Fenugreek and other lesser known legume galactomannan-polysaccharides: Scope for developments

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Among useful plants, legumes stand next only to grasses, yet the variety of products produced from legumes are more numerous than those from grasses, which mainly contain glucans (starches & cellulose), hemicelluloses and sugars as carbohydrates. Some legume seeds additionally have galactomannans and amylloids as endosperm reserve polysaccharides, while many legume trees exude variety of gums. Because of unique industrial applications, legume galactomannans have acquired commercial value. There are many unutilized tropical legume galactomannans that could be exploited. This review presents galactomannans of some less common legumes.

Keywords: Legume galactomannans, Underutilized plant resources, Fenugreek, Sesbania bispinosa, Casia tora, C. fistula, Galactomannan derivatives

Introduction

Polysaccharides based hydrocolloids are high molecular weight polymers, generally used as thickening or gelling agents in food and other industries. Galactomannans, an important group of polysaccharide hydrocolloids, are produced in certain plants as cell wall and storage polysaccharides¹. Industrial applications of galactomannans arise due to their interactions with molecules of their own kind as well as with other molecules. A large group of galactomannans is produced from seeds of Leguminoseae family. Thus, out of 163 legume-seeds examined², 119 seed endosperm mucilage contained galactomannans as the major, reserve polysaccharide constituent. Leguminoseae family embraces full sized trees as well as herbaceous plants, amenable to annual agriculture cropping.

About a decade ago, following seed endosperm galactomannans were approved for food use³: (1) Guar gum (Cyamopsis tetragonoloba); (2) Locust bean gum (Ceratonia siliqua, LBG) or the Carob tree; and (3) Tara gum (Caesalpinia spinosa, locally called Huarango or Guararnga or the Peruvian carob tree. Guar is an annual crop, mainly cultivated in the Indian subcontinent. Locust bean and tara seed gums come from evergreen perennial trees, which grow in the Mediterranean region of Europe, North Africa, and South America³.

Major portion of any dry plant material consists of polysaccharides. The cost of raw material and process-cost of extraction of different polysaccharides in commercially pure form vary considerably. Thus the manufacture of many seaweeds, microbial gums and pectin polysaccharides involve their extraction into water followed by precipitation with alcohol (to be recovered by fractional distillation and reused) and drying. In contrast to such wet methods, legume endosperm consisting mainly galactomannans of commercial grade are separated by differential grinding, sifting and sieving of the seed. These are purely mechanical operations and keep the process cost low⁴,⁵.

Potential Polysaccharide Gums from Agricultural Resources⁶

A newly investigated gum is adopted for industrial production and use when: (1) It has some special functional properties such as a good viscosity builder or a good gelling agent, for which it is being considered for commercialization; (2) Gum cost is low and is likely to be reasonably constant for several years to come. Galactomannans from annual crops are cheaper compared to those from full-grown trees; (3) The supply of high quality product (gum) is well assured, particularly when there is an increased world demand. This again is true of gums from land
cultivated annual crops; and (4) When a gum is meant for human consumption, consideration for its acceptance as a food, cosmetics or drug additive by the government agencies (FDA) becomes an important consideration. In many cases a modified or chemically derivatised gum provides better functionality.

Chemotaxonomy studies of Leguminoseae plants based on mannose:galactose (M:G) ratio, showed that galactomannans from full grown legume trees have lower percentage of galactose. M:G in galactomannans determines the functional properties of a gum. A gum with higher percentage of galactose has good cold water dispersability and higher viscosity, but poor gelling property. M:G for some gums is as follows: carob, 4:1; tara, 3:1; guar (Sasbania bispinosa, SB-Gum), 1.8:1; CT-Gum (Cassia tora), 2:1; and fenugreek, 1:1. This observation can form a guideline for exploitation of new galactomannans of desired M:G.

Less exploited Seed Gums of Indian Subcontinent
Indian subcontinent is a major producer of guar crop, which is the source of galactomannan guar gum. There has been frequent short supply and cost escalation of guar gum due to variation in crop production because of climatic changes. In case of short supply of guar gum, certain other seed galactomannans are used as substitutes in nonfood applications of guar gum, such as a thickener for print paste in textile printing. Alternative gums include SB and CT gums, which are products from non-irrigated wild plants growing in wastelands and have slowly gained some commercial value. However, seed gum of fenugreek, which is an established agriculture crop, commercialized only recently.

Fenugreek Galactomannan (FG) as an Emerging Industrial Polysaccharide
Fenugreek seed-endosperm galactomannan was not in industrial production till 1993. Currently some industries are producing and marketing a sizable amount of fenugreek gum, and other fenugreek products are finding increasing applications. Guar and LBG have been in industrial use for quite some time. Similar to guar gum, FG is also a product from a widely grown annual agriculture crop, and hence its sustainable supply is well assured. Besides, fenugreek seed also contains spicy oil, saponins and edible protein, thereby making it a cost-effective agricultural crop. According to the Ayurvedic System of Medicine, fenugreek seed has been used in condiments and as a spice component in food in India, the Middle- and Far-East for centuries and its nontoxic and innocuous nature as a food additive is well established. Considering these factors, the Research Branch of Agriculture Canada started a project to produce fenugreek crop of an improved seed yield. A breeding program to produce seeds yielding higher levels of saponins (diosgenin) from fenugreek seed was undertaken in England, but no breeding program to improve gum yield from fenugreek seed appears to have been undertaken so far. Improvement of seed quality and its yield can result in increased yield of most of the seed components and value of the crop.

Fenugreek Crop
Fenugreek or Methi (Trigonellafoenum-graecum Linn) is an old cultivated spice-bean crop, indigenous to India, Middle East and Southern Europe. Currently, it is cultivated in Middle East, North Africa, Southern Europe and more recently in North America. India is the major fenugreek exporting country, followed by France, Egypt and Argentina. It is an erect annual plant, 30-60 cm high. It has thin 10-15 cm long sword-shaped pods, with 10-20 seeds. The oblong, yellow-brown seeds (2-3 mm long) are hard with a wrinkled surface. The plant grows in well-drained soil under mild climate. The seed crop matures in 3-4 months and yields 300-400 kg seeds per acre. In India, besides the seed, green fenugreek leaves are used as a vegetable.

Seed Composition
Dicotyledonous fenugreek seed consists of a wrinkled brown-yellow seed coat or the husk, enclosing two whitish translucent endosperm halves mainly composed of soluble galactomannan polysaccharide. Seed contains (dry basis): moisture, 3.6; protein, 25-30; ether extract, 7-9; steroidal saponins, 5-7; galactomannans, 25-30; insoluble fiber, 20-25; and ash, 3-4%. Between the two endosperms is sandwiched yellowish germ portion, which is mainly composed of good quality of edible protein, and also contains ether extractable (7-9%, fatty and flavoring essential oil) and alcohol extractable (5-7%, consisting of disogenin, yellow pigments, trigonelline, free amino acids, vitamins of B-complex group and flavonoids). Fibrous material mainly contains insoluble cellulosic fiber, while the endosperm galactomannan is soluble.
In legumes, the endosperm galactomannans are the reserve seed polysaccharides, which are used up during the germination and growth of the plant embryo till it starts photosynthesis. Germinating legume seeds produce enzymes (β-mannase, α-galactase) to break down polysaccharide. Galactomannan gums have a strong tendency to bind and hold moisture. Solvent defatted and protein free fenugreek gum is whitish and odorless, and it is effective in lowering blood sugar and blood lipid level. It promotes growth of prebiotic colon bacteria, which are good for human health.

### Chemical Structure of Galactomannans

Galactomannans generally consist of a β, 1-4-linked linear mannan backbone, to which single galactose grafts are linked randomly by α, 1-6 glycoside bond. Galactomannans from different legume seeds differ in M:G, molecular weight and mode of placement of the galactose grafts, which are generally not spaced regularly but placed randomly on the linear backbone.

Fenugreek polysaccharide contains small amount of sugars other than mannose and galactose. This discrepancy might be due to less efficient methods of purification, contamination and the analytical method employed. M:G in fenugreek gum, in most cases, is ~1. Thus FG has the highest galactose (~48%; M:G, 1.02:1) in its molecule, and its linear mannan backbone has α, 1 → 6 linked single galactose grafts on nearly all the mannose groups of the main chain. Besides fenugreek, lucerne (Medicago sativa), and clover (Trifolium pratense), less common galactomannans, have ~48 percent galactose. Molecular weight of FG is ~30,000 Daltons, corresponding to an average presence of 180-190 monosaccharide (mannose + galactose) units in a molecule. On an average, the linear mannan backbone of fenugreek polysaccharide is built up of 90-95, β, 1 → 4 linked mannopyranosyl units and each backbone monomer carries an α, 1 → 6 linked galactopyranosyl group (Fig. 1).

In case of galactomannans having less galactose, there exists portions of unsubstituted, also called smooth regions of mannan backbone, while the grafts are clustered in other (called hairy) regions. In case of LBG, the unsubstituted blocks of the backbone can be as large as 50 mannose, while in case of guar these are estimated to be ~5-6 mannose. These smooth regions of the backbone come closer and form intra-chain hydrogen bonds, which reduce hydration of the gum, so that cold water solubility of LBG is less (~30%) and that for guar gum is high (~60-70%). Cold-water solubility of fenugreek gum is nearly complete (~80%). Linear mannans and cellulose, being not grafted, are insoluble due to extensive intra-chain hydrogen bonding.

### Conformation of Galactomannans

The monosaccharide units in galactomannans possess a cis-pair of hydroxyl groups on their pyranosyl ring structure (2,3-positions in case of mannose and 3,4-positions in case of galactose). In contrast, glucose, cellulose and starch have trans-hydroxyl groups. Presence of cis-hydroxyl pair along with an essentially linear chain configuration leads to many unique interactions of galactomannans. The hydrogen bonding property of galactomannan polymers is far stronger compared to glucan. Galactomannans in solution have an extended rod like conformation, and occupies large volume of gyration. Fenugreek polysaccharide has nearly completely substituted mannan backbone, leaving little chance of interchain association. The gyrating molecules of such linear polysaccharides colloid with each other and with clusters of solvent molecules to produce solutions of high viscosity, which is dependent on molecular weight. Wide-angle X-ray diffraction pattern of hydrated FG shows its crystallization into orthorhombic lattice, with \(a=9.12 \text{ Å}, b=33.35 \text{ Å}, \text{ and } c=10.35 \text{Å}\), and the density corresponds to four chains passing through unit cell. This crystal structure is similar to those of other galactomannans.

### Physical Properties and Rheology of Fenugreek Gum

Fenugreek gum solutions decrease in viscosity with rise in temperature and exhibit non-Newtonian (shear thinning) viscosity behaviour, except in very dilute (~0.2%) solutions. Its viscosity at a given concentration is lower than guar and LBG, due to lower molecular weight. Because of a fully substituted backbone, it does not interact with other
polysaccharide (carrageenan or xanthan) to show synergetic viscosity increase and gelling. It is, however, gelled by borate ions, which must be attributed to the interaction of cis-hydroxyl groups.

Complete shielding of mannose backbone by galactose grafts makes the fenugreek polysaccharide backbone difficult to be approached and cleaved by β-mannase enzymes, which are normally used for partial depolymerization of other galactomannans. In such galactomannans with highly substituted mannose backbone, an initial reaction with α-galactocidase removes some galactose grafts, exposing bare sites of mannanose chain for attack by β-mannase enzymes to cleave the polysaccharide, thus causing a decrease solution viscosity.

Most soluble polysaccharides have a certain degree of emulsion stabilizing action, which has been attributed to their solution thickening properties, and not reducing interfacial- and surface tension. In contrast, fenugreek solution shows unique property of reducing interfacial and surface tension, which is comparable to that of gum arabic. It has been suggested that fully extended polysaccharide molecules are deposited on emulsified oil droplet in water protecting them against coalescence and flocculation. This property, coupled with its moisture holding capacity, opens up interesting possibility of using of fenugreek gum in cosmetics.

Medicinal and other uses of Fenugreek Gum

Fenugreek seed has a bitter taste and strongly aromatic odor. The plant has been respected for its various medicinal benefits for last 2500 years. It has been described in Ayurvedic and Unani Systems of Health Care. Medicinal properties of seed are due to soluble dietary fiber. Being non-metabolized in human system, the galactomannans act as dietary fibers and promote the beneficial pre-biotic colon bacteria. FG, compared to other dietary fibers, shows maximum efficacy in lowering blood glucose and lipids including LDL and cholesterol. In India, many persons regularly take whole fenugreek seed powder, in spite of its bitter taste and odor. In the western countries, isolated, odorless and tasteless gum is used as fiber rich food additive. Clinical studies have shown that 2-3 g/day fenugreek gum is effective in controlling blood sugar, whereas the requirement of other food fibers is in larger (~20g) amounts.

Fenugreek fibres thicken ingested food to form a gel in stomach trapping fat, sugars and starch hydrolyzing amylase enzymes to slow down sugar absorption. Thus, it is good for obese and diabetic persons. The gel, which appears like 'fat' inside the body, signals the brain to send message to gall bladder to empty bile in the stomach. The gel then irreversibly traps lipid-emulsifying bile salts and prevents their reabsorption. Thus, emulsification and absorption of lipids including cholesterol results in lowering of blood lipid. This in turn reduces hypertoness and chance of heart attack.

**Sesbania bispinosa and Cassia tora Galactomannan Gums**

Both these gums are products from wild, but annual herbs of Indian origin. Traditionally, these plants are used for wasteland reclamation and for soil fertility improvement. These plants are a source of fodder and wood fiber similar to jute cellulose for making paper-pulp, fuel and gum. The seed yield from these plants is ~1000-1600 kg/ha, and the yield of seed gum (28-30%) is comparable to that of guar (Table 1).

During early seventies, when guar emerged as most widely used galactomannan, these legume plants were considered as its cheaper alternatives. These were investigated as guar gum substitute in non-food applications or even for adulteration of guar gum. Around 50,000 ton of seeds from these plants are currently exported from India, suggesting their use elsewhere in the world.

Since S. bispinosa and C. tora, continue to grow wild, their suitability in food applications remains questionable. No systematic studies have been carried on toxicity of these two gums and these are not approved as food additives. Presence of chrysophenic acid (anthraquinone type) in these gums at concentration higher than the permissible limit (<15 ppm) certainly indicates the toxicity in these gums. There is a need for independent study in India to study toxicity of minor constituents of these gums. M/S B.F. Goodrich Co. of Germany, which has patented gum extraction process from C. tora and installed a gum-processing unit in India, claims that

<table>
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<tr>
<th>Seed constituents</th>
<th>Seed coat (16-20%)</th>
<th>Endosperm (30-42%)</th>
<th>Kernel (42-46%)</th>
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<td>(a)</td>
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<tr>
<td>Seed coat</td>
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<td>Oil</td>
<td>0.5</td>
<td>1.2</td>
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<td>Fiber</td>
<td>24.6</td>
<td>28.6</td>
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<tr>
<td>Ash</td>
<td>4.5</td>
<td>3.8</td>
<td>0.6</td>
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<tr>
<td>Carbohydrate</td>
<td>3.5</td>
<td>4.6</td>
<td>73</td>
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the toxic constituents in CT-gum are reduced in their extraction process to a certain limit, and gum can be used in pet-food.

Reasonable success has also been achieved in replacing guar gum by derivatised (carboxymethyl or CM-derivatives) SB- and CT-gum in textile printing and paper industry. Thus, there is a good possibility of using these gums for technical applications, where the functional properties permit their use as a suitable substitute of guar gum. Collection and processing of these gums is only carried in an unorganized, small scale or cottage industry level. There shall be a need for further study and R&D efforts, particularly for their derivatization, which can result in improved solubility of these gums for textile use. There is also a need to install a dedicated R&D center for overall development in the field of polysaccharides and gums.

**Processing of S. bispinosa and C. tora Gums**

Because of smaller seed size, the existing split making plants for guar do not produce pure split from these seeds as desired, with the result that product gum contained higher percentage of protein, ‘acid insoluble residue’ or AIR, which is mainly cellulose arising from contamination of seed coat and other impurities.

**Properties and Rheology of SB-gum and CT-gum**¹²

An apparent viscosity (measured by Brookfield Viscometer) of gums is as follows: SB-gum, 400-1200 cps (1.0 % moisture); and CT-gum, 3000-12000 (2.0 % moisture). The viscosity is stable over pH range 4 to 9. Like most hydrocolloids, these gums have shear thinning flow behavior and viscosity decreases with rise in temperature. When blended with xanthan polysaccharide, these gums do not form gel, but there is synergic increase in viscosity: A behavior similar to guar gum. SB-Gum has [η]₅₀ = +22 (0.5% in water). SB-gum¹² has usual legume seed galactomannan structure. For both these polysaccharides, M: G ratio (2:1) is not much different from that of guar gum. Because of the lower molecular weight, viscosity of these gums is lower compared to guar gum. These properties make these gums suitable as print paste thickeners in textile industry. Major limitation of commercial grade gums in textile printing is higher AIR and poor filterability of the paste through 50-60 micron-mesh cloth resulting in choking of printing screen in flat bed and rotary screen-printing machines. This limitation is overcome by derivatising these gums into anionic products e.g. sulfonated or CM-gum. Carboxymethylation of these polysaccharides lowers the insoluble impurities (cellulose, proteins) and AIR and also improves solubility, filterability and after-wash of the printed cloth to produce a soft and supple handle for the finished cloth.

**Cassia fistula Gum**

*C. fistula* (Amaltas), also called the ‘Golden Shower’ or the ‘Indian labunum’, is an ornamental, perennial tree, growing all over India. Its seed galactomannan gum was found comparable to tara-gum and to a lesser extent to LBG. M:G in tara gum and in *C. fistula* gum is 3: 1, which is intermediate between guar gum (2: 1) and LBG (4: 1). In physical and functional properties, it behaves like a blend of LBG and guar gum. Blends of either *C. fistula* or tara gum with xanthan gum, has a rheology, which can open new vistas for their use in food, but till now *C. fistula* gum has not found favor for commercialization.

Structural studies of *C. fistula* gum revealed the usual structural features of legume galactomannans. Distribution of galactose grafts on tara-gum backbone has not been studied in full details, but its binary gelling of xanthan gum and agar suggests that, galactose stubs are present in blocks. This is also true of *C. fistula* gum. Tara gum (M: G = 3:1) gives weak gelling reactions compared to LBG (M: G = 4:1), yet both these gums have applications in food industry. After toxicity studies, *C. fistula* gum could be approved as food additive. It has weak self-gelling properties and forms mixed gels similar to LBG. In many food applications, it is suitable as a stabilizer and mixed gel systems.

Tara gum is produced in a small amount (1000-1500 ton/y), which is primarily used in food industry, as a thickener and stabilizer. It is used in dessert gels, seafood, meat, ice cream and yogurt. It has an improved rheology, water binding, and emulsifying properties. Patents related to tara gum claim it to be a water-soluble polymer, which has applications comparable to guar or LBG, but the price structure of tara gum does not justify its functional application, other than food.

The manufacturers of tara gum are powerful MNC’s, Caesalpina SpA and Nutragum SpA of Italy and Unpectin A.G. Company in Switzerland. Compared to *C. fistula*, the processing of tara gum is
more problematic due to poor dehusking of the seed and problem in powdering of hard endosperm. Tara pod, which is rich in pyrogallol tannins, was earlier processed only for its tannin, used in specialty leather. Recovery of another commercial product (gum) from its seed resulted in a good value addition. Processing problems do not exist with *C. fistula* seed.

**Galactomannan from Pods of *Prosopis juliflora***

*P. juliflora*, introduced into India from Central America, has spread all over the country, particularly in the semi-arid and wastelands. Currently, it is being used as a source of firewood, while its pods are used as fodder for cattle, particularly for sheep and goats in Rajasthan, Gujarat and Haryana. The dried, flattened yellow pod consists of an outer layer of pulp, rich in sugars, and underneath lies the hard and protein rich seed. Just like many tree legumes, the endosperm portion of the seed contains a galactomannan gum, similar to guar gum. Currently the low seed volume does not make processing of gum economical. Another reason of this gum, not getting favor from the consumers, is that supply of guar gum is better assured and consumers do not want to use an alternate product, unless it is very cheap.

**Sulfonate Group Substitution in Polysaccharides: A New Class of Polysaccharide Derivatives and their Uses**

The authors have carried out a new derivatising reaction of several polysaccharides (guar, starch, *C. tora*, *S. bispinosa* and tamarind), in which a strongly anionic, sodium sulfonate group is introduced into the polysaccharide molecule. The new derivative is O-2-hydroxy-propyl-1-sulfonic acid salt, produced by reacting a polysaccharide with the derivatising reagent, which is sodium-3-chloro-2-hydroxy-propane-sulfonate. The reaction takes place via initial formation of an epoxide form (2-hydroxypropyl-trimethyl-ammonium, cationic derivative), of the reagent (Fig. 2). The degree of substitution can be varied and the reaction efficiency in such etherification reaction, carried at 60-70°C, in presence of alkaline catalyst is ~ 70 percent.

Unlike CM-group, sulfonic salts remain ionized over a wide range of pH and do not form insoluble salts with bivalent metals (Ca & Mg ions) and have better tolerance to hard water. This makes sulfonic acid derivatives of polysaccharides such as *S. bispinosa*, guar, tamarind and most other polysaccharides better suited for textile applications.

**Applications of Sulfonate Derivatives of Polysaccharides**

Most of the textile dyes are anionic due to the presence of sulfonic acid group in their molecules to induce water solubility. Sodium alginate is considered as the most versatile anionic polymer as print paste thickener. Because of its good water solubility and viscosity and above all, being anionic, it has poor affinity for anionic dyes. This results in preferential transfer of a dye to cotton fabric and a high color yield. Anionic sulfonate group bearing polysaccharide thickeners behave similar to alginate. They are economical and more abundant than alginates of seaweeds.

For exterior water based paints (distemper), anionic polysaccharides (CMC) are used as rheology control agents. Such paints are suspension of solids (pigments, fillers, vinyl and acrylic polymers) in water. Linear-anionic gum acts as emulsion stabilizer for paints, by getting adsorbed on the surface of finely divided solids, and impart a negative charge to them. Charged particles form a stable suspension. Authors have found sulfonate polysaccharide derivative to be even better stabilizer than CMC for such paints.

**Conclusions**

Small and medium scale industries, concerned with manufacture and utilization of agro-products, such as hydrocolloid gums, do not have their own R&D facilities and do not get guidance from experts about the developments in this field. At the international level, the panel of ‘Scientists and Advisory
Committees on Technology Innovation of the ‘National Academy of Science, USA’, in collaboration with the UNO, have made a compilation, “Under Exploited Legumes as Resource for Future”53. Similarly, National Botanical Research Institute (CSIR), Lucknow, has published a database on legumes of eight South-Asian countries54. Still there shall be a need for an institutional cell such as those of CSIR and ICAR, where developments related to industrial polysaccharides can be made and passed on to industries. India is fortunate to have many less-used natural resources and it is the high time that the country starts undertaking developments on utilization of such resources.

References