ELSEVIER

Carbohydrate Polymers **24** (1994) 133–138

0144-8617/94/$07.00

Partial characterization of cacao pod and stem gums

Antonio Figueira,[a] Jules Janick[a] & James N. BeMiller[b]

[a]*Department of Horticulture,* [b]*Department of Food Science, Purdue University, West Lafayette, Indiana 47907, USA*

(Received 1 March 1993; revised version received 18 January 1994; accepted 21 January 1994)

Histochemical analysis confirmed the ubiquitous presence of gums in *Theobroma cacao*. Gums from cacao stems and pod husks were prepared and partially characterized. Both gum preparations contained 2–3% protein after treatment with a cation-exchange resin, indicating that they are protein-polysaccharides. They both contained Gal, Glc, Rha, Ara, Xyl, GalA, and GlcA, stem gum in molar ratios of 1·0:2·8:1·9:0·3:0·1:2·4:1·2 and pod gum in molar ratios of 1·0:0·2:1·9:0·2:trace:4·2:1·3. Both cacao gums contained considerably more uronic acid than did gum karaya, and were approximately ten times less viscous than gum karaya.

INTRODUCTION

Gum karaya, produced from various *Sterculia* species, mainly *Sterculia urens* Roxb., Sterculiaceae, has in the past been used in the food and medical industries but its use has now diminished because its supply is variable, unreliable, and at best static (Whistler, 1993). In the US its use is now essentially restricted to colostomy ring and denture adhesives. Gums of cacao (*Theobroma cacao* L.), a related species in the same family, might serve as a substitute.

The original interest in cacao gums arose from its suspected role in the recalcitrance of cacao growth *in vitro* (Figueira *et al.*, 1989). The objectives of the investigation reported here were to characterize cacao gums from stems and pod husks and to evaluate their potential as a replacement for gum karaya or as a new commercial product.

Lysigenous cavities filled with mucilaginous substances occur in roots, stems, flowers, and leaves of cacao (*Theobroma cacao*, Sterculiaceae) (Brooks & Guard, 1952). The presence of large amounts of gums in cacao tissues implies an important physiological role, which could be related to storage, osmoprotection, defense mechanisms, or other processes.

Polysaccharides of cacao were first characterized by Whistler *et al.* (1956), who found differences in hot-water-soluble polysaccharides between seed and pod husks. Blakemore *et al.* (1966) examined the hot-water-soluble fraction of husk polysaccharides and concluded that the major part of this fraction was pectic material. Dittmar (1958) described the hot-water extract as pectin contaminated with a glucan and a mannan. Cacao pod husks (mature fruits without seeds or pulp) were examined as a source of pectin by Adomako (1972, 1975) and Berbert (1972), but yields were low and the gum was inferior to apple or citrus pectin in gel-forming ability.

EXPERIMENTAL

Histological examination

Mature cacao pod husk sections (1 cm^3) and primary and secondary stem tissues (1 cm in length) were fixed in formalin/acetic acid, dehydrated with ethanol-*tert*-butanol (Sass, 1951), and embedded in Paraplast (Monoject Scientific, St Louis, MO). Sections 15 μm thick were cut and then double stained with safranin-fast green for observation by light microscopy.

Polysaccharide extraction and purification

Stem tissue (mostly nonlignified stems, i.e. without secondary growth, cut into *c.* 1-cm pieces) was obtained from greenhouse-grown mature trees, and pods were either harvested from greenhouse trees or collected at the Centro Agronomico Tropical de Investigacion y Enseñanza (CATIE), Turrialba, Costa Rica. Tissue (stem and pod husk without seeds) was extracted twice

for 30 min with boiling 70% ethanol (100–120 g fresh wt/l), then twice extracted (30 min and 10 min) with boiling methanol. The tissue pieces were left overnight in methanol and air-dried.

The alcohol-extracted tissue was blended with distilled water (*c.* 175 g original tissue wt/l), then centrifuged at 1500 × *g* for 30 min. The supernatant was decanted, filtered and concentrated under reduced pressure at 50°C. Gum was precipitated with three volumes of 95% ethanol and collected by centrifugation for 25 min at 3500 × *g*. The resulting pellet was redissolved in water and freeze-dried.

Proteins and other cations were removed from the crude gum by passing a solution of it through a column of Amberlite IR-120[H^+] cation-exchange resin and washing the column with distilled water. The carbohydrate-containing eluate was concentrated under reduced pressure at 50°C, dialyzed (MW cutoff 12,000–14,000 daltons) for 48 h, and freeze dried; total gum recovery was 33%.

Stem and Pod Husk Tissue

1) Extract with hot 70% EtOH
2) Extract with hot MeOH
3) Extract gum with r.t. water
4) Precipitate gum with 3 volumes EtOH

▼

Crude gum
(Yield = 1·5% of fresh wt and 8·0% of dry wt from stem tissue
= 0·8% of fresh wt and 9·1% of dry wt from pod husk tissue)

1) Treat with cation-exchange resin
2) Dialyze

▼

Purified (Deproteinized) Gum
(Recovery *c.* 33%)

Chromatography

Deproteinized cocoa stem gum (0·3048 g) was loaded onto a column of DEAE-cellulose (*c.* 135 g; Sigma Chemical Co., St Louis, MO) which was then developed with water and five salt gradients (0–1 M NaCl) and one acid gradient (0–0·02 M HCl). Tubes containing carbohydrate were combined into four peaks. Each fraction was dialyzed for 48 h against distilled water and freeze dried; total gum recovery was 0·0603 g (20%).

Constituent determination

Colorimetric assays for total neutral sugars (Dubois *et al.*, 1956), protein (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA), and uronic acids (York *et al.*, 1986) were performed on stem and pod gums before and after deproteinization. Samples (3 mg) of cacao gums and gum karaya (Sigma) were solvolyzed with liquid HF (5 ml) for 2 h with stirring; hydrolysis was then effected by addition of Millipore-filtered water (5 ml) (Yadav *et al.*, in press). The samples were dried at 50°C under nitrogen gas, redissolved in 0·5 ml of filtered water, passed through a 0·2-μm filter, and analyzed using a Dionex (Sunnyvale, CA) BioLC Gradient Pump Module HPLC with a Model Pad 2 pulsed amperometric detector (Hardy *et al.*, 1988). Samples from crude and purified preparations were digested using a hydrogen peroxide–perchloric acid procedure (Adler & Wilcox, 1985); potassium, calcium, and magnesium ion concentrations were estimated using a Varian Model Spectra AA 10 Atomic Absorption Spectrophotometer (Varian Techtron Pty. Ltd., Victoria, Australia).

Viscosity

The viscosities of purified cacao stem gum solutions were compared with those of gum karaya solutions using a Brookfield (Stoughton, MA) model LVTD viscometer with small sample adapter (10 ml solution) and spindle SCH-31. Crude pod gum solution (600 ml) viscosities were also compared with those of gum karaya using a Brookfield model LVTDV-II viscometer with spindles LV-04/64 (for solutions from 3–5% concentration) and LV-02/62 (for solutions from 0·5–2·5% concentrations).

RESULTS AND DISCUSSION

Histology

Histochemical analysis confirmed the ubiquitous presence of gums in cacao. The gum was found in lysigenous cavities (as noted by Brooks & Guard (1952)) which stained a deep magenta with safranin. Cavities were located throughout the pith and cortex of stems, constituting a significant portion of the cross-sectional area of nonlignified shoots, and were found throughout the pericarp of husks. In *Sterculia urens* Roxb., the major karaya producing species, gum ducts develop lysigenously and lysi-schizogenously (Nair & Shah, 1984). In *Sterculia*, gum formation is a product of lysis of the epithelial cells in the pith ducts; in the cortical ducts, it originates from transformation of part of the inner tangential wall followed by lysis of epithelial cells. The anatomical site and mechanism of cacao gum formation is unknown.

Gum preparation and analysis

From greenhouse-grown trees, crude gum yields by extraction with room-temperature water averaged 1·5% of fresh weight and 8·0% of dry weight of stems, and 0·8% of fresh weight and 9·1% of dry weight of pod husks. Pods collected from field-grown trees in

Turrialba, Costa Rica, averaged similar yields on dry and fresh weight bases (Table 1). Polysaccharide yields from husks reported in the literature range from 8–11% on a dry weight basis (Blakemore *et al.*, 1966; Adomako, 1972; Berbert, 1972). Although carbohydrates play a major role in controlling rhythmic shoot growth (i.e. flushing) of cacao (Machado & Hardwick, 1988), no attention has been placed on the potential role that polysaccharides play in carbohydrate balance or host–pathogen interaction. Figueira *et al.* (1989) hypothesized that the gum is responsible for the recalcitrance of cacao shoots to in-vitro culture.

Colorimetric assays indicated that cacao gums were acidic protein-polysaccharides (since cation-exchange did not remove all the protein) and that gums from pod husks were distinct from gums taken from the stems (Table 2). Pod gum had a higher percentage of uronic acids. The uronic acid content of gum karaya ranges from 35–40% (Aspinall & Nasir-ud-din, 1965; Goldstein & Alter, 1973; Glicksman, 1982); a similar amount was found in cacao pod gum. Cacao stem gum contained *c.* 2% protein and pod gum contained 3–5% protein, while the protein content of gum karaya is *c.* 1% (Anderson *et al.*, 1985).

Table 1. Gum yields from pods obtained from different genotypes grown under field conditions

Genotype	DW/DW (%)	DW/FW (%)
UF613 × SCA6	15·2	1·2
CC42 × P7	5·1	0·6
CC42 × Catongo	4·7	0·5
Catongo × UF296	10·0	ND[a]
UF296 × Catongo	12·2	1·4
Average	9·4	0·9

[a]Not determined.

Table 2. Preliminary analysis of cacao stem and pod husk gum before and after cation exchange and dialysis

Component	Content (%)			
	Stem gum		Pod gum	
	Crude	After cation exchange	Crude	After cation exchange
Neutral sugars[a]	49	64	47	50
Uronic acids	20	20	44	39
Protein	1	2	5	3

[a]As D-glucose.

The monosaccharide composition of gum karaya varies with species, gum quality and gum type (Hirst *et al.*, 1949*a*; Goldstein, 1954; Anderson *et al.*, 1982). The sugar composition of gum karaya obtained here (Table 3) agrees with previous reports (Phillips *et al.*, 1980; Goldstein & Alter, 1973), except for the detection of trace amounts of arabinose and glucose. Hirst *et al.* (1949*b*) reported that gum karaya from at least one source contained rhamnose, galactose, tagatose, and galacturonic acid in a molar ratio of 1·0:1·0:0·2:1·6. Both cacao gums contained basically the same monosaccharides as gum karaya (Table 3), but with greater amounts of rhamnose, galacturonic acid, and glucuronic acids. Cacao pod gum was closer in composition to gum karaya than was cacao stem gum.

The major component of cacao stem gum was glucose, not found in any appreciable amounts in cacao pod gum or gum karaya. Cacao stem gum contained twice the amount of galacturonic acid present in gum karaya, and four times the amount of glucuronic acid. Cacao pod gum did however contain four times as much glucuronic and galacturonic acid than gum karaya. The relative proportions of monosaccharides in cacao pod gum found in this study were similar, although not identical, to those reported by Whistler *et al.* (1956), and Blakemore *et al.* (1966) (see Table 3). Adomako (1972) extracted pod husk with dilute acid according to the procedure used to isolate pectin and obtained a product with a similar ratio of neutral sugars, but a much higher concentration of galacturonic acid (see Table 3). By hot water extraction, Dittmar (1958) isolated 11% of a product described as pectin contaminated with a glucan and a mannan. Room-

Table 3. Monosaccharide compositions of purified[a] cacao stem and pod husk gums and gum karaya[b]

Gum	Sugar composition (molar ratio)							
	Rha	Gal	Ara	Glc	Xyl	Man	GalA	GlcA
Gum karaya[b]	1·0	1·0	0·1	trace	0·0	0·0	1·0	0·3
Cacao stem gum[a]	1·9	1·0	0·3	2·8	0·1	0·0	2·4	1·2
Cacao pod gum[a]	1·9	1·0	0·2	0·2	trace	0·0	4·2	1·3
Cacao pod gum[c]	1·0	1·0	0·3	0·0	0·0	0·3	0·0	0·0
Cacao pod gum[d]	0·4	1·0	0·2	0·4	trace	0·3	1·3	0·0
Cacao pod pectin[e]	0·6	1·0	0·4	trace	0·3	0·0	13·4	0·0

[a]Treated with cation-exchange resin and dialyzed.
[b]Not purified.
[c]Whistler *et al.* (1956).
[d]Blakemore *et al.* (1966).
[e]Adomako (1972).

temperature water extraction used in the present study found glucose in the stem extract only. Mannose, as reported by Whistler *et al.* (1956) and Blakemore *et al.* (1966), was not detected. Larger amounts of rhamnose were observed than were previously reported, probably because the hydrolysis procedure which was employed minimizes decomposition of sensitive sugars; for the same reason uronic acid concentrations were likely to be higher than previously reported (Yadav *et al.*, in press). Uronic acids were not reported by Whistler *et al.* (1956); while Adomako (1972) reported more than 85% galacturonic acid, probably because a mild acid extraction was used to isolate the pectin. Glucuronic acid, previously unreported, was found by us. Some differences in proportions of monosaccharides were not unexpected because of the variability of source material and differences in isolation and analysis procedures.

Crude cacao gums contained a high concentration of potassium ions (Table 4). Purification by a cation-exchange column successfully reduced the cation content. Total ash content previously observed for cacao pod gum and pectin was 9% (Whistler *et al.*, 1956; Adomako, 1972), or 22·7% when based on dry husks (Blakemore *et al.*, 1966). Adomako (1972) identified calcium and potassium ions as the major ions present in a cacao pod pectin preparation. Gum karaya usually occurs in a salt form, containing calcium and magnesium ions (Kubal & Gralén, 1948). The high concentration of potassium ions in cacao pod husks explains the use of pod ash for soap production in Nigeria (Oduwole & Arueya, 1990).

Table 4. Cation composition of cacao stem and pod husk gum

Gum	Cation concentration (% dry wt)			
	Calcium	Magnesium	Potassium	Total
Crude pod gum	0·10	0·40	9·64	10·14
Purified pod gum[a]	0·07	0·02	0·01	0·10
Crude stem gum	0·42	1·34	3·81	5·57
Purified stem gum[a]	0·19	0·04	0·03	0·26

[a]Treated with cation-exchange resin and dialyzed.

Four major fractions and one minor fraction were obtained by DEAE-cellulose chromatography of stem gum. The protein content of the fractions recovered after anion-exchange chromatography was much less than that in the crude gum, and only one-third of the preparation was recovered, suggesting that the protein moiety was acidic. Aspartate and glutamate are reported to constitute >25% of the amino acid composition of the proteinaceous part of gum karaya (Anderson *et al.*, 1985). The percentage of uronic acids also decreased (see Table 5).

Viscosity

Cursory rheological examination of the two cacao gums revealed a similarity in rheological properties which were in turn similar to gum karaya's rheological properties (Figs 1–4). Solutions of all three gums exhibit marked shear-thinning behavior, however gum karaya produces greater viscosity. To achieve the same viscosity with the same Brookfield spindle and spindle speed as given by gum karaya, required about twice the concentration of cationized cacao stem gum (Fig. 1) and a little more than twice the amount of cacao pod gum (Fig. 2).

Decationized stem gum, i.e. stem gum in the free-acid form, was examined with a Brookfield viscometer using the small sample adapter. Compared to gum karaya, it produced a much lower rate of increase in viscosity with increasing concentration (Fig. 3). Viscosities at 5% concentration were about the same as those of gum karaya at 2·5% concentration (Fig. 1). Both gums displayed pseudoplasticity.

The comparison in viscosities of gum karaya and cacao pod gum solutions was made with a Brookfield viscometer using a larger volume and pod gum without cation removal. Again, gum karaya produced a much greater increase in viscosity with increasing concentration (Fig. 4), with the two cacao gums producing similar viscosity versus concentration profiles. Gum karaya is water-swellable rather than water-soluble and forms

Table 5. Composition of cacao stem gum fractions after anion-exchange chromatography

Fraction	Gradient	Yield[a] (%)	Content (% dry wt.)			
			Neutral sugars[b]	Uronic acids	Esters[c]	Protein
3	0·05–0·12 M NaCl	6·9	55	28	6	0·3
4a	0·12–0·35 M NaCl	3·7	45	12	6	1·7
4b	0·12–0·35 M NaCl	7·6	46	27	10	0
7a	0–0·2 M HCl	0·4	ND[d]	ND	ND	ND
7b	0–0·2 M HCl	1·1	40	17	17	1·5
Total		19·8	—	—	—	—

[a]Percent of free-acid form of gum loaded.
[b]As D-glucose.
[c]Calculated as acetate.
[d]Not determined.

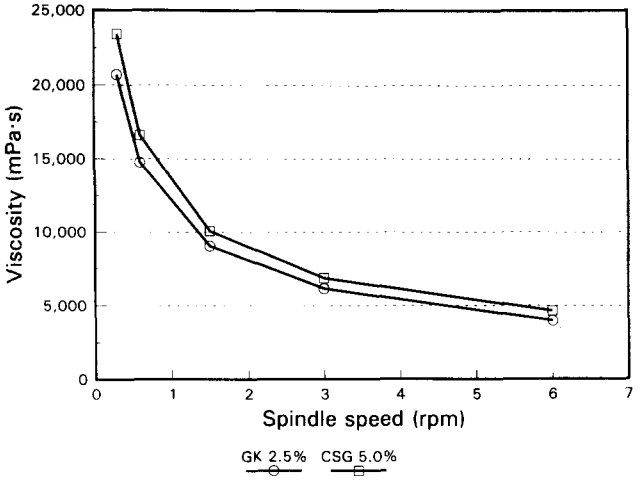

Fig. 1. Brookfield viscosity as a function of spindle speed of solutions of decationized cacao stem gum (5·0%) and gum karaya (2·5%); small sample adapter, spindle SCH-31.

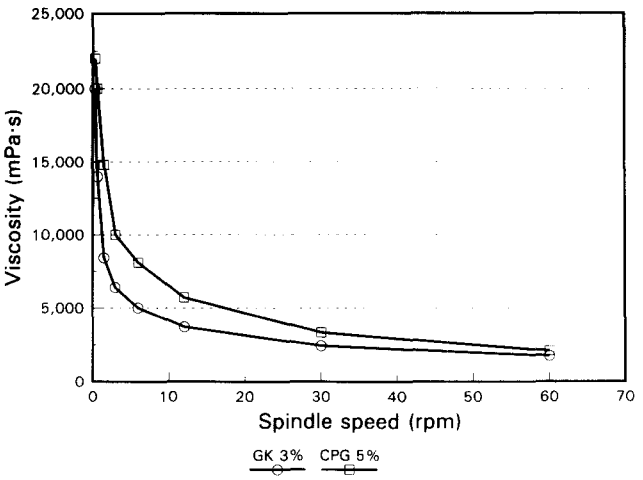

Fig. 3. Brookfield viscosity as a function of concentration and spindle speed of solutions of decationized cacao pod gum and gum karaya; small sample adapter, spindle SCH-31.

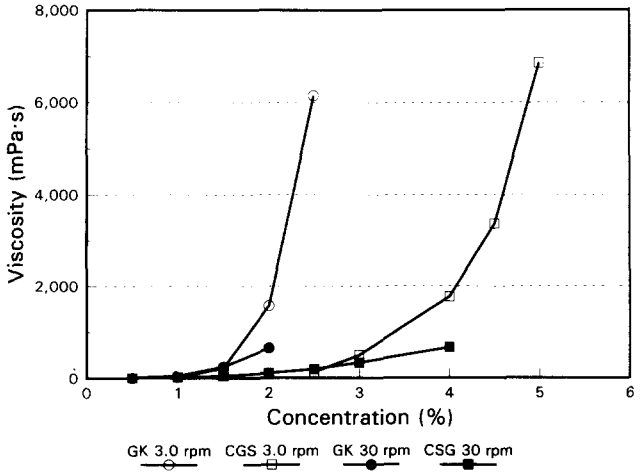

Fig. 2. Brookfield viscosity as a function of spindle speed of solutions of crude cacao pod gum (5·0%) and gum karaya (3·0%); spindle LV-04/64.

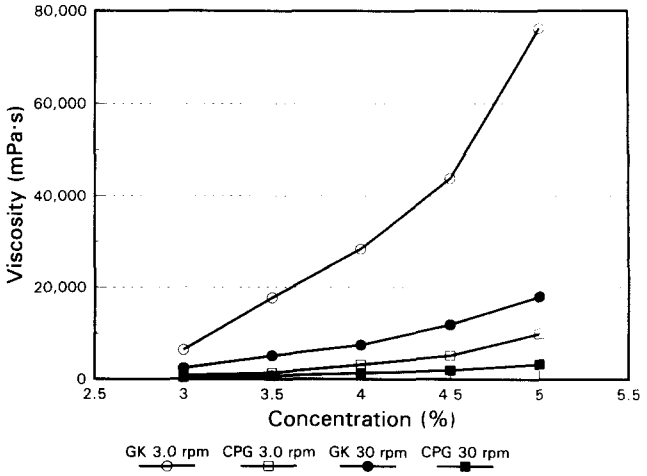

Fig. 4. Brookfield viscosity as a function of concentration and spindle speed of solutions of crude cacao pod gum and gum karaya; spindle LV-04/64.

viscous colloidal dispersions (Goldstein & Alter, 1973). This phenomenon probably accounts for its higher viscosity, as the cacao stem and pod husk extracts were centrifuged so that only soluble material was obtained.

Potential

Pod husks are a waste product of the cacao industry and present a serious disposal problem. They become a significant source of disease inoculum when used as a mulch inside the plantation. Husks may be used as livestock feed, however the theobromine content reduces the proportion that can be consumed, and its use has been restricted (Wood & Lass, 1987). Stem tissue is available from pruning.

Gum karaya, once an important gum, has been used as an emulsifier, stabilizer, and viscosifier for food products, and as a fixative/adhesive in the pharmaceutical industry (Anderson *et al.*, 1982). In recent years, gum karaya has become relatively expensive due to tapping restrictions in India (Anderson *et al.*, 1985). A substitute gum would be of interest (Aslam *et al.*, 1978). Water extracts of cacao stems and/or pods could be a new source of gum which might serve as a replacement for gum karaya, and/or have other commercial uses, thus providing an additional source of revenue to the cacao industry.

ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance of M.P. Yadav. One of the authors (A.F.) would like to thank the Brazilian National Research Council (CNPQ) and the Executive Commission for the Development of the Cacao Industry (CEPLAC) for financial support.

REFERENCES

Adler, P.R. & Wilcox, G.E. (1985). Rapid perchloric acid digest methods for analysis of major elements in plant tissue. *Commun. Soil Sci. Plant Anal.*, **16,** 1153–63.

Adomako, D. (1972). Cocoa pod husk pectin. *Phytochemistry,* **11,** 1145–8.

Adomako, D. (1975). A review of researches into commercial utilization of cocoa by-products, with particular reference to the prospects in Ghana. *CMB Newsletter,* **61.**

Anderson, D.M.W., McNab, C.G.A., Anderson, C.G., Brown, P.M. & Pronguer, M.A. (1982). Studies of uronic acid materials, Part 58: Gum exudates from the genus *Sterculia* (gum karaya). *Intern. Tree Crops J.,* **2,** 147–54.

Anderson, D.M.W., Howlett, J.F. & McNab, C.G.A. (1985). Studies on uronic acid materials, Part 74: The amino acid composition of the proteinaceous component of gum karaya (*Sterculia* spp.). *Food Addit. Contam.,* **2,** 153–7.

Aslam, M., Pass, G. & Phillips, G.O. (1978). Properties of *Khaya grandifoliola* gum. *J. Sci. Food Agric.,* **29,** 563–8.

Aspinall, G.O. & Nasir-ud-din (1965). Plant gums of the genus *Sterculia*, Part I: The main structural features of *Sterculia urens* gum. *J. Chem. Soc.,* 2710–20.

Berbert, P.R.F. (1972). Estudo da pectina do mel e da casca do fruto do cacau. *Rev. Theobroma,* **2**(2), 49–51.

Blakemore, W.R., Dewar, E.T. & Hodge, R.A. (1966). Polysaccharides of the cocoa pod husk. *J. Sci Food Agric.,* **17,** 558–60.

Brooks, E.R. & Guard, A.T. (1952). Vegetative anatomy of *Theobroma cacao. Bot. Gaz.,* **113,** 444–54.

Dittmar, H.F.K. (1958). Untersuchungen an kakao-fruchtschalen. *Gordian,* **58,** 48–9.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.,* **28,** 350–6.

Figueira, A., Whipkey, A. & Janick, J. (1989). Gum in *Theobroma cacao* L.: A potential deterrent for micropropagation. *86th Annual Meeting Am. Soc. Hort. Sci.,* Abstract No. 469, p. 121.

Glicksman, M. (1982). Gum karaya (*Sterculia urens*). In *Food Hydrocolloids*, Vol. 2, ed. M. Glicksman. CRC Press, Boca Raton, FL, pp. 39–60.

Goldstein, A.M. (1954). Chemistry, properties, and application of gum karaya. In *Natural Plant Hydrocolloids.* American Chemical Society, Washington, DC, pp. 33–7.

Goldstein, A.M. & Alter, E.N. (1973). In *Industrial Gums*, eds. R.L. Whistler & J.N. BeMiller, 2nd edn. Academic Press, New York, pp. 273–87.

Hardy, M.R., Townsend, R.R. & Lee, Y.C. (1988). Monosaccharide analysis of glycoconjugates by anion exchange chromatography with pulsed amperometric detection. *Anal. Biochem.,* **170,** 54–62.

Hirst, E.L., Hough, L. & Jones, J.K.N. (1949*a*). Composition of the gum of *Sterculia setigera:* Occurrence of D-tagatose in nature. *Nature,* **163,** 177.

Hirst, E.L., Hough, L. & Jones, J.K.N. (1949*b*). Structure of *Sterculia setigera* gum. I. An investigation by the method of paper partition chromatography of the products of hydrolysis of the gum. *J. Chem. Soc.,* 3145–51.

Kubal, J.V. & Gralén, N. (1948). Physiochemical properties of karaya gum and locust-bean mucilage. *J. Colloid Sci.,* **3,** 457–71.

Machado, R.C.R. & Hardwick, K. (1988). Does carbohydrate availability control flush growth in cocoa? In *Proc. 10th Intern. Cocoa Res. Conf.,* Santo Domingo, Dominican Republic, 1987, pp. 151–7.

Nair, G.M. & Shah, J.J. (1984). Ultrastructure of gum and gum-resin secreting tissues in some tropical trees. In *Developmental and Comparative Aspects of Plant Structure and Function*, ed. D. Nautiyal. Society of Indian Plant Taxonomists, Allahabad, India, pp. 27–39.

Oduwole, O.O. & Arueya, G.L. (1990). An economic analysis of soap production from cocoa pod husk. *Café Cacao Thé,* **34,** 231–4.

Phillips, G.O., Pass, G., Jeffries, M. & Morley, R.G. (1980). Use and technology of exudate gums from tropical sources. In *Gelling and Thickening Agents in Foods*, eds. H. Newton & W. Pilnik. Foster-Verlag, Zurich, pp. 135–61.

Sass, J.E. (1951). *Botanical Microtechnique.* Iowa State College Press, Ames, IA.

Whistler, R.L. (1993). Exudate gums. In *Industrial Gums*, eds. R.L. Whistler & J.N. BeMiller, 3rd edn. Academic Press, San Diego, CA, pp. 309–39.

Whistler, R.L., Masak, Jr. E., & Plunkett, R.A. (1956). Cacao polysaccharides. *J. Am Chem. Soc.,* **78,** 2851–3.

Wood, G.A.R. & Lass, R.A. (1987). *Cocoa*, 4th edn. Longman Scientific & Technical, London; John Wiley & Sons, New York.

Yadav, M.P., BeMiller, J.N. & Embuscado, M.E. Compositional analysis of polysaccharides via solvolysis with liquid hydrogen fluoride. *Carbohydr. Polym.,* (in press).

York, W.S., Darville, A.G., McNeil, M., Stevenson, T.T. & Albersheim, P. (1986). Isolation and characterization of plant cell walls and cell wall components. *Meth. Enzymol.,* **118,** 3–41.