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review

Gellan Gum: Fermentative Production, Downstream Processing and Applications

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Summary

The microbial exopolysaccharides are water-soluble polymers secreted by microorganisms during fermentation. The biopolymer gellan gum is a relatively recent addition to the family of microbial polysaccharides that is gaining much importance in food, pharmaceutical and chemical industries due to its novel properties. It is commercially produced by C. P. Kelco in Japan and the USA. Further research and development in biopolymer technology is expected to expand its use. This article presents a critical review of the available information on the gellan gum synthesized by *Sphingomonas paucimobilis* with special emphasis on its fermentative production and downstream processing. Rheological behaviour of fermentation broth during fermentative production of gellan gum and problems associated with mass transfer have been addressed. Information on the biosynthetic pathway of gellan gum, enzymes and precursors involved in gellan gum production and application of metabolic engineering for enhancement of yield of gellan gum has been specified. Characteristics of gellan gum with respect to its structure, physicochemical properties, rheology of its solutions and gel formation behaviour are discussed. An attempt has also been made to review the current and potential applications of gellan gum in food, pharmaceutical and other industries.

Key words: gellan gum, *Sphingomonas paucimobilis*, fermentation

Introduction

Microbial exopolysaccharides have found a wide range of applications in the food, pharmaceutical and other industries due to their unique structure and physical properties. Some of these applications include their use as emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening and suspending agents (*1*). These biopolymers are rapidly emerging as industrially important, and are gradually becoming economically competitive with natural gums produced from marine algae and other plants.

Microbial polysaccharides are water-soluble polymers and may be ionic or non-ionic. The repeating units of these exopolysaccharides are regular, branched or unbranched, and are connected by glycosidic linkages. Some microbial polysaccharides are commercially accepted, while others are at various stages of development. Currently a small number of biopolymers are produced commercially on a large scale (*2*). Among the biopolymers which are either currently commercial products or which have been the subject of extensive studies are xanthan from *Xanthomonas campestris*, gellan and a range of structurally related polysaccharides from the strain of *Sphingomonas paucimobilis*, bacterial alginates secreted by *Pseudomonas* sp., *Azotobacter vinelandii* and *Azotobacter chrococcum*. Small amounts of bacterial cellulose from *Acetobacter xylinium*, hyaluronic acid from *Streptococcus equii* and succinoglycan from *Rhizobium* have also found application (*3*).

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Gellan gum is one of the industrially useful exopolysaccharides due to its various functional properties. It is a sphingan group of heteropolysaccharides secreted by members of the bacterial genus *Sphingomonas* (*4*). It is currently produced by C. P. Kelco in Japan and the USA. It is marketed with four different trade names: Kelcogel, Gelrite, Phytagel and Gel-Gro. Kelcogel is widely used in food industry as a thickener and gelling agent, whereas Gelrite, Phytagel and Gel-Gro are used as solidifying agent, a substitute for agar in media for microbial growth and plant tissue culture.

History

Gellan gum is the generic name for extracellular polysaccharide produced by bacterium *Pseudomonas elodea*. Kaneko and Kang (*5*) discovered the polymer in the laboratory of the Kelco Division of Merck and Co., California, USA in 1978. It had previously been referred to by the code names S-60 or PS-60. The gellan gum-producing microorganism was isolated from the *Elodea* plant tissue. Further studies revealed that the bacterium was a new strain of the species *Pseudomonas*, and hence termed as *Pseudomonas elodea* (*6*). In 1994, it was discovered that gellan-producing bacterium was *Sphingomonas paucimobilis*, and classified in the α-4 subclass of the *Proteobacteria* (*7*). Successful toxicity trials were completed and gellan gum received approval for use in food in Japan in 1988. The US FDA approved gellan gum for use as a food additive in 1992 (*8*).

Specifications for gellan gum were prepared at the 46th Joint Expert Committee on Food Additives (JECFA) in 1996 (*9*) and published in FNP 52 Add 4 in 1996. These are summarized in Table 1.

Strains Producing Gellan Gum

Sphingomonas is a group of Gram-negative, rod-shaped, chemoheterotrophic, strictly aerobic bacteria containing glycosphingolipids (GSLs) in their cell envelopes, and they typically produce yellow-pigmented colonies (*10*).

Some *Sphingomonas* species are not motile and not capable of fermentative metabolism (strictly aerobic), but they all contain a series of unusual components, that is, 18 or 21 carbon straight chain saturated or monosaturated dihydrosphingosines, or cyclopropane-containing dihydrosphingosines in ceramide glycolipid. The glycolipid contains an amide-2-hydroxyfatty acid, which is an indicator of novel lipid composition (*11*).

The bacterium used for the industrial production of gellan gum is *Sphingomonas paucimobilis* ATCC 31461 (*12*). Some researchers have isolated new strains producing gellan gum, but their use for commercial production has not been reported. Different strains producing gellan gum are enlisted in Table 2 (*12–16*).

Table 1. Specifications for gellan gum (*9*)

Property	Value
Definition	Gellan gum is high molecular mass polysaccharide gum produced by a pure culture fermentation of carbohydrates by *Pseudomonas elodea*, purified by recovery with isopropyl alcohol, dried, and milled. The high molecular mass polysaccharide is principally composed of tetracyclic repeating unit of one rhamnose, one glucuronic acid, and two glucose units and is substituted with acyl group as the O-glycosidically-linked esters. The glucuronic acid is converted to potassium, sodium, calcium and magnesium salt. It usually contains small amount of nitrogen-containing compounds resulting from fermentation procedures
Molecular mass	Approximately 500 000
Description	Off-white powder
Functional uses	Thickening agent, gelling agent, stabilizer, *etc*.
Solubility	Soluble in water, forming viscous solution; insoluble in ethanol
Loss during drying	Not more than 15 % (105 °C, 2.5 h)
Lead	Not more than 2 mg/kg
Nitrogen	Not more than 3 %
Gel test with calcium ion	Add 1.0 g of sample to 99 mL of water, and stir for about 2 h. Draw a small amount of this solution into a wide bore pipette and transfer to a 10 % solution of calcium chloride. A tough worm-like gel will be formed immediately
Gel test with sodium ion	To the 1 % solution of the sample, add 0.5 g of sodium chloride, heat to 80 °C by stirring and hold at 80 °C for 1 min. Allow solution to cool to room temperature. A firm gel will form
Isopropyl alcohol	Not more than 750 mg/kg
Microbiological criteria	
1. Total plate count	Not more than 10 000 colonies per gram
2. *E. coli*	Negative by test
3. *Salmonella*	Negative by test
4. Yeasts and moulds	Not more than 400 colonies per gram

Table 2. Organisms producing gellan gum

Strain	Gellan gum yield g/L	Reference
Sphingomonas paucimobilis ATCC 31461	35.70	*12,13*
Sphingomonas paucimobilis E2 (DSM 6314)	8.73	*14*
Sphingomonas paucimobilis NK2000	7.33	*15*
Sphingomonas paucimobilis GS1	6.60	*16*

Composition of Different Types of Gellan Gum

The repeating unit of gellan polysaccharide is composed of β-D-glucose (D-Glc), L-rhamnose (L-Rha), and D-glucuronic acid (D-GlcA). The composition is approximately: glucose 60 %, rhamnose 20 % and glucuronic acid 20 %. In addition, considerable amount of non-polysaccharide material is found in gellan gum (cell protein and ash) that can be removed by filtration or centrifugation (*17,18*). An example of chemical composition of different type of gellan gum is illustrated in Table 3.

Table 3. Composition of different types of gellan gum (*12*)

Gellan gum	Neutral sugars Glc/Rha=6/4	*w*(uronic acid) %	*w*(acetyl group) %	*w*(protein) %	*w*(ash) %
Native	69.0	11	3	10	7.0
Deacetylated	62.0	13	0	17	8.0
Deacetylated and clarified	66.5	22	0	2	9.5

Biosynthetic Pathway of Gellan Gum

Many researchers have investigated the pathway for the synthesis of repeating tetrasaccharide units of gellan gum by *Sphingomonas paucimobilis* (*19,20*). The route of gellan synthesis, role of enzymes involved and some process conditions supporting optimum production of gellan gum have been described.

The route of gellan synthesis

Vartak *et al.* (*20*) studied gellan gum biosynthesis in two strains of *Sphingomonas paucimobilis*, the wild type and a polyhydroxybutyrate (PHB) deficient mutant. Enzyme analysis suggested that in both strains glucose utilization was initiated by the action of glucokinase and glucose dehydrogenase. No exogenous gluconate utilization was observed.

Sphingomonas paucimobilis catabolizes glucose *via* the Embden-Meyerhof pathway (glycolysis), or the pentose phosphate pathway. The Embden-Meyerhof pathway apparently does not have a role in glucose degradation, because no phosphofructokinase activity, a key enzyme in glycolysis, has been detected (*20*). Fig. 1 illustrates the proposed scheme for glucose catabolism in *Sphingomonas paucimobilis.* According to the proposed pathway glucose has to enter the cell before it is degraded. Either of the following steps is used for glucose uptake:

$$\text{glucose}_{out} \rightarrow \text{glucose}_{in} \rightarrow \text{gluconate} \rightarrow \rightarrow \text{gluconate-6-phosphate}$$

or

$$\text{glucose}_{out} \rightarrow \text{gluconate-6-phosphate}_{in} \rightarrow \rightarrow \text{gluconate-6-phosphate}$$

Mutant strain lacking G6PD (glucose-6-phosphate dehydrogenase) showed no difference in rates of glucose utilization, gellan production or CO_2 production suggesting that this enzyme is not essential for glucose metabolism in *Sphingomonas* (*19,20*). This indicates that either the main route of glucose utilization involves glucose dehydrogenase or gluconate kinase, or the absence of G6PD induces a compensatory increase in these enzymes. As yet, however, there is no clear indication of which mechanism occurs (*21*).

Martins and Sá-Correia (*19*) proposed a possible pathway for the synthesis of repeating tetrasaccharide unit of gellan gum. They assumed that gellan synthesis requires activated precursors before the repeating unit is assembled, similar to other exopolysaccharides synthesized in the cell wall of microorganisms. These gellan precursors were detected by enzyme assays, and were found to be nucleotide diphosphate sugars, *viz*. UDP--glucose, TDP-rhamnose and UDP-glucuronic acid. The proposed biosynthetic pathway is shown in Fig. 2 (*14*).

Glucose-6-phosphate seems to occupy a key position from which two routes commence, one leading to uridine-5-diphosphate glucose (UDPG) and the other leading to thymine-5-diphosphate glucose (TDPG). In turn, UDPG induces D-glucose and D-glucuronic acid synthesis and TDPG leads to the synthesis of rhamnose. The combination of these three compounds presumably results in the synthesis of gellan (*22*). However, the reactions leading to binding of these three monomers have not been clearly elucidated.

Specific activities of gellan synthetic enzymes

Conditions that favour gellan gum formation might be expected to increase the levels of the enzyme responsible for the formation of precursors. Phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM) possess the highest activities in cell-free extracts (*in vitro*) as they play multiple roles in the cell metabolism. The enzymes UDPG phosphorylase (UGP) and TDPG phosphorylase (TGP) appeared to have values of speci-

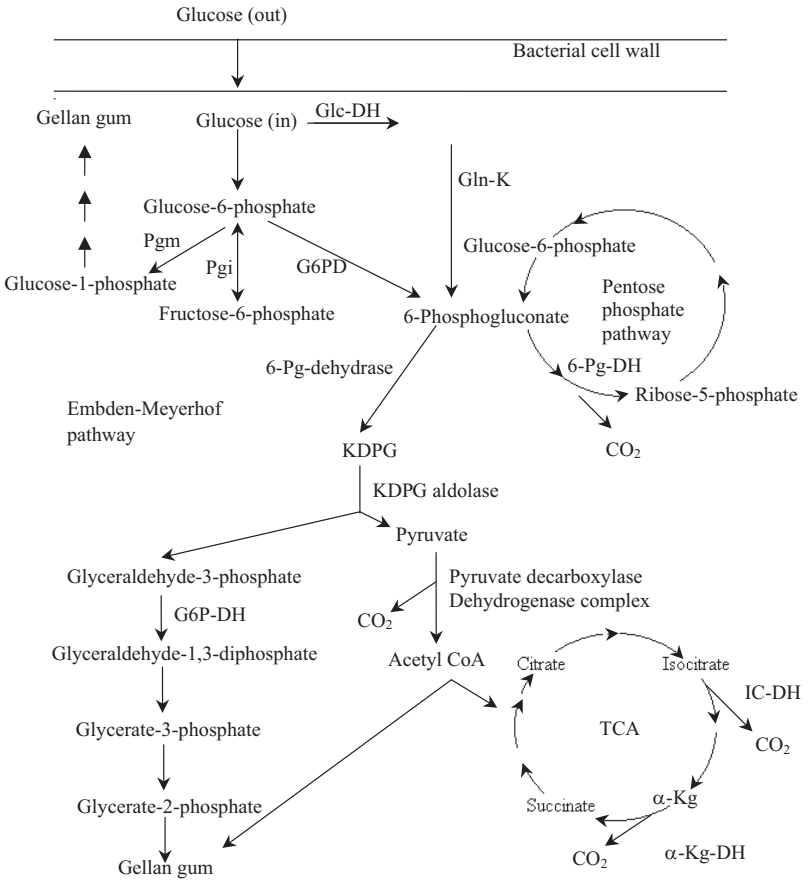

Fig. 1. Proposed pathway for glucose catabolism in *S. paucimobilis* (*19*)
α-Kg, α-ketoglutamate; **Glc-DH,** glucose dehydrogenase; **Glc-K,** glucose kinase; **Gln-K,** gluconate kinase; **IC-DH,** isocitrate dehydrogenase; **G6PD** glucose-6-phosphate dehydrogenase**, TCA,** tricarboxylic acid cycle; **KDPG,** 2-keto-3-deoxy-6-phosphogluconate; **Pg,** phosphogluconate; **Pgm**, phosphoglucomutase; **G3P-DH**, glyceraldehyde-3-phosphate dehydrogenase; **Pgi**, phosphoglucoisomerase

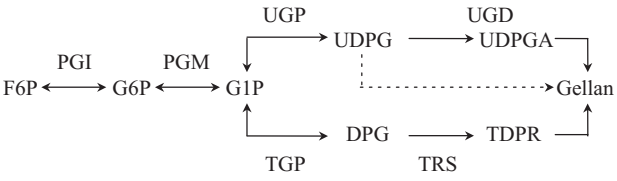

Fig. 2. Postulated pathway leading to the nucleotide-sugar precursors presumed to be involved in biosynthesis of gellan gum (*19*)
F6P, fructose-6-phosphate; **G6P,** glucose-6-phosphate; **G1P,** glucose-1-phosphate; **UDPG,** uridine-5-diphosphate-D-glucose; **TDPG,** thimidine-5-diphosphate-D-glucose; **UDPGA,** uridine-5-diphosphate-D-glucuronic acid; **PGI,** phosphoglucose isomerase; **TDPR,** thimidine-5-diphosphate-L-rhamnose; **PGM,** phosphoglucose mutase; **UGP,** UDPG phosphorylase**; TGP,** TDPG phosphorylase; **TRS**, TDPR synthetase

fic activities lower than PGI and PGM. TDPR synthetase (TRS) and UGD are the least active and the most thermosensitive enzymes above 30 °C. They are essential for synthesis of rhamnose (TRS) and glucuronic acid (UGD). The activity of these enzymes presumably limits gellan synthesis, especially at temperatures higher than 30 °C (*22*). Additionally, it has been found that isocitrate isomerase, which is involved in CO_2 production through the carboxylic acid cycle, has very high specific activity *in vitro* (*20*). This might represent an unfavourable reaction for industrial purposes, because glucose is not channelled towards gellan gum production. Apart from the enzymes mentioned, there must be other enzymes that influence gellan synthesis after the formation of gellan precursors.

Genetic engineering of the gellan pathway

The most exciting prospects for gellan modification and increasing production yield are found in genetic engineering. Some attempts have been made to increase the relatively low conversion efficiency of gellan from glucose in *S. paucimobilis* ATCC 31461. By site-specific mutagenesis, the G6PD gene encoding glucose-6-phosphate dehydrogenase was inactivated, envisaging diversion of the carbon flow toward gellan synthesis, apparently without the expected results (*20*).

Identification of a few genes and elucidation of crucial steps of the gellan biosynthesis pathway indicated some possibilities of exerting control over gellan production at any of the three levels of its biosynthesis: (*i*) at the level of synthesis of sugar-activated precursors, (*ii*) at the level of the repeat unit assembly and of gellan, (*iii*) at polymerization and export. By modifying expres-

sion of any of the individual, or of a group of these genes the conversion efficiency and gellan gum yield can be increased.

In spite of recent advances in the elucidation of the gellan biosynthetic pathway, a better knowledge of the poorly understood steps and of the regulation and bottlenecks of the pathway is crucial for the eventual success of the metabolic engineering of gellan production.

Fermentative Production of Gellan Gum

The growth media suitable for the production of different exopolysaccharides by microorganisms vary widely, and this probably reflects the differing role of each exopolysaccharide in nature. It is instructive to consider the effect on polymer biosynthesis rates, yields and composition of varying growth media during fermentative production of these exopolysaccharides (*23*).

Factors affecting gellan gum production

Media components

The media used for production of gellan gum are simple media containing carbon source, nitrogen source and inorganic salts. The exact quantity of carbon utilization depends in part upon the other ingredients of the medium (*12*). A copious secretion of exopolysaccharide is usually most noticeable when the bacteria are supplied with an abundant carbon source and minimal nitrogen (*4*). Sometimes complex medium ingredients supplying vitamins can also enhance the cell growth and production (*21,24*). Effects of various medium components on gellan gum production are as follows.

Effect of carbon source on gellan gum production

Carbon source is the most important component of the media used for the production of exopolysaccharides because it directly affects the production yields, compositions, structures, and properties of bacterial exopolysaccharide (*25*). According to Kang *et al*. (*12*), carbohydrates such as glucose, fructose, maltose, sucrose and mannitol can be used either alone or in combination as carbon source. The amount of carbon source usually varies between 2–4 % by mass. Kang *et al*. (*12*) and Lobas *et al*. (*26*) used glucose as carbon source for production of gellan gum with approximate yields of 8–10 g/L. Ashtaputre and Shah (*14*) studied sucrose as carbon source for gellan gum production using *Sphingomonas paucimobilis* GS1 and obtained yield of 6.6 g/L of gellan gum. Fialho *et al*. (*25*) compared gellan gum production by using glucose, lactose and sweet cheese whey as carbon source and yields obtained were 14.5, 10.2 and 7.9 g/L, respectively. Nampoothiri *et al*. (*16*) and Bajaj *et al*. (*13*) compared soluble starch, glucose, lactose, maltose and sucrose as carbon source for gellan gum production and found soluble starch to be the best carbon source for gellan gum production with yields of 24–28 g/L.

Banik *et al*. (*27*) developed a molasses-based medium for the production of gellan by *Sphingomonas paucimobilis* ATCC 31461. They applied Plackett–Burman design criterion to study the effect of various nutrient supplements on gellan production using molasses. Among the 20 variables tested, molasses, tryptone, casamino acid, disodium hydrogen orthophosphate and manganese chloride showed significant effect on gellan production. Molasses 112.5 g/L, tryptone 1 g/L, casamino acid 1 g/L, disodium hydrogen orthophosphate 1 g/L and manganese chloride 0.947 g/L produced maximum (13.81 g/L) gellan gum.

Effect of nitrogen source on gellan gum production

Following carbon source, nitrogen is the most important medium component for gellan gum production. In general, the type and concentration of nitrogen source in the medium influenced the flow of carbon to either biomass or product formation (*14*). Abundant secretion of the exopolysaccharide is usually most noticeable when bacteria are supplied with abundant carbon source and minimal nitrogen (*4*).

The choice of the nitrogen source has strong effect on gellan broth characteristics. Dreveton *et al*. (*28*) reported that organic nitrogen accelerates cell growth and biosynthesis of gellan gum. Hence broth with organic nitrogen is more viscous as compared to the broth without organic nitrogen, and therefore requires proper impeller system to provide enough oxygen transfer during gellan gum production.

Organic nitrogen sources like corn steep liquor (*12*) and inorganic nitrogen sources like ammonium nitrate (*26*) and potassium nitrate (*14*) have been tried for gellan gum production. Hyuck *et al*. (*29*) compared bactopeptone and soybean pomace (an agroindustrial by-product) for gellan gum production from *Sphingomonas paucimobilis* NK 2000 and achieved maximum yield of 3.27 and 7.33 g/L, respectively. Nampoothiri *et al*. (*16*) compared various organic and inorganic nitrogen sources for gellan gum production from *Sphingomonas paucimobilis* ATCC 31461, and reported maximum gellan gum production of 32.1 g/L with tryptone. Bajaj *et al*. (*13*) studied the effect of different nitrogen sources on gellan gum production. Among the various nitrogen sources used, yeast extract supported the maximum gellan gum production.

Effect of the addition of precursors

Addition of precursor molecules is of considerable importance in the polysaccharide synthesis in terms of metabolic driving force. In case of polysaccharides, higher intracellular levels of nucleotide phosphate sugars under nitrogen-limited conditions reportedly enhance metabolite flux of exopolysaccharide synthesis (*15*).

Many researchers have described the pathway for the synthesis of the repeating tetrasaccharide unit of gellan gum by *Sphingomonas paucimobilis* (*19,20*). It is assumed that gellan synthesis requires activated precursors before the repeating unit is assembled. These gellan precursors were detected by enzyme assays, and they were found to be nucleotide phosphate sugars (*21*). The repeating unit of gellan gum is a tetrasaccharide composed of the glucose, rhamnose and glucuronic acid. The sugar nucleotides providing the activated precursors for synthesis of this tetrasaccharide are assumed to be respectively UDP-glucose, TDP-rhamnose and UDP--glucuronic acid. Bajaj *et al*. (*13*) studied the effect of the addition of guanosine-5′-monophospate (GMP), uridine-

-5′-diphospate (UDP), adenosine-5′-diphospate (ADP), cytidine-5′-monophospate (CMP), and adenosine-5′-triphospate (ATP) on gellan gum production, and observed that ADP at 1 mM to give maximum gellan gum (32.15 g/L) production.

Media used for production of gellan gum usually contain complex medium ingredients that supply vitamins and amino acids to enhance cell growth and gellan production (*21*). Amino acids have been used by some researchers as nitrogen source or as stimulator for improving gellan gum production (*14*,*16*). Studies carried out by Bajaj *et al*. (*13*) demonstrated that tryptophan at 0.05 % concentration gave maximum (39.5 g/L) yield of gellan gum.

pH

pH plays a very important role in production of gellan by *Sphingomonas paucimobilis*, as it significantly influences both cell growth and product formation. In general, optimal pH value for bacterial exopolysaccharide production is somewhat higher than that of the fungal glucan production (*21*). The pH value usually recommended for gellan production ranges from 6.5 to 7 (*12*, *13*,*28*,*30*). More acidic or more alkaline environment reduces the cell growth, and consequently gellan production (*13*,*16*).

Agitation rate

Dreveton *et al*. (*28*) studied the effect of agitation rate on gellan gum production. Fermentations were carried out in a 14-liter vessel with an initial working volume of 10 L. Culture temperature was maintained at 30 °C and pH of the broth was regulated at pH=6.5.

An agitation of 250 rpm using a helical ribbon impeller is adequate for the mixing of gellan gum broth. Lower levels of agitation were insufficient for homogenous conditions and the broth exhibited gelling characteristics. On the other hand, the same authors observed high stirring rates (600 to 800 rpm) with Rushton turbines to lead to cavitations in impeller zone suggesting that high shear thinning properties of the broth result in formation of stagnant zone. Consequently, the medium became heterogeneous with increasing agitation rate. This is a major drawback as it causes limitations in heat and mass transfer, and substrate exhaustion could occur in stagnant zones (*28*).

Giavasis *et al*. (*31*) investigated the effects of agitation and aeration on the synthesis and molecular mass of the gellan gum in batch fermentor cultures of the bacterium *Sphingomonas paucimobilis*. High aeration rates and vigorous agitation enhanced the growth of *S. paucimobilis*. Although gellan formation occurred mainly parallel with cell growth, the increase in cells able to synthesize gellan did not always lead to high gellan production. For example, at very high agitation rates (1000 rpm) growth was stimulated at the expense of biopolymer synthesis. Maximal gellan gum concentration can be obtained at the agitation of 500 rpm at 1 and 2 vvm aeration (12.3–12.4 g/L gellan). At low agitation rates (250 rpm), an increase in aeration from 1 to 2 vvm enhances gellan synthesis.

Banik and Santhiagu (*32*) studied the effect of agitation rate on cell growth and gellan gum production. Growth of *Sphingomonas paucimobilis* increased up to 5.4 g dry cells/L with an agitation rate of up to 700 rpm. Specific growth rate was high at 700 rpm (0.38 h^{-1}) and was comparatively low at 1000 rpm (0.29 h^{-1}). This was contrary to the report given by Giavasis *et al*. (*31*), in which the authors reported higher cell growth at 1000 rpm. Gellan production increased up to 500 rpm (14 g/L) due to increased mass and oxygen transfer and decreased at 700 rpm (13 g/L) because of stimulation of cell growth.

Dissolved oxygen and oxygen transfer capacity

Rho *et al*. (*33*) suggested O_2 to be vital for gellan synthesis, as depletion in oxygen concentration decreased the growth, and hence gellan gum production. The best gas dispersion conditions of the turbine systems were accomplished by high gellan production (*28*). In contrast to the above, Rau *et al*. (*34*) observed an improvement of exopolysaccharide production when cultures of *Sclerotium glucanicum* were grown under limited oxygen supply. Clearly the high oxygenation rate that promotes optimal gellan synthesis is in distinct contrast with the low or limiting oxygen levels which contribute to high concentrations of fungal glucans. One explanation for these observations may be that in the case of glucans, exopolysaccharide synthesis follows the growth phase; whereas with gellan, biopolymer is produced at a higher rate during the growth phase.

Banik and Santhiagu (*32*) studied the effect of dissolved oxygen tension (DOT) on cell growth and gellan gum production, and found that DOT levels above 20 % have no effect on cell growth; gellan gum yield, however, increased to 23 g/L with increase in DOT level to 100 %. DOT level acts as a driving force for increasing oxygen uptake rate by the cells, which resulted in higher gellan production. Higher DOT levels reportedly improve the viscosity and molecular mass of the polymer with change in acetate and glycerate content of the polymer (*32*).

Temperature

Most of the fermentations involving gellan gum production are carried out at 30 °C (*15*,*29*). However, it is reported that gellan yield reaches its peak at 20 °C, remains quite high at 25 °C, and significantly decreases above 30 °C (*35*).

Influence of fermentation hydrodynamics on the physicochemical properties of gellan gum

Dreveton *et al*. (*36*) revealed that the degree of esterification, the average molecular mass and the intrinsic viscosity of the gellan polymer depend on the fermentor hydrodynamics. Comparing several helical ribbon impellers with Rushton turbine impellers, they found that degree of esterification with acetate and glycerate was higher for products produced by process using HR250 and HR125 impellers, both of which are characterized by oxygen limitation. Hence, it was assumed that acetate and glycerate substitutes are related to oxygen limitation or the physiological state of the cells.

Using a range of impellers and dissolved oxygen regimes, Dreveton *et al*. (*36*) noted that gellan molecular mass is related to the degree of homogeneity in the fer-

mentor. Under the most homogeneous conditions, the average molecular mass of gellan gum doubled compared to heterogeneous conditions. They also noted that the least viscous broth had the lowest molecular mass biopolymer, and intrinsic viscosity of gellan gum broth seemed to be a function of molecular mass of gellan gum. However, oxygen limitation did not seem to influence the molecular mass of gellan gum.

Recently, Wang *et al*. (*37*) have proposed kinetic model for understanding, controlling, and optimizing the fermentation process for gellan gum production. Fermentation was carried out by *Sphingomonas paucimobilis* ATCC 31461. Logistic and Luedeking-Piret models were confirmed to provide a good description of gellan gum fermentation. Analysis of kinetics in batch fermentation process demonstrated that gellan gum production is largely growth associated. Based on the model prediction, fed--batch fermentation for gellan gum production was carried out. Higher gellan gum production and higher conversion efficiency were obtained at the same total substrate concentration.

Rheology of the Fermentation Broth

The rheology of the fermentation fluid during gellan gum production exhibits strongly pseudoplastic behaviour, even at 0.1 % (by mass per volume). The initial broth viscosity is that of Newtonian fluid with a viscosity close to that of water, but the broth rapidly becomes non-Newtonian with strong shear thinning properties. This pseudoplastic behaviour during the exopolysaccharide accumulation phase is also common in the production of other microbial polymers and described by power law model (*28*):

$$\eta = \tau/\gamma = k\gamma^{n-1} \qquad /1/$$

where η stands for broth viscosity, τ is the shear stress and γ is the shear rate. The model has two independent parameters: the shear thinning index n (equal to 1 for Newtonian fluid and decreasing to 0 with increasing degree of shear thinning) and consistency index k. Dreveton *et al*. (*28*) found that the value of the index n dropped quickly from an initial value of around 1 to 0.30 within 9 h of culture and remained constant thereafter. The consistency index, k, increased steadily with polymer concentration. The power law is, however, valid only if the stress τ exceeds the critical value τ_c (which is extremely difficult to determine). The yield stress, τ_0, is another important factor that describes the shear stress at the very beginning of the pseudoplastic behaviour, corresponding to the first non-zero shear rate. The square root of this parameter was found to be linear function of the gellan concentration.

Dreveton *et al*. (*28*) reported that viscosity of fermentation broth during production of gellan gum depends on media constituents. Growth without organic nitrogen source resulted in a broth of low consistency and intense shear thinning behaviour. Fermentation parameters were also reported to influence the viscosity of fermentation broth. Viscosity of fermentation broth appears higher at higher agitation rate.

Isolation and Purification of Gellan Gum

Optimization of fermentation parameters alone is not enough to ensure high yield of gellan gum. The next crucial step after the completion of successful fermentation is the recovery and purification of gellan.

Recovery of gellan gum

In the recovery process described by Kang *et al*. (*12*), the culture broth is first heated to 90–95 °C for 10–15 min. The heating step not only kills the cells, which remain with the capsular polysaccharide, but also gently reduces the viscosity of the broth and this facilitates mixing during precipitation. The polysaccharide is separated from the cells by filtration or centrifugation. Cell-free supernatant was added to ice-cold isopropyl alcohol and the mixture was kept at 4 °C for 12 h for complete precipitation of gellan gum. The precipitate formed was then recovered by centrifuging. After gellan recovery, the product was dried at 55 °C for 1 h. Perhaps lyophilization of gellan could offer another alternative for formulating dry gellan powder (*29*). Clarified gellan gum was obtained by filtration of the hot fermentation broth with cartilage filters (0.2 μ), followed by precipitation with isopropyl alcohol (*12*).

Purification of gellan gum

The gellan gum obtained after alcohol precipitation was washed repeatedly with acetone and ether, dissolved in deionised water and dialyzed against deionised water by using dialysis tubing with molecular mass cut-off of 12 000–14 000. After dialysis for 2–3 days with four or five changes of deionised water, the solution was lyophilized to formulate dry gellan powder (*29*). Chromatographic methods like gel filtration chromatography (GFC) can also be used for the purification of gellan gum, although any such report has not yet been available.

Deproteinization of gellan gum

Deproteinization is a technical bottleneck in the purification of viscous water-soluble polysaccharides. Wang *et al*. (*38*) investigated the effectiveness of several methods of deproteinization including Sevag method, alkaline protease, papain and neutral protease for deproteinization of crude gellan gum. The results revealed that using Sevag method deproteinization efficiency of 87.9 % was achieved, but recovery efficiency of gellan gum (28.6 %) was unsatisfactory, making it unsuitable in industrial applications. Deproteinization by alkaline protease was most suitable with high polysaccharide recovery (89.3 %) and high deproteinization efficiency (86.4 %).

Types of Gellan Gum

Native gellan gum

Native gellan gum consists of a backbone of repeating unit of β-1,3-D-glucose, β-1,4-D-glucuronic acid, β-1,3--D-glucose, α-1,4-L-rhamnose, and two acyl groups, acetate and glycerate, bound to glucose residue adjacent to glucuronic acid (*18*).

Deacetylated gellan gum

The acetyl groups in native gellan gum are removed by alkaline treatment to produce deacetylated gellan gum. Acyl substituents affect the rheology, and deacetylation of native gellan results in a change from soft, elastic thermoreversible gels to harder, more brittle gels with higher thermal stability (*39*).

Steps involved in deacetylation of native gellan gum are as follows (*16*,*39*). The fermentation broth was immersed in boiling water bath for 15 min, cooled and pH increased to 10.0 using 1.0 M NaOH. The broth was then kept at 80 °C for 10 min and the pH was brought down to 7.0 using 1.0 M HCl. Cell mass from the broth was separated by centrifugation at 8000 rpm for 30 min at 4 °C. The supernatant was then added into three volumes of ice-cold alcohol to precipitate the deacetylated gellan. The precipitated gellan gum was then dried to constant mass in hot air oven at 80 °C for 12 h.

There are two types of deacetylated gellan gum differentiated on the basis of degree of deacetylation: high acyl gellan gum (partially deacetylated) and low acyl gellan gum (highly deacetylated) (*40*).

Clarified gellan gum

Clarified gellan gum results from filtration of hot, deacetylated gellan gum for enhanced removal of cell protein residue. Clarification of gellan gum is of value especially when the gum is used as agar substitute (*41*). Dreveton *et al.* (*36*) described the method for clarification of gellan gum. Initially, 0.1 % solutions of gellan gum were prepared by mechanical stirring at 40 °C for 16 h in deionised water. Then the solutions were heated at 95 °C for 30 min. These heated solutions were then centrifuged at 13 000 × *g* for 30 min. The supernatants obtained were heated to 95 °C and then totally clarified by filtration (0.7 μ). Clarified gellan gum is suitable for some confectionary products where clarity is a crucial quality issue. It is also used as gelling agent for microbial growth media. Fig. 3 illustrates the repeating unit of chemical structure of acetylated and deacetylated gellan gum.

Physicochemical Properties

Gelling characteristics and texture properties of gellan gum

Gelation of gellan solutions occurs abruptly upon heating and cooling of gellan gum solutions in the presence of cations. Such sol-gel transitions are considered as phase transition. The gelation of gellan gum is a function of polymer concentration, temperature, and presence of monovalent and divalent cations in solution (*42*). At low temperature gellan forms an ordered helix of double strands, while at high temperature a single-stranded polysaccharide occurs, which significantly reduces the viscosity of the solution. The transition temperature is approximately 35 °C, but can range from 30–50 °C. Below transition temperature, a stiff structure is obtained (setting point), and results in gel formation. The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (*43*). Addition of monovalent or divalent cations during cooling markedly increases the number of salt bridges at junction zone, thereby improving the gelling potential of gellan gum. Various studies have been carried out to study the effect of different factors on the gel strength. Some of the important factors affecting gel strength are discussed bellow.

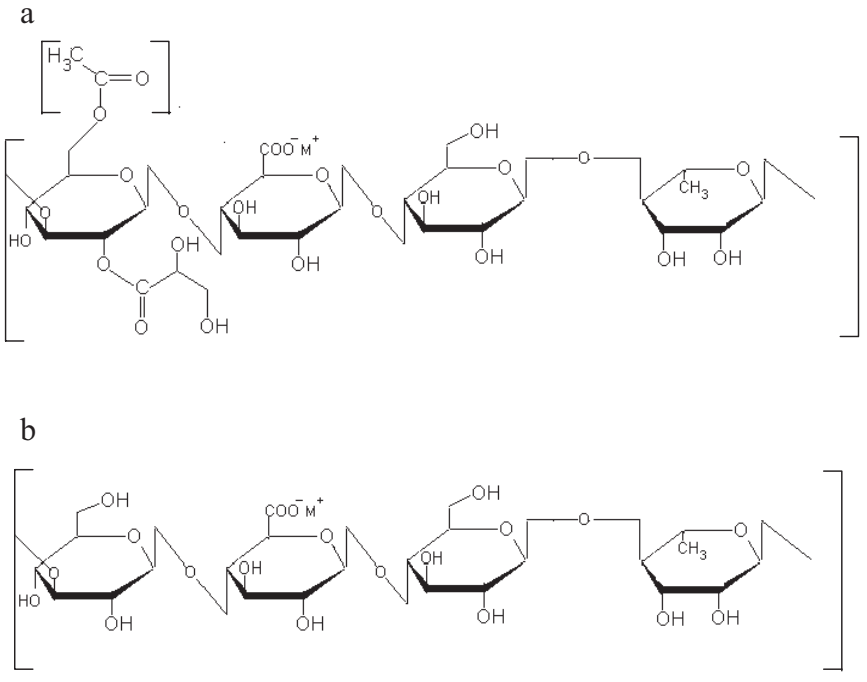

Fig. 3. Repeating units of chemical structure of native (a) and deacetylated (b) gellan gum (*18*)

Acetyl content

Acetyl content is the most important factor affecting the gel strength. Gellan gum with different acetyl content gives gels with different properties. Native gellan gum provides soft, elastic, thermoreversible gels, and is very weak because of bulky acetyl and glyceryl groups that prevent close association between gellan polymer chains in bulk-helix formation, and hinder compact packing of the cross-linked double helix. Deacetylated gellan gum forms firm, brittle and thermoreversible gel because of the absence of acetyl and glyceryl groups (*44*).

Type and concentration of ions

Ions have an impact on gel strength and brittleness. Gellan does not form gel in deionised water, but the addition of salts of calcium, potassium, sodium, and magnesium causes an increase in these two properties (*40*). Notably, divalent cations are more effective in achieving this; even in gellan gels of very low concentration (0.2 %, by mass per volume), a high strength was achieved with a maximum at about 0.004 % (by mass per volume) calcium and 0.005 % (by mass per volume) magnesium. Similar gel strength can be achieved with 0.16 % sodium or 0.12 % potassium (by mass per volume) (*45*). Gellan gels with KCl or NaCl had lower gel strength, even at high salt concentration (1 %, by mass per volume) (*39*). A concentration of 0.1–0.2 % gellan is suitable for many food systems. It is important economically that strong gels can be obtained at low concentration of gellan, with the incorporation of trace amount of salt.

Gel pH

Sanderson and Clark (*46*) showed the gel strength to be enhanced within pH range of 3.5 to 8, which corresponds to the natural pH range of most foods. Change in pH does not alter the setting point of the gel, but affects melting temperature in some cases. For example, gels prepared with very low levels of monovalent ions melt at around 70 °C at neutral pH, but at pH=3.5 the melting temperature is slightly increased. This trend is not seen for divalent ions.

Presence of hydrophilic ingredients

Addition of hydrophilic ingredients like sucrose (at about 10 %, by mass per volume) tends to decrease the ion concentration required for optimal gellan gel strength (*47*). Kasapis *et al*. (*48*) used transmission electron microscopy to examine the changing nature of a polysaccharide network with increasing levels of sugar. Mixtures of deacylated gellan (<1 %) with low (0–20 %) and high (80–85 %) levels of sugar were prepared and studied. Micrographs of the high sugar/gellan gels produced clear evidence of reduced crosslinking in the polysaccharide network, which exhibits a transition from rubber to glass-like consistency upon cooling.

Tang *et al*. (*49*) studied the effects of fructose and sucrose on the gelling temperature, clarity, and texture properties of gellan gels cross-linked with calcium or sodium ions. They reported the gelling temperatures of gellan solutions to generally increase on the addition of sucrose, whereas addition of fructose up to 35 % (by mass per volume) had no effect. Incorporation of fructose and sucrose markedly increased the gel clarity. Effect of sucrose on gel strength was found to be dependent on cation concentration. At low cation concentrations, sucrose strengthened the gels; but at high cation concentrations, sucrose weakened them.

Temperature stability and flexibility of the melting point

Gellan gum is stable at higher temperatures and maintains its strength at 90 °C, whereas xanthan gum looses 74 % of its original strength after heating up to 90 °C (*14*). According to Sanderson and Clark (*40*), the melting temperature can be below or above 100 °C, depending on the conditions of gel formation. The most important factor responsible for the flexibility of the melting point is concentration of cations in the gels because monovalent and divalent cations markedly increase the number of junction zones in gels and make them more resistant to temperature. Modification of the melting point can successfully replace other conventional thickeners/stabilizers, while used in much lower concentration (*21*).

Effect of the presence of other hydrocolloids on textural properties of gellan gum

Various studies to find out the changes in the textural properties of gellan gum when mixed with other food hydrocolloids have been carried out.

Sodium alginate

Sodium alginate dissolved in calcium chloride solution at 90 °C shows weak gel properties similar to those of ordered xanthan. The solutions show a sharp increase in rigidity on cooling, and convert to permanent gels on storage at low temperature. The gels attain maximum hardness at about 40 % calcium conversion (for alginate with a polyguluronate content of 58 %), and their elasticity can be readily controlled by adjustment of Ca^{2+} concentration around this optimum value. Papageorgiou *et al*. (*50*) observed that incorporation of moderate concentrations of gellan (0.1–0.3 %, by mass per volume, in combination with 2 %, by mass per volume, alginate and 5 mM trisodium citrate, sequestrant) increased the strength of the gels, but did not significantly change their elasticity, indicating that the gellan acts as strong 'filler' in an alginate matrix.

Gelatin

Lau *et al*. (*51*) carried out texture profile analysis on mixed gellan-gelatin gels to assess the effect of the ratio of the two components and calcium ion concentration. Hardness, brittleness, cohesiveness and springiness were measured. The results suggested that there was a weak positive interaction between gellan and gelatin when no calcium was added; at higher concentrations, gellan formed a continuous network and gelatin the discontinuous phase. Hardness was dependent on the concentration of gellan gum in the mixture, whereas brittleness, springiness and cohesiveness were very sensitive to low levels of calcium (0–10 mM), but less sensitive to higher calcium concentrations and gellan/gelatin ratio.

Carrageenan and xanthan

Rodríguez-Hernández and Tecante (*52*) studied texture properties of gellan-carrageenan and gellan-xanthan mixtures in order to determine the contribution of both polysaccharides to the viscoelastic behaviour of the mixture. Admixtures having a constant total concentration of 0.5 % (by mass) with different proportions were prepared in the presence of 0.01 mol/kg $CaCl_2$. It was observed that gel strength of 0.5 % gellan alone was the highest, and gel strength of the two-component gels decreased as the proportion of gellan was reduced. Mixed gels having a gellan concentration equal to or lower than 50 % mass of the total concentration were less stiff and brittle, hence were more elastic.

Effect of chelatants on textural properties of gellan gum

Camelin *et al*. (*53*) studied the effect of various concentrations of sequestrants (sodium citrate, sodium metaphosphate, and EDTA) on gellan gel setting temperature and rheological properties. Addition of EDTA between 0 and 0.8 % (by mass per volume) progressively decreased the setting temperature. Citrate and metaphosphate decreased this parameter when added up to 0.4 or 0.6 %, depending on gellan gum concentration, eventually resulting in the absence of gel formation at room temperature for the 1.5 % gellan solution containing 0.4 % citrate. This effect was accompanied by a significant decrease of gel strength, and might be attributed to the binding of divalent cations required for chain association during gelatinisation by chelatants.

Rheological Analyses and Molecular Characteristics

Dreveton *et al*. (*36*) studied viscosity profile of gellan gum with Couette rheometer Low Shear 30. The analysis was performed at 25 °C, low concentration and at lowest shear rate. The intrinsic viscosity (η) was calculated by using the following formula:

$$\eta = \lim_{c \to 0} \left[(\eta_{\text{solution}} - \eta_{\text{solvent}}) / \eta_{\text{solvent}} \cdot c \right] \qquad /2/$$

where c is the concentration of the polymer.

Intrinsic viscosity is the characteristic property of an isolated polymer molecule in a given solvent, and represents measurement of its hydrodynamic volume. Viscosity results as a function of polymer concentration are expressed conveniently by means of Huggins equation (*54*):

$$\frac{\eta_{\text{solution}}}{\eta_{\text{solvent}}} - 1 = \eta \cdot c + k' \cdot \eta^2 \cdot c^2 \qquad /3/$$

where k' is the Huggins constant and is representative of the interaction of the polymer with its solvent. Experimentally, k' is close to 0.3-0.4 in a good solvent and is greater than or near to 1 in case of occurrence of aggregation phenomenon in the solution.

The intrinsic viscosity of native gellan gum was approximately 8000 mL/g in 0.1 M KCl. The Huggins constant varied between 0.7-1.8. These values of Huggins constant suggest that aggregation phenomena occurred. Dentini *et al*. (*55*) studied physicochemical characteristics of deesterified gellan gum in 0.025 M tetramethyl ammonium chloride (TMAC). In this case, no aggregation phenomena were detected. On the other hand, Dreveton *et al*. (*36*) showed significant aggregation to occur in case of native gellan gum in TMAC. The results of viscometric analysis were correlated by using Mark-Houwink relationship:

$$\eta = K \cdot M_r^a \qquad /4/$$

where η is intrinsic viscosity, M_r is average molecular mass, and K is the Mark-Houwink relationship constant. Dreveton *et al*. (*36*) determined the Mark-Houwink relationship for gellan gum as:

$$\eta = 7.48 \cdot 10^{-3} M_r^{0.91} \qquad /5/$$

Toxicological Aspects

In 1991, toxicological evaluation of gellan gum was done at the thirty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in Geneva (*56*). The comments of the committee were as follows:

Gellan gum was shown to be poorly absorbed and did not cause any deaths in rats, which received a single large dose (5 g per kg of body mass) in the diet or by gavage. Short-term (90-day) exposure of rats to gellan gum at levels up to 60 g/kg in the diet did not cause any adverse effects. In prepubertal monkeys, toxicity of gellan gum was studied for 28 days at the highest dose level of 3 g per kg of body mass per day. Signs of any overt toxicity were not observed during this study. In reproduction and teratogenicity studies in rats in which gellan gum was given up to 50 g/kg in the diet, there was no evidence of interference with the reproductive process, and no embryotoxic or developmental effects were observed. Gellan gum was also shown to be non-genotoxic in a battery of standard short-term tests.

Study in dogs, which were treated at dose levels up to 60 g/kg in the diet for 1 year, showed that there were no adverse effects that could be attributed to chronic exposure to gellan gum. In long-term carcinogenicity studies, gellan gum did not induce any adverse effects in mice or rats at the highest dose levels of 30 and 50 g/kg in the diet, respectively.

Results from a limited study on tolerance to gellan gum in humans indicated that oral doses of up to 200 mg per kg of body mass administered over a 23-day period did not elicit any adverse reactions, although faecal bulking was observed in most subjects.

Toxicological studies show that gellan gum is relatively nontoxic. An acute oral toxicity test on rat found that the LD_{50} of gellan is higher than 5000 mg/kg, while a similar inhalation toxicity test caused no death in a group of 10 animals, and an eye irritation test indicated gellan to be safe for eye contact. Such evidence led to gellan gum being given FDA approval for use in foods on November 25, 1992 (*8*).

Applications of Gellan Gum

Applications in foods

Table 4 illustrates the potential application of gellan gum in various food products. Gellan gum is not only applicable in foods, which require a highly gelled structure, but may also be suitable for uses in systems to provide body and mouth-feel rather than gelatin. In some products, it may be desirable to use gellan gum in combination with other hydrocolloids like locust been gum, xanthan gum, guar gum and modified starches to obtain optimal product texture and stability.

Confectionery

Gellan gum can be utilized in confectionery and bakery products (*53*). The major function of gellan gum in confectionery products is to provide structure and texture to reduce the set time of starch jellies. Starch jellies normally take 24 to 48 h to set, while the introduction of gellan reduces setting time to 10–12 h. In addition, gellan can prevent moisture fluctuations in sugary foods, icings and toppings, and the required gellan gum concentration in these products is only one fifth of the commonly used agar (*40*).

Water based gels

Gellan gum provides dessert gels with mouth-feel characteristics similar to those of gelatin. The use of high clarity gellan gum is preferred in this application and results in a gel with clarity of water. This is highly desirable in meat and vegetable aspic. Moreover, the increase of melting temperature due to addition of gellan gum helps such gels to remain soft and juicy without melting and loosing their visual appearance. For these types of applications, a level of around 0.3 % gellan gum was found to be ideal (*40*).

Jams and jellies

Gellan can successfully replace pectin in jams and is effective at lower concentrations (about 0.4 % of gellan as compared to about 0.6 % of high methoxypectins and 0.8 % of low methoxypectins). In these products, syneresis is minimized, while jams have good organoleptic characteristics and good spreadability (*57*). Low solid and reduced calorie jams with excellent sheen can be prepared with only 0.15 % of clarified gellan (*40*).

Pie fillings and puddings

Gellan gum can be used as a structuring agent to partly replace starches in pie fillings and puddings. Alternatively, gellan in mixture with modified starches can be used as a stabilizer and water-binding agent (*40*), preventing the 'blunting effect' starches can have on food flavour.

Fabricated foods

Fabricated fruit pieces or meat chunks, including pet food, fall into this category. Many gums have been used to provide a structured form after heating and cooling (*58*). Since the gellan gum provides matrix which does not melt during pasteurization, the pieces retain their characteristic shape under the processing conditions. For this, a level of 0.7 % gellan is required in contrast to 1 % needed with carrageenan/locust been gum (*40*).

Dairy products

Negatively charged hydrocolloids like gellan or carageenan interact with the positively charged milk proteins, leading them to precipitate. The effect is undesirable when homogenous solutions/gels of milk proteins and gellan are required, and can be averted if hydrocolloids are pretreated to neutralize their negative charge (*59*). However, in other dairy products like cheese, the interactions of gellan with milk proteins, especially casein and whey lactoglobulins, increase the total yield of cheese and reduce the loss of solids (mainly proteins) in whey. Also, water retention during cheese making was enhanced after the addition of gellan to milk. The used levels of gellan gum are again very low (250–750 ppm). Ice cream is another dairy product that can be improved by the addition of gellan, where it acts as an effective bulking agent (*40*).

Pet foods

A wide variety of pet foods are commercially available in dry, semisolid or canned form. Gelling polysaccharides are generally used for solidification of certain canned products. The purpose of gelling polysaccharides in these cases is to provide continuous matrix, which retains the shape during processing. Gellan gum's gel forming properties and efficacy at low concentration levels make it an ideal material for this application (*40*).

Table 4. Applications of gellan gum in food (*39*)

Food area	Example of products	Conventional agents used
Water-based gels	Dessert gels, aspic	Gelatin, alginate, carrageenan
Confectionery	Pectin jellies, fillings, marshmallow	Pectin, gelatin, starches, agar, xanthan, locust been gum
Jams and marmalades	Diet jams, imitation jams, bakery fillings	Pectin, carrageenan, algin
Pie fillings and puddings	Desserts, pie fillings, canned/precooked puddings	Alginate, carrageenan, starches
Fabricated foods	Restructured meat, fruits and vegetables	Alginate, carrageenan, locust been gum
Pet foods	Canned/gelled pet foods	Alginate, carrageenan, locust been gum
Dairy products	Yogurt, milk shakes, gelled milk, ice cream	Alginate, carrageenan, gelatin

Reduction of oil uptake during frying

The potential use of gellan gum for reducing oil uptake has been reported (*60*). Oil uptake during frying is surface phenomenon. An increased hydrophobic character of the surface would result in increased oil uptake during frying. The ability of gellan gum to reduce oil uptake can be attributed to its hydrophilic character. Bajaj and Singhal (*61*) studied the use of gellan gum for reducing oil uptake in a traditional Indian deep-fat fried product called sev, which is based on chickpea flour. Addition of 0.25 % (by mass) gellan gum to chickpea flour decreased oil content of the sev by 24.6 %. Effect of the addition of gellan gum on texture of dough and sev was also studied. Addition of gellan gum significantly altered the texture of dough, but not the texture of sev.

Applications in pharmaceutical industry

The potential role of gellan in controlled drug release and adsorption in stomach has also been examined. Wataru *et al*. (*62*) studied sustained delivery of paracetamol by gellan and sodium alginate formulations, and reported that the bioavailability of paracetamol from the gels formed *in situ* in the stomach of rabbits following oral administration of the liquid formulations prepared from gellan gum and sodium alginate was similar to that of a commercially available suspension containing an identical dose of paracetamol. Use of gellan gum for controlled bioavailability of ophthalmic formulations has also been proposed. Sanzgiri *et al*. (*63*) have shown that gellan-based ophthalmic solutions have longer residence time in tear fluid than saline solution. Sidda *et al*. (*64*) formulated *in situ* gellan gum-based gels with ciprofloxacin hydrochloride as a drug and studied their diffusion characteristics. The gel formulation containing both ciprofloxacin and gellan gum showed a prolonged drug release pattern. Gellan gum was evaluated as a binding agent in lactose-based tablets containing metronidazole or paracetamol. The binding properties of the gum were compared with acacia and gelatin. Granules were prepared by the conventional wet granulation method. The results indicated that though the hardness of tablets containing gellan gum was lower than that containing gelatin or acacia, gellan gum can be employed in the formulation of normal release of metronidazole and paracetamol with moderate hardness, low friability and good disintegration and dissolution properties (*65*).

Solid culture media for growth of microorganisms and plants

A clarified grade of gellan (Gelrite™) is used as an agar substitute to solidify nutrient media for growth of microorganisms (*41*). The product withstands several autoclave cycles and is also resistant to a variety of enzymes (*66*). It has a texture that resembles that of agar. As against 15 g/L of agar, the required concentration of Gelrite™ is only 6 g/L of the medium (*67*). There is also evidence that gellan gum is an ideal medium for plant tissue culture (*68*). Gelrite™ media showed superior shoot proliferation, rooting and embryogenesis when compared with agar medium (*21*).

Phytagel™ is an agar substitute, which is manufactured by Sigma, USA, by using gellan gum. It produces a clear, colourless, high-strength gel, which aids in the detection of microbial contamination. It is an economical alternative to agar as a gelling agent. Arregui *et al*. (*69*) compared Phytagel™ with Difco bacto agar for *in vitro* tuberization of six potato cultivars. Chemical analyses of both gelling agents revealed a higher mineral content and organic impurities in Difco bacto agar than in Phytagel™, which is therefore recommended for microtuber production.

Gel electrophoresis in biological research

Gels of gellan can be used as a solid matrix for separating DNA fragments on the basis of size by electrophoresis (*70*). Gel electrophoresis is a widely practiced and key procedure in molecular biology. Gellan-based electrophoresis gels must include a second polymer such as hydroxymethylcellulose or polyethylene oxide to reduce electroosmosis. In this application gellan can replace highly refined agarose, which is very costly and used at about 1 %. By contrast, gellan costs much less than agarose and is required only at 0.125 %.

Cell immobilization

Camelin *et al*. (*53*) found gellan gels to provide a mechanically stable matrix for the immobilization of *Bifidobacterium longum* (gels were stable for over 150 h of fermentation of a whey medium). In addition, biocatalyst activity (lactic acid production) was very high corresponding to the values reported by *Bifidobacterium longum* entrapped in carrageenan/locust bean gum.

Wenrong and Griffiths (*71*) evaluated the ability of the gellan–xanthan beads to protect bifidobacteria under different conditions including peptone water, pH=4.0, pasteurized yogurt, and simulated gastric juice and they found that immobilization of bifidobacteria in beads of gellan–xanthan gum mixtures increased their tolerance of high acid environments. This approach may be useful for use of gellan gum in delivery of probiotic cultures to the gastrointestinal tract of humans.

Uses of gellan gum in microencapsulation techniques (*72*) have also been proposed. Gellan gum can be used for the encapsulation of cultures for wastewater treatment. Moslemy *et al*. (*73*) demonstrated that encapsulation of activated sludge in gellan gum microbeads enhanced the biological activity of microbial populations in the removal of gasoline hydrocarbons.

Conclusion

Gellan gum has gained importance in the food or pharmaceutical industries, gel electrophoresis, immobilization of cells and enzymes, and bioremediation. Its potential use as a replacement for gelatin and agar makes it the most important polysaccharide. Comparatively low productivity and requirement for costly downstream processing steps impair the economic viability of microbial production of gellan. Use of cheaper raw materials like agricultural waste or dairy waste can help reduce the cost of fermentative production of gellan gum. Certain metabolic precursors that increase the conversion of carbon sources to gellan gum can also improve the yield of gellan gum. The most exciting prospects for gellan modi-

fication and increasing production yield are found in genetic engineering. A great deal of research is needed on gellan gum fermentation to overcome the mass transfer limitation caused by high viscosity. Similarly, improvement of the existing purification processes or using new approaches for gellan gum purification like gel permeation chromatography need to be developed.

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