Extraction, Purification, and Physicochemical Characterization of Seed Galactomannans

Wancheng Sittikijyothin*

Department of Chemical Engineering, Faculty of Engineering, Burapha University
Chonburi, Thailand

Abstract

The galactomannans as endosperm mucilage were extracted from seeds of *Caesalpinia pulcherrima* and *Delonix regia*. Crude galactomannans were then purified by using isopropanol precipitation, the purified galactomannan yields of 75 and 82% of crude mass were obtained for *Caesalpinia pulcherrima*, *Cassia fistula*, and *Delonix regia*, respectively. The physicochemical properties of both crude and purified galactomannans were analyzed. The results showed that the crude galactomannans revealed high protein content which eliminated after purification. Crude galactomannans obtained 3.46 and 6.12 of mannose to galactose (M/G) ratio for *Caesalpinia pulcherrima* and *Delonix regia*, respectively. After purification the M/G ratio exhibited lower: 2.92 for *Caesalpinia pulcherrima* and 4.72 for *Delonix regia*. The intrinsic viscosity and viscosity average molecular mass of galactomannan samples were estimated by using Huggins' and Kraemer equations and the Mark-Houwink relationship, respectively. The obtained results showed that the intrinsic viscosity of purified galactomannans increased when compared with crude galactomannans. It indicated that the purification process used alter the chemical structure. Since the intrinsic viscosity directly resulted in chemical structure as M/G ratio.

Keywords: Galactomannan, Extraction, Purification, Intrinsic viscosity, *Caesalpinia pulcherrima*, *Delonix regia*

I. Introduction

Galactomannans are plant carbohydrates that occur in the endosperm of the seeds of leguminous plants. They are heteropolysaccharides whose structural components are two monosaccharides: D-mannose and D-galactose. The mannose units form a linear chain consist of (1→4)- β-D-mannopyranosyl residues with (1→6) linked α-D-galactopyranosyl residues [1], [2]. In general, galactomannans from seeds of different botanical sources differ by the mannose to galactose (M/G) ratio [1], [3], [4]. The different M/G ratios make galactomannans versatile materials used to modify textural attributes as thickening, water holding, stabilizing, and emulsifying agents in the food and other industrial applications [1], [2], [5]. In principle, the M/G ratio causes significant changes in the solubility, viscosity, and ability of galactomannan to interact with other carbohydrates. For seed galactomannan is classified in weakly branched classical galactomannans [2] due to high mannose. For example, guar gum (M/G ~ 2) disperses more rapidly in water than locust bean gum which has lower galactose content (M/G ~ 4) [2], [5]. Because galactomannans with higher mannose content consist of long blocks of unsubstituted mannose units that can either covalently interact with or chemically bind other polysaccharides (cross-linking) [6].

In Thailand, there are many plants that belong to the leguminous family which obtain seed galactomannan. *Caesalpinia pulcherrima* and *Delonix regia* are some of them and native Caesalpiniaceae species (Leguminosae-Caesalpinioideae) which widely cultivated as ornamental trees in many regions of country. Since, the industry trends demand the opening of alternative sources of seed galactomannans. For that reason, the physicochemical properties of seed galactomannans from *Caesalpinia pulcherrima* and *Delonix regia* as alternative sources were interested to characterize and get more information in this work.

II. Meterial and Methods

A. Plant Materials

Pods of *Caesalpinia pulcherrima* and *Delonix regia* were collected in Chonburi province, Thailand. Mature seeds were manually separated from the pods and maintained in a cool dry place until the extraction process.

B. Extraction and Purification

The sample seeds were crushed before they were soaked. The endosperms were manually removed from the germ and the hull. Then, they were dried, milled and ground through a 355 micron mesh sieve.

The extraction and purification of galactomannans is shown in Fig. 1. The ground powder of crude galactomannan was extracted in water under mechanical stirring at room temperature (time varying from 0.5 to 3 hours). Sodium azide was added

* Corresponding author: E-mail: wancheng@buu.ac.th

(5 ppm) to avoid bacterial growth. The solutions were heated for 2 hours at 80 °C. After cooling, the non-dissolved material was removed by centrifugation at 6,000 g for 1 hour. The solubilized galactomannan was precipitated with excess of isopropanol. The precipitate was washed with acetone and dried in vacuum oven at 30 °C. Then it was ground to a fine powder (purified sample) by the 355 micron mesh sieve.

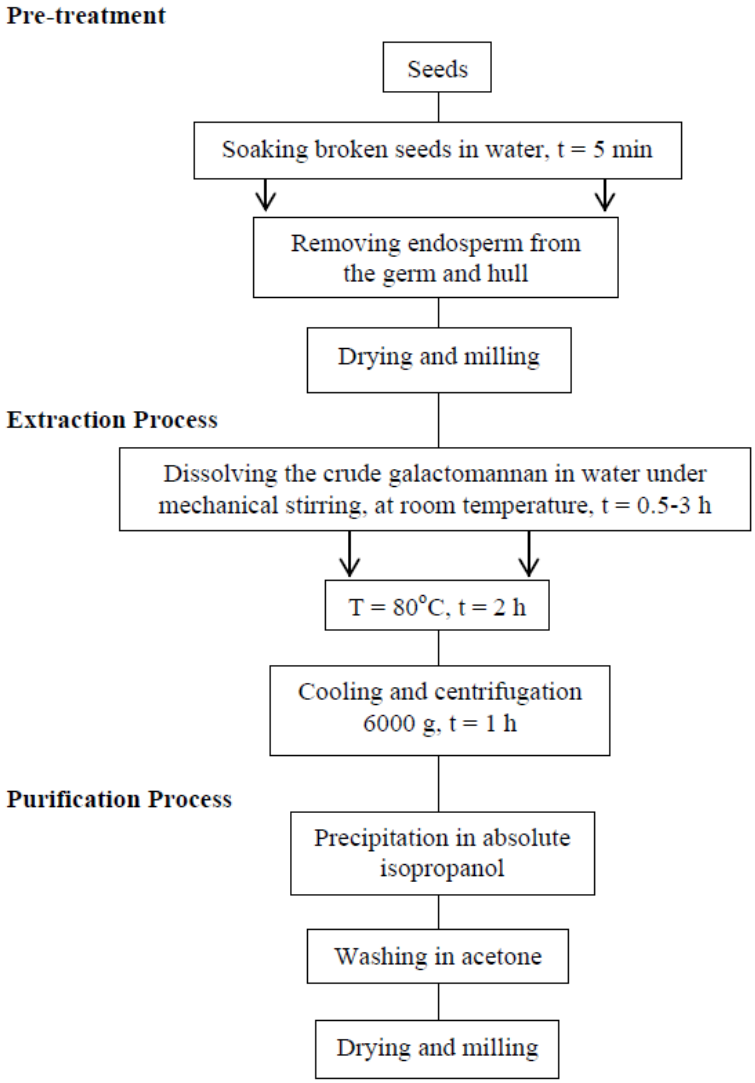


Figure 1 Extraction and purification processes of seed galactomannans from *Caesalpinia pulcherrima* and *Delonix regia*

C. Chemical Composition

Moisture and ash contents were determined according to the American Society for Testing and materials methods (ASTM-D2974-87) and AOAC Official Method 923.03, respectively. Protein content was obtained from the total nitrogen content (N×5.7) by the Kjeldahl method, as described in the AOAC Official Method of Analysis 981.10. Fat content was determined according to the AOAC Official Method of Analysis 923.06.

The main monosaccharide component was analyzed by GC-FID (Agilent Technologies 6890N Network GC system) fitted with flame ionization detector, equipped with a 30×0.25 mm. DB-225 column [7].

D. Intrinsic Viscosity

The stock galactomannan solution (0.1 wt%) was prepared. The galactomannan powder was slowly added to the appropriate amount of distilled water in the presence of sodium azide (5 ppm) in order to prevent bacterial degradation. The dispersion was moderately stirred for 1 hour, at room temperature, and then heated at 80 °C in the water bath for 30 min, under continuous stirring. After cooling, a clear solution was, normally, obtained. The stock solution was centrifuged. Then, supernatants recovered. Further dilutions were made to obtain a wide range of solution concentrations.

Viscosities of dilute solutions were measured at 20.0±0.1°C with a Cannon-Fenske Routine Viscometer (9721-A53) (ASTM-D2515, and Series

100). The limiting viscosity number ("intrinsic viscosity"), $[\eta]$, is conventionally obtained by double extrapolation to zero concentration of Huggins' and Kraemer equations, respectively.

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \quad (1)$$

$$\frac{(\ln \eta_{rel})}{C} = [\eta] + k''[\eta]^2 C \quad (2)$$

Where η_{sp} and η_{rel} are the (dimensionless) specific and relative viscosities, k', k'' are the Huggins' and Kraemer's coefficients, respectively, and C is the solution concentration.

E. Viscosity average molecular mass

Viscosity average molecular masses ($\bar{M}_v$) were calculated using the Mark-Houwink relationship that account the different mannose to galactose ratios of galactomannans [8].

$$[\eta] = 11.55 \times 10^{-6}[(1-\alpha)\bar{M}_v]^{0.98} \quad (3)$$

where $\alpha = 1/[(M/G)+1]$ and $[\eta]$ is expressed in dL/g.

III. Results and Discussion

A. Extraction and Purification

The crude galactomannans obtained by milling the seeds of *Caesalpinia pulcherrim* and *Delonix regia*, were extracted as in the following Fig. 1. The crude galactomannan yields of 34 and 27% were obtained for *Caesalpinia pulcherrima* and *Delonix regia*, respectively. According to extraction process, only extraction period at room temperature was varied from 0.5 to 3 hours while the extraction time at 80°C was fixed for 2 hours. Then, crude galactomannan was purified by precipitation with isopropanol. Yields of purified galactomannans at different extraction periods were presented in Fig. 2. The obtained results show that increasing of solubility period at room temperature enhanced more yields for both galactomannans.

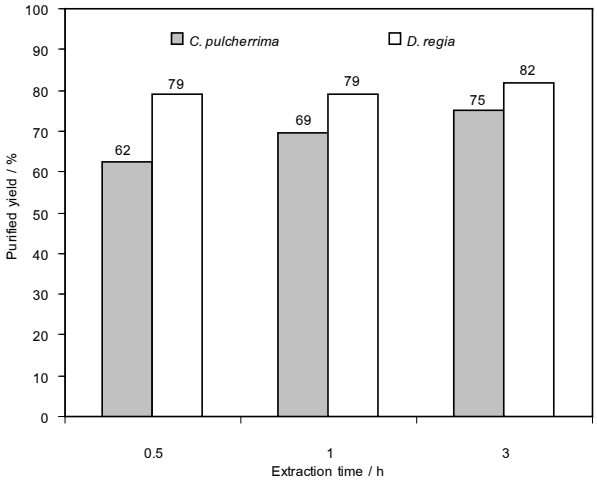


Figure 2 Purified yields of galactomannans at different extraction periods at room temperature and fixed for 2 hours at 80°C

B. Chemical Composition

In Table 1, the main characteristics of both crude and purified galactomannans from three legume plants are presented. It can be seen that the seed galactomannans are rich source in polysaccharide with some amount of protein content. However, the purification process seems efficient to eliminate protein and other impurities from crude galactomannans [9-12]. Regarding the impurities, they are the important factor to estimate the purity of gums, particularly in food applications [4]

TABLE I. Chemical composition of galactomannans

	Caesalpinia pulcherrima		*Delonix regia*	
	Crude	Purified	Crude	Purified
Moisture (%)	10.9	12.86	11.17	12.99
Ash (%)	0.06	0.05	0.11	0.10
Protein (%N×5.7)	4.62	1.52	2.86	1.62
Fat (%)	1.45	0.18	0.90	0.17
Polysaccharide[a] (%)	93.87	98.25	96.13	98.11
M/G ratio	3.46	2.92	6.12	4.72

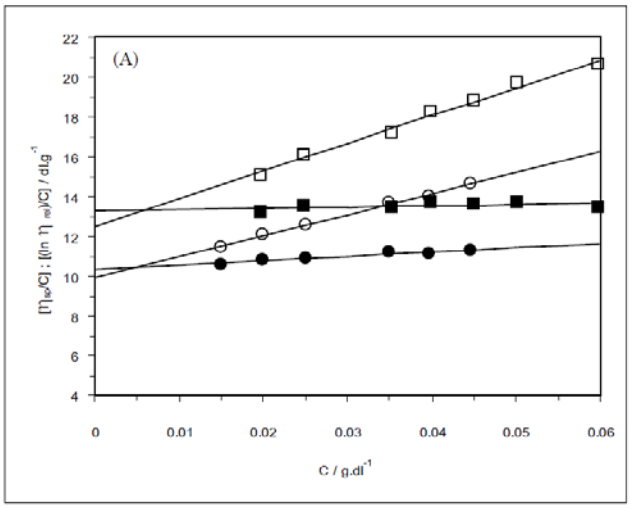
All values (%) on a dried weight basis are mean ± standard deviation of three determinations.
[a]Polysaccharid values (%) were calculated by difference.

Then, the mannose to galactose (M/G) ratio of galactomannan samples was analyzed by GC-FID. The obtained result confirms that the M/G ratio varied for the different leguminous species. Hence, the M/G ratios for crude galactomannans from *Caesalpinia pulcherrima* and *Delonix regia* seeds were about 3.46 and 6.12, respectively, higher than the values obtained by previous researchers who found

M/G ratio for *Caesalpinia pulcherrima* of 2.70, 2.83, and 2.88 [4], [8], [13] and for *Delonix regia* of 4.28 [2], [4]. According to purification process by isopropanol precipitation in this work, the presence of M/G ratios for purified galactomannans were reduced. It can be evidently pointed out here that the M/G ratio differs based not only on the source but also on isolation procedure [12], [13].

C. Intrinsic Viscosity and Viscosity Average Molecular Mass

Intrinsic viscosity of the galactomannan samples was estimated at 20 °C. Fig. 3 displays the determination of $[\eta]$, by extrapolation to zero concentration of Huggins' (Eq.1) and Kraemer (Eq.2) plots.



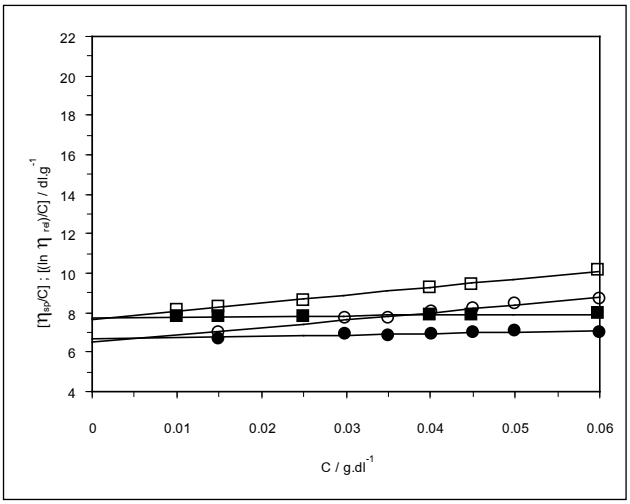


Figure 4 Determination of the intrinsic viscosity for crude (circles) and purified (squares) galactomannan solutions by combined Huggins' (open symbols) and Kraemer's (full symbols) extrapolations to zero concentration, at 20°C; (A) *Caesalpinia pulcherrima* and (B) *Delonix regia*

For all samples presented good linearity to infinite dilution. Table 2 represents the attained parameters. Values of these parameters obtained for purified galactomannan samples, were higher than for crude galactomannan samples, while the value for the Huggins' coefficient, k_H', was lower. The k' value depends on solute-solvent interactions and on the state of aggregation of macromolecules; in theory, value is independent of molecular mass. In a good solvent and for flexible macromolecules, k' ~ 0.35 [14]; but, it can be higher than 1.0 in case if aggregation. For our samples, the k' values of 0.70 – 1.07 may reflect some intermolecular aggregation in the solutions.

TABLE II. PHYSICAL-CHEMICAL PARAMETERS OF GALACTOMANNANS, AT 20°C

TABLE III.	*Caesalpinia pulcherrima*		*Delonix regia*	
	Crude	Purified	Crude	Purified
$[\eta]H^a$, (dl/g)	9.91	12.46	6.51	7.65
$[\eta]H^b$, (dl/g)	10.32	13.27	6.64	7.76
Huggins' coefficient, k_H'	1.07	0.90	0.88	0.70
$\overline{M}_v{}^c$, (Da × 10^{-6})	1.46	1.92	0.86	1.06

[a] Intrinsic viscosity (Huggins' extrapolation).
[b] Intrinsic viscosity (Kraemer extrapolation).
[c] Viscosity average molecular mass.

In principle, the difference of M/G ratio of galactomannans directly relates to the viscosity of the solution. The intrinsic viscosity of galactomannan from seed of *Caesalpinia pulcherrima* with lower M/G ratio (3.46) was higher than that of *Delonix regia* with higher M/G ratio (6.12) as shown in Tables 1 and 2. The presence of a large number of galactose units on a linear polysaccharide induces water solubility, which can result in more viscous solution [6]. Regarding the purified galactomannans, their intrinsic viscosities increased since their M/G ratios were altered by purification method which is same as in another report [12].

Then, the viscosity average molecular mass of galactomannan was calculated using the Mark-Houwink relationship (Eq. 3). The obtained results show that the molecular mass for galactomannan from *Caesalpinia pulcherrima* seed was higher than another (Table 2) due to the higher galactose substitution.

IV. Conclusion

Galactomannans from *Caesalpinia pulcherrima* and *Delonix regia* seeds are rich in polysaccharide and protein contents. They differ in M/G ratio which directly relates to the viscosity of solution: the higher values of intrinsic viscosity of galactomannan due to possessed lower M/G ratios. The purification methodology as isopropanol precipitation used in this work not only removes the impurities from the crude galactomannans but also alter their M/G ratios. For this case, the M/G ratio of purified galactomannans was decreased resulting in the intrinsic viscosity increasing.

Acknowledgement

The authors acknowledge the financial support from Faculty of Engineering, Burapha University for research project (12/2554).

References

[1] Sharma, B. R., Dhuldhoya, N. C., Merchant, S. U. and Merchant, U. C. A glimpse of galactomannans. Science Tech Entrepreneur, February, 2008, 1-10.

[2] Srivastava, M., and Kapoor, V. P. Seed galactomannans: an overview. Chemistry & Biodiversity, 2, 2005, 295-317.

[3] Lazaridou, A., Biliaderis, C. G. and Izydorczyk, M. S. Structural characteristics and rheological properties of locust bean galactomannans: a comparison of samples from different carob tree populations. Journal of Science of Food and Agriculture, 81, 2000, 68-75.

[4] Rana, V., and Bachetii, R. Seed galactomannans: a natural source of industrial potential. International Transactions in Applied Sciences, 2, 2010, 201-211.

[5] Batlle, I., and Tous, J. Carob tree. Ceratonia siliqua L, Promoting the conservation and use of underutilized and neglected crops. 17. Rome, Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, 1997.

[6] Mathur, N. K., ed. Industrial galactomannan polysaccharides. New York, CRC Press, 2012.

[7] Blakeney, A. B., Harris, P. J., Henry, R. J., and Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydrate Research, 113, 1983, 291-299.

[8] Andrade, C. T., Azero, E. G., Luciano, L., and Gonçalves, M. P. Solution properties of the galactomannans extracted from the seeds of Caesalpinia pulcherrima and Cassia javanica: comparison with locust bean gum. Internaitonal Journal of Biological Macromolecules, 26, 1999, 181-185.

[9] Amin, A. M., Ahmad, A. S., Yin, Y. Y., Yahya, N., and Ibrahim, N. Extraction, purification and characterization of durian (Durio zibethinus) seed gum. Food Hydrocolloids, 21, 2007, 273-279.

[10] Azero, E. G., and Andrade, C. T. Testing procedures for galactomannan purification. Polymer Testing, 21, 2002, 551-556.

[11] Bouzouita, N., Khaldi, A., Zgoulli, S., Chebil, L., Chekki, R., Chaabouni, M. M., and Thonart, P. The analysis of crude and purified locust bean gum: a comparison of samples from different carob tree populations in Tunisia. Food Chemistry, 101, 2007, 1508-1515.

[12] Youssef, M. K., Wang, Q. Cui, S. W., and Barbut, S. Purification and partial physicochemical characteristics of protein free fenugreek gums. Food Hydrocolloids, 23, 2009, 2049-2053.

[13] Cerqueira, M. A., Pinheiro, A. C., Souza, B. W. S., Lima, A. M. P., Ribeiro, C., Miranda, C., Teixeira, J. A., Moreira, R. A., Coimbra, M. A., Gonçalves, M. P., and Vicente, A. A. Extraction purification and characterization of galactomannans from non-traditional sources. Carbohydrate Polymers, 75, 2009, 408-414.

[14] Wang, Q., Ellis, P. R., and Ross-Murphy, S. B. The stability of guar gum in an aqueous system under acidic conditions. Food Hydrocolloids, 14, 2000, 129-134.