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Role of bacterial exopolysaccharides in edible films for food safety and sustainability. Current trends and future perspectives

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REVIEW ARTICLE

Abstract

Bacterial biopolymers are changing the concepts in the food industry as customer demands the addition of no artificial additives and want products "Clean labelled." Exopolysaccharides (EPS) produced by some bacterial strains are the best replacements for these artificial additives. The monomeric composition, quality, quantity, structure, molecular weight, and charge on these bioparticles decide the qualities, that is, rheology, texture, shelf life, and physiochemical properties of the product. Exopolysaccharides are the best biothickeners and are being widely used in the food industry. They are widely used as coatings and edible films in food substances, especially fruits and vegetables. These consist of substances that are Generally Recognized as Safe. These coatings and edible films promise improved shelf life and better nutritional properties and act as a protective barrier for food items. Bacterial EPS are widely used in edible films, yogurt-like and plant-based beverages, the meat industry, and fermented low-fat meat, sourdoughs, and low-fat cheese. It interacts with other components and improves the texture, stabilization, viscosity, and mouthfeel of end products. The structural and functional properties of these substances still need to be explored. The main objective of this review is to focus on the importance of EPS produced by various strains, its structure and uses as edible coatings and films, functionality, and applications in various food products. Overall, the future of bacterial EPS in the food industry appears promising, with avenues for innovation and application across multiple facets of food production, including texture modification, nutritional enhancement, and sustainability.

Keywords: biopolymers; edible film; EPS; EPS applications; food packaging

Introduction

The trends of food production, food safety, sustainability, and utility are changing over time. These new trends are minimizing the use of synthetic ingredients (Adame *et al.*, 2024; Shi *et al.*, 2024). This industry has always welcomed the use of innovative products to enhance the quality of food and to promote the increased nutrition supply (Sakr *et al.*, 2021). For this reason, multiple products are being formed and they are used in the food industry. To promote the function and quality value of food products, many researchers are designing their works on probiotics and their possible combinations (Korcz and Varga, 2021). Majorly, the research is being done on prebiotics, probiotics, post-biotics and para-probiotics to add value to the food industry. These biopolymers have revolutionized the food industry with their extraordinary benefits. Biopolymers are polymers that are biodegradable, bio-based or both in nature. There are three types, namely, microbial substances, synthetic, or natural (Zannini *et al*., 2016). The polymers that originated from microbes such as mold, yeast, and bacteria have gained much more attention due to their possible use in various industries. Exopolysaccharides (EPS) are getting more attention due to their wide range of applications in multiple industries, including food, pharmaceutical, and cosmetic industries. One of the most important classes of EPS-producing bacteria is the lactic acid bacteria (LAB) (Xu *et al*., 2019).

For the protected production of bacterial EPS, biofilm confers the bacterial cells with resistance against antibiotics and a higher level of invasiveness capability (Silva *et al*., 2019). The cell-surface-associated PsI are present there, and they help in the process of adhesion in the initial phase of biofilm formation (Pecoraro *et al*., 2022, 2023). The bacteria-produced EPS can be either secreted into extracellular spaces or loosely attached to bacterial surfaces. Several varieties of EPS are produced by different strains of LAB including *Lactiplantibacillus, Lacticaseibacillus, Fructilactobacillus, Lentilactobacillus, Pediococcus, Lactococcus, Weissella, Latilactobacillus, Limosilactobacillus, Lactobacillus, Streptococcus*, and *Leuconostoc* species (Patel and Prajapati, 2013). The products of *Weissella* have not been recognized as safe so far by the European Food Safety Authority and the US Food and Drug Administration, but they are being widely used in the final finishing of products. It is thought that the EPS of this species can contribute to the sensory characteristics of food due to a wide range of functional and technological properties (Patel *et al*., 2021).

In the same way, EPS production is strain-dependent, and they play a critical role in EPS-producing cells due to their structural diversity. The EPS is not used as an energy source, rather it helps to sustain cellular integrity in harsh conditions like osmotic stress, dehydration, and in the presence of pathogenic microbes (Sanalibaba and Çakmak, 2016). It also maintains the property of cell recognition and biofilm production to colonize the natural habitat. They also maintain no entry signs for toxic chemicals like lysozyme, nisin, and detergents and protect cells from phagocytosis, bacteriophage, and antibiotics (Daba *et al*., 2021). These chemicals usually enter the cell by disrupting the cell wall. Lysozymes and other chemicals cleavage the bonding between them and can easily get entry into cells. EPS protect this cleavage by making a layer and not allowing them to encounter cells (Ferraboschi *et al*., 2021). This is positively proved by the fact that almost all commercialized probiotic bacteria are producers of EPS (Abarquero *et al*., 2022).

One important characteristic of EPS is that it can replace commercially available food additives as it is a natural alternative to artificial additives, and it possesses physiochemical characteristics. They confer multiple properties in food material including stabilizing, emulsifying, and thickening properties along with rheological properties such as reduced syneresis, increased viscosity, and improved texture (Abarquero *et al*., 2022). There is a wide range of uses of EPS that are used as bio-thickeners in the food industry. They also possess some important techno-functional properties that make them unique to be used in the food industry (Ryan *et al*., 2015). Moreover, they also ensure creaminess, mouthfeel, and better food texture, and have application in food packaging. Likewise, this satisfies the customer demands and food optimization by replacing the use of artificial additives. On the other hand, the commercially available food hydrocolloids need labeling of additive names and their added quality which is against the customer desires and demands. On the other hand, there is no need to label EPS products if they are added (Hu *et al.,* 2023; Laorenza *et al*., 2022). Based on monomers variety, exopolysaccharides are of two types: 1) homopolysaccharides (HoPS) having all the identical monosaccharides, and 2) heteropolysaccharides (HePS) having different types of monomers usually ranging from 2–8 monosaccharides (Silva *et al*., 2019; Yildiz and Karatas, 2018). The complete elaboration of HoPS and HePS is given in Figure 1. This article highlights the various strains involved in EPS production along with their structure and properties. The article also enlightens on the application of EPS in edible films and coatings.

Edible Films, Functionalities, Characteristics, and Role of EPS

Edible films and coatings are consumable coatings on fruits or vegetables that can be directly used, and their thickness is about 0.3 mm. These coatings are widely applied to enhance the protection of processed fruits and vegetables (Moradi *et al*., 2021). These films can be of three types: polysaccharides, proteins, and lipid based. The complete information on these types is given in Figure 2. There is a difference based on their formation as edible films are formed separately and are applied on the surface of fruits and vegetables, while edible coatings are directly formed on the surface of foodstuffs (Zikmanis *et al*., 2021). Both are direct contact layers that protect the matrix from direct touch (Alizadeh-Sani *et al*., 2019).

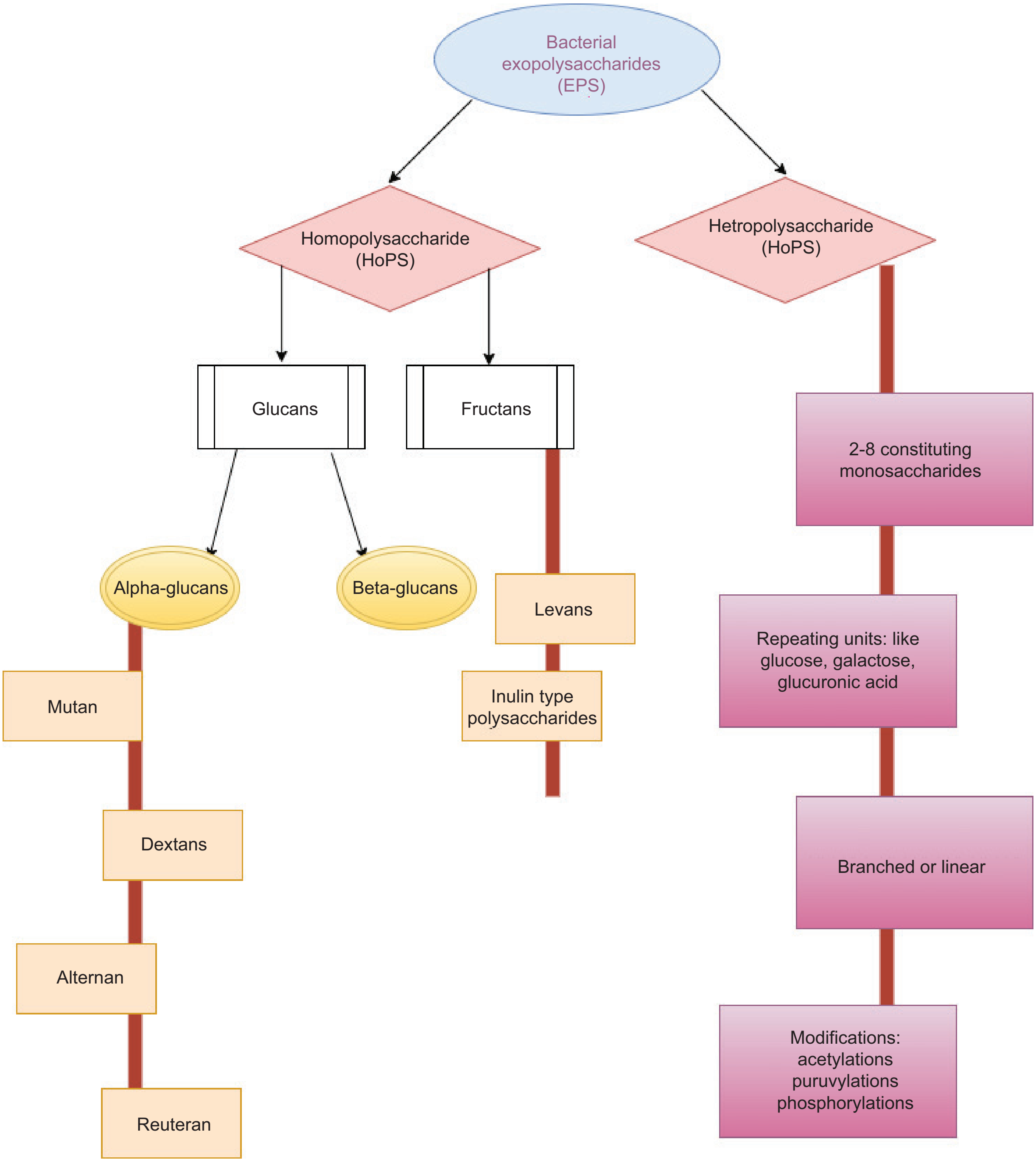

Figure 1. Bacterial exopolysaccharides, types, and characteristics.

While developing films and coatings, the following points should be given utmost importance.

a. There should be direct contact with the surface of fruits or vegetables.

b. The performance and shelf life of the contact food should increase after their application (Li *et al.*, 2020).

c. These coatings should not alter the flavor, color, or major characteristics of foodstuffs.

d. Processing conditions such as color, temperature, and thickness of the coating should be under control (Singh and Kaur, 2015).

Sometimes it becomes very difficult in the case of artificial film and coatings. As these are directly consumed by people and are not acceptable. So, the nature of these films and coatings must be completely organic. For this reason, bacterial EPS provides the best opportunity to go through the formation of these films and coatings (Daba *et al.*, 2021).

Cellulose is considered the most abundant material on Earth. It is present in the cell walls of some bacteria, algae, and plants. This possesses a homopolymer, linear structure and possesses 40–70% crystallinity. This is very susceptible to film coatings. Its goodness is determined by the level of crystalline nature of the fruits and vegetables (Li *et al.*, 2020). This coating is usually done with the help of cellulose and glucose units by combining them with glycosidic bonds. These cellulosic films provide optical and physiochemical properties and act as a barrier to the external environment. This film may alter the solubility level of hydrocolloids as it is insoluble in aqueous solutions (Alizadeh-Sani *et al.*, 2019). These cellulosic films also provide good blending dispersion properties and formation of film due to the presence of strong hydrogen binding. Moreover, sago starch and carboxymethyl cellulose (CMC) nanoparticles provide good mechanical strength in vegetables and fruits. Besides CMC, olive oil has also proved to provide a good antimicrobial coating for tomatoes and oranges. Some other important edible film sources are carrageenan, pectin, chitin and chitosan, gums, and starch (Ibrahim, 2015). Complete information and characteristics of these edible films are given in Table 1.

Different strains of EPS-producing bacteria and biosynthesis of exopolysaccharides

Owing to their unique and diverse structure, EPS are getting more attention from researchers and the industrial sector. Different strains belonging to the gram-negative and gram-positive bacteria are capable of forming EPS (Kumar and Micallef, 2017). Gram-negative bacteria may include Alphaproteobacteria, betaprotobacteria, and gammaprotobacteria. Some important genera included in the class *Alphaproteobacteria* are *Agrobacterium, Rhizobium, Gluconacetobacter, Komagataeibacter, Acetobacter*, *Gluconobacter*, *Kozakia*, *Zymomonas,* and *Neoasaia*. Some important examples of class *betaprotobacteria*

Table 1. Applications of bacterial based EPS coatings and films in fruits and vegetables.

Coating/Film material	Uses	Properties	References
Cellulose or their derivatives	Coatings/edible film	They provide strong adhesion between the cellulosic interface and fiber	(Moradi *et al*., 2021)
Essential oils (plant-based) encapsulated with cellulose acetate butyrate or cellulose acetate	Coating/edible film	They possess air-freshening properties and have fragrance	(Ibrahim, 2015)
Cellulose esters	Edible film	Maintain hydrophobicity, enhance oxygen and water barrier.	(Regubalan *et al*., 2018)
Oil enclosed with cellulose ester	Edible film	Provide plasticizing effect, improve physiochemical properties, improve gas and water barrier and increase water holding capacity.	(Singh and Kaur, 2015)
Steric acid/CMC	Edible film	Drop the water vapor transmittance rate (WVTR) and improve the barrier of water.	(El-Sharoud *et al*., 2012)
Bacterial cellulose with ginger and olive oil	Edible coating	Antimicrobial properties.	(Berthold-Pluta *et al*., 2019)
Stearic acid/rice starch	Edible film	Possess WVTR and mechanical properties.	(Padmanabhan and Shah, 2020)
Glycerol/pearl millet starch	Edible films	They reduce the permeability of water vapors and improve mechanical strength.	(Hashemi *et al*., 2022)
Agar maltodextrin blends	Edible films	Due to aggregations of hydrophobic and hydrogen bonding presence, they act to enhance the properties of the barrier.	(Bibi *et al*., 2021)
Oregano essential oils/pectin	Coatings/edible films	Improve antibacterial properties related to food-based micro-organisms.	(Trollope, 2015)
Starch	Edible films and coatings	Improve oxygen barrier properties, mechanical properties, oil-based properties.	(El-Sharoud *et al*., 2012)
Modified starch	Edible films	Improve physiochemical and physio-mechanical characteristics; antimicrobial agents, antioxidants, etc. are trapped in the matrix of starch due to its high level of hydrophilicity.	(Hashemi *et al*., 2022)

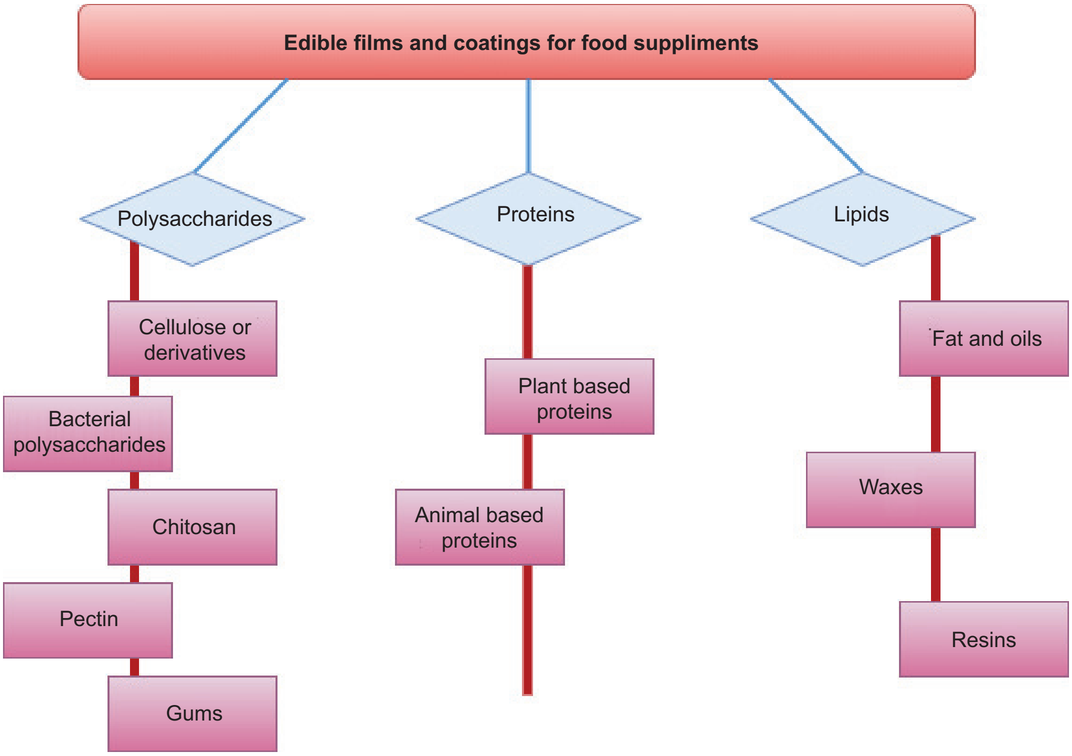

Figure 2. **Different sources of edible films in coatings.**

include *Achromobacter* and *Alcaligenes* (Donot *et al.*, 2012). Some important EPS-producing genera in class *gammaprotobacteria* are *Pseudomonas, Alteromonas, Xanthomonas, Klebsiella, Vibrio, Erwinia, Halomonas Pseudoalteromonas, Azotobacter*, and *Enterobacter* (Oleksy and Klewicka, 2018). Some major EPS-producing classes of gram-positive bacteria include *Bacilli* and *Clostridia*. Some major genera in the class *Bacilli* are *Lactobacillus, Paenibacillus, Bacillus, Streptococcus*, and *Leuconostoc*. *Sarcina* sp. and *Bifidobacterium* are some important genera of *Clostridia* and *Actinomycetia* classes, respectively (Jurášková *et al.*, 2022). Some of the most important EPS are xantham, dextran, alginate, curdlan, gellan, hyaluronan, levan, bacterial cellulose, and others. All these EPS are products of different microbes. To widen the range of applications and achievement of new desired results, scientists are trying to explore novel and excellent EPS having some unique properties. Recently, researchers have proved the high importance of EPS produced by probiotic bacteria, especially in the field of medicine (Madhuri and Vidya Prabhakar, 2014).

In the same way, LAB is the major class of bacteria that forms the EPS that are used in the food industry. The formation of EPS is catalyzed by some enzymes. These enzymes can be divided into two groups. The first group is meant to synthesize basic sugars and nucleotides and some proteins catalyze the reaction (Dertli *et al.*, 2016). However, these proteins are not specific to EPS. The other group possess EPS-based specific enzymes such as acetyltransferases and glycosyl transferases. These use the monomeric substances as catalysts during reaction and perform their work more efficiently (Dertli *et al.*, 2016). Here lies a limitation, the biological function of some EPS based enzymes is still a burning question. These EPS-based enzymes are encoded by some specific genes and are usually present on chromosomal DNA, that is, genophores in the case of thermophilic bacteria. On the other hand, it can also be found on plasmid, especially in the case of mesophilic LABs (Oleksy-Sobczak *et al.*, 2020).

The *eps* genes that encode the exopolysaccharides are usually arranged in a unique cluster and are oriented in a direction. These are transcribed in a single mRNA. These *eps* genes perform four different functions including EPS production regulation, repeating units biosynthesis, determination of chain length, and polymerization and export. The production of HoPS is usually simple as compared to HePS (Amao *et al.*, 2019). There is no need for different stages of active transport, hence no energy is required. Energy is only required for the synthesis of necessary extracellular enzymes called fructosyl and glucosyl transferases. They use fructose and glucose as glycosyl donors during the regulation and synthesis of EPS (Zayed *et al.*, 2022).

The formation of HePS is relatively more complex and demands more amount of energy during production. Their formation demands different and separate reactions which include (1) the internalization of sugar molecules, (2) sugar molecules precursor synthesis, (Naseem *et al.*, 2018), (3) monosaccharides repeating subunits assembly, and (4) the export of EPS (Wu *et al.*, 2023). All these are regulated by different enzymes, hence there is a high energy demand. In its case, the transportation of EPS from cytoplasm to extracellular spaces is unknown (Wu *et al.*, 2023). Some researcher thinks that flippase enzymes are responsible for carrying out this process. In the case of nonoptimized cultural conditions, the yield of EPS is about 10–400 mg/L; and in the case of optimized conditions, this yield can be multiplied by this amount depending upon the strain and cultural conditions (Zayed *et al.*, 2022). Under optimum conditions, *Lactobacillus* strains yield 160–740 mg/L, *Streptococcus* strains yield 126–319 mg/L, and *Pediococcus* strains yield 132–134 mg/L EPS (Polak-Berecka *et al.*, 2013). In the same way, the EPS yield of *Lactiplantibacillus plantarum* is 515 mg/L. The highest EPS is reported as 2.8 g/L, which is produced by *Lacticaseibacillus rhamnosus* strain, that is, RW-9595M. The yield of the second best EPS producer is 2.5 g/L, produced by *Lactobacillus kefiranofaciens*, WT-2BT (Pourjafar *et al.*, 2023).

The purification process is of high importance in the application process of EPS in the food industry. There are different methods involved in the purification of these biopolymers. Another important point is the quantification of EPS (Pourjafar *et al.*, 2023). There are many processes involved, which are summarized in Figure 3. While giving high importance to quantity, quality cannot be ignored as it possesses much importance especially when it is going to be used in the food industry as an additive to achieve important physiobiological properties (Fox, 1991). Some researchers have proved that the final quality of yogurt is not affected by the quantity of EPS formed, rather it is affected by the structural chains and bacterial strains forming the EPS (Dimopoulou *et al.*, 2014). When the EPS interact with other food additives, the textural, rheological, and sensory characters are affected in different ways depending upon the type of EPS used. In a nutshell, the selection of EPS-producing strain will be selected with much care and it will improve the product characteristics and food processing. Moreover, a lot of aspects are still needed to be explored (Badel *et al.*, 2011).

Applications of EPS Bacteria in Edible Films and Coatings

The yield of EPS production by LAB is of less quantity. The high yield is observed in HoPS bacteria. These can convert the sucrose to dextran in very high proportions. Over time, a very small number of EPS-producing LAB can meet the commercial requirements (Shori *et al.*, 2021). The problem is to find out the way to increase the EPS yield in LAB. This could be achieved by the phenomenon of genetic engineering. For this technique, a complete understanding of EPS-producing pathways is crucial (Hickisch *et al.*, 2016). After this, some genetic regulations could be carried out such as the over-expression of genes responsible for EPS production. Another limitation falls in the pathway of HePS as they must compete for the common enzymes by achieving several household routes. Hopefully, this is the reason that most food additive biopolymers are usually not produced by LAB, such as cellulose, gellan gum, and xanthan gum (Lorusso *et al.*, 2018). Exopolysaccharides derived from LAB find their roots deep in the food industry and are used as stabilizers, gelling agents, thickeners, and moisture retention agents. They also improve the mouthfeel, syneresis, rheology, firmness, texture, and sensory properties when they are used as edible films and coatings. Exopolysaccharides have a wide range of applications when they are used as edible films and coatings (Pourjafar *et al.*, 2023). Some important HoPS and HePS are listed in Tables 2 and 3 along with their properties and applications as edible films and coatings.

There are multiple properties of EPS when it is used as edible films or coatings. First, they act as a barrier to gases and moisture loss. In this way, the process of oxidation and spoilage is retarded. This adds up to the shelf life of the food products. For example, gellan and xanthan gums are excellent and commonly used to attain gas and moisture barrier properties (Lynch *et al.*, 2018). Along with this, they are used to enhance the flexibility and mechanical strength of the films and coatings. This protects the outer layer from breakage and reduces the risk of exposure to the external environment. Curdlan and alginates are commonly used to increase the mechanical strength and flexibility of the food product (Mollakhalili Meybodi and Mohammadifar, 2015). Moreover, their usage as an edible film can prove helpful in enhancing the nutritional value of the product. They promote the growth of beneficial gut bacteria by adding up the dietary fibers in the foodstuff (Altun, 2018). One important example is kefiran. This not only helps in the protection of foods but also provides some important health benefits. The engineering of these bioactive compounds can be done to prepare essential oils, antioxidants, and antimicrobial agents. Products aim to enhance and improve the shelf life of edible products (Cui *et al.,* 2024). Gellan and dextran are important examples, which are used in improving food preservation to enhance its shelf life (Darwish *et al.*, 2022). In the same way, these are widely used in food packaging as they are biodegradable. The packaging made by xanthan gums can decompose more rapidly as

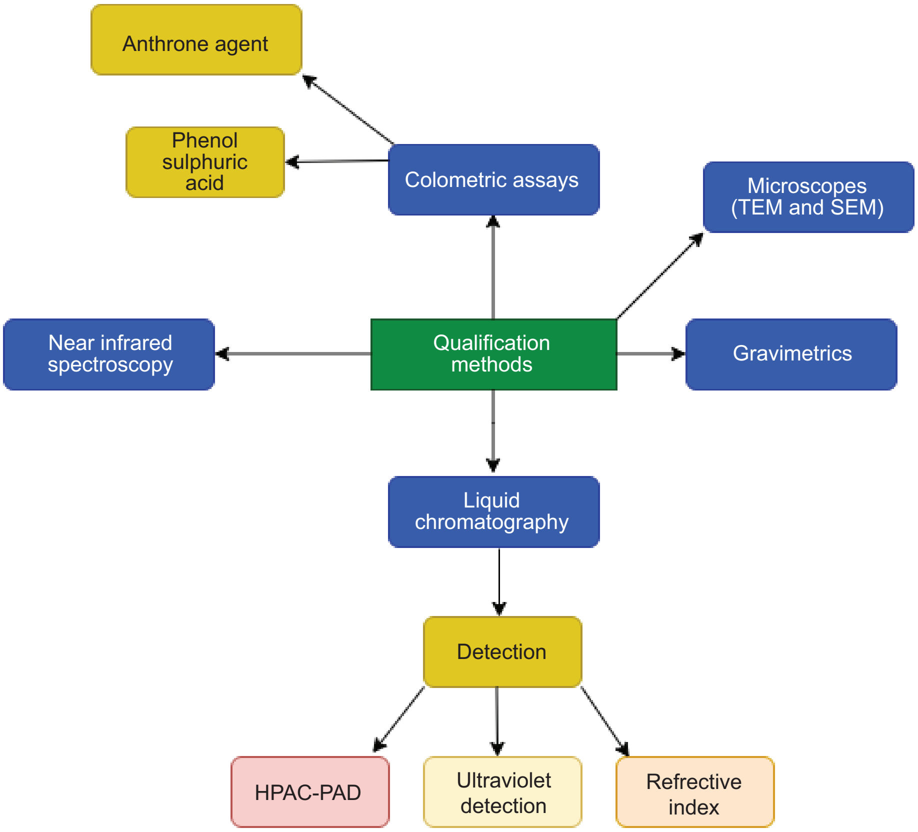

Figure 3. **Bacterial EPS quantification techniques.**

Table 2. **Structure, properties and molecular weight of HoPS and their applications as edible films and coatings.**

HoPS	EP S	Strain	Structure and molecular weight	Applications in edible films and coatings	Reference
α-D-glucans	Dextran	*Weissella cibaria,* *Pediococcus pentosaceus,* *Lacticaseibacillus casei,* *Lentilactobacillus parabuchneri,* *Leuconostoc mesenteroides*	α-D-Glcp (1 → 6) Mw: 103–107 Da	Used as edible films to increase their shelf life and act as a protective layer.	Bibi *et al*., 2021; Hassnain *et al*., 2021
	Alternan	*Leuconostoc citreum,* *Leuconostoc mesenteroides*	α-D-Glcp (1 → 3)/ α-D-Glcp (1 → 6) Mw: > 106 Da	Used as an edible coating and possesses certain properties such as quality preservation, bioactive compound delivery, and enhanced sensory attributes.	Hassnain *et al*., 2021; Jamil *et al*., 2018
	Reuteran	*Limosilactobacillus reuteri*	α-D-Glcp (1 → 4) Mw: 107 Da	Used as an edible coating and enhances shelf life and it is biocompatible. It is used in sustainable packaging solutions.	Rizvi *et al*., 2020
β-D-fructans	Inulin	*Lactobacillus johnsonii,* *Leuconostoc citreum,* *Limosilactobacillus reuteri*	β-D-Frup (2 → 1) Mw: 103–107 Da	It possesses moisture and gas barrier properties. Along with this, they are important to enhance shelf life.	Rathore *et al*., 2018; Rizvi, 2020, p. 139
	Levan	*Leuconostoc mesenteroide,s* *Limosilactobacillus reuteri,* *Streptococcus salivarius,* *Limosilactobacillus frumenti*	β-D-Frup (2 → 6) Mw: 104–108 Da	It is biocompatible and possesses moisture barrier and antimicrobial properties.	Hassnain*et al*., 2021; Mujahid and Noman, 2015

Table 3. Structure, properties and molecular weight of HePS and their applications in food industry.

Strain name	Monomer information	Molecular weight (Da)	Techno-functional properties in edible films and coatings	References
Lactobacillus delbrueckii subsp. *bulgaricus* DGCC291	Galactose, glucose	1.4×10^6	Moisture barrier enhancers and add up in nutrition value.	Saleem *et al*., 2023
Lactobacillus helveticus MB2-1	Glucose, galactose, rhamnose, arabinose, mannose	1.8×10^5	They are used to enhance the flavor and texture of fruits and vegetables.	Shah *et al*., 2016
Lactobacillus spp. (*Lactobacillus kefiranofaciens* and *Lentilactobacillus kefiri*)	Glucose, galactose, Kefiran	7.6×10^5	Carrier of bioactive compounds, enhances the nutritional value.	Shori *et al*., 2021
(Tabibloghmany and Ehsandoost, 2014)	Fucose, xylose, glucose, galactose, mannose, rhamnose, arabinose	1.2×10^4	They act as Stabilizing agents, antimicrobial properties, moisture loss barrier etc.	Siddiqui *et al*., 2020
Lactiplantibacillus plantarum C88	Glucose and galactose	1.2×10^6	Nutrition value addition, moisture retention, etc.	Alizadeh-Sani *et al*., 2019
Lactiplantibacillus plantarum JLK0142	Glucose and galactose	1.3×10^5	They help retain moisture and add in the nutritional value.	Saleem *et al*., 2023)
Streptococcus thermophilus S-3	Glucose, galactose, N-acetyl-galactosamine	5.7×10^5	Plasticizers, mechanical properties, and flexibility enhancers.	Mehmood *et al*., 2012
Streptococcus thermophilus NIZO 2104	Glucose, galactose, N-acetyl-galactosamine, ribose	9.0×10^5	Sustainability, improved sensory attributes, energy source, textural improvement, antimicrobial properties, gel formation etc.	Tabibloghmany and Ehsandoost, 2014
Streptococcus thermophilus CH101	Glucose, galactose	8.5×10^5	Improve viscosity, add nutritional value and flavor enhancers.	Siddiqui *et al*., 2020

compared to any other artificial packaging (Kim *et al.,* 2024). Likewise, they are used as flavor and texture enhancers in foods. They can also improve the mouthfeel of the food products. For example, gellan enhances the texture and acts as a gelling agent to make the foodstuff more attractive to the user (Malik and Iqbal, 2011).

Conclusion

Now it is the need of the hour to eliminate artificial additives and ingredients from the food industry. This is the demand of customers to add natural and safe additives to food products. Microbial EPS specially formed in in-situ conditions is widely being used in this industry, thanks to their special techno-functional and rheological characteristics. Depending upon their structure and characteristics of chains, different types of exopolysaccharides possess different and emerging characteristics as in a variety of ways, viscoelastic properties of food are being modified. There are a wide range of examples of EPS applications in edible films and coatings. They possess a wide variety of applications such as in food preservation, act as moisture loss barriers, enhance nutritional content, improve texture, mouthfeel, plasticizers, mechanical properties, and flexibility.

EPS formed by LAB are mostly used in the food industry. They have some drawbacks in that their production volume is relatively low but have many benefits. Problems with low quantity can be solved by selecting appropriate strain in predefined optimized parameters. Another burning question is the understanding of the structural and functional relationship between EPS and other components. Further studies on this will help open new areas of applications.

Conflicts of Interest

The authors declare no conflicts of interest.

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