J Sci Food Agric 85:2321–2324 (2005)
DOI: 10.1002/jsfa.2279

Short Communication

Cytochemical characterization and structural approach to *Prosopis juliflora* (Sw) D.C. seed gum extraction

Maria I Gallão,[1] Ricardo S Furtado[2] and Edy S de Brito[2]*

[1]Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil
[2]Embrapa Agroindústria Tropical, R Dra Sara Mesquita, 2270, Fortaleza, CE, 60511 110, Brazil

Abstract: Mesquite seed is a good gum source. In this work seed structure was characterized in order to help seed gum extraction. Seed gum was located between cotyledon and seed coat. Milling process yielded, basically, two fractions: endosperm and seed coat plus gum. This fact was supported by the cytochemical observation of a dense layer near the seed coat and a loose layer near the endosperm. Cells containing seed gum had thick cell walls and a proteic core. Seed gum extraction implications are discussed.

Keywords: gum; mesquite; *Prosopis juliflora*; structure

INTRODUCTION

Mesquite tree (*Prosopis juliflora* (Sw) D.C.) is a leguminous tree native to Central America, but now widely distributed elsewhere. The ground endosperm of mesquite seed consists mainly of galactomannan-type polysaccharides, similar to those in locust bean and guar gums.[1] Mesquite pod is used to produce syrup[2], flour[3], drinks[4] and also used as a coffee substitute.[5] However, the most extended use of this fruit nowadays is as animal forage[4]. Mesquite seed gum is not yet produced on a commercial scale, but *P. juliflora* is widely grown as a source of animal feed, fodder and fuel in some countries, such as Brazil and India, and since some research has been carried out involving pilot-scale processing of the seed with a view to recovering the gum, it is possible that mesquite may be produced commercially in the future.[6]

The objective of this work was to characterize and study some structural aspects of the mesquite seed in such a way as to support mesquite seed gum extraction.

MATERIAL AND METHODS

Mesquite pods (*Prosopis juliflora* (Sw) D.C.) were purchased at Ceará state, Brazil and identified in the Prisco Bezerra Herbarium from Universidade Federal do Ceará (voucher number 29 424). The capsules containing the seeds were obtained by crushing the pods with water (1:2, w/w) into a helicoidal mill with a 25-hp motor (Agrometal, Fortaleza, Brazil). The capsules containing the seeds were sun dried and passed through a manual disc mill to release the seeds, which were milled using a shorter disc distance (2 mm).

Seed rupture force was measured in a TA.XT2i (Stable Micro Systems, Godalming, UK) using a P/2 probe (3.140 mm^2). Forty seeds were used. Test velocity was 1 $mm\,s^{-1}$ and the probe was adjusted to an 80% penetration in the sample.

Mesquite seed gum content was determined gravimetrically. Seed fractions were extracted with water at 80 °C (1 g: 50 ml), precipitated with three parts of ethanol, centrifuged and dried at 45 °C.

For cytochemical study mesquite seeds were fixed with 40 $mg\,ml^{-1}$ paraformaldehyde in 0.1 $mol\,l^{-1}$ phosphate buffer, pH 7.2 and 10 $mg\,ml^{-1}$ glutaraldehyde for 24 h at ambient temperature.[7] The material was dehydrated in an ethanol series, and embedded in Historesin Embedding Kit (Jung) (Leica Instruments, Nussloch, Germany). The tissue blocks were sectioned at 3–4 μm on a Leica RM 2065 microtome (Leica Instruments, Heidelberg, Germany). The following cytochemical reactions were carried out: (a) xylidine ponceau pH 2.5 for the detection of total cationic radicals;[8] (b) periodic acid–Schiff reagent (PAS) for polysaccharide.[9] Sections were observed under a light microscope.

RESULTS AND DISCUSSION

In Fig 1 we can observe different aspects of the mesquite seeds. In Fig 1A can be seen a general view

* Correspondence to: Edy S de Brito, Embrapa Agroindústria Tropical, R Dra Sara Mesquita, 2270, Fortaleza, CE 60511 110, Brazil
E-mail: edy@cnpat.embrapa.br
Contract/grant sponsor: Banco do Nordeste do Brasil
(*Received 23 April 2004; revised version received 15 December 2004; accepted 5 April 2005*)
Published online 17 August 2005

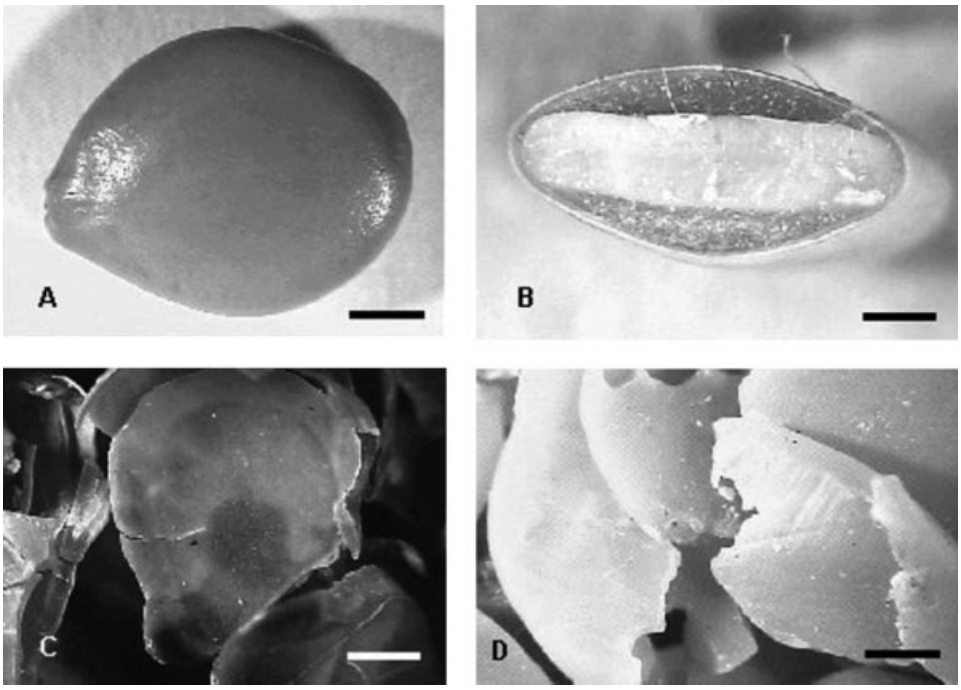

Figure 1. Different aspects of *Prosopis juliflora* seeds: (A) whole seed; (B) cross-section; (C) seed coat plus gum layer; (D) endosperm. Bars = 1 mm.

of the seed. Polysaccharide is localized between the seed coat and the cotyledon (Fig 1B). After milling, a separation was observed into two fractions, one consisting of the seed coat and polysaccharide layer and other containing the endosperm (Fig 1C and D).

In mesquite seeds a well-developed endosperm, comprised largely of the storage carbohydrate galactomannan, lies between the seed coat and the cotyledons (Fig 2A). Cells from this region have thick cell walls, which were stained with PAS (Fig 2B). A dense region was also verified near the seed coat and a loose region near the endosperm. This fact could explain the two fractions obtained after milling. Other compounds were present in the endosperm, namely proteic bodies, whose identity was confirmed by strong positive staining with Xilidine Ponceau (Fig 2C). The galactomannan plays an important role in seed physiology, due its high solubility, absorbing a great amount of water and delivering it to the embryo during the first stages of germination. The embedded endosperm protects the embryo against water loss in dry periods by an effect known as water buffer.[10] Polysaccharide can also be mobilized during germination, as detected in many species such as *Trigonella foenum-graecum*,[11] *Sesbania marginata*[12] and *Cyamopsis tetragonolobus*.[13]

One limiting factor for Prosopis seed extraction is the seed separation from the pods.[6,14] In this case pod drying is the critical step because, if the pods contain more than 40 mg g^{-1} moisture, they become flexible and sticky and this makes milling impossible with most mills. A Bauer disc mill was chosen amongst different mills for *P. velutina*, *P. chilenenses*, *P. tamarugo* and *P. pubescens* pods milling after drying.[15] In our work the use of an helicoidal mill eliminates the pod drying, since milling was performed in a wet environment (pod/water, 1:2, w/w). Capsules and seeds obtained were dried and milled with a disc mill.

The profile of seed rupture can be observed in Fig 3. A seed coat deformation followed by rupture was observed. The force necessary to rupture seed coat was 316.8 ± 35.3 N. Immediately after seed rupture the probe resistance was decreased, followed by an increase until 80% of the sample height was achieved. This could be associated with the different layers observed in cytochemical studies (seed coat, endosperm and cotyledon), the endosperm layer being the least resistant.

Many efforts have been made to extract mesquite seed gum using water extraction followed by ethanol precipitation, fractionated milling or both.[1] In all cases, an efficient separation of the endosperm layer has not been achieved. It was suggested that

Table 1. Proportion of mesquite seed gum of different fractions after seed milling

	Seed + capsule		Seed	
Screen Tyler	Retained (g kg^{-1})	Gum in retained material (g kg^{-1})	Retained (g kg^{-1})	Gum in retained material (g kg^{-1})
9 mesh	693 ± 7 a	nd	466 ± 23 a	nd
20 mesh	159 ± 3 b	49 ± 1 c	414 ± 15 b	48 ± 4 c
48 mesh	136 ± 3 b	68 ± 11 b	106 ± 26 c	72 ± 10 b
60 mesh	12 ± 1 c	99 ± 3 a	6 ± 1 d	nd
Bottom	nd	nd	8 ± 1 d	106 ± 4 a

Values in the same column followed by different letters are significantly different at $P \leq 0.05$.
nd: not determined.

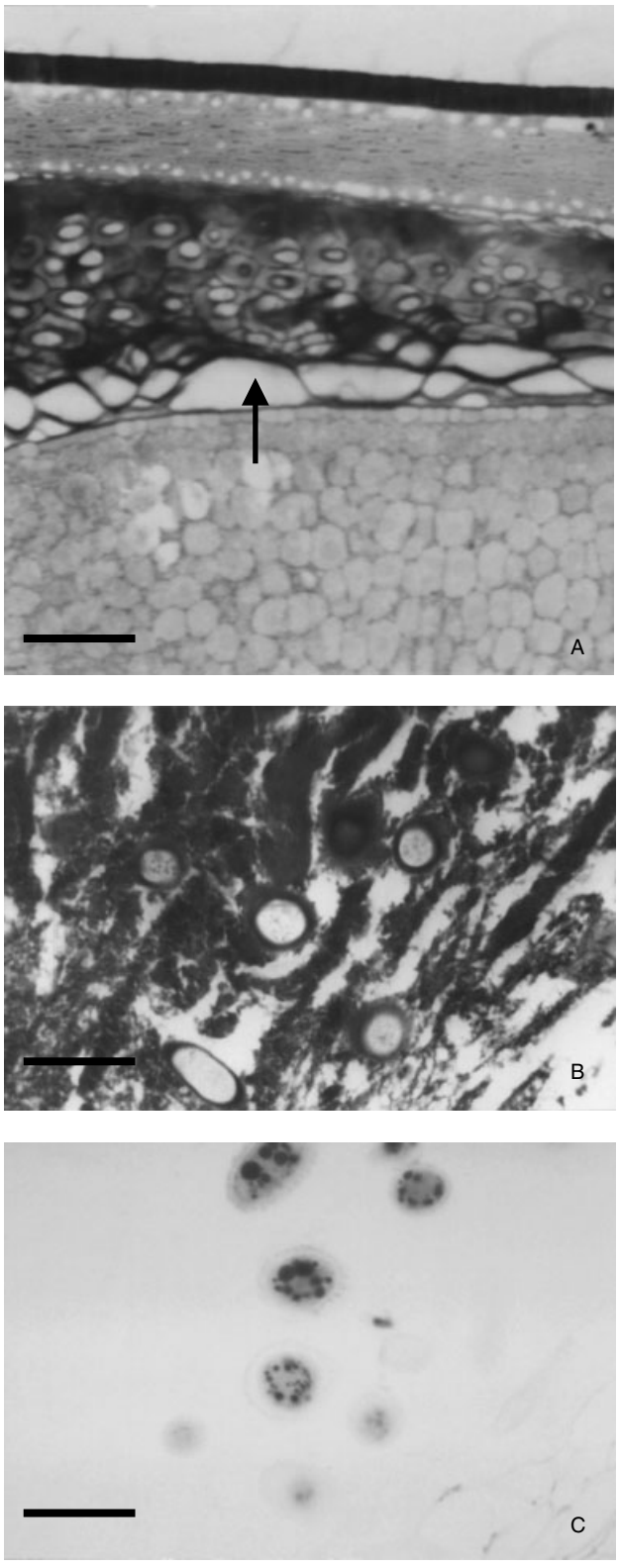

Figure 2. Cytochemical aspects of *Prosopis juliflora* seeds: (A) section stained with PAS showing seed coat, polysaccharide layer with a dense and a loose layer (vertical arrow) and endosperm; (B) polysaccharide layer stained with PAS with thick cell walls; (C) polysaccharide cells stained with XP showing proteic bodies. (Bars: A = 100 μm, B and C = 10 μm.)

the more appropriate process seems to be a 'dry process', in which the endosperm was mechanically separated from the rest of the seed.[1] Good gum yields

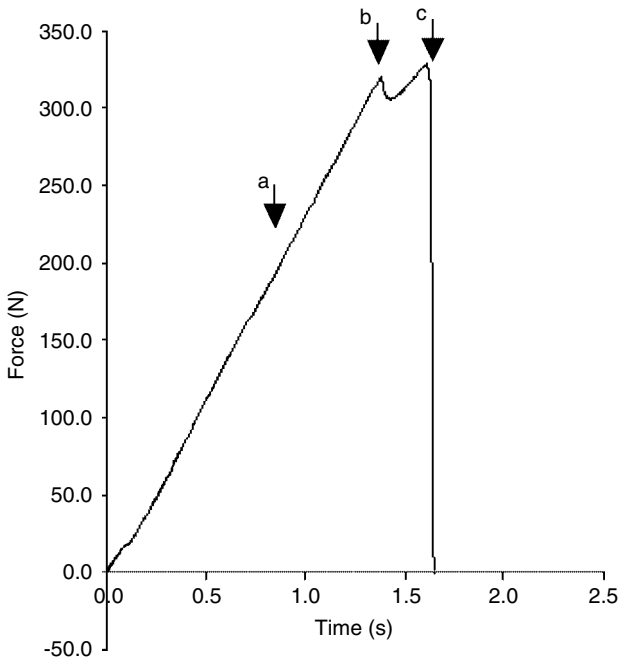

Figure 3. Rupture profile of mesquite seed: a, seed coat deformation; b, seed coat rupture; c, probe release at 80% of the sample height.

were obtained with alkaline and acid extractions of *P. chilensis* seeds, but the process is still dependent on temperature.[16] The milling process with a disc mill was useful to release seeds from the capsule and to grind the seeds. Material retained at 9 mesh was mainly intact seeds which can be submitted to a second grinding process. The 60 mesh fraction presented the highest gum concentration (Table 1), but part of the gum was still attached to the seed coat. The total gum recovery of the milled fractions was 226 g kg^{-1}, which represents 83.7% of the total gum present in the sample. In conclusion, the milling process with a disc mill led to different fractions containing the *P. juliflora* seed gum, but it was adhered to the seed coat. A further treatment to separate them should be investigated.

ACKNOWLEDGEMENT

We are grateful to Banco do Nordeste do Brasil for financial support.

REFERENCES

1 Figueiredo AA, Mesquite: History, composition, and food uses. *Food Technol* **44**:118–128 (1990).
2 Larrauri Garcia JA and Saura Calixto F, Evaluation of CIE-Lab colour parameters during the clarification of a sugar syrup from Mesquite pods (*Prosopis pallida* L.). *Int J Food Sci Technol* **35**:385–389 (2000).
3 Lima CAA and Lima EDA, Estudo da utilização da farinha integral da algaroba (*Prosopis juliflora* SW) D.C.) Na alimentação humana. *Agropecuária Técnica* **6**:8–17 (1985).
4 Bravo L, Grados N and Saura-Calixto F, Characterization of syrups and dietary fiber obtained from mesquite pods (*Prosopis pallida* L.). *J Agric Food Chem* **46**:1727–1733 (1998).

5 Vieira RL, Guerra NB and Freitas EM, Sucedâneo de café a partir de *Prosopis juliflora* D.C. *Pesquisa Agropecuária Brasileira* **30**:121–124 (1995).
6 FAO, Non-Wood Forest Products 6: Gums, resins and latexes of plant origin. [Online]. (1995). Available: www.fao.org/docrep/V9236E/V9236e00.htm [01 March 2004].
7 Karnovisky MJ, A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* **27**:137–138 (1965).
8 Vidal BC, Dichroism in collagen bundles stained with xylidine Ponceau 2R. *Ann Histochem* **15**:289–296 (1970).
9 Maia V, *Técnica histológica*. Atheneu, São Paulo (1979).
10 Reid JSG and Bewley JD, A dual role for the endosperm and its galactomannan reservs in the germinative physiology of fenugreek (*Trigonella foenumgraecum* L.), an endospermic leguminous seed. *Planta* 147 (1979).
11 Dirk LMA, Krol AL, Vreugdenhil D, Hilhorst HWM and Bewley JD, Galactomannan, soluble sugar and starch mobilization following germination of *Trigonella foenum-graecum* seeds. *Plant Physiol Biochem* **37**:41–50 (1999).
12 Buckeridge MS and Dietrich SMC, Mobilisation of the raffinose family oligosaccharides and galactomannan in germinating seeds of *Sesbania marginata* Benth. (Leguminosae-Faboideae). *Plant Sci* **117**:33–43 (1996).
13 McCleary BV and Matheson NK, Galactomannan utilization in germinating legume seeds. *Phytochemistry* **15**:43–47 (1996).
14 Souza SM, Lima PCF and Araújo MS, Sementes de algaroba: Métodos e custos de beneficiamento. *Revista Brasileira de Sementes* **5**:51–61 (1983).
15 Meyer D, Becker R, Gumbmann MR, Vohra P, Neukom H and Sauders RM, Processing, composition, nutritional evaluation and utilization of Mesquite (*Prosopis* spp.) pods as a raw material for food industry. *J Agric Food Chem* **34**:914–919 (1986).
16 Estévez AM, Sáenz C, Hurtado ML, Escobar B, Espinoza S and Suárez C, Extraction methods and some physical properties of mesquite (*Prosopis chilensis* (Mol) Stuntz) seed gum. *J Sci Food Agric* **84**:1487–1492 (2004).