ISOLATION AND IDENTIFICATION OF GUAR SEED (CYAMPOSIS TETRAGONOLOBA) GUM

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ABSTRACT

Guar polysaccharide was extracted from its grounded seeds by using hot water, alkali and acetic acid treatments. The highest yield of this polymer (11.55%) was obtained with hot water and its $(\alpha)_D^{25}$ in water was (+74°) while cupperammonium complex reached (-224°).

Silyl derivatives on G.L.C. technique showed that the monomer residues were identified as D (+) galactopyranose and D (+) mannopyranose with molar ratio 1:2, respectively.

I.R., spectrum of the polysaccharide indicated that the linkages between the units were of B-type.

The average molecular weight of guar gum was determined physically and chemically the first value was 22×10^3, while the second was 166×10^3.

Periodate oxidation technique indicated that the guar polysaccharide has (1 ---> 4) glycosidic linkages and a branched structure with Ca 16.7% of the units in terminal positions.

The proposed structure of guar polysaccharide might consist of 171 repreating segments, each segment composed of a main chain of four manno-opyranose units jointed by (1 ---> 4) glycosidic linkages. Each segment is also jointed with two units of galactopyranose through B (1 ---> 6) glycosidic bonds.

The technological and jelling examination of guar gum proved that it has high jelling quality and a good capacity (stability) for preservation.

INTRODUCTION

Guar belongs to leguminosae family (Gyamopsis tetragonoloba L. tab.), known as (Cyamposis psaralioides Dc.). It was originally grown in India and recently in other many countries (Khater, 1977). Sandford and Baird (1983), reported that the total world production of guar gum reached to 90,000 tonnes (1980) since the gum has a wide range of functional characteristics exhibited by its polysaccharide. Khater (1977), stated that the guar polysaccharide has 5-8 times the thicking power of starch and it is used as a filter aid in the mining industry, textile industry, pharmaceuticals as a thickner in cosmetics and as a strengthing agent in paper industry. Also, Ibrahim et al. (1983), found that guar gum can be used as a pectin substitute in jam making and preservation.

In Egypt, nowadays several attempts for planting guar were sucessfully carried out, its yield amounted to 1.2 tonne per feddan (Khater, 1977)

Rees (1972), reported that galactomannans occur as reserve polysaccharide in seeds of many leguminous plants and guar powdered seeds contain large amounts of these galactomannans.

Smith and Montgomery (1959), McCleary et al. (1976), and Dey (1978), have confirmed that galactomannans are composed of mannopyranose residues. These residues are joined through B- (1 ---> 4) glycosidic linkages. On the other hand, the galactopyranose units were present as branches and linked through (1 ---> 6) glycosidic bonds.

McCleary (1979, 1983) concluded that D-gal-p- groups in locust bean, guar and other galactomannans of similar galactose content could be randomly distributed.

Tewari et al. (1984), have extracted a galactomannan polysaccharide from Cassia carymbosa (Leguminoseae) seeds with cold, acidulated water and purified it to give a water soluble product having $(\alpha)_D^{25}$ + 72, ash content 0.2% and containing D-galactose and mannose in molar ratio 4: 7. Acid catalyzed fragmentation, periodate oxidation and methylation showed that the seed gum has a branched structure consisting of a linear chain of B- (1 ---> 4) linked mannopyranosyl units, some of which are substituted at 0-6 by two -D-(1 ---> 6) galactopyranosyl units mutually linked glycosidically.

The aim of the present investigation is to study the polysaccharide content of guar gum in a trial to elucidate the skeletal of this polymer accompanied with some technological examination of this substance for better use in industrial aspects.

MATERIALS AND METHODS

The seeds of guar gum were supplied by Agricultural Research Centre at Giza.

Guar polysaccharide was isolated by three different methods, i.e. hot water; Manzi et al. (1984), aqueous alkali, Metry (1977) and acetic acid (1.5%) as recommended by Tewari et al., (1984). The highest yield of guar polysaccharid was obtained with hot water (11.55%) on dry weight basis while extraction with alkali and acid gave lower values, 9.77% and 7.11%, respectively.

Purification of the obtained polysaccharide was accomplished via the formation of its cupperammonium complex as mentioned by Saad (1980). This complex gave $(\alpha)_D^{25}$ -224 (0.01% in water) for different samples. The polysaccharide was recovered from the cupperammonium complex with N. Hcl. The product gave $(\alpha)_D^{25}$ + 74 (0.01% W/W in water).

The following tests were applied on the obtained polysaccharide according to A.O.A.C. (1980). Molish test (+), Fehling's test (-), Bendict's test (-), Furfural test (-), Iodine test (-) and Lassaigne's test (Nitrogen (-), sulphur (-), phosphorous (-) and Halogens (-)).

After acid hydrolysis, the hydrolyzate product gave positive result with reducing tests.

Moisture and ash contents were determined according to the method of A.O.A.C. (1980), while estimation of cations were carried out as reported by Saad (1985), by using a Pye Unicam atomic absorption spectrophotometer (Sp 1900).

The hydrolysis of the polysaccharide was accomplished as described by Saad (1980) and the ions were removed from the hydrolyzate using ion exchange chromatography as reported by Guindi et al. (1977), cation resin: IR- 120; anion resin: Dowex IX- 400. Sugars identification of the hydrolyzate were achieved using the method reported by Farag et al. (1985), using G.L.C. (model sp 2100) and silylation of the soluble sugars. The G.L.C. was set up under the conditions of flow rates of the nitrogen, hydrogen and air 25,

30 and 300 ml/min, the chart speed was one cm/min; temperatures for injector, column and detector were 220, 190 and 220 respectively.

Peak identification was performed by comparison of the relative retention time for each peak with those of standard chromatogram.

The relation of the individual sugars were therefore, obtained by determination the partial areas relative to the total area.

Periodate oxidation was carried out according to Whistler (1965) and applied by Attia (1983), a blank experiment was run at the same time under the same conditions. The amount of periodate consumed by each mole of polysaccharide was calculated in moles per gram corresponding to each mole of anhydroglycose units.

Estimation of the liberated formaldehyde was carried out using the method mentioned by Metry (1977).

The I.R. spectrum of guar gum was recorded on pressed disc of potassium bromide (100-150 mg) using Pye Unicam (Sp 1000) spectro-photometer.

The average molecualr weight was determined as described by Christensen (1954) and modified by Sarhan (1975).

Determination of technological and jelling properties of guar gum was carried out according to the method described by Kertesz (1951). The prepared jellies were compared with jelly of a standard pectin sample which was supplied from the United Kingdom (Bulmer Limited company). Gel strength of guar gum was also evaluated comparing with Egyptian Standards as mentioned by Wasef (1979).

RESULTS AND DISCUSSIONS

Analysis of Seeds:

Different constituents of guar (Cyanposis tetragonoloba L. tab.) seeds were determined. The results are shown in table (1).

Table (1): Different components of guar seeds (G/100 gm of guar seeds).

Moisture	10.43	
Ash	4.93	5.51*
Fats	3.92	4.38*
Total carbohydrates	36.09	40.30*
Proteins	43.80	48.91*

* These values were calculated on dry weight basis.

The values in table (1) illustrate that the seeds of guar, which are grown in Egypt, contain a high protein content i.e. 48.91%. Consequently, the cake after removing the gum might be used as a rich source of proteins. This result is in agreement with that obtained by Misra *et al.* (1984). As mentioned before the isolation of this polymer from guar seeds using hot water gave the highest amount (11.55%) while the extraction with acid gave the lowest amount (7.11%).

This observation might be attributed to the partial degradation of this polysaccharide, resulting from both acid hydrolysis and alkali treatments. Alkali treatment leads to the conversion of aldehydic terminal unit to ketose rearrangement, followed by saccharinic acid formation (Aspinall, 1982), this process mostly occurred for the polysaccharides containing 3-linked main chains and for 4-linked glycans. Also, the side chain units remotely attached at C-6, might be degraded as for the linear glycan forming 0-glycosylsaccharic acids.

Such alkaline degradation causes the reduction of the polysaccharide yield. Similar explanation have been reported by Aspinall (1982), with the galactomannan of guaran (4-linked B-D-mannan chain). Also acidic conditions cleavage of glycosidic bonds can be occurred during the extraction of this polysaccharide.

Preliminary tests and properties of the guar gum (guaran):

The extracted guar gum was free from starch, reducing sugars, proteins and contains traces of halogens (chloride), its $(a)_D^{25}$ was + 74 (in water). This result indicates that there is a homogenity of this polysaccharide. Such results were obtained by Tewari *et al.*, (1984).

The precipitate of cupper complex with the polysaccharide gave $(a)_D^{25}$ -224. This observation has been shown by Reeves (1979), these reactions involving the hydroxyl groups occur at C_2 and C_3 of the type galactopyranoside