Functional biopolymers produced by biochemical technology considering applications in food engineering

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Abstract–Polysaccharides are widely used in foods as thickening, gelling, stabilizing, emulsifying, and water-binding agents. The majority of the polysaccharides currently employed in the food industry are derived from plants and seaweeds. Recently, microbial polysaccharides have emerged as an important set of biothickeners for foods. These biopolymers have overcome to great extent the flaws associated with the plants and seaweeds polysaccharides. This relatively new class of biopolymers has unique rheological properties because of their potential of forming very viscous solutions at low concentrations and pseudoplastic nature. This review deals with the current applications of these microbial biopolymers in the food industry with a special focus on the commonly used important exopolysaccharides in this area.

Key words: Microbial Exopolysaccharides, Foods Industry, Dextran, Xanthan, Bacterial Cellulose, Gellan, Curdlan, Pullulan

INTRODUCTION

Polysaccharides present a wide array of potential applications, particularly for the food industry (Scheme 1). Produced by plants, animals and microorganisms and constitute a distinct group of biopolymers, they possess a variety of unique and in most cases rather complex chemical structures and different physiological functions [1]. Although the majority of the polysaccharides currently employed in industrial use are obtained from plants and seaweed, microbial synthesis has recently emerged as an important source of novel biopolymers of this group. Examples of the microbial polysaccharides are glucans, bacterial cellulose, pullulan, xanthan gum, gellan gum, and bacterial alginates [2]. These biopolymers have unique rheological properties attributable to their high purity and regular structure and, therefore, the food industry frequently uses these products as thickening, gelling, or stabilizing agents [3]. Other novel applications of the microbial polysaccharides include immune modulation and tumouristasis by β-D-glucans, the use of bacterial cellulose in audio membranes, and hyaluronic acid in cosmetics [4-6].

In short, microbial polysaccharides have a variety of food and non-food applications, but in this review we will concentrate on the literature related to the current uses of microbial exopolysaccharides (EPSs) in the food industry.

## MICROBIAL AND CHEMICAL PRODUCTION OF POLYSACCHARIDES

Most polysaccharides have complex structures and thus are difficult to synthesize through conventional synthetic methods [7,8]. There are many possibilities for the combination of monomeric units (due to the presence of several hydroxyl groups of similar reactivity) as well as for glycosidic linkages to be formed stereospecifi-

cally (α- or β-anomers). In conclusion, it is always difficult to control the stereospecificity and the regiospecificity of these products [8,9] and thus hinder their efficient chemical production. These difficulties in the synthesis of polysaccharides are also coupled by the disadvantages of synthetic production compared to natural ones including high cost of the requisite chemicals and their hazardous effects towards health and environment.

Due to these problems associated with chemical synthesis of polysaccharides, the scientific and industrial community has always relied on natural sources for these products. The major natural sources for the production of polysaccharides include plants and microorganisms. Production of polysaccharides from plants and seaweed requires certain geographical and climatic conditions; thus their production is limited only to certain parts of world [10,11]. Similarly, land plant growth takes place in months and in some cases in years. The type and composition of plant polysaccharides may be sensitive to seasonal variations, which are difficult to control. Moreover, the plant polysaccharides occur with other uncountable secondary polysaccharides, which makes their isolation and purification difficult. Similarly, the plant polysaccharides may not always be readily available in the requisite quantity, quality or their rheological properties may not exactly match the desired ones and thus require chemical or enzymatic modifications [12-15], which strongly restricts their use in food [14], and medical related products.

Syntheses of polysaccharides via microbial fermentations offer several benefits compared to synthetic and plants polysaccharides [7-18], which are summarized as follows:

1. Microbial production is not restricted due to regional and climatic conditions.

2. It is independent of seasonal variations and EPS recovery and purification is relatively easy.

3. Microorganisms exhibit high growth rate and are more open to manipulation of conditions for enhancing growth and/or EPS production and thus the EPS can be prepared readily in requisite quanti-

Polysaccharides

Thickening and gelling agents

Secondary effects

Film formation | Inhibition of syneresis | Encapsulation | Control of crystallization | Stabilization | Suspension of Particulates | Emulsification
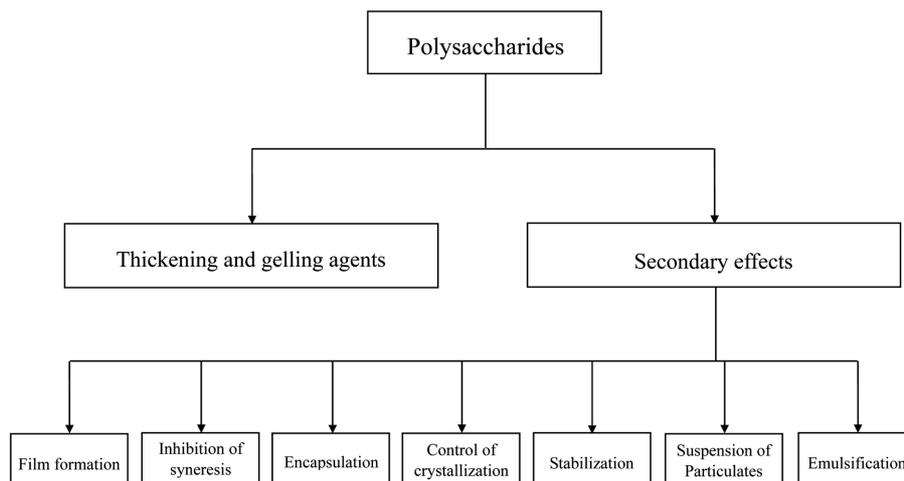
Scheme 1. Major applications of polysaccharides in the food industry.

ties in a relatively short span of time (hours or days).

4. Better yielding microorganisms can be genetically modified to produce polysaccharides of desired properties.

5. It does not involve any hazardous materials for synthesis and requires only simple and safe ingredients for microbial growth medium.

6. Several successful efforts have been made to use certain industrial food wastes as growth medium for the producer organisms, which is not only a cheap way but also works as a basin for environmental cleaning.

7. Sometimes these polysaccharides can be produced directly with the food system for the desirable functions, thus leading to a reduction of time and labor, e.g., yogurt etc.

MICROBIAL EPSs AND FOOD INDUSTRY

As mentioned above, most viscosifiers and texture enhancers, the so-called biothickeners, currently in food industry use are derived from plants and seaweed (e.g., starch, pectin, agar and alginates) [2] and the animal proteinaceous hydrocolloids (e.g., gelatin and casein) [19]. These polysaccharides, however, may not always be readily available in the requisite quantity, quality or their rheological properties may not exactly match those required. The structure and rheological properties of most of these carbohydrates (e.g., cellulose, starch, pectin, alginate and carrageenan) are therefore improved by way of chemical or enzymatic modifications [12-15] and thus their use is strongly restricted in food products. For example, the European Union allows their addition only in some food products [14].

In response to these technical problems encountered in the use of plant and animal products in food commodities as biothickeners, over the past 20 years a new class of polymers, the microbial polysaccharides, have grown in industrial importance. These include xanthan from *Xanthomanas campestris* [20] and gellan from *Sphingomonas paucimobilis* [21]. They can be prepared in reliable quantities by using conventional biotechnological processes [22]. Many bacteria, yeasts and fungi can produce polysaccharides, some of which have been proven to be useful industrial products that com-

pete with plant and algal polysaccharides [13]. The microbial EPSs produced by fermentation are examples of high-tech products that have several nutritional, medical and pharmaceutical applications. These EPSs particularly suit food industry needs [12] where these are used for their unique properties as viscosifiers, stabilizers, emulsifiers or gelling agents [23]. These unique rheological properties are mainly related to their capabilities of forming very viscous solutions at low concentrations and their pseudoplastic nature [15]. Moreover, the microbial polysaccharides are less toxic, well biodegradable, environmentally friendly, highly selective and particularly active at extreme temperatures, pH, and salinity [24]. Due to their superior properties, these products can be used as alternatives to other synthetic or natural water-soluble polymers or as novel polymers in thickening, suspending and gelling applications.

IMPORTANT EPSs AND THEIR APPLICATIONS IN THE FOOD INDUSTRY

The physical and chemical characteristics of important microbial EPSs and their major uses in the food industry are summarized in Table 1.

1. Dextran

Dextran, which is the first industrial microbial polysaccharide, was discovered in 1880 as a fermentation product of *Leuconostoc mesenteroides* in sugar cane, or beet syrups where it caused thickening and gelation of the syrups [25]. The term 'dextran' collectively refers to a large class of extracellularly formed glucans produced by the genus *Lactobacillus, Leuconostoc*, and *Streptococcus* and consist of α-D-glucopyranosyl units polymerized predominantly in α-1 $\rightarrow$ 6 linkage with a smaller proportion α-1 $\rightarrow$ 2, α-1 $\rightarrow$ 3, or α-1 $\rightarrow$ 4 linkages resulting in a highly branched molecule (Fig. 1) [26,27]. Dextrans are synthesized outside the cell by dextransucrase, which catalyzes sucrose to produce D-fructose and D-glucose, and transfers the latter to an acceptor to form dextran [27].

Lower molecular weight dextrans have less branching and narrower range of molecular weight distribution, while those with molecular weight greater than 10,000 are highly branched. Dextran molecules attain greater symmetry as the molecular weight increases;

those with molecular weight lower than 2,000 are more rod-like, while those from 2,000 to 10,000 exhibit the properties of expandable coils [28].

Due to their structural differences, some dextrans are water-soluble and others are insoluble [27] but still most have greater than 30 mg/ml solubility in water. The terminal reducing end groups of dextrans can be oxidized in alkaline solutions [28]. Generally, dextran has high solubility characteristics and promotes low solution viscosities. It (mol. wt 500,000) exhibits Newtonian behavior at concentrations <30% w/w. However, higher molecular weight dextran

Table 1. Physicochemical properties and major food applications of industrially important microbial exopolysaccharides

Microbial polysaccharide	Physicochemical properties	Important uses in foods
Dextran	Consists of α-D-glucopyranosyl units mainly with α-1→6 linkage. Synthesized extracellularly by dextransucrase. Terminal reducing end groups can be oxidized in alkaline solutions. Highly water-soluble and promotes low solution viscosities. Branching and molecular symmetry increase with molecular weight. Exhibits Newtonian behavior at concentrations <30% w/w.	Used in various foods as conditioners, stabilizers and bodying agents. Used in confectionary for improvement of moisture retention, viscosity and inhibition of sugar crystallization. In gum and jelly candies used as gelling agent. Crystallization inhibitor in ice cream. Provides the desirable body and mouth feel in pudding mixes.
Xanthan gum	Anionic polyelectrolyte of (1→4)-β-D-glucan cellulose backbone. The alternate glucose residue is substituted at C-3 with a trisaccharide. A pyruvic acid residue is linked to the 4 and 6 positions of the terminal D-mannose residues. Forms double-stranded helix and has high tendency to aggregate. Does not form gels. When heated above its transition temperature, it changes from an ordered helical structure to a disordered (random coil).	Imparts good flavor release and is a good suspending and stabilizing agent for oil/water emulsions. Used in salad dressing to prevent oiling off and separation of insoluble solid particles. Used in baked goods, confectionery products, syrups, toppings, dry beverage mixes, frozen foods, dairy products, weight loss formula and ice creams.
Bacterial cellulose	Unbranched polymer of β-1→4-linked glucopyranose residues. The chemical structure is similar to plant cellulose except the degree of polymerization. Macromolecular structure and properties of bacterial cellulose also differ from the plant cellulose. The fiber diameter of bacterial cellulose is about 1/100 of that of plant cellulose while its Young's modulus is almost equivalent to that of aluminum. The bacterial cellulose has a high crystallinity and high water holding capacity (up to 700 times its dry weight).	Potential uses include pourable and spoonable dressings, sauces, and gravies; frostings and icings; sour cream and cultured dairy products; whipped toppings and aerated desserts; and frozen dairy products. Its potential food applications also include a low-calorie additive, thickener, stabilizer, texture modifier, pasty condiments, and in ice cream. A bacterial cellulose gel, called *nata*, is eaten as a dessert delicacy.
Gellan	Heteropolysaccharide. The repeating unit is a tetrasaccharide [→3]-β-D-Glc$p$(1→4)-β-D-GlcAp(1→4)-β-D-Glc$p$ (1→4)-α-L-Rha$p$(1→). The native polysaccharide is partially esterified. The glucuronic acid moiety form salts with cations (K, Ca, Mg). Its molecules are in disordered coil states at higher temperatures, which on cooling changed into double helical form. At high concentrations the double helices transform into thicker rod-like aggregates and form macroscopic gel. Soluble in water and insoluble in ethanol. The acylated form produces soft, flexible and thermoreversible gels. The de-acylated type form hard, non-elastic brittle gels. Form gels at concentrations as low as 0.05 wt%. Gels are transparent, heat and acid resistant with good flavor release.	Used in foods as a thickening or gelling agent. Improve the texture, physical stability, flavor release and water-holding capacity in food systems. Gels are used as adhesive to apply seasonings to the surfaces of foods. Utilized in making a food material processable. Used as a suspending agent and texture modifier. Used for oil reduction in fried foods. The de-acylated gellan's gels impart the 'melting in the mouth' sensation with the release of water and associated flavors from the weak gel network instantly. Used in puddings, dessert gels, frostings, beverages, dairy products, fruit spreads, bakery fillings, glazes, confections, icings, sauces, batters and breadings.

Table 1. Continued

Microbial polysaccharide	Physicochemical properties	Important uses in foods
Curdlan	Neutral, essentially linear, $(1\rightarrow3)$-β-glucan with few intra- or inter-chain $(1\rightarrow6)$-linkages. Can exist in a triple helix, single helix or single chain. Colorless, odorless and has a bland taste. Insoluble in water, alcohols and majority of organic solvents. Dissolves in dilute bases, DMSO, formic acid, and aprotic reagents. Morphologies range from endless microfibrils to spindle-shaped fibrils of various lengths. Forms a high-set, thermo-irreversible or low-set, thermo-reversible gel from aqueous suspension depending on heating temperature. Curdlan can take up to about 100 times its weight of water.	Used as a biothickening and gelling agent in foods. Used as textural agent in food such as meat, dairy, baking, and nutraceutical products. High-set gel is used to mimic meat and seafood in vegetarian foods. Used for the improvement of elasticity and strength of noodles. Used to maintain oil-moisture barriers in batter and coating systems. Used in tofu products having freeze thaw and retort stability. Used in delicately flavored foods like dairy preparations and sauces. Mimics the rheological behavior of fat in processed liquid foods such as low-fat dressings, sauces, and gravies.
Pullulan	Neutral, water-soluble and unbranched homopolysaccharide. Consists of maltotriose and maltotetraose units with both α-$(1\rightarrow6)$ and α-$(1\rightarrow4)$ linkages in regular alteration. Average molecular weight varies from hundreds to thousands kDs. Non-toxic, non-mutagenic, odorless, tasteless, edible white colored powder. Easily soluble in water and makes clear and viscous solution. Have high adhesion, sticking, lubrication, and film forming abilities. Biodegradable, heat resistant, and has wide range of elasticities and solubilities.	Used as a food additive, as a component of flour, as a texturizer for tofu, ham and sausage, and as a substrate for flavors and as a mean of protecting flavors through micro encapsulation. Applied in low-calorie foods and drinks and as a preservative. Its films and fibers are applied for packaging of food products. Pullulan film is used for decorations of candies and bakery goods. It is applied as a binder for seasoning and a sheet for wrapping various food items, and as edible packaging material for instant noodles or packages of tabletop sweeteners.
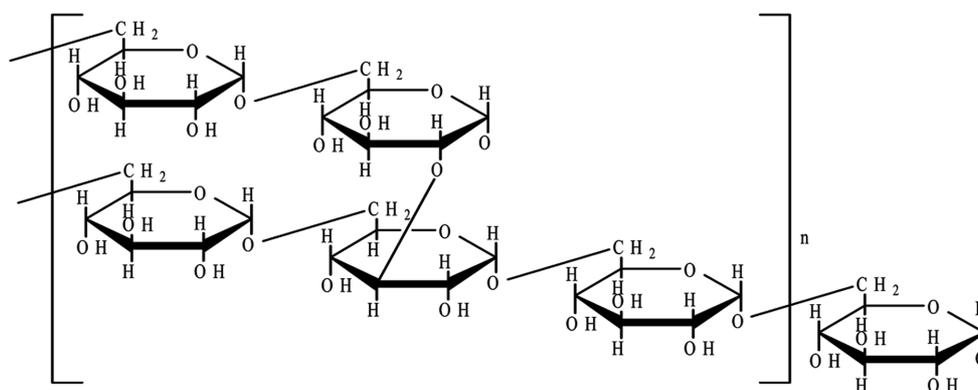
Fig. 1. Chemical structure of dextrans.

solutions possess slight pseudoplasticity at concentrations >1.5% w/w [29].

The dextrans have great potential for applications in various food products as conditioners, stabilizers, bodying agents or related uses [27]. These are used in confectionary for improvement of moisture retention, viscosity and inhibition of sugar crystallization. In gum and jelly candies these are used as gelling agent. In ice cream these are applied as crystallization inhibitor, while in pudding mixes they provide the desirable body and mouth feel [30].

2. Xanthan

Xanthan gum, the second microbial EPS, was approved for use in foods. It was discovered in the 1950s and reported in 1961 from *Xanthomonas campestris* by the research laboratories of the US department of agriculture [31]. In 1969 it was approved as a food additive by the FDA for use as a stabilizer and a thickener. In 1980 the European Commission approved it in the list of permitted thickeners and stabilizers, and in 1988 the AD1 (acceptable daily intake) of xanthan gum was altered into 'not specified', which confirmed it as a safe food additive [31].

Xanthan gum is a high molecular weight extracellular non-gelling polysaccharide. Its structure consists of an anionic polyelectrolyte with a backbone chain consisting of $(1\rightarrow4)$-β-D-glucan cellulose,
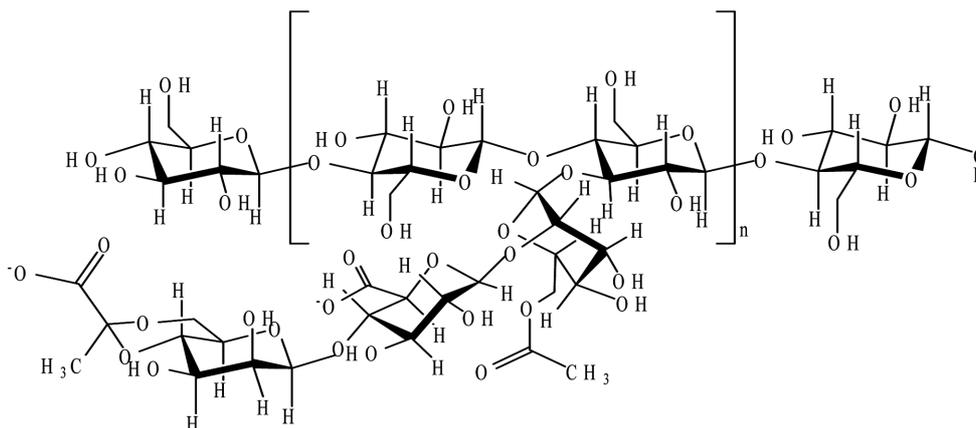
Fig. 2. Structure of xanthan.

the alternate glucose residue of which is substituted at C-3 with a trisaccharide side chain which consists of β-D-mannopyranosyl-$(1\rightarrow4)$-$(\alpha$-D-glucuropyranosyl)-$(1\rightarrow2)$-β-D-mannopyranoside-6-cetate (Fig. 2). A pyruvic acid residue is linked to the 4 and 6 positions of the terminal D-mannose residues (Fig. 2). The acetate (60-70%) and pyruvate (30-40%) contents vary with culture conditions and post-fermentation processing [31,32]. Xanthan gum easily forms a double-stranded helix and has high tendency to aggregate, but does not form gels by itself [33,34]. When xanthan is heated above its transition temperature, it changes from an ordered helical structure to a disordered (random coil) form [35].

Xanthan gum is a major commercial biopolymer; its production exceeds 20,000 tones per year from several commercial sources [4]. It is a white to cream colored free flowing powder soluble both in hot and cold water, but insoluble in most organic solvents. It is unique to other polysaccharides because it gives high viscosity solutions at low concentrations; its viscosity is little affected by temperature variation, stable over a wide pH range and forms pseudoplastic but not thixotropic dispersions in water [36,37]. The xanthan solution is compatible with many other ingredients in food, imparts good flavor release (pseudoplasticity enhances sensory qualities), and is a good suspending and stabilizing agent for oil/water emulsions [36].

The major use of xanthan gum is in salad dressing, but is also used in baked goods, confectionery products, syrups, toppings, dry beverage mixes, frozen foods, and dairy products. Xanthan gum is synergistic with locust bean gum and guar gum, and they are often used together for enhanced gelation or viscosity. It is an ingredient in weight loss formulas [37]. In ice cream the xanthan gum provides viscosity to the liquids in the processing. Further, xanthan gum reduces the freezing point of the product and enhances both the taste and texture of the final product [38]. In salad dressing it prevents oiling off and separation of insoluble solid particles because of the three-dimensional network formed by the associated xanthan gum chains. Its shear-thinning flow properties cause the industrially produced dressings and sauces easy to mix, pump and pour. It prolongs the shelf life of these oil-in-water emulsions because of its rheological properties and high tolerance towards acids and salts [31].

In bakery products, xanthan gum improves the cohesion of starch granules, thus providing a structure to these products. It is used to increase water binding during baking and storage and thus prolongs the shelf life of baked goods and refrigerated dough. Xanthan gum can also be used in soft baked goods for the replacement of egg white content without affecting appearance and taste. It is also used in prepared cake mixes to control rheology and gas entrainment and to impart high baking volume. Solid particles like nuts are prevented from settling during baking. Gluten-free bread with a fine pored structure and elastic surface can be baked with xanthan gum [31].

Many frozen foods show syneresis after one or two freeze-thaw cycles. Xanthan gum can enhance the stability in such products by binding free water and thus limiting the ice crystal growth and providing the desired texture [31]. Xanthan gum is used as a stabilizer alone or in combination with other hydrocolloids in desserts, toppings, and dairy products [31].

In beverages xanthan gum is effective for suspending fruit pulp for long periods of time and also imparts the drink enhanced mouth feel with full-bodied taste and good flavor release. This property of xanthan gum can be easily exploited in low-calorie drinks where sugars are totally or partially replaced by artificial sweeteners, resulting in a 'thinner' consistency [31].

3. Bacterial Cellulose

Cellulose is the most abundant biopolymer on the earth and is of significant importance globally. It is the major constituent of cotton and wood, which are the key resources for all cellulose products such as paper, textiles, construction materials, as well as such cellulose derivatives as cellophane, rayon, and cellulose acetate [39-42]. Cellulose is the main constituent of plant cell wall, but some bacteria can also produce cellulose (called biocellulose or bacterial cellulose). The bacterial cellulose has the same chemical structure as that of plant cellulose but has different physical and chemical properties. For example, the fiber diameter of bacterial cellulose is about 1/100 of that of plant cellulose (Fig. 3) while its Young's modulus is almost equivalent to that of aluminum [40-43].

Cellulose-producing bacteria includes members of the genera Acetobacter, Rhizobium, Agrobacterium, and Sarcina [44]. *Acetobacter xylinum* is the most efficient synthesizer of bacterial cellulose. It has been applied as a model microorganism for basic and applied studies on bacterial cellulose [45].

Bacterial cellulose (X 20,000)   **Plant cellulose (X 200)**
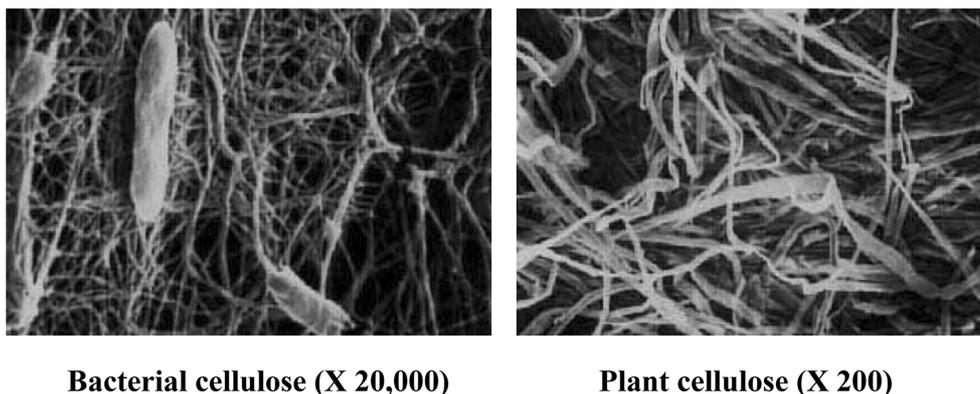
**Fig. 3. Bacterial cellulose and plant cellulose.**
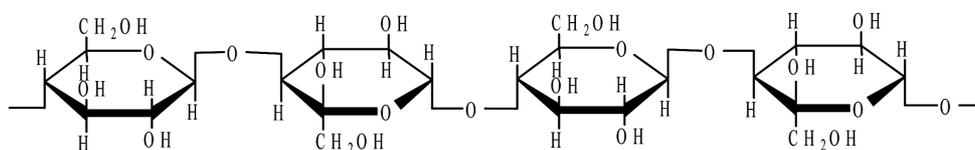
Fig. 4. Structure of cellulose.

The bacterial cellulose is the chemically pure form of cellulose and is free from hemicellulose, pectin, and lignin that are associated with plant cellulose and are difficult to eliminate [46,47]. The bacterial cellulose has a higher crystallinity, tensile strength, moldability and water holding capacity (up to 700 times its dry weight) [46,47]. Its strength and toughness makes it appropriate in applications for which common cellulose is unsuited [46].

Cellulose is an unbranched polymer of β-1→4-linked glucopyranose residues (Fig. 4). The chemical structure of plant and bacterial cellulose is the same except the degree of polymerization, which is about 13,000 to 14,000 for plant and 2,000 to 6,000 for bacterial cellulose [44]. Moreover, the macromolecular structure and properties of bacterial cellulose also differ from the latter [46]. The small chains of bacterial cellulose combine together and form subfibrils. The subfibril having a width of approximately 1.5 nm is one of the thinnest naturally occurring fibers. These subfibrils are crystallized into microfibrils, which in turn into bundles, and the latter into ribbons [46].

Because of its unique properties and structure, bacterial cellulose has important applications in a variety of food formulations. It is especially used when low use levels, lack of flavor interactions, foam stabilization, and stability over wide pH range, temperature, and freeze-thaw conditions are required. Potential uses include pourable and spoonable dressings, sauces, and gravies; frostings and icings; sour cream and cultured dairy products; whipped toppings and aerated desserts, and frozen dairy products. The bacterial cellulose in combination with other agents like sucrose and carboxymethylcellulose improve the dispersion of the product. Besides the above, its potential food applications also include a low-calorie additive, thickener, stabilizer, texture modifier, pasty condiments, and in ice cream [48-51].

Bacterial cellulose has been determined to be "generally recognized as safe" (GRAS) and accepted for by the Food and Drug Administration in 1992. A bacterial cellulose gel, called *nata*, is eaten as a dessert delicacy in the Philippines [52].

4. Gellan

Gellan gum was discovered in 1977 through a screening program of more than 30,000 bacterial isolates in search of polysaccharides that could impart high solution viscosity [53]. It received limited FDA approval in 1990 and the final approval was granted on Nov. 25, 1992 [54,55].

Gellan gum is a high molecular weight (greater than 70,000 with 95% above 500,000) polysaccharide produced by *Pseudomonas elodea*. It is a linear heteropolysaccharides and its repeating unit is composed of D-glucose, (D-Glc), L-rhamnose (L-Rha), and D-glucuronic acid (D-GlcA) and is the tetrasaccharide [→3]-β-D-Glc$p$ (1→4)-β-D-GlcAp(1→4)-β-D-Glc$p$(1→4)-α-L-Rha$p$(1→) (Fig. 5) [56,57].

The native polysaccharide is partially esterified; the 1,3-D-Glc residue can be linked to L-glycerate at C-2 and/or to acetate at C-6, and contains 1 mol of glycerate and 0.5 mol of acetate per repeating unit [58]. The glucuronic acid form salts with potassium, calcium, and magnesium ions. The relative concentrations of these ions control the physical properties such as gel strength, melting point and setting point of the gum. Gellan gum is normally de-esterified by alkali treatment before use in food [59,60]. Gellan molecules are in disordered coil states at higher temperatures in an aqueous solution, while on cooling these are changed into double helical form [61,62]. If the concentration of the gellan gum is sufficiently high, then these double helices can further transform into thicker rod-like aggregates leading to the formation of a macroscopic gel. Gellan gels have a wide range of applications due to their transparent appearance, resistance against heat and acid, and good flavor release properties [62,63].

Gellan gum is soluble in water, forming a viscous solution, and is insoluble in ethanol. It is used as thickening and gelling agent and stabilizer [64]. As mentioned above, the functionality of gellan gum depends on the degree of acylation and the ions present. The
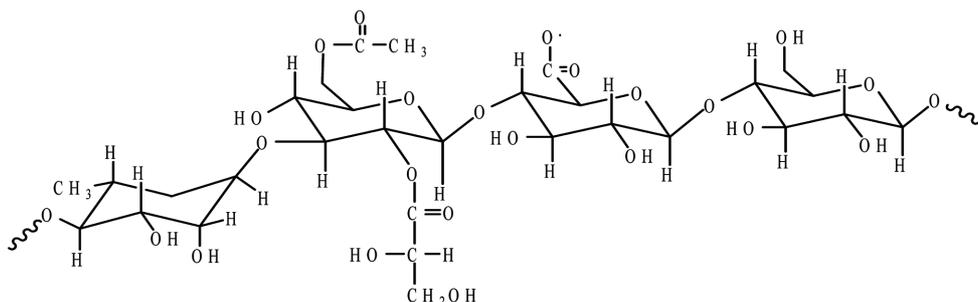
Fig. 5. Chemical structure of gellan.

acylated form of gellan produces soft, elastic, transparent, flexible, and thermoreversible gels while its de-acylated form leads to the formation of hard, non-elastic brittle gels. The glycerate substituents cause significant changes in rheology after deacylation of gellan [65]. In short, the gelation by gellan is dependent on the type of cation, ionic strength, temperature, and polymer concentration [66]. Gellan is able to form gels at concentrations as low as 0.05 wt%. The concentrations commonly employed in food formulations range from 0.2 to 0.4 wt% [67].

Gellan gum has been applied to a very wide variety of foods. Its primary use in these foods is a thickening or gelling agent. Commercially it is available in two forms, low acyl or Kelcogel and high acyl or Kelcogel LT 100. As discussed earlier, low acyl gellan gum forms firm brittle gels while the high acyl gellan gum forms soft elastic gels. By blending of these two forms a wide range of novel texture of gellan gum can be produced for various applications [68]. Gellan gum imparts a good flavor release and is stable over wide range of pH. It has been used alone and in combination with other hydrocolloids to improve the texture, physical stability, flavor release and water-holding capacity in food systems [69-71]. Gellan is compatible with a number of other gums (xanthan, locust bean), starches and gelatin to manipulate the type of gel, elasticity and stability [55].

Gellan microgel is a collection of very fine particles of gellan gum gel and has intermediate characteristics between sol and gel. It makes full use of the gelling characteristics, heat resistance, good flavor release of the gum. Gellan gum form gels immediately in the presence of appropriate cations and therefore it can be used as an adhesive to apply seasonings to the surfaces of foods such as potato chips, crackers and arare. Moreover, this gel formation property of gellan gum in the presence of cations is utilized in making a food material processable which is, otherwise, not suitable for such purposes [63]. Gellan gum is also used as a suspending agent and texture modifier in a wide range of foods. It is also used for oil reduction in fried foods [72].

The firm but brittle and delicate gels formed with the de-acylated form of gellan gum crumble in the mouth. This imparts the 'melting in the mouth' sensation with the release of water and associated flavors from the weak gel network instantly [60,73]. This property makes gellan gum versatile when used alone or in combination with other hydrocolloids, for example guar gum, xanthan and K-earrageenan, in food applications [66].

In conclusion, gellan gum is a versatile compound that is tolerant to large variations in environmental conditions, and is therefore an excellent multifunctional agent. It is used in a wide range of food
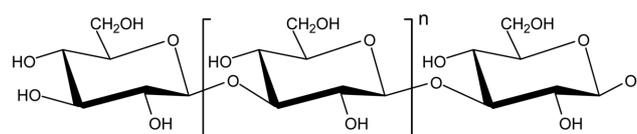
Fig. 6. Chemical structure of curdlan.

products such as puddings, dessert gels, frostings, beverages, dairy products, fruit spreads, bakery fillings, glazes, confections, icings, sauces, batters and breadings [53].

5. Curdlan

Curdlan is a high molecular weight homopolymer. It is neutral, essentially linear, $(1 \rightarrow 3)$-β-glucan. It may have a few intra- or inter-chain $(1 \rightarrow 6)$-linkages (Fig. 6). It is produced by the bacterium *Alcaligenes faecalis* var. *myxogenes*, which is a non-pathogenic and non-toxicogenic organism. In December 1996 it became the third microorganism-fermented hydrocolloid after xanthan gum and gellan gum to be approved by the FDA. Curdlan has been extensively studied for toxic effects and has been evaluated as a safe product [74,75].

Curdlan molecules may have up to 12,000 glucose units. It is insoluble in water, alcohols and the majority of organic solvents. It dissolves in dilute bases, dimethylsulphoxide (DMSO), formic acid, and aprotic reagents such as N-methylmorpholino-N-oxide and lithium chloride in dimethylacetamide. Curdlan shows different morphologies when precipitated from NaOH or DMSO solutions. These range from endless microfibrils to spindle-shaped fibrils of various lengths, depending on the method of preparation [74]. Curdlan molecules can exist in a triple helix, single helix or single chain. The form in which curdlan exists mainly depends on the degree of hydration, heating temperature and solvent conditions [76-78].

The term 'curdlan' has been derived from 'curdle' - describing its gelling behavior at high temperatures with differing characteristics [79,80]. Curdlan forms a high-set, thermo-irreversible gel when its aqueous suspension is heated above 80 °C followed by cooling. On the other hand a low-set, thermo-reversible gel can be obtained by heating it to 55 °C and subsequently cooling to ambient temperatures. The latter type of gels can alternatively be formed in the absence of heating, simply by the neutralization of such solutions with an alkali [74,78].

Curdlan has most unusual rheological properties among natural and synthetic polymers and therefore it is widely used as a bio-thickening and gelling agent in foods [74]. It has a high β-glucan content and can form gel only with heating. Both these properties

are of interest to the food and dietary supplements industry. Moreover, it is tasteless, colorless and odorless and has a high water holding capacity. All these characteristics make it an ideal textural agent in various food applications such as meat, dairy, baking, and nutraceutical products [80]. Curdlan has two types of applications in food systems: i) direct addition at low levels (less than 1%) in regular food systems, and ii) as a building block of the food structure in the creation of new types of food products requiring higher usage levels and preparation of the gel [79].

These different forms of curdlan, obtained with heating, have different textural qualities, physical stabilities and water-binding capacities. Moreover, gels of different strength are formed by variation in heating temperature, time of heat-treatment and curdlan concentration. Gelation is possible over a wide pH range (2-10), in the presence of sugars (sucrose, glucose, fructose), starches, salt, fats and oils [74,81]. This broad range pH stability makes it suitable for applications such as dressings and dips, where the pH is often as low as 3.5 and some nutraceutical ingredients (e.g., spirulina), which have a very high pH [81]. In the majority of foods, curdlan is used in the high-set, thermo-irreversible gel form and is stable during retorting, deep fat frying and cycles of freeze thawing [74,80]. The texture of gel obtained with curdlan is intermediate between the soft, elastic texture of gelatin and the hard brittle texture of agar-agar [82].

Curdlan can take up to about 100 times its weight of water. It absorbs juices released from meat and hydrates and swells. It entraps the juices in the helical configuration of its molecule. This process leads to the formation of a moister product with an improved yield. The texture and the irreversible character of a curdlan high-set gel can be used to mimic meat and seafood. Therefore, it has numerous applications in formulating vegetarian foods (such as crab meat analogue, vegetarian frankfurter sausages, etc.). It eliminates the need for reducing the levels of soy proteins or wheat gluten (a potential source of allergenic compounds) in some of these applications [80].

Curdlan is used in Japan mainly in the meat processing industry to improve the texture by enhancement of the water binding capability of the products. It is also used for the improvement of elasticity and strength of noodles. Besides this, curdlan is used in food for the integrity of oil-moisture barriers in batter and coating systems, imitation seafood delicacies, vegetarian meat analogues, tofu products having freeze thaw and retort stability, and fat replacing systems [74,79,83].

As mentioned earlier, curdlan is colorless, odorless and has a bland taste. These properties make it applicable for delicately flavored foods such as dairy preparations and sauces. Curdlan can be used in fat replacement. When hydrated and heated, curdlan's particles mimic the mouthfeel of fat-containing products. Curdlan also binds the additional water in the system so that it becomes unavailable for ice crystallization and moisture migration upon storage, freeze thawing, or deep fat frying. In addition, aqueous suspensions of curdlan exhibit thixotropic properties. This thixotropic nature of curdlan is exploited in mimicking the rheological behavior of fat in processed liquid foods such as low-fat dressings, sauces, and gravies [84].

In brief, curdlan can impart diverse food textures, depending upon the type of heat treatment, concentration, and other ingredients present. The versatility of curdlan in combination with its nutritional and health benefits is certainly a valuable asset for product designers in producing innovative food systems [80].

6. Pullulan

Pullulan was described for the first time in early 1960s from the fermentation medium of a strain of the fungus *Aureobasidium pullulans*. It is an extracellular, neutral water-soluble polysaccharide [85]. Pullulan is an unbranched homopolysaccharide, which consists of maltotriose and maltotetraose units with both α-(1→6) and α-(1→4) linkages in regular alteration (Fig. 7) [86]. It may also contain a small proportion of α-(1→3) linkages depending on culture conditions and strain differences [87,88]. The average molecular weight of pullulan varies from hundreds to thousands of kiloDaltons [88].
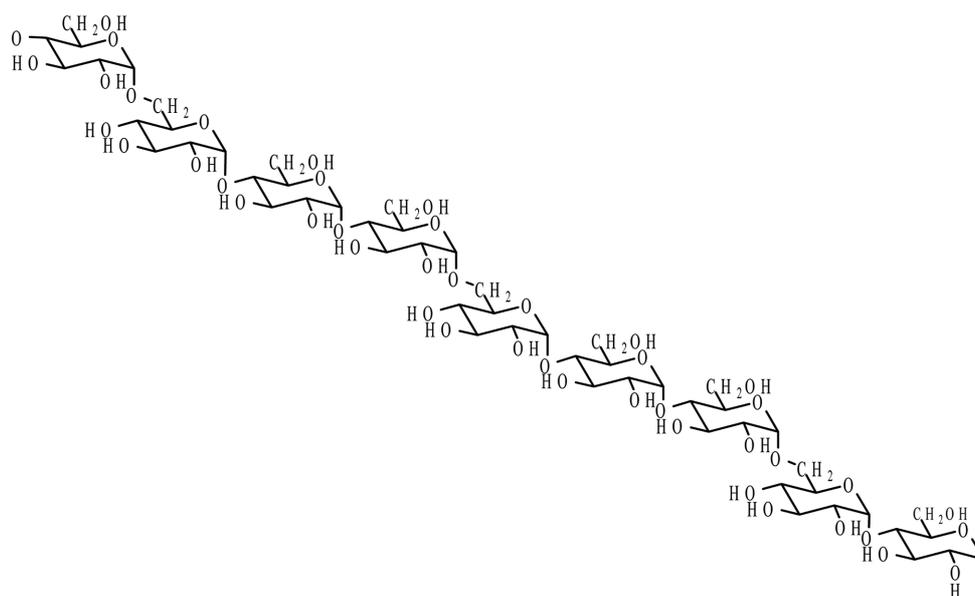

Fig. 7. Chemical structure of a portion of pullulan.

The regular alternation glycosidic linkages in pullulan molecule result in structural flexibility and enhanced solubility [86]. It also produces high-viscosity solutions at relatively low concentrations. Due to these properties, pullulan possesses several unique physical characteristics including adhesive properties, moldability into fibers and capacity of formation of strong, oxygen-impermeable films. It is also used as a thickening or extending agent. Because of these distinctive properties, pullulan and its derivatives have a wide range of applications in foods [86,89,90].

Pullulan is non-toxic, non-mutagenic, odorless, tasteless, edible white colored powder, easily soluble in water and makes clear and viscous solution. It has high adhesion, sticking, lubrication, and film forming abilities [91,92]. Its unique properties like biodegradability, high heat resistance, and wide range of elasticities and solubilities make it applicable in the food industry in many ways. It can be used as a food additive, providing bulk and texture [93]. It is used as a component of flour, as a texturizer for tofu, ham and sausage, and as a substrate for flavors and as a mean of protecting flavors through micro encapsulation [91]. It is resistant to mammalian amylases, provides only few calories [94,95] and thus can be applied as an additive in low-calorie foods and drinks, in place of starch or other fillers [88,93]. In addition, pullulan inhibits fungal growth and has good moisture retention, and thus can be used as a preservative [87, 93]. As mentioned above, pullulan has regular α-(1 →6) linkages in its structure, which may be responsible for its structural flexibility and solubility, resulting in unique film- and fiber-forming characteristics. These films and fibers are edible, biodegradable, highly water-soluble, [90] clear, highly oxygen-impermeable and have excellent mechanical properties [96] and, therefore, can be applied for packaging of food products [86,93]. Pullulan coating forms an airtight membrane that can also be used in the packaging of oil-rich food products that are susceptible to oxidation. As pullulan is edible, removal of its coating before eating or cooking is therefore not mandatory. Tobacco packing with pullulan film prolongs the shelf life of the product as well as aroma retention. Moreover, it protects the product from oxidation and mold attack. Using esterified [97,98] or etherified forms of pullulan, water insoluble coatings can be prepared. In Japan, pullulan film is used for decorations of candies and bakery goods. It is also used in beverages. It is applied as a binder for seasoning and a sheet for wrapping various food items; and as edible packaging material for instant noodles or packages of tabletop sweeteners. Currently in the US, the commercial interests are focused on pullulan film as a flavor substrate for a mouth-refreshing item [91,93].

7. The Future Outlook

If we look at the current widespread uses of microbial EPSs in the various areas of food industry, it is not difficult to make some predictions about their future usage in this business. The increased interest in the physical properties of these biopolymers, along with a much better understanding of the correlation of physical properties to the chemical structure and the continued search for new EPSs, will certainly lead to new discoveries. Additionally, EPSs are biodegradable, prepared from renewable resources and inherently safe products. These properties alone may lead to further increase in their already broad-spectrum applications in foods in place of non-renewable materials or in a search for a safer and cleaner environment.

However, legislative and financial are the two important con-straints that may restrict most of the future microbial EPSs to find application in the food industry. Therefore, there is a need to search for some alternate inexpensive sources for the production of these EPSs. Waste from the food industries may contain good nitrogen and carbon content and thus have the potential to be used for such purposes. Moreover, emphasis should be given to the screening of food grade microorganisms for the production of microbial polysaccharides for food industry. We hope that this way will lead to the discovery of more safe products, which may be acceptable from the legislative point of view.

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