Food and Agriculture Organization of the United Nations

87th JECFA - Chemical and Technical Assessment (CTA), 2019

GELLAN GUM

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*1. **Summary***

This Chemical and Technical Assessment (CTA) summarizes data and information on gellan gum submitted to JECFA by two sponsors [1] [2] in dossiers dated 30 November 2018. Upon request by the 50th Codex Committee on Food Additives (CCFA) information was submitted to the Committee for the evaluation of all data to assess the safety, dietary intake and specifications related to the use of gellan gum as thickener for use in formulas for special medical purposes for infants (FSMP) (GSFA Food category no. 13.1.3), and for the reevaluation of the specification for residual ethanol in the existing monograph. This document discusses published information relevant to the identity and manufacture of gellan gum, manufacturing specifications, use and use levels. The Committee previously reviewed gellan gum at its thirty-seventh meeting for use as a gelling agent, stabilizer and thickener in a wide range of foods and beverages; an ADI "not specified" was allocated (WHO, 2016; Annex 1, reference 95). The Committee prepared specifications for gellan gum at its forty-ninth and seventy-ninth meeting. At the seventy-ninth meeting, the Committee established a numerical limit of 50 mg/kg for residual ethanol after a request to include it as an additional extraction solvent during the processing of gellan gum with ethanol (WHO, 2016; Annex 1, reference 222).

At the present meeting the Committee evaluated the safety of gellan gum for use as a thickener and to maintain homogeneity for better delivery of nutrients in formulas for special medical purposes for infants (FSMP) at levels up to 50 mg/L in the fed products. [3] Gellan gum would also be used as a stabilizer along with octenyl succinic anhydride (OSA)-modified corn starch (INS No. 1450). Additionally, the Committee reevaluated the limit for specification for residual ethanol.

2. Description

Gellan gum is a high-molecular weight, extracellular anionic polysaccharide (>500, 000 Da). The primary structure of gellan gum, is comprised of a linear tetrasaccharide backbone repeating unit (Figure 1) consisting of D-glucose, L-rhamnose, and D-glucuronic acid in molar ratios of 2:1:1. Native gellan gum also contains an acetyl and a glyceryl group bound to the glucose adjacent to the glucuronic acid residues.

[→3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→]n

Figure 1: Repeating units of gellan gum (Prajapati et al., 2013)

[1] *Zheijang DSM Zhongken Biotechnology Co Ltd, 314515 Gaoqiao Economic Zone, Tongxiang city, Zhejiang, PR China.*
[2] *Abbott Nutrition, 3300 Stelzer Road Dept. 104070, Bldg. RP3-2 Columbus, OH 43219 USA.*
[3] *The target concentration for gellan gum in the final FSMP is 40 mg/L*

It is produced by the controlled fermentation of a carbohydrate source in a pure culture of the non-pathogenic *Pseudomonas elodea* (reclassified as *Sphingomonas elodea*), a well characterized aerobic Gram-negative bacterium.

Commercially, gellan gum is available as three basic forms, namely high acyl (native), unclarified, low-acyl, unclarified, and low acyl, clarified forms. It is distinguished by its polysaccharide content, the percent substitution of O-acetyl functional groups, and/or the protein content. The low acyl clarified form of gellan gum is proposed for direct addition to ready-to-feed FSMPs or as a component of concentrated liquid fortification products formulated with hydrolyzed protein and/or amino acids.

3. Manufacturing

Gellan gum is produced by inoculating a fermentation medium with non-pathogenic *S. elodea*. *S. elodea* is deposited in the American Type Culture Collection (ATCC 31461) and its genome has been sequenced (Gai et al., 2011). Fermentation is performed under controlled conditions in the presence of a medium that contains sources of carbon, nitrogen and appropriate mineral salts. The process is stopped by heat treatment to kill viable cells. By-products of fermentation include polyhydroxybutyrate, enzymes and viable cells of the production organism, which are removed and/or inactivated during processing. Gellan gum can be recovered in multiple ways: precipitation with food grade isopropanol or ethanol yields the high acyl form; treatment with alkali prior to precipitating with alcohol results in deacylation and yields the low acyl form; further filtration is applied to produce low-acyl clarified gellan gum. The gelling properties of the articles of commerce are controlled by the addition of metal ions such as sodium, potassium and calcium to neutralize the glucuronic acid. The resulting gellan gum is separated, dried and milled. The glucuronic acid is neutralized by the addition of potassium, sodium, calcium or magnesium salts.

4. Chemical characterization

4.1 Composition

In its native form, gellan gum is substituted with two acyl groups, acetate and glycerate, as O-glycosidically linked esters (Figure 2). The weight average molecular weight of gellan gum is >70 000 Da, with 95% of the polysaccharide >500 000 Da.

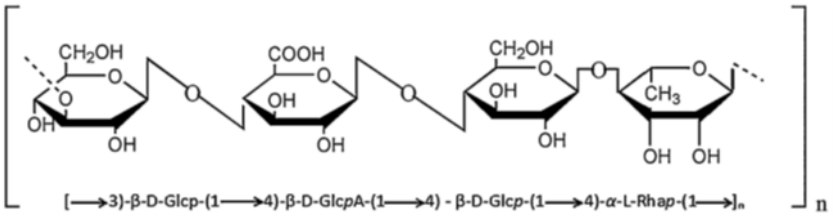

Figure 2: Structural formula of deacetylated gellan gum backbone

The Committee established specifications for gellan gum at its thirty-seventh meeting, and subsequently amended the monograph at the seventy-ninth meeting. One of the sponsors submitted results from three batches of gellan gum intended for use in FSMPs for infants to demonstrate compliance with the current specifications for gellan gum.

4.2 Identification

Gellan gum is soluble in water and insoluble in ethanol. It is identified based on the formation of a worm-like gel when a stirred aqueous solution of gellan gum is treated with a 10 % solution of calcium chloride. It is also identified based on the formation of a firm gel when a stirred aqueous solution of gellan gum is treated with sodium chloride, heated to 80° with stirring, and held at 80° for 1 minute.

4.3 Possible impurities

Possible impurities from manufacturing can include polyhydroxybutyrate (PHB), enzymes, the nitrogen source from the fermentation medium, viable cells from the fermentation, residual alcohol used for the precipitation, heavy metals, and microbiological contaminants. The sponsor states that appropriate filtration steps are put in place to remove cell debris and PHB. Any residual enzyme activity or viable cells from the production organism are expected to be inactivated during the alkali-heat treatment step. In addition, downstream purification steps are put in place to remove residual protein matter from fermentation; this is verified by testing for nitrogen content in the final gellan gum product.
The specifications monograph for gellan gum cites specific tests from Volume 4 for the determination of residual isopropanol and microbiological criteria.

4.4 Analytical methods

The specifications monograph for gellan gum cites general tests included in the *FAO Combined Compendium of Specifications* (FAO JECFA Monographs 1, Vol. 4, 2006) and specific tests for the determination of residual alcohol remaining from the precipitation step (isopropanol or ethanol).
The Committee at the present meeting evaluated the method of assay that predicts the purity of gellan gum based on results obtained from a decarboxylation method. The Committee noted that this method is empirical in nature and does not accurately provide the purity of the gellan gum that is the article of commerce. The Committee also noted that the decarboxylation method cannot differentiate between the three types of gellan gum that are produced as described in the Definition of the specifications monograph.
The Committee at the present meeting proposed to make the specifications monograph *tentative*, pending submission of new method or methods for characterizing the three forms of gellan gum in commerce.
The Committee requested specific information regarding the method or methods to characterize the three gellan gums that are commercially available:

- A method to differentiate the three commercial forms of gellan gum, i.e., high-acyl, low-acyl, and low-acyl clarified.
- A method to determine the degree of acylation.
- Validation data for the above methods of including detailed description of the sample preparation.
- Data from five non-consecutive commercial batches of material using the proposed validated methods.
  The Committees at previous meetings included a head-space gas chromatography (HS-GC) method with flame ionization detection to measure residual isopropanol and ethanol. Limits of 750 mg/kg and 50 mg/kg, respectively, were set based on the data provided to those Committees.
  At the present meeting, the Committee was requested to reevaluate the specification for ethanol based on
- the technological limit of ethanol used in the manufacturing process (0.8%),
- dietary exposure of ethanol, and
- harmonizing with other regulatory bodies.

In addition to the request to reevaluate the limit for residual ethanol, the sponsor also proposed a change to the sample preparation for the HS-GC method for detection of isopropanol and ethanol.

Based on analytical data provided by the sponsor for three different batches of gellan gum, lead levels are below the 2mg/kg limit set in the specifications. Isopropanol levels were reported at 71 mg/kg, 103 mg/kg, and 123 mg/kg, well below the specification limit of 750 mg/kg. Data supporting the increase of limit for residual ethanol were presented by the second sponsor, for five batches at 5125 mg/kg, 5265 mg/kg, 7692 mg/kg, 4810 mg/kg, and 7188 mg/kg, respectively; these results were obtained by the sponsor using their proposed sample preparation for HS-GC detection of residual alcohols.

5. Levels of lead from the use of gellan gum in infant formula

The Codex Committee on Contaminants in Foods (CCCF) had agreed at its 8th session to a maximum level (ML) of 0.01 mg/kg (as consumed, in the ready-to-use product) for lead in infant formula (CCCF, 2014). Consistent with the Committees' previous consideration of the contribution of infant formula ingredients, the Committee at this meeting considered the level of lead that could result in infant formula from the proposed uses of gellan gum. At the maximum level of gellan gum in infant formula of 50 mg/L as consumed and the existing maximum permissible level of lead in gellan gum (2 mg/kg, per the existing specifications), the maximum level of lead in infant formula from gellan gum would be not more than 0.0001 mg/L of formula. Therefore, gellan gum at the proposed maximum use level in infant formula, would not exceed lead levels in infant formula as established at the eighth session of the CCCF (2014). Furthermore, as noted by the Committee at the eighty-second meeting, "it is the responsibility of the infant formula manufacturers to ensure that the lead levels in the final infant formula (as consumed) comply with the maximum limit for lead as set by the Eighth Session of CCCF" (WHO, 2016; Annex 1, reference 230).

6. Rationale for proposed specifications

Upon review of the submitted data from the sponsors, the Committee at this meeting made the following modifications to the specification monograph for gellan gum that was last prepared by the Committee at the 79th meeting and made it *tentative*.

1. Updated the description in the specification monograph to include the use of gellan gum as a stabilizer and thickening agent in FSMP.
2. Requested validated method/s and supporting data to differentiate the three commercial forms of gellan gum, i.e. high-acyl, low-acyl, and low-acyl clarified.
3. Removed the limit for residual ethanol.

7. Functional uses including technological function, food categories, and use levels

Gellan gum is listed in the CODEX General Standard for Food Additives (GSFA) for use as a thickener, gelling agent, and stabiliser in several food categories (GSFA, 2015). The Committee at its thirty-seventh, forty-ninth, and seventy-ninth meetings, evaluated the safety of gellan gum and allocated an acceptable daily intake (ADI) of "not specified" (Annex 1, references *94, 95, 96, 124, 131, 220*, and *222*). At this meeting, the Committee was requested to evaluate the safety of gellan gum for use as a thickener at a level of 50 mg/L (as consumed). in food category no. 13.1.3, 'Formulae for special medical purposes for infants.' This includes concentrated liquid fortification products based on hydrolysed protein and/or amino acids.

8. Reactions and fate in foods

The sponsor provided stability data for four lots of gellan gum stored for 3 years, and two lots stored for 5 years, at ambient temperature. Parameters including solid content, solution pH and gel strength were monitored. The results demonstrated stability of gellan gum when stored at ambient temperature for extended periods of time. Literature data also supported stability of gellan gum at temperatures as high as 90 °C (Bajaj et al., 2007).

The sponsor also provided stability data that exhibited heat stability of gellan gum in infant formula and in concentrated fortification products intended for addition to formula.

9. References

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