GELLAN. FOOD APPLICATIONS

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Gellan is an anionic polysaccharide, which is produced by *Sphingomonaspaucimobilis* bacterium through aerobic fermentation. One of its most important properties is the capacity to form gels through ionic reticulation in the presence of polyvalent cations. The gellan gels have various bioapplications in the technologies based on fermentation processes in medicine, pharmaceutical and cosmetic industries. The aim of this paper is to present this biopolymer's structure, physico-chemical and gelling properties, as well as to review the applications of gellan gels in the food industry.

Keywords: gellan, gel, food applications, gelling properties

INTRODUCTION

Microbial polysaccharides are water-soluble polymers, which can be ionic or non-ionic. Exopolysaccharides are synthesized from microorganisms that can be found attached to the surface of the cell or to the extracellular medium in an amorphous mixture. Branched or unbranched units of exopolysaccharides repeat regularly and are connected by glycosidic linkages.

Dextran was the first glycopolymer synthesized by microbial fermentation in 1880. Xanthan was obtained in 1960 through research conducted in several laboratories.[1-4]

In 1978, in Kelco Merck & Co laboratory (California, USA), Kaneko and Kang found a water soluble polymer – gellan, which has gained great importance in the pharmaceutical, food, and chemical industries due to its functional properties. The success of gellan production in the laboratory was first reported in 1982.[5]

Gellan is an anionic polysaccharide with a high molecular weight produced in an aerobic environment by *Auromonas* (*Pseudomonas*) *elodea* bacterium, renamed *Sphingomonas paucimobilis.*

Currently, gellan is obtained on an industrial scale by using several varieties of *Sphingomonas paucimobilis* strains capable of producing it:

ATCC31461 [7] – the most efficient, E2(DSM6314), NK 2000, and GS1.[6-9]

In 1988, after performing toxicology studies, Japan approved gellan gum for use in food products. Also, the Food and Drug Administration approved the use of gellan gum as an additive in 1992.[10,11]

Gellan gum is branded as Kelcogel, Gelrite, Phytagel,and Gel-Gro. Kelcogel is used primarily in the food industry as a thickening and gelling agent, while Gelrite, Phytagel, and the Gel-Gro are used as solidification agents, as substitutes for agar and also as media for the growth of bacteria and plant tissue cultures.[12] Food industry uses gellan as a stabilizer, gelling agent, as film-forming and encapsulation agent. Moreover, gellan represents a vehicle for drug transportation in pharmaceutical technology. These applications are possible due to its reactivity with the cations and its ability to form a gel at remarkably small concentrations in comparison with other hydrocolloids, such as carrageenan, alginate, pectin, or gelatine.[13]

Functional gellan properties recommend it in many applications in the pharmaceutical, cosmetics and food industry. The basic properties of gellan, which make it a polymer widely used in bioapplications including the food industry are:

biocompatibility, lack of toxicity, biodegra-dability, the stability in an acidic environment and against enzymes in the gastrointestinal tract, temperature resistance, the ionotropic gelation ability with formation of mechanically stable gels, the ability to form films that act as barriers to oil absorption; al of them will be detailed further in the review. These properties allow a wide range of applications in products that can include gellan in their composition, such as liquid gels (used in juices or liquids containing cocoa, fibers, insoluble minerals), products creating a satiety effect, gelatin desserts and jams, particles that encapsulate beer yeast with applications in wine and beer fermentation and alcohol production, nutrients, nutraceuticals, and food additives immobilized in particles, etc.

Though there are studies reporting on each of the applications of this polysaccharide in the food industry, there are few reviews dedicated to this subject.[14] Therefore, we consider that our intention to propose a review in this area is justified and also it could be useful forthose working in the food industry.

GELLAN PRODUCTION AND STRUCTURE
Gellan production

Many researchers have investigated methods of gellan synthesis, the role of the enzymes involved in the process, and the optimal process conditions. Gellan is an anionic heteropolysaccharide produced by the *Sphingomonas paucimobilis* bacterium, using aerobic fermentation through the immersion method as production process.[15,16]

Gellan production is stimulated by media containing carbon sources, nitrogen, and inorganic salts.[17] Medium composition plays an essential role in gellan production.

Carbohydrates, such as glucose, fructose, maltose, sucrose, and mannitol (alone or in combination), are used as carbon sources. The amount of carbon source varies between 2-4% of the fermentation medium mass.[12]

Another important component of the culture medium in gellan production is the source of nitrogen. Nitrogen can stimulate or limit gellan production. If bacteria have anincreased carbon source and the nitrogen source is reduced, then gellan secretion is abundant.[18] Corn extract is used as an organic nitrogen source,[17] while ammonium nitrate[19] and potassium nitrate[20] as inorganic nitrogen sources.

Polysaccharide synthesis also depends on the addition of precursors. Enzyme tests were performed to determine the gellan precursors and results have identified them as sugar phosphate nucleotides. The repeating units of gellan are tetrasaccharides consisting of glucose, rhamnose and glucuronic acid. Sugar nucleotides are expected to be UDP-glucose, UDP-rhamnose, and UDP-glucuronic acid.[20] Their role is to provide active precursors for the synthesis of these tetrasaccharides.

The agitation speed is very important in gellan synthesis. Optimal agitation is carried out at 250 rpm using a helical screw rotor. The gellan mixture presents gelling characteristics at lower stirring rates as well.

Viscosity is reduced when the mixture containing gellan is sheared; therefore higher stirring rates (600-800 rpm) lead to a heterogeneous environment. This fact is a major disadvantage and it causes heat and mass transfer limitations.[21,22]

The yield of gellan production also depends on some cations present in the culture medium. The presence of magnesium in the culture medium determines a higher gellan gum yield. Thus, gellan yield is of 9.3 g/L in the absence of magnesium and of 18.3g/L in the presence of 0.44g Mg/L.[23] Also, gellan concentration decreases with increasing concentration of copper ions in the culture medium.[24]

In order to control viscosity, to facilitate the transfer of mass and energy and to improve the yield of extraction, surfactants are also added in theculture medium. A non-ionic surfactant in concentrations of up to 1.5% at the beginning or during fermentation[25,26] is usually preferred.

Other important factors in gellan production are: oxygen transfer capacity,[25,27]temperature, and pH; the latter has an important role in cell proliferation.[20] The optimum pH value is between 6.5-7.[8,17,21]Acidic or alkaline media reduce cell proliferation and, consequently, gellan production. The temperature of the fermentation process is 30ºC.[12]

Gellan structure

Gellan is the latest biopolymer available on the market and it has been used by the food industry as gelling agent. It is an anionic polymer, linear,with repeating tetrasaccharide units, which consist of two residues of β-D-glucose, one of β-D-glucuronicacid and the other of α-L-rhamnose(Fig. 1) in the ratio is 2:1:1.[28,29]This

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structure was first proposed by Jansson *et al.*[28]and then by M. A.O'Neill *et al.*[29] The native polysaccharide presents a L-glyceril group at O-2 from the third residue of glucose moiety bonded to thetetrasaccharide unit and, by at least several glucose units, one acetyl group at O-6 from the same residue.[30]

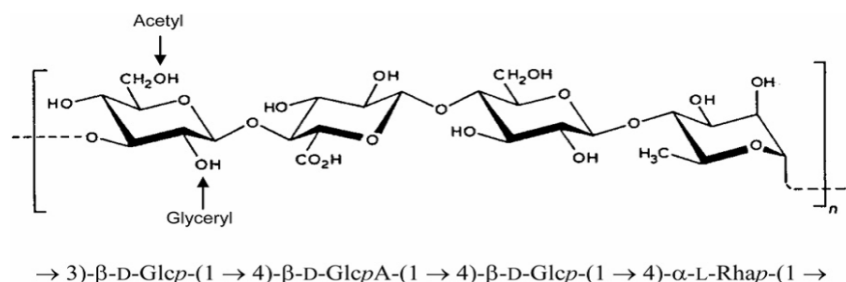→ 3)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*A-(1 → 4)-β-D-Glc*p*-(1 → 4)-α-L-Rha*p*-(1 →

Figure 1: Repeating tetrasaccharide units of deacetylated gellan (this figure indicates the positions where glyceryl and acetyl substituents are attached to gellan rich in acyl groups).[29] (Reprinted from *Carbohydrate Research*, M. A. O'Neill,R. R. Selvendran,V. J. Morris, Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*, **124**(1), 123-133 (1983), with permission from Elsevier)
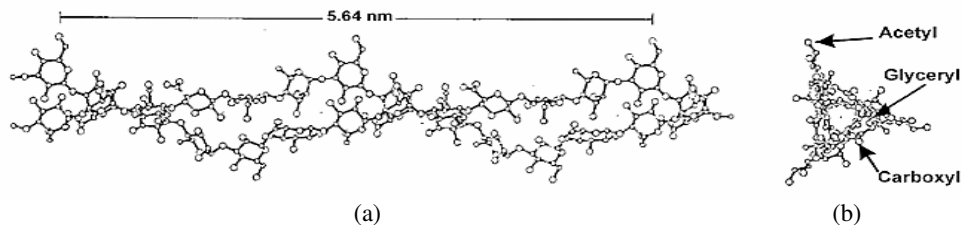

(a) (b)

Figure 2: Double helix of deacetylated gellan structure: (a) viewed perpendicular to the helix axis and (b) viewed along the helix axis (the positions where glyceryl and acetyl substituents are attached to gellan rich in acyl groups, in relation to the carboxyl groups position near the glucuronide residue.[31](Reprinted from *Carbohydrate Research*, R.Chandrasekaran, R. P. Millane, S.Arnott, E.D.T. Atkins, The crystal structure of gellan, **175**(1), 1-15 (1988), with permission from Elsevier)

Industrially, for market purposes, both substituents are removed by treating the fermentation medium with hot alkaline solution. Thus, a deacetylated polymer also known as "gellan gum", branded and sold as Kelcogel (in food industries) or Gelrite (other non-food industries), is obtained.

Gellan rich in acyl groups is marketed as well. The acyl groups can be retained by using mild extraction procedures.The branched chains have been analysed by atomic force microscopy to observe the polymer network resulted from drying the gellan solution on freshly ground mica.

The length among branching points within the gellan linear regions varies considerably, but overall it is 150nm. Gellan macromolecules are paired into a double helix structure (Fig. 2), as proposed by Chandrasekaran *et al.*[31] for the first time in *Carbohydrate Research*, **175**, 1 (1988). The two polymer chains are parallel to each other and are joined exactly in the middle (with a180º chain rotation).

Therefore, the helix has a repeating distance that is half the individual chain, a similar arrangement to that observed in carrageenan.[32]

Gellan gelling properties

Gellan is a microbial anionic exopolysaccharide, water-soluble, and with a molecular weight between $1\text{-}2 \times 10^6$ Da for gellan rich in acyl groups and $2\text{-}3 \times 10^5$Da for deacetylated gellan.[32] Acetylated gellan is soluble in hot water, while deacetylated gellan or gellan containing fewer acyl groups is soluble in both hot andcold water. Gellan properties depend on the number of acyl groups/mole.[14,33]

The heating ofgellan rich in acyl groups at 85-95 ºC is enough to make it soluble in water.When the dispersion of high acyl gellan is heated at 40-50 ºC, it swells and forms a pasty suspension. If the heating process is continued, the suspension suddenly disappears and solubilization is

complete. By cooling, macromolecules undergo a conformational change from an irregular shape to a regular helical structure. In the presence of acetyl groups, the aggregation of helical structure is limited and soft, elastic gels are formed. Acetylated gels do not exhibit syneresis or thermal hysteresis. Gellan gels richin acyl groups have low viscosity. Gelling properties of gellan rich in acyl groups are similar to those of xanthan and locust bean.[14,34]

In the case of deacetylated gellan with few acyl groups, hydration depends on the type and the concentration of the ions. It is soluble in deionized water at room temperature, similar to its sodium salt. The gellan solution turns into a hydrogel when the temperature is changed and by adding a very small concentration of sodium ions in the solution. Compared to other polysaccharide gels, gellan gel is more resistant to temperature and less sensitive to pH.[35,36]

Gellan gelling has been investigated by many researchers, who concluded that it is a two-step process. In the first stage, a conformational change from a disordered conformation to an ordered one consisting of a double helix occurs. Then, the double helix is aggregated and the junction points are formed.[37,38]

Gellan gelling depends on the polymer solution concentration, temperature, and on the presence of monovalent or divalent cations within the solution. Gellan forms an orderly double helix structure at low temperatures, while at higher temperatures it appears as a single polysaccharide chain, which reduces significantly the viscosity of the solution.The transition temperature for the sol-gel phase transformation is 35 ºC, but can vary between 30 and 50 ºC. Gellan gel forms below this temperature. The gelling mechanism consists in the formation of double helical junction zones, followed by the aggregation of double helical segments to create a three dimensional network by complexation with cations and by hydrogen bonds with water.[32,39] It has been suggested that the mechanism for deacetylated gellan gelling (gellan containing fewer acyl groups) in the presence of bivalent ions is different from the mechanism of gelling in the presence of monovalent ions.[40]

In the presence of monovalent cations, gelling occurs with subsequent aggregation of the double helix and at a temperature lower than the temperature necessary to transform the irregular structure into the ordered structure of a double helix. However, when cooling occurs, it is assumed that divalent cations interact immediately with the chain segments of gellan, forming an ordered structure at a temperature above the transition temperature. Thus, the gel networks become very stable to temperature after the gradual addition of cations.[41,42] The monovalent cations decrease electrostatic repulsion by their binding to specific locations in the helical structure around the carboxylic groups of the polymer. Bond strength increases with increasing ionic radius($Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$). While higher concentrations of monovalent cations are needed to obtain gellan gels with optimal strength, lower concentrations of divalent cations are necessary to obtain a gel with the same strength.[32,37]

The gelation with divalent cations is performed by binding them to the sites of the double helix structure of the polymer. High salt or acid concentrations cause excessive aggregation and therefore a decrease in gel strength.

These ordered structures are very stable at high temperature when divalent cations are gradually added. Thus, divalent ions are more effective than the monovalent ones.[40] The divalent cations of transition metals (Zn^{2+}, Cu^{2+} and Pb^{2+}) create stronger gels than those of metals from Group 2.[43] It has been noticed that gels containing Ca^{2+} cations are 1.1-1.4 times more powerful than those containing Mg^{2+} cations, for the same salt concentration.[37]

When monovalent or divalent cations are added during the cooling process, the gellan gelling potential is enhanced. This fact leads to an increase in the number of salt bridges in the junction area. In order to elucidate the relationship between the structure of macromolecules and cations, studies on the mechanism of aggregation of gellan chains during the gelling process and on the rheological properties of polymer solutions and gels have been made.

Circular dichroism spectroscopy was used to observe conformational changes of gellan chains from a disordered structure to a double helical ordered one during the gelling process. A maximum value of the molar ellipticity appears at 201 nm, and the temperature at which the conformational transition occurred becomes higher, while the cations concentration is increased. NMR spectra were also recorded to observe the changes in the mobility of gellan chains during phase transformation (sol-gel transition) in the presence of monovalent and

divalent cations. The formation of hydrogen bonds can also be noted.[44]

Rheological methods are used to monitor the gelling process of viscoelastic fluids because the viscoelasticity of the polymer solution changes considerably at the gel point. Gelling temperature increases with increasing cation concentration.[42,45-47]
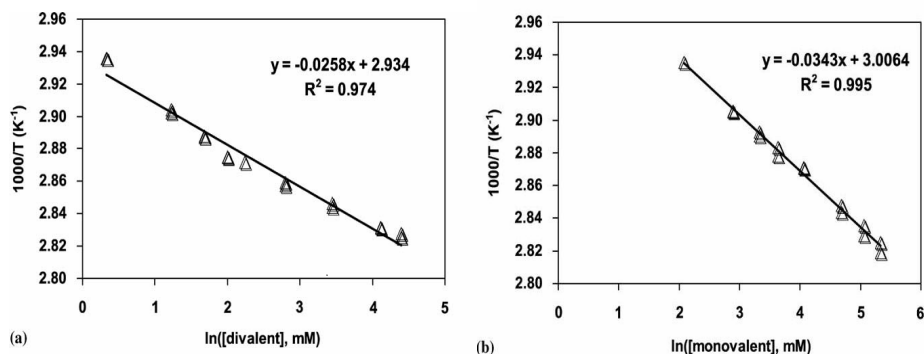

Figure 3: Relationship of 1% HA gellan gelling temperatures with concentrations of (a) divalent cations, and (b) monovalent cations.[42] (Reprinted from *Carbohydrate Polymers*, Y. Huang, J. Tang, B. G. Swanson, B. A. Rasco, Effect of calcium concentration on textural properties of high and low acyl mixed gellan gels, **54**(4), 517-522 (2003), with permission from Elsevier)
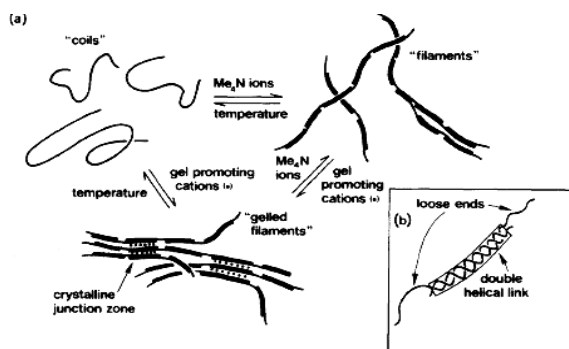

Figure 4: Gelling model proposed by Gunning and Morris (1990).[49] The cations that promote double helical structure aggregation are represented by the filled circles.[49] (Reprinted from *International Journal of Biological Macromolecules*, A.P. Gunning, V.J. Morris, Light scattering studies of tetramethyl ammonium gellan, **12**(6), 338-341 (1990), with permission from Elsevier)

Rheological studies and confocal laser scanning microscopy demonstrate that gellan solutions with concentrations between 0.005-0.05% lead to a gel network formation in the presence of 10 mM CaCl$_2$. At lower gellanconcentrations, the mechanical spectrum shows a gel conformation and the microscopic observations indicate the formation of a three-dimensional network, even if the viscosity of the solution is significantly reduced. The progressive increase of gellan concentration determines an elastic character of the gel systems obtained, but the gel properties are improved depending on the cation concentration.[36,48]

Figure 4 presents a gelling mechanism model proposed by Gunning.[49]

Various studies have been conducted to investigate the factors that influence the gel strength. The most important factors are described below.

Content of acyl groups

Gellan gels with different properties can be obtained depending on the concentration of the acyl groups present in the polysaccharide composition. Researches have proved that this is the most important factor that influences the properties and gel strength.

Native gellan, rich in acyl groups, forms soft elastic, thermoreversible and very weak gels. Acetyl and glyceryl groups prevent the close association between the polymer chains necessary to form the helical structure, as well as the compact packing double helix. Deacetylated gellan forms firm, brittle, and thermoreversible gels due to the absence of acetyl and glyceryl groups.[50]

Type and concentration of ions

The gel strength and its fragility are influenced by ions.Gellan does not form gels in deionized water, but when adding calcium, potassium, sodium, and magnesium salts an improvement of these two properties can be observed. Divalent cations, in particular, are much more effective in influencing the gel strength. High strength gel with a concentration of 0.004% (m/v) calcium ions and of 0.005% (m/v) magnesium ions has been obtained even at very low gellan concentration (0.2% m/v). A similar strong gel can be obtained using a concentration of 0.16% sodium ions and of 0.12% (m/v) potassium ions. A gellan concentration of 0.1-0.2% is suitable for many food systems. From an economical point of view, it is important to get strong gels at low gellan concentrations, using small amounts of salt.[42,51]

Gel pH

Sanderson and Clark[52]showed that the gel strength can be improved when the pH value is 3.5-8. This pH corresponds to the natural pH variation of most food. Changing the pH value does not change the point of gel formation, but in some cases affects the melting temperature. For example, in the case of neutral pH, gels prepared with very low concentrations of monovalent ions are melted at 70°C, but if the pH is 3.5 the melting temperature is slightly higher. This observation has not been madefor divalent ions.[20,52]

Presence of hydrophilic components

The addition of hydrophilic components, such as sucrose (with a concentration of about 10% m/v) decreases ions concentration needed for a strong gellan gel. M. Paoageorgion and S. Kasapis[53] used transmission electron microscopy to examine the change within the nature of a polysaccharide network once sugar levels increase.

Mixtures of deacetylated gellan (<1%) with low (0-20%) and high (80-85%) sugar concentrations have been prepared and studied. Micrographs of gellan gels containing high sugar levels showed clear evidence of cross-linking reduction within the polysaccharide network, which after cooling presented a transition from an elastic consistency to a glassy one.[53]

Tang *et al.*[54] studied the effect of fructose and sucrose on the properties (gelling temperature, gel clarity, and texture) of gellan gels cross-linked with calcium and sodium ions. They observed that increasing with up to 35% the sucrose and fructose concentration within the gels had no effect on the gelling temperature.

Incorporation of fructose and sucrose within the gels has increased their clarity. The effect of sucrose on the gel strength is dependent on the cation concentration. Therefore at low cation concentration, sucrose reinforces gels, but at high concentrations it forms weaker gels.[54]

The sugar influence within the gellan solution was investigated by Miyoshi *et al.*[55,56] In both studies, sugar concentration varied from 0 to 72%. Gel properties were studied using characterization methods such as DSC and rheology. Bayarri *et al.*[57] studied the sucrose effect (0-25%) on three gellan samples with different concentrations (0.3, 0.75, and 1.2%), using compression tests. When the amount of sucrose increased, gels became stronger and less brittle. When higher gellan concentrations were used, the changes were more evident.[57]

Sworn and Kasapis[58] studied the influence of sucrose on the gel strength using sucrose concentrations of more than 40%; they observed that under these conditions the gel strength increased.[58] The addition of glucose or fructose together with sucrose to a concentration of 60% sugars gave a stronger gel, unless only sucrose was added. Furthermore, for gellan gels with added fructose the value of the breaking modulus was higher than for gellan gels with only sucrose added at the same concentration.[59]As a conclusion,from the research conducted by Gibson,[59] the efficiency of sugars in obtaining gellan gels with optimal strength is as follows: fructose> glucose> sucrose.

Miyoshi and Nishinari[55] proposed another order of sugar effectiveness, in contrast to Gibson's results:sucrose> glucose> fructose.

Morris *et al.*[37] reported that this difference is caused by the gellan types used in the two researches. Marketed gellan has higher divalent

cations amounts than the gellan high in sodium ions used by Miyoshi and Nishinari.[54] Marketed gellan containing higher divalent cations used to obtain gels manifested excessive aggregation in the presence of sugar. This aggregation causes a gel strength decrease, leading to the following order of sugar effectiveness in developing stronger gellan gels: fructose> glucose> sucrose.[37]

Temperature and melting point

The gellan is stable at high temperatures and maintains its strength up to 90°C, while the xanthan loses 74% of its original strength after heating at 90°C. According to Sanderson,[52] the melting temperature may be lower or higher than 100°C, depending on the conditions of gel formation. The most important factor responsible for the melting point flexibility is the cation concentration of the gels, because monovalent and divalent cations increase significantly the number of junction zones within the gels and make them more resistant to temperature. Through the possibility of modifying the melting point other conventional thickeners/stabilizers may be replaced, while using much lower polymer concentrations.[33,52,60]

Water absorption

The ability to absorb water is an important parameter for materials used in various food and biomedical applications. The capacity of water absorption of physical and chemical gellan hydrogels has been studied in various environments. Physical hydrogels showed similar behavior in different environments, which means that water absorption is not influenced by the different composition of gellan hydrogels. Only the physical hydrogel with 2% gellan concentration showed low values for swelling parameters and water absorption ratio, especially in a solution simulating gastric fluid (HCl 0.1 M; pH=1). Low pH environment leads to this action and to the development of a more stable junction area. In fact, when pH is 1, polyelectrolyte properties are reduced within the gellan chains, because carboxylic groups are present in their acid form. The polymer chains can be close to each other, leading to the macroscopic reduction of the gel. For chemically cross-linked samples, water absorption is strongly dependent on the composition of both the hydrogel and the solution. Water absorption is inversely proportional to the crosslinking degree of the sample. When the hydrogels are swollen in water,

their reaction is similar to that of superabsorbent materials, showing very high values of swelling parameters.[51]

In physiological saline solution (0.9% g/v) and in a solution that simulates intestinal fluid (phosphate buffer, pH = 7.4), cross-linked and physical hydrogels show low values of swelling degree due to the presence of large amounts of sodium ions. These are counterions that reduce the electrostatic repulsion between carboxylic groups within the polymer chains and thus create a more compact structure. An exception to this rule is the sample with a concentration of 2% chemically crosslinked gellan with 0.1% lysine. In this case, the water absorption is high because the polymer chains display helix conformation and the network, with a relatively low crosslinking, is able to absorb large amounts of water. In a solution that simulates intestinal fluid, there is low water absorption.[61]

Besides molecular weight, chemical structure and functional groups, another important parameter that determines gellan solution properties is the intrinsic viscosity. Higher intrinsic viscosity leads to increased capacity to induce solution viscosity.[62] Intrinsic viscosity depends on molecular weight, molecular structure, pH, ionic strength of the solution, and temperature. It decreases with increasing salt concentration and temperature.[63]

FUNCTIONAL APPLICATIONS OF GELLAN IN FOOD INDUSTRY

The hydrocolloids or gums are natural polymers that form aqueous dispersions or gels when dispersed in water. They form bonds with water molecules due to the numerous hydroxyl groups they possess.[64,65,66] Due to their functional properties, hydrocolloids are very useful in the food industry. They are used as thickening, gelling, emulsifying and stabilizing agents, as coatings (food films), and they have other applications as well. Food industry uses gellan mainly due to its property to modify food viscosity and texture.[67-69]

An increasing incidence of obesity and chronic diseases has been remarked in recent years. This is caused by unhealthy lifestyles and diets low in nutrients and rich in fat foods, which can affect health. Alternative therapies and functional foods are important for the prevention and treatment of these diseases. Numerous products based on hydrocolloids have been developed to replace fat foods.[70]

Gellan is one of the latest hydrocolloids added to the list of permitted food additives in 1988 approved by Japan, and later by the USA (1990) and Europe (1995).[71] Gellan is branded and sold both in deacetylated and acetylated form. Each forms gels with different structures and properties.[59]

Gellan is widely used in different industries, such as pharmacy, food, and biomedicine.[72] In the food industry, due to its functional properties, gellan is frequently used as a polymer to obtain liquid gels, food films, jelly desserts, jams, and as a polymer system to encapsulate many bioactive components.[73] This polysaccharide can be used not only as thickening, gelling, emulsifying, stabilizing and food film formation agent, but also to replace undesired ingredients, especially fat, obtaining low calorie foods, or satiety increasingproducts.[74-76] It can be used as a biopolymer support to encapsulate various bioactive compounds, to protect them and to ensure controlled release and also to obtain functional foods with various benefits in the prevention and treatment of chronic diseases.[77]

As a substitute for gelatin, gellan ensures the structure, texture and flavor in many foods better than gelatin. It is used in pastry and it replaces successfully jellies made of starch. It has lower gelling time and confers an optimal structure and texture to these products. Gellan gels prevent loss of moisture in sweet foods, and confer greater clarity to gelatin desserts. The melting temperature of a gellan gel can be increased, thus keeping foods soft and juicy.[12]

Because gellan is a water-binding agent, it is used to increase stability in foods based on modified starch. It removes the starch effect on food flavor; gives shape and structure after heating-cooling processes applied to many foods (from meat, fruit, and confectionery).[78,79]

Liquid gels

Liquid gels are viscous solutions that display similar properties to those of strong gels when low shear speeds are applied and act as liquids when high shear speeds are applied.

The possibility of creating certain textures and the unique rheological properties of gellan gels have led to the production of liquid gels utilized in the food industry. Because gellan gels prevent particle sedimentation, they are used in fruit pulp juice or liquid foods containing cocoa, fibers, or insoluble minerals. The particles are captured in the gel structure.[80]

The methods for producingliquid gels using gellan gum gels are the following:
- gellan heating at 70-95 ºC, followed by gradual cooling and stirring;
- solution heating and sudden cooling;
- gellan dissolution in cold water and gel formation by adding ions and stirring.[81]

The factors that influence gel formation comprise temperature, gellan concentration, cations concentration, cations type, stirring, and shear stress applied. Any change in these factors leads to different rheological properties for a product that has a constant composition, resulting in elastic gels or fluids.[82]

The mentioned gels are considered physical gels because the connection points are formed by physical bonds, such as hydrogen bonds, hydrophobic bonds, and butt joints using cations. There have been numerous studies on the use of gellan gels. In this regard, gellan was used to stabilize Doogh, a drink produced by milk fermentation containing peppermint particles or wheat insoluble fiber. In all studies, the use of gellan liquid gel prevented the phase separation of Doogh, with changes of the rheological properties. Thus, an appropriate texture for the traditional Iranian drink was developed. The addition of gellan and fiber changed the rheological properties of Doogh from a Newtonian behavior to a pseudoplastic one. Interactions between milk proteins and gellan during the pasteurization process also led to a change of rheological properties.[83,84]

Researches have been made for developing a gel used as food ingredient. This gel was formed from a deacetylated and acetylated gellan mixture to help structure the stomach content and thus, have an effect on satiety. The gellan mixture based gel is composed of separate phases, and DSC analysis shows separate conformational transitions for the two gellan variations at neutral pH. An acid pH affects the gelling properties and the structure of the mixture becomes a semi-interpenetrated network. In obtaining such a gel, the ratio between the biopolymer quantities is of major importance. Acetylated gellan is used in a proportion of about 60%. The storage conditions of these gels can affect their properties. Supplementary studies are needed before using them as satiety increasing food agents.[85]

Mixed gels obtained from many hydrocolloids, used in the food industry, have been investigated as well. The results have shown that food

containing mixed gels exhibits superior features and a gel mixture is much more efficient than using a single hydrocolloid.[86] These gels can provide economic and health benefits as they are used to obtain functional foods. Oxidized cellulose, along with other gelling agents, reduces cholesterol in functional foods.[87,88]

The studies performed on the nutritional gels with carrot pulp proved that they can be prepared with gellan.[89,90]

Soumya Banerjee et al.[91] have studied the properties of a nutritional gel based on agar, gellan, and carrot juice. For gel preparation, equal amounts of hydrocolloid solution (1%), carrot juice, and an amount of sucrose, which ranged from 7.5% to 15%, were used.[91] Alginate gels with carrot juice showed that gel strength decreases with an increase of carrot pulp concentration and increases when a higher polymer concentration is used.[90] Tear strength, compression energy, and syneresis of the gel increase due to carrot juice pectin. Agar gel has a breaking tension smaller than that of the gellan gel and has no effect in the presence of pectin. Mixing the two polymers results in the formation of a more brittle gel. Sucrose improves the formed gel cohesion. These gels are healthy because they are rich in β-carotene and they also seem profitable for marketers.[91]

Food films

Food films are thin biopolymer layers covering food. The properties of biopolymers, such as biodegradability and ability to control dehydration, slow down undesired textural changes and have the capacity to form an adequate barrier against oil absorption into various products. These characteristics have led to using polysaccharides to obtain edible food films.[92]

Biopolymers have a significant role in reducing oil absorption within fried foods. Studies on using biopolymers in food frying have become popular in recent years.[93,94] Gellan used in preparing a traditional Indian fried snack known as *sev* has been researched, and results showed a decrease in oil absorption. As a conclusion, due its thermal gelling property, gellan has the ability to reduce oil absorption.

Gellan reduces dehydration and offers less space for oil absorption in food texture during frying.[95]

Gelatin desserts and jams

Gellan is an economic biopolymer suitable for producing gelatin desserts and jams, since it can be easily gelled and its humidity can be controlled. Gelatin desserts obtained using gellan have a great clarity (transparency) similar to that using gelatin. Because gellan gels have a high melting point, desserts can be kept at room temperature. The gellan concentration is normally of 0.3%.[12]

In jam production, pectin can be replaced by gellan. Gellan offers products with lower syneresis, with desired sensory properties, and fewer calories compared to pectin.[96]

Gellan is suitable to be used in various gelatin desserts due to its functional properties in the presence of sugars. The commercial gellan has a divalent cation content and the gel gets stronger by adding sugars, also the clarity of the gel increases. Gellan is also stable at high temperatures (it resists up to 90 °C) and the jelly confectionery maintains shape and consistency for a longer period of time.

Water absorption is a very important property for the use of gellan gels in foods. Gellan has the ability to absorb large quantities of water and thus, the foods can keep the shape and freshness for a longer period of time.

Microencapsulation

Release systems may be used within food industry to encapsulate and protect the bioactive components like nutraceutics, enzymes and cells. An essential quality of these systems is that they must be compatible with both the bioactive component and the product that will incorporate them to create a functional food or drink. Other system properties include increased stability or controlled release of bioactive components.[77]

Probiotics are microorganisms that can provide health benefits to the human body by keeping bacterial balance inside the digestive tract.[97] The benefits that probiotics provide are: they are antimicrobial agents that maintain healthy intestinal flora, inhibit the growth of pathogenic bacteria, help stimulate the immune system growth, improve vitamin synthesis, and increase calcium absorption when there are enough probiotics in the colon. *Bifidobacterium* and *Lactobacillus* are the best known and the most important probiotics that maintain healthy gastrointestinal tract.[98,99]

Probiotic cell viability in food depends on various factors, such as the pH, the acidification

during food fermentation processes, the production of hydrogen peroxide, and the storage temperature. The microencapsulation method increases cell survival and stability under adverse environmental conditions and release is controlled and targeted to certain sites in the gastrointestinal tract.

Foods that have probiotics incorporated are generally milk products, such as yogurt, cheese, ice cream, dairy desserts, and other foods: chocolate, cereals, juices. Cell viability of microencapsulated probiotics is influenced by a number of factors such as particle size, initial number of cells, bacterial species, type and concentration of the cover substances.[100]

Researches have demonstrated that gellan is a suitable biopolymer for probiotics encapsulation due to its functional properties: biodegradability, resistance to many enzymes in the gastrointestinal tract, high resistance to pH and temperature; also, gel strength can be controlledby the concentration of the ions used.

This biopolymer is not only resistant to acidic conditions, but also it is stabilized by calcium ions, and is able to protect cells in an acid environment. Gellan combined with xanthan was used to cover *Bifidobacterium* species. The results proved the resistance of the microencapsulated bacteria to acidic conditions.[101]

Studies have been reported on encapsulating *Lactobacillus casei* cells in a gel matrix formed by a mixture of biopolymers (sodium caseinate and gellan), using a water-in-oil emulsionas producing method. The gelling of the biopolymer mixture was achieved by progressive pH decrease with glucono-δ-lactone. An 89.5% encapsulation efficiency was obtained. *In vitro* tests carried out to observe the survival rate of the cells were performed by incubating the cells for 30 min in simulated gastric fluid. It was observed that the viability of theencapsulated cells was higher than that of free cells. Even if the results are promising, further studies are needed to use these biopolymers as encapsulating agents for probiotic bacteria in foods.[102]

Supports and immobilization techniques have been proposed and tested in various applications for the alcoholic beverage production. Due to gellan's lack of toxicity, its capacity of resisting to high differences of pH and to its ability to form gels in the presence of different types of ions, it was used as support for immobilization of beer yeast. The gellan matrix in which the cells were immobilized protects them against the toxic metabolites produced by ethanol and maintains the viability of the immobilized cells. A biocatalyst consisting of spherical gellan particles with immobilized yeast cells, with real prospects of being used in sparkling wine production technology, was proposed. The beads were developed by extruding the gel formed through a capillary in a bath of 2% $CaCl_2$ solution, used as ionic cross-linking agent.[103]

Tan *et al.*[104] have evaluated the feasibility of encapsulating yeast cells into a gellan gum support, obtained through the emulsification method, and the reuse of the resulted microbio-reactors. Ethanol production during the first three cycles using the microbioreactors was comparable to that of using free yeast. The microbioreactors are stable and can be easily recovered from the fermentation medium by filtration. Therefore, they can be reused for at least 10 fermentation cycles with a relatively high yield of ethanol.[104]

Protein-based ingredients can be administered orally. They have a reduced capacity to overcome the epithelial intestinal barrier since they are sensitive to the enzymes from the gastrointestinal tract.[105,106] Studies have shown that delivery systems based on biopolymers are the most suitable for transport and controlled release of proteins inside the gastrointestinal tract. Polymeric systems can protect proteins against the enzymes of the gastrointestinal tract. Thus, the proteins and peptides are absorbed into the colon and not in the preceding digestive tract segments.[107]

Gellan is a biodegradable and non-toxic polymer; it has a very stable pH value between 2 and 10.[108] It is resistant to the action of enzymes, such as pectinase, amylase, cellulase, papain and lipase.[5] Significant gellan degradation occurs in the presence of galactomanase, which allows the release of bioactive components from the polymeric system into colon fluids.[109]

Fei Yang *et al.*[110] have prepared and evaluated spherical particles of chitosan and gellan obtained by ionotropic gelation and polyelectrolyte complexation for encapsulation and controlled release of proteins by using calcium ions to induce ion gelling. They incorporated albumin in chitosan and gellan spherical particles with an encapsulation efficiency between 65-85%. The encapsulation efficiency and protein release rate depend on the chitosan, gellan, and calcium concentration. Higher gellan concentrations combined with vacuum drying decrease the rapid release of proteins at pH = 1.2. A sustained

release of proteins occurs at pH = 6.8, and an effective protein release is observed at pH = 7. The spherical biopolymer particles thus obtained can be successfully used for controlled release of functional ingredients based on proteins.[110]

CONCLUSION

Gellan is an exopolysaccharide, which has a polyanionic character and presents conformational modifications depending on temperature and pH. Gellan physical gels present weak mechanical properties, but this feature can be improved by using chemical cross-linking of gellan alone or in combination with other synthetic polymers. Due to its biocompatible and biodegradable properties, gellan is used in various applications, especially in the food industry (biochemical technologies, encapsulation systems of nutraceutics, and obtaining of functional ingredients based on proteins), pharmaceutical industry and medicine.

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