g. Cellulose

Cellulose is a GRAS homopolysaccharide produced by a broad range of bacterial species, including *Komagataeibacter* (former *Gluconacetobacter*), *Agrobacterium*, *Rhizobium*, *Salmonella*, and *Sarcina*. *Komagataeibacter* is the most active strain in cellulose production with high yield and purity (1,18). The chemical composition of bacterial cellulose is indistinguishable from the plant one; however, it is free of hemicellulose, lignin, and pectin, which simplifies its extraction. Bacterial cellulose shows a higher water holding capacity and longer drying time (75), both of which make it a good candidate for use in food systems (1,76,77).

Bacterial cellulose as a thickener and gelling agent has several applications in increasing water binding capacity of surimi (78), improving the gel strength of tofu (79), replacement of fat in meatballs (80), emulsion and foam stabilization of ice cream (81) and immobilization of probiotic bacteria (82). As a dietary fiber, it can help to reduce food calories and improve body health.

Fructans

The fructans are made from sucrose by fructosyltransferase enzyme and can be separated into two groups of levan-type and inulin-type.

a. Levan

Levan is a non-toxic homofructan found in plants and some yeasts, fungi, and bacteria (83,84). Levan sucrase (also called sucrose 6-fructosyltransferase. EC 2.4.1.10) is responsible for levan biosynthesis (85).

Levan is water and oil-soluble polymer and insoluble in almost all organic solvents (86). It has low intrinsic viscosity and does not dissolve or swell in water at room temperature. It is resistant to amylase and invertase (43,87). It has some beneficial applications in medicine such as a plasma volume expander (88), anti-obesity agent (89), antitumor agent (90), and hyperglycaemic inhibitor (91). Levan can be used as a thickener, emulsifier, stabilizer, film-forming agent, encapsulating agent, and carrier for flavor in the food industry (92).

A study on animals showed that the intake of levan can stimulate the growth of lactic acid bacteria and increases their number in the feces (83). Levan heptose can also cause an increase in the fecal counts of *Bifidobacterium* sp. (93).

Levan can be used for film packaging; however, pure levan films are too brittle for practical use due to the lack of long flexible moieties in levan, which can be solved by the addition of plasticizers (84). Using more than 10 wt% glycerol plasticizer can reduce the fragility of the films (94). Levan-based films are good oxygen barriers (84). Usually, biopolymer nanocomposites have greater properties than the corresponding pure biopolymers. Due to the high molecular weight, and the highly branched and dense globular structure of levan, significant intermolecular entanglement is not possible. At the same time, using exfoliated montmorillonite clay blended with levan facilitates the hydrogen bonding between levan (hydroxyl groups) and montmorillonite, which leads to the formation of transparent, elastic, and strong film (95).

b. Inulin

Inulin-type EPS are fructooligosaccharides which have many applications in the food industry. It can increase the viscosity of water, which is dependent on the molecular weight and temperature (10). It can be used as a fat replacer in sausages (96,97) and non-fat functional dairy foods (98) and also a sugar replacer in chocolate (99). Generally, inulin gels are based on the interactions occurring between dissolved inulin chains. High molecular weight inulins are better gel formers than their lower molecular weight counterparts (10).

Inulin is a soluble fiber fermented by intestinal bacteria, resulting in the generation of large amounts of short-chain fatty acids; therefore, it can be used as a prebiotic in human and animal foodstuffs (100). Besides, it is effective in reducing food calories and blood triglycerides, lowering the risk of irritable bowel diseases, and preventing colon cancer (101,102).

Heteropolysaccharides

Heteropolysaccharides consist of various types of monosaccharides. The most widely used varieties in the food industry are listed below.

Kefiran

Kefiran is a water-soluble branched glucogalactan which consists of about equal amounts of D-galactose and D-glucose residues (103). It is excreted from kefir grains and is a potential food-grade thickener in fermented dairy products. It improves the rheological properties and viscosity of acidified milk and yogurt, which can be intensified by heat treatment (104). The viscosity of kefiran is lower than some polysaccharides such as locust bean or guar gum (105) and higher than some dextrans (106).

At low concentrations (less than 1 g/L), it shows the Newtonian behavior, while at higher concentrations, the pseudoplastic or shear-thinning flow is seen. Kefiran can form a translucent gel during cryogenic treatment (freezing, frozen storage, and thawing) (107) and transparent edible films. The plasticizers such as glycerol and sorbitol at low concentrations are needed to decrease the stiffness of this polysaccharide-based film (103,108). Kefiran film has a good water vapor barrier property. An excessive amount of glycerol (25 g/100 g) reduces the water vapor permeability, improves flexibility, and decreases the glass transition temperature of films. Kefiran films are soluble in water, which correlates with water temperature and glycerol addition (103,108). Using γ radiation (up to 9 kGy) can improve surface hydrophobicity, water sensitivity, and water vapor permeability of kefiran film; however, it changes the color of films (109). Probiotic organisms (*Lactobacillus plantarum* CIDCA 8327 and *Kluyveromyces marxianus* CIDCA 8154) can be incorporated into edible kefiran films, which can increase the resistance of organisms to acid (110). These features of plasticized kefiran films improve their potential uses, especially in the food industry.

Surveys show the role of kefiran in controlling blood pressure, lowering serum cholesterol and sugar levels, increasing fecal wet weight in constipated rats (111), promoting antimicrobial activity, and improving wound healing properties (112).

Xanthan

Xanthan is a high molecular weight, water-soluble, neutral, and non-toxic gum. This GRAS (38) heteropolysaccharide consists of repeating pentasaccharide units of D-glucose, D-mannose, and D-glucuronyl acid residues (molar ratio of 2:2:1) and variable proportions of O-acetyl and pyruvyl residues which can form a highly viscous solution in cold or hot water at low concentrations. It is resistant to enzymatic degradation and pH and temperature changes (113).

There are different opinions regarding the antioxidant properties of xanthan. Gawlik-Dziki revealed the strong antioxidative effect of xanthan gum (114). However, Sun et al stated that adding xanthan to whey protein isolate (WPI) stabilized oil-in-water emulsions prevented the antioxidant activity of WPI due to its interaction with xanthan, followed by the acceleration of lipid oxidation (115).

Xanthan is primarily used in the food industry due to its viscosifying and stabilizing properties. Its solution shows a shear-reversible pseudoplastic behavior. The high molecular weight xanthan shows high Newtonian viscosity at lower shear rates due to the formation of complex superstructures through hydrogen bonding. By increasing the shear rate, the network separates, and individual macromolecules are aligned in the shear direction; therefore, the viscosity decreases (116). Synergistic interactions between xanthan and plant galactomannans (such as locust bean and guar gum) at room temperature result in enhanced viscosity (117). Low concentrations of xanthan (up to 3 g/L) do not affect the yogurt viscosity, while as the concentration increases, the viscosity increases

(118). The viscosity of xanthan strongly depends on salt or sugar concentration in the solution (105).

Thickening and emulsion stabilizing effects of xanthan are due to the formation of a fragile gel-like structure in the continuous phase of the emulsion, which prevents the oil droplets from creaming. However, due to the weak gel structure, xanthan alone cannot stabilize the emulsion unless it is combined with the proteins. Exclusively, adding xanthan to oil/water emulsions stabilized with lupin and soy protein isolates enhances the emulsion stability, which is associated with an increase of protein at the interface, and builds a polysaccharide network in the water phase (119). It can also reduce the oil uptake in deep frying foods (120).

Gellan

Gellan gum is a high molecular weight anionic polysaccharide composed of a tetrasaccharide backbone consisting of 2 β-D-glucose, L-rhamnose, D-glucuronic acid, and acyl (glyceryl and acetyl) substituents (29). It is available in a substituted or unsubstituted form. The polymer is produced from two acyl substituents present in the 3-linked glucose; namely, L-glyceryl positioned at O(2) and acetyl at O(6) (121). It is resistant to heat and relatively to pH. As the gellan gum is relatively non-toxic, it is approved by the FDA for use in foods (122).

It acts as a stabilizer, binder, thickener, and perfect gelling agent in different types of foods (123). Gellan gum is insoluble in cold water but can disperse in milk and reconstituted milk. A gel is produced rapidly by heating and cooling gellan solutions in the presence of cations. The rheological characteristics of gel depend on the level of acyl substituent. The low acyl one requires acid (H^+) and ions such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and potassium (K^+) to produce the gel. Divalent cations are more efficient than monovalent ions (121). Gellan gum can be used as a gelling agent in desserts and jams to provide gelatin with mouth-feel characteristics and a more potent gel (at a lower concentration) compared to pectin.

Interaction between gellan (negative charge) and milk protein (positive charge) leads to protein precipitation. Therefore its use in the solutions/gels of milk proteins is not reasonable unless by neutralizing the negative charges (124). However, its interaction with casein and lactoglobulins increases the yield of cheese and reduces the loss of proteins in whey. Both types of gellan can be used in a stirred yogurt; however, using the low-acyl type gives a lumpy consistency to the yogurt, which must be thoroughly mixed to achieve a smooth texture. High-acyl gellan is the only form that can be used in set yogurts (121). Adding low-acyl gellan can increase the heat stability of fermented cream so that it keeps the structure after being added to hot foods (125). It can also be used as a bulking agent in the ice cream, texture, and flavor release in jellies and improve the efficiency of other hydrocolloids in confections (125). Combinations of low acyl gellan and carrageenan can be used to produce gelatin-free confectionery which is suitable for halal (121).

Gellan film has excellent oil barrier properties, and conversely, it is a poor moisture barrier, which can be improved by adding lipids (126). Coating foods with gellan can reduce fat absorption during deep-frying, resulting in a reduction of fat in the final product (120).

Konjac glucomannan–gellan gum blend films are suitable for the release of active agents such as nisin. They were found to have antimicrobial activity against *Staphylococcus aureus,* which can be enhanced by increasing the content of gellan gum (127). A composite film composed of the gellan and cassava starch shows relatively good mechanical and barrier properties (128). Gellan film can act as a carrier of vitamin C (129) and as a matrix for encapsulation of heat-sensitive and probiotic bacteria (130) and essential fatty acids in the food (131).

Alginate

The alginates are linear anionic biocompatible polysaccharides produced from seaweed and bacteria (132). Intake of alginates as dietary fiber can decrease the intestinal absorption and destructive potential of gastrointestinal luminal contents, increase satiety, modulate the colonic microflora, and promote the colonic barrier function (133). It is used as a viscosity regulator, stabilizer, and packaging material in the food industry, and has applications in wound healing, drug delivery, and cell microencapsulation in medical sciences (32,133-136). It is well known that the M/G ratio, the degree of acetylation, and the molecular weight determine their rheological properties (137). As the gelling properties are linked to the G subunits interacting with divalent ions, such as calcium, increasing the G-blocks leads to the formation of stronger gels with higher viscosity in the presence of Ca^{2+} (87).

Viilian

The viilian is the linear heteropolysaccharide isolated from a ropy fermented milk product "viili" and is composed of glucose, galactose, rhamnose, and phosphate with a molar ratio of 2:2:1:1, respectively (31). Viilian decreases the syneresis of fermented milk products. It can be used as a thickener in food systems and is also correlated to the lowering of serum cholesterol levels in rats (138).

Acetan

The acetan (or xylinan) is an anionic heteropolysaccharide produced by *Acetobacter xylinum*. It is a good viscosifier and gelling agent in sweet confectionery products (139).

The main applications of various EPSs in the food system are summarized in Table 2.

Isolation and Purification of EPS

Due to the favorable effects of EPS mentioned above, in recent years, interest in the isolation of these compounds and their use in different industries has increased. The isolation method should not affect the chemical and physical properties of the polysaccharides (180). Microbial

Table 2. The Applications of EPS in the Food Industry

EPS	Food industry	Applications	References
Dextran	Bakery	Improves the softness, crumb texture, and loaf volume	38
	Dairies	Ice cream: cryoprotectant and stabilizer Cheese: improves water binding Butter: fat replacer (polydextrose)	140-142
	Confectionery	Prevents sugar crystallization, gelling agents in jelly candies	43, 143
	Frozen and Dried	Retard oxidation and chemical changes	141
	Functional foods	Prebiotic: stimulates the growth of probiotics *Bifidobacterium lactis*, *B. infantis*, and *Lactobacillus acidophilus*	144
	Oil	Oil recovery enhancement, emulsion stabilizer	39, 145
	Food packaging	Dextran-coated silver nanoparticles: reduces oxygen transfer and inhibition of *Escherichia coli*	146
Pullulan	Food packaging	Reduces respiration rates of vegetables, extends the shelf life of fresh foods, antimicrobial films	49, 57
	Functional foods	Prebiotic: enhances the variability of *Bifidobacterium* and *Lactobacillus* in yogurt	147
	Dairies	Yogurt: thickener, increases viscosity, fat replacer	147, 148
	Confectionery	Starch replacer, reduces retrogradation	149
Curdlan	Meat products	Fat mimetic, increase water holding capacity, increase adhesiveness and viscosity of meatballs	70, 66
	Confectionery	Reduces oil uptake, gelling agents	68, 150
	Dairies	Improves texture of tofu, yogurt, Cream: fat mimetic	151
	Functional foods	Prebiotic	151
Alternan	Functional foods	Prebiotic	152
	Artificially sweetened foods	Bulking agents	153
Reuteran	Bakery	Improve the bread quality (from gluten-free sorghum flours) Dietary fiber: enhances the nutritional properties of bread	154
Scleroglucan	Dairies, Confectionery, Frozen food	Thickener, gelling or stabilizing agent	75
Cellulose	Meat products	Keeping water binding capacity, thickener, stabilizer, fat replacer	155, 156
	Dairies	Yogurt: stabilizer, decrease syneresis, increase viscosity Ice cream: fat substitute, stabilizer, reduces the melting rate, increase fiber content	157-159
	Food packaging	Tough, biodegradable, and acceptable levels of water vapor permeability	160
	Confectionery	Biscuits: fat replacer, increases the hardness	161
Levan	Functional foods	Prebiotics: increases *Bifidobacterium* spp. count, assist in the absorption of calcium and magnesium in the gut	94
	Beverages	Stabilizer, emulsifier, flavour enhancer	162
Inulin	Meat products	Sausage and burgers: fat substitute, higher fiber content	163
	Dairies	Yogurt: fat replacer, improves overrun, viscosity and melting properties of frozen yogurt Ice cream: reduce the melting rate, increases fiber content	159, 164-166
	Functional foods	Prebiotics: increases availability of probiotics (*L. acidophilus, Bifidobacterium lactis*) in food	164,165
	Confectionery	Sugar replacer in chocolate	100
Kefiran	Dairies	Stirred fruit yogurt: fat replacer, decreases syneresis, decreases yeast and mold growth Acidified milk: gelling agent, increases viscosity, shelf life.	108, 167
	Food packaging	Compostable and biodegradable	168
	Functional foods	Prebiotic	168
Xanthan	Dairies	Increases viscosity, thickener and emulsion stabilizer	119
	Frying foods	Reduce oil uptake	121
	Bakeries	Thickener, stabilizer, and suspending agent	169
	Food packaging	Biodegradable, inhibits the growth of aerobic microorganisms, extends the shelf life of meat and fish	170
	Sauce & dressing	Better mouthfeel, egg yolk substitute in mayonnaise	171, 172
	Confectionery	Cakes, muffins, biscuits: uniform distribution of moisture, increases water-binding and air stability in batter Chocolate: cocoa substitution, increases the melting point	173, 174

Table 2. Contined.

EPS	Food industry	Applications	References
Gellan	Confectionery	Gelling agents	150
	Functional foods	Encapsulation of probiotics such as *Lactobacillus paracasei* in yogurt	175
	Food packaging	Monitoring seafood freshness	176
Alginate	Food packaging	Preserves volatile flavor compounds, incorporation with antimicrobials, prolong shelf life	177
	Functional foods	Encapsulating active enzymes and live bacteria	178
	Dairies	Ice cream: thickener, stabilizer, increases viscosity, increases heat–shock resistance, reduces crystal formation, and improves melting characteristics	178
	Restructured foods	Thermo-irreversible gels	178
Viilian	Dairies	Thickener, decreases the syneresis of fermented milk products	138
Acetan	Confectionery	Viscosifier and gelling agent	139

Table 3. Typical Processes for Purification of Important Microbial Exopolysaccharides (EPS)

EPS	Isolation Process	Reference
Dextran	Cell removal by centrifugation or filtration Precipitation by water-miscible organic solvents (ethanol, acetone, etc.) Re-precipitation and dialysis Purification by size-exclusion chromatography (high molecular weight dextran), or ultrafiltration (low molecular weight dextran)	142
Pullulan	Cell removal (centrifugation or filtration) Melanin removal (activated charcoal/Alcohols in combination with salts) Precipitation (propyl alcohol, isopropyl alcohol, tetrahydrofuran, dioxane) Purification (ultrafiltration, ion exchange chromatography)	52
Levan	Cell removal (centrifugation) Deactivation of enzyme in supernatant Precipitation (isoelectric point, organic solvent, salting out, polyelectrolytes flocculation) Separation of levan (filtration, dialysis) Purification	163
Xanthan	Pasteurization of the fermented broth (sterile bacteria and deactivate enzymes) Precipitation of xanthan or cell free xanthan by alcohol. Washing with water and re-precipitation	172
Kefiran	Heating the bacterial culture and cell removal (centrifugation) Precipitation (cold ethanol) Washing with water and re-precipitation	183
Cellulose	Harvesting the pellicles (centrifugation) Washing with water to remove the residual culture medium Lyse the microbial cells (alkali at 80°C) Filtration (remove the dissolved materials) Neutralization with 5% acetic acid and rinsing Washed with deionized water	184

EPS production occurs during the bacterial growth stages. The quality, molecular characteristics, and yield of EPS depend on the nutrient status and bacterial growth condition. Therefore, choosing the appropriate culture medium is the first step in isolating an adequate amount of high-quality EPS. An optimal balance between carbon (for energy production) and nitrogen (for cell synthesis) is needed to achieve high yields (181). Various media were used to culture EPS-producing LAB, most of which are skim milk and whey-based media (182). The concentration and type of simple sugars in the culture media affect the EPS yield (181).

The simplest method of EPS isolation involves three stages of centrifugation (for cell removal), dialysis against water, and lyophilization. In some cases, ethanol precipitation may be used before dialysis to concentrate the EPS. As the culture media components become more complex, the extraction method becomes more sophisticated. For example, in high-protein environments, it may be necessary to reduce protein levels by trichloroacetic acid, proteases, or a combination of both. Other techniques such as membrane filtration (microfiltration, ultrafiltration, and diafiltration) may be used to purify the EPS (183). Table 3 presents the extraction process of some important microbial EPSs. The isolation method has an impact on the total amount of EPS obtained; therefore, different methods should be analyzed to determine the best method for isolation of EPS.

Conclusion

Nowadays, the ability of microorganisms to produce EPS has been the focus of attention. These natural compounds have different applications in various industries, including the food industry. The rapid growth of microorganisms, high productivity rate, and safety approval of EPS have enabled them to be used as inexpensive compounds to improve the texture, sensory, and nutritional attributes of foods and make functional food to treat some human diseases especially gastrointestinal disorders and metabolic syndromes.

Conflict of Interest Disclosures
None.

Ethical Issues
None.

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