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A new polysaccharide from a traditional Nigerian plant food: *Detarium senegalense* Gmelin [1]

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Abstract

The seed flour of an African leguminous plant, *Detarium senegalense* Gmelin, is used traditionally in Nigeria as a thickening agent in foods. Recent studies have shown that the detarium seed contains a large amount of water-soluble, non-starch polysaccharide (s-NSP), which suggests it has important nutritional properties. The aims of the present study were to characterise the structure and solution properties of purified s-NSP. The main monosaccharide residues of the extracted s-NSP were glucose, xylose, and galactose in the ratio of 1.39:1.00:0.52, suggesting structural similarity to the xyloglucan group of cell wall storage polysaccharides. This was confirmed by comparing the oligosaccharides released on *endo*-(1 → 4)-β-D-glucanase digestion with those obtained from tamarind seed xyloglucan. The intrinsic viscosity [η] of a sample of the detarium polysaccharide was found to be 8.9 dl/g, indicating that the sample was of high molecular weight, a result confirmed by light scattering. Histochemical examination of detarium seed using bright field and epifluorescence microscopy showed the presence of xyloglucan in highly thickened cell walls, which were particularly prominent at the cell junctions. © 1996 Elsevier Science Ltd.

Keywords: Detarium seed; Polysaccharide; Glucanase; Xyloglucan

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[1] In this manuscript an unambiguous shorthand nomenclature for xyloglucan oligosaccharides is used [1]. Each (1 → 4)-β-linked D-glucosyl residue in the backbone is given a one-letter code according to its substituents. Thus G = unsubstituted glucose residue; X = xylose-substituted glucose residue; L = galactosylxylose-substituted glucose residue; sequences always read towards the reducing end of the molecule.

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1. Introduction

The seed flour of the African leguminous plant, *Detarium senegalense* Gmelin, is used traditionally in Nigeria as a food condiment for the thickening of soups and stews [2]. The yield and ease of harvesting time from planting to maturity etc. do not appear in the usual literature. Indeed it may be that the seeds are a potential cash crop ripe for exploitation. Recent human and animal studies have shown that the seed flour also has important physiological properties [3,4], which may have clinical use in the treatment of diabetes mellitus and other metabolic disorders [5]. Preliminary chemical and physical analysis has indicated that the detarium seeds have a high level of water-soluble, non-starch polysaccharide (s-NSP $\approx 43\,g/100\,g$) [3]. Monosaccharide analysis of s-NSP indicated that the main polymer was similar in composition to tamarind seed (*Tamarindus indica* L.) xyloglucan [6]. The solution properties of a purified extract of this polymer appear to be similar to those of a commercial grade of guar gum [3,7]. Since guar gum is known to reduce plasma cholesterol and improve blood glucose control when given orally to healthy and diabetic individuals [8,9], it seemed likely that the s-NSP fraction of the detarium seed was, at least in part, responsible for its biological activity.

The purpose of this study was to further characterise the structure and solution properties of purified s-NSP extracted from the seed cotyledons of detarium, and to investigate its location within the seed using histochemical techniques.

2. Experimental

Characteristics of detarium seed and preparation of seed flour.—Detarium senegalense Gmelin is an under-exploited and largely uncharacterised leguminous seed crop belonging to the subdivision Caesalpinoideae [10]. Its tree is small to medium-sized, normally 5–7 m high, occasionally larger, and it is mainly found in West Africa, Chad and Sudan. The pods, each of which contain one seed, are usually rounded, oval or flattened and are about 4 cm in diameter. The seed samples used in the present study were purchased at a local market in Nsukka, Enugu State, Nigeria and then stored at −20 °C for later analysis.

The seed coats (testae), which are a deep brown–purple colour, were removed after boiling in water for about 1 h. The cotyledons were soaked in cold water for 1 h, washed three times and left to soak in cold water overnight. The cotyledons were then air-dried for about 24 h and ground into a fine powder (to pass through a 1 mm screen) using a coffee grinder. The powder were further dried at room temperature for 24 h and stored at −20 °C.

Chemical composition of legume flour.—Flour samples were analysed for moisture (104 °C for 16 h) and crude fat (Soxhlet; light petroleum, bp 40–60 °C, diethyl ether extraction), according to AACC methods 44-15A and 30-26 [11]. Crude protein ($N \times 5.7$) was estimated by the Kjeldahl method [12]. Ash (total minerals) was analysed by slowly heating a 5 g sample in a muffle furnace to 525 °C and leaving for 12 h at that temperature [12]. The Englyst procedure [13] was employed to determine the total and

insoluble NSP. Soluble NSP was determined as the difference. The insoluble fraction was hydrated overnight in phosphate buffer, as opposed to the 40 min suggested by Englyst et al. in order to allow complete hydration of any s-NSP. This method produced a significantly lower concentration of insoluble NSP than the standard technique.

Extraction and purification of s-NSP.—The s-NSP was extracted and purified by a modification of the method of Girhammer and Nair [14]. Detarium seed flour (20 g) was boiled with 80% (v/v) ethanol (70 mL) for 1 h under reflux to inactivate enzymes, denature protein and remove ethanol-soluble substances. The residue obtained by filtration was washed with 95% (v/v) ethanol and air-dried at room temperature. The dried residue was then extracted with 7 vol of distilled water followed by centrifugation at 9000*g* for 10 min. The supernatant was collected, adjusted to pH 7.5 with 40% NaOH, and digested for removal of protein and starch with porcine pancreatin (1% of the weight of the boiled samples), and pullulanase (0.5 units) at 34 °C for 24 h with continuous stirring in the presence of 0.05% (w/v) NaN_3, which was added to inhibit microbial growth. Both enzymes were obtained from Sigma Chemical Co., St Louis, USA. The solution was then centrifuged at 9000*g* for 15 min and the s-NSP in the supernatant was precipitated by the addition of absolute ethanol to give a final concentration of 80% (v/v) ethanol. The precipitate was collected by filtration (Whatman No. 541 filter paper), washed with 95% (v/v) ethanol, acetone, and diethyl ether, then freeze-dried and stored at 4 °C until analysis.

Analysis of constituent sugars by gas–liquid chromatography.—Total acid hydrolysis [13] of the purified s-NSP fraction was carried out to produce the constituent sugars, which were then converted to the alditol acetate derivatives. The derivatives were analysed by GLC (Pye Unicam Series 204) fitted with flame ionisation detector and computing integrator using a Supelco Sp-2330 wide-bore capillary column [13]. The uronic acid content was determined by a sulfuric acid–dimethylphenol colorimetric assay [13]. Absorption was measured at 400 and 450 nm. The reading at 400 nm was subtracted from that at 450 nm to correct for the interference from hexoses.

Structural analysis of polysaccharide of detarium.—Cellulase from *Trichoderma* species was purchased from Megazyme Pty. Ltd. (North Rocks, NSW, Australia). It was dialysed exhaustively against an ammonium acetate buffer (50 mM, pH 5.0) at 4 °C. 200 μL (12 mg/mL) of detarium polysaccharide and xyloglucan from tamarind seed (Glyloid 3S, Dainippon Pharmaceutical Corporation, Osaka, Japan) were incubated with 80 μL cellulase (approximately 8 units) in 200 μL ammonium acetate buffer (50 mM, pH 5.0). Aliquots of the incubation were taken at 0.5, 1, 2, and 4 h and the enzyme reaction was terminated by heating the samples in a boiling water bath for 2 min. The extent of hydrolysis was monitored by TLC [15]. The hydrolyses were considered complete when there was no apparent change in the pattern of oligosaccharides produced. The oligosaccharides of the final hydrolysate were analysed by Dionex HPAE chromatography [15].

Intrinsic viscosity measurement.—Solutions were prepared by dispersing the known weights of a freeze-dried sample of purified detarium polysaccharide in deionized water for 1 h at 80 °C and then mixed overnight by magnetic stirring at room temperature. The solution was filtered through a 0.45 μm syringe filter before measurements were taken. The concentrations of s-NSP selected were based on the polysaccharide content of the

purified samples rather than the dry matter content. Viscosity measurements were performed in a dilution capillary viscometer [Cannon Ubbelohde Dilution B glass viscometer, size 50, 0.8–4.0 cst. Glass Artefact (Viscometers), UK] immersed in a water bath to maintain the temperature as 25 ± 0.1 °C. Precautions were taken to ensure the viscometer was aligned vertically, and flow times (> 250 s) were measured in triplicate using a simple computer timing system; agreement between triplicates was within ± 1 s. Under the prevailing flow conditions no flow kinetic energy correction was required.

Anatomical observations.—Detarium seed cotyledons were immersed in 75% (v/v) ethanol and cut into 1 mm^3 cubes. Samples were fixed in 4% (v/v) paraformaldehyde in phosphate buffered saline for 24 h at room temperature, dehydrated by graded ethanol serial dilution (5, 25, 40, 55, 70, 95, 100%, v/v) and finally infiltrated and embedded in glycolmethacrylate (GMA) using a JB-4 embedding kit (Polyscience Ltd.). Sections were cut at 7 μm thickness on a Reichert–Jung 150 Autocut Microtome fitted with a glass knife. Then the sections were stained with an iodine–potassium iodide reagent [16,17] or with the plant lectin from *Bandeiraea simplicifolia* (BS-1; Sigma Chemical, Poole BH17 7BR, UK), which is labelled with fluorescein isothiocyanate (FITC) and is highly specific for α-D-galactose residues [18]. Samples were examined under epifluorescence using a Leitz Dialux 22 FB microscope with appropriate barrier filters (A2, excitation range 270–380 nm). Images were photographed with a Wild MP551 camera system.

3. Results and discussion

Chemical compositions of detarium flour and purified s-NSP extract.—The macronutrient composition of the detarium seed flour (Table 1) indicated that most of the polysaccharide material was in the form of water-soluble, non-starch polysaccharide (59.8 g/100 g dry matter). A high yield of s-NSP was extracted from the original detarium seed flour (51.4 ± 1.7 g/100 g dry matter). The s-NSP content of the extract was 91.8 ± 1.7 g/100 g dry matter. The results of the compositional analysis are presented in Table 2 and Fig. 1. The main monosaccharide components of s-NSP were glucose, xylose, and galactose in the ratio of Glc:Xyl:Gal ~

Table 1
Macronutrient composition of detarium flour

Component	Amount (g/100 g dry matter)	SD [a] (±)
Protein	13.1	0.03
Fat	8.2	0.8
Starch	0.4	0 [b]
s-NSP	59.8	0.9
Total NSP	62.2	1.1
Ash	2.7	0.03

[a] SD = standard deviation.
[b] Single value only.

Table 2
Monosaccharide composition of the water-soluble, non-starch polysaccharide extract and flour from *Detarium senegalense* Gmelin

Monosaccharides	Polysaccharide extract (g/100g dry matter)		Flour (g/100g dry matter)	
	Mean	SD [a] (±)	Mean	SD (±)
Arabinose	1.75	0.14	2.24	0.04
Xylose	30.49	0.53	18.84	0.58
Mannose	0.72	0.14	0.55	0.04
Galactose	15.91	0.52	10.90	0.93
Glucose	42.19	1.21	26.81	1.23
Uronic acid	0.75	0.14	2.81	0.56
Total	91.81	1.71	62.15	0.63

[a] SD = standard deviation.

1.39(±0.04):1.00(±0.04):0.52(±0.02). This was close to the values reported for tamarind seed xyloglucan [19].

Structural analysis.—To determine whether or not the s-NSP extract of detarium was a xyloglucan similar to that from tamarind seeds, samples of both polysaccharides were digested with a pure *endo*-(1 → 4)-β-D-glucanase of fungal origin. This enzyme is known to hydrolyse tamarind and other seed xyloglucans to a mixture of the four subunit oligosaccharides XXXG, XLXG, XXLG, XLLG [15,19,20] (Fig. 2). TLC analysis of the progress of the hydrolysis of tamarind xyloglucan showed that a 4 h incubation was sufficient for the complete conversion of the polysaccharide to three components, previously identified as XXXG, XLXG and XXLG (unresolved), and XLLG [15] (Fig. 3, lanes 5–8). Exactly the same components were obtained from the

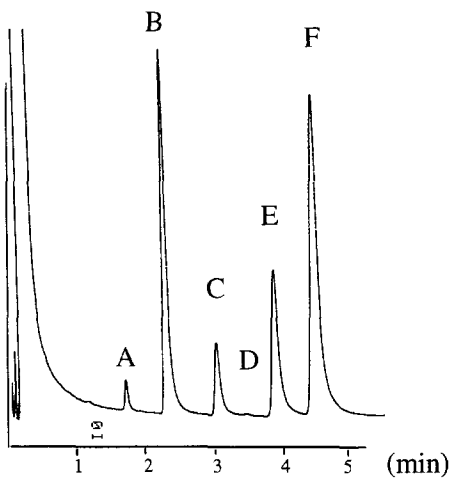

Fig. 1. Gas–liquid chromatogram of the alditol acetates from a hydrolysate of water-soluble, non-starch polysaccharide extracted from detarium seeds, where A = arabinose, B = xylose, C = allose, D = mannose, E = galactose, F = glucose.

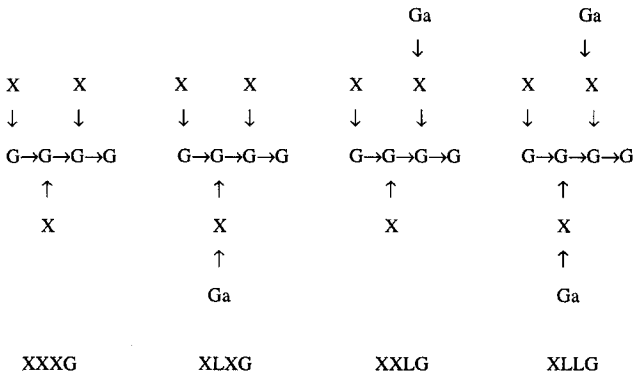

Fig. 2. Structure of seed xyloglucan subunit oligosaccharides, where X = xylose, G = glucose, Ga = Galactose.

s-NSP of detarium over the same time period, indicating that the s-NSP was also a xyloglucan (Fig. 3, lanes 9–13). To confirm this, and to obtain fine-structural information, the two 4 h digests were subjected to HPAE chromatography (Fig. 4). As has been observed previously [15,20], the tamarind digest contained peaks corresponding to XXXG, XLXG, XXLG, XLLG in the relative proportions 1.00:0.42:2.07:6.20. The detarium s-NSP contained peaks indistinguishable from those of tamarind, but in the relative proportion 1.00:0.30:5.60:6.20. These results confirm that the detarium s-NSP is a xyloglucan, consisting of a cellulosic backbone with single-unit α-D-xylopyranosyl

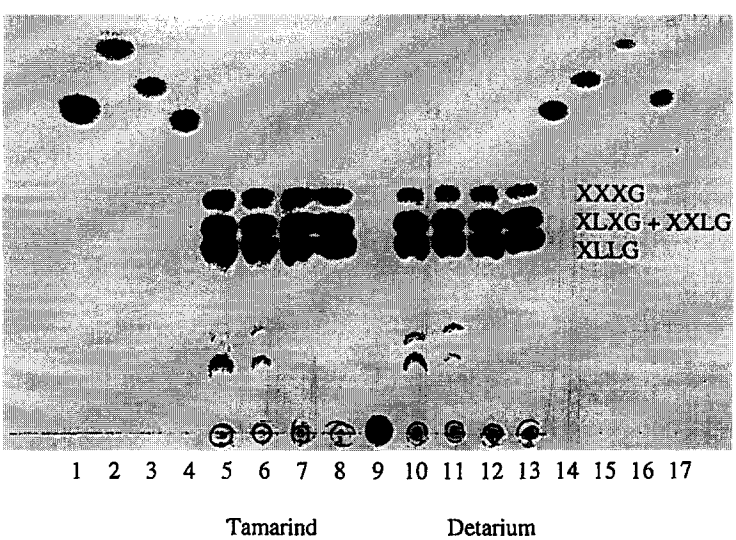

Fig. 3. TLC plate showing time-course of *endo*-(1 → 4)-β-glucanase hydrolysis of detarium and tamarind polysaccharides. Lanes 1, 17, galactose standard; 2, 16, xylose standard; 3, 15, glucose standard; 4, 14, cellobiose standard. Lanes 5, 6, 7, 8, digest of tamarind xyloglucan after 0.5, 1, 2, 4 h, showing conversion to XXXG, XLXG and XXLG (unresolved), XLLG; 9–13, digest of s-NSP after 0, 0.5, 1, 2, 4 h.

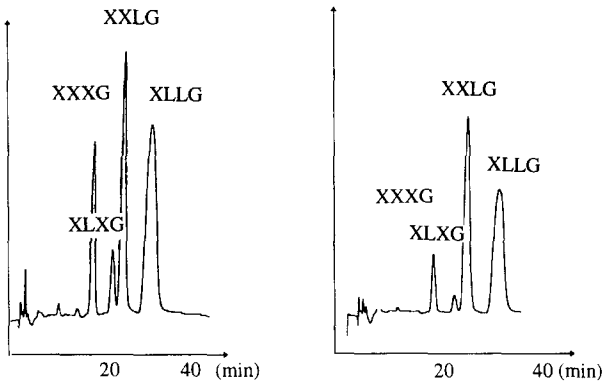

Fig. 4. HPAE trace showing oligosaccharides converted from tamarind xyloglucan and detarium xyloglucan when treated with *endo*-1,4-β-glucanase.

substituents attached to carbon-6 of the glucosyl residues, and with some of the xylose residues further substituted at carbon-2 by β-D-galactopyranosyl residues. The quantitative information on the relative amounts of the four oligosaccharides given by Dionex HPAE analysis allowed deduction of the Glc:Xyl:Gal ratio of the polysaccharides. They were in close agreement with the value obtained by direct hydrolysis (Table 3). The degree of galactose substitution of the xyloglucan core is lower in detarium than tamarind. It is interesting to note that in both polysaccharides there is a high amount of the subunit XXLG relative to XLXG. This indicates a similar non-random distribution of galactosyl substituents in both polysaccharides.

Intrinsic viscosity and molecular weight.—The intrinsic viscosity was determined with the glass capillary viscometer at polysaccharide concentrations ranging from 0.01 to 0.1% (w/v). In this case, the viscosity relative to that of the solvent (water) lies in the range $1.2 < \eta_r < 2.0$. Other experiments (to be published) carried out at higher concentrations and at a range of different shear rates suggest that under these conditions (i.e. $1.2 < \eta_r < 2.0$) the solution viscosity is essentially Newtonian. The intrinsic viscosity (or limiting viscosity number) $[\eta]$ was conventionally obtained from the double extrapolation of the Huggins and Kramer equations:

$$\eta_{sp}/c = [\eta] + K'[\eta]^2 c + o(c^2) \quad (1)$$

Table 3
Results of HPAE showing quantitatively oligosaccharide fractions converted from tamarind xyloglucan and detarium xyloglucan when treated with *endo*-1,4-β-glucanase

	Oligosaccharides ratio				Deduced monosaccharides		
	XXXG	XLXG	XXLG	XLLG	Xylose	Galactose	Glucose
Tamarind	1.00	0.42	2.07	6.20	1.00	0.51	1.34
Detarium	1.00	0.30	5.60	6.20	1.00	0.46	1.33

$$\ln(\eta_r)/c = [\eta] + (K' - 0.5)[\eta]^2 c + o(c^2) \quad (2)$$

where c is the polysaccharide concentration, and K' is the Huggins coefficient. $[\eta]$ was estimated as the average of the two ordinate intercepts from the two extrapolations to $c = 0\%$ as presented in Fig. 5. The notation $o(c^2)$ is used to denote second and higher order terms of such power series expansion of η_{sp}/c and $\ln(\eta_r)/c$ around $c = 0\%$ [21]. Since the plots are essentially linear such terms are of negligible importance in this case.

The intrinsic viscosity of the polysaccharide from detarium was found to be $\sim 8.9 \pm 0.2$ dl/g, which is significantly higher than the value reported for tamarind seed xyloglucan ($[\eta] = 6.0 \pm 0.5$ dl/g) [22]. Since an increase in the intrinsic viscosity of any polymer reflects both the short range intramolecular interactions (chain flexibility) and its molecular weight, this difference could be due either to the fact that a decrease in galactose residues attached to the xyloglucose core of detarium increases chain stiffness (persistence length or chain characteristic ratio) or, more likely that the detarium xyloglucan has a relatively higher molecular weight. Note that such an increase need not indicate that the molecular weight of the native material in situ is higher, in so far as this is valid for cell wall material. However, it is clear that the detarium xyloglucan is of high molecular weight.

The so-called Mark–Houwink relationship is given by:

$$[\eta] = KM_r^{\alpha} \quad (3)$$

where M_r is the (viscosity) average molecular weight and the parameters K and α are related to local stiffness of the polymer (i.e. structure chain flexibility) and the long distance structure (i.e. the excluded volume), respectively. For most random coil linear polymers, including polysaccharides, the exponent should lie in the range 0.5–0.8 [23]. Recent studies show that xyloglucan exhibits a strong tendency to aggregate in aqueous solution [21,24]; this was described as bundle-shaped lateral aggregation of single

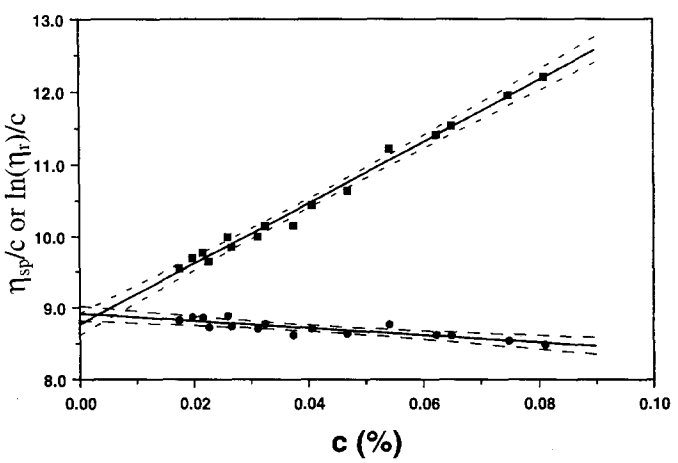

Fig. 5. Estimating intrinsic viscosity of detarium polysaccharide from plots η_{sp}/c vs. c (%) (■) and $\ln(\eta_r)/c$ vs. c (%) (●). Dotted lines indicate 95% confidence intervals.

polymer strands. This behaviour can obviously result in marked increase in the polymer stiffness, but generally such aggregation effects, although they may affect the small angle light scattering behaviour, tend not to influence the value of $[\eta]$. However, if for detarium xyloglucan we assume that α is 0.5 we can estimate the average molecular weight of the detarium xyloglucan, M_D, using eq 4 and the data obtained for the tamarind xyloglucan by Gidley and colleagues [21] (i.e. $[\eta]_T = 6.0$ g/dl, $M_T = 8.8 \times 10^5$ g/mol), then M_D was found to be $\sim 1.9 \times 10^6$ g/mol:

$$M_D = ([\eta]_D \times M_T^{\alpha}/[\eta]_T)^{1/\alpha} \quad (4)$$

On the other hand, if we use an exponent value of 0.8, a somewhat lower estimate of $M_D \sim 1.3 \times 10^6$ g/mol is obtained.

Preliminary light scattering measurements carried out at the Institüt für Makromolekulare Chemie, Universität Freiburg, Germany, courtesy of Prof. Walther Burchard, confirm that the weight-average molecular weight is indeed high, but is actually ~ 2 times greater than we estimate above [$(2.69 \pm 0.08) \times 10^6$, from four experiments]. However, this is actually consistent with our calculation above, because this was evaluated assuming that the xyloglucan was an essentially linear molecule. The value of c^* (or equivalently the volume of space swept out by the chain) from light scattering is quite close to that from intrinsic viscosity measurement, but there is clear evidence from the angular dependent light scattering data that the chain is not linear, instead there is apparently an amount of long chain branching. This obviously has a very large effect on the chain profile in solution, but, at the same time, is probably well below that detectable by (bio)chemical methods. Further details will have to be deferred to a subsequent paper.

Anatomical observations.—Light microscopic examination of the detarium cotyledonary sections showed highly thickened cell walls, which stained bright blue with iodine reagent and were particularly prominent at the cell junctions (Fig. 6). This and other morphological characteristics are remarkably similar to those seen for cotyledonary material of tamarind seed (ungerminated), which like many other species of seed xyloglucan, show a blue colour (amyloid reaction) with iodine [16,19]. The positive staining of the fluorescein-labelled lectin (BS-1), which is specific for α-D-galactose residues [18], confirms the presence of xyloglucan in the thickened cell walls of detarium cotyledon. These results suggest strongly that the xyloglucan of detarium seed, which is clearly present as large deposits, functions as a cell-wall storage polysaccharide like other seed xyloglucans [6], although germination experiments need to be carried out to confirm this.

4. Conclusions

Studies of a seed flour extracted from the leguminous plant *Detarium senegalense* Gmelin indicate that it is rich in s-NSP, comprising mainly glucose, xylose, and galactose in the ratio 1.39:1.00:0.52. The s-NSP component is structurally similar to tamarind seed xyloglucan, but with less galactose substitution on the detarium xyloglu-

Fig. 6. Bright field image of storage cell-wall xyloglucan in detarium seed endosperm stained with iodine–potassium iodide (magnification 400×).

can core than that found in tamarind. The molecular weight and intrinsic viscosity of detarium xyloglucan was found to be higher than for tamarind seed. Histochemical examination of the detarium seed, using bright field and epifluorescence microscopy, indicated that the xyloglucan was located in highly thickened cell walls.

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