

Characterisation of Rosa Mosqueta seeds: cell wall polysaccharide composition and light microscopy observations

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Abstract: The utilisation of enzymes for the extraction of vegetable oils from seeds has been a topic of growing interest in recent years. Knowledge of the cell wall polysaccharide composition is important to select the enzyme(s) necessary for the most effective degradation of the cell walls. The purpose of the present work is to characterise the seeds of Rosa Mosqueta (*Rosa aff rubiginosa*) by light microscopy (where several differential staining methods were applied to analyse the seed structure) and by the isolation of cell wall polysaccharide extracts. The mature seed of Rosa Mosqueta has a very thick and structurally complex seed coat comprising heavily lignified tissue. The embryo has two cell layers of remaining endosperm tissue (indicating that this is an exalbuminous seed), two voluminous cotyledons that contain the oil, and bundles of provascular tissues distributed perpendicularly to the transverse axis of the embryo. The major non-cellulosic polysaccharides from the non-lignified tissues are glucuronoxylans and pectic polysaccharides; glucans are also present in small amounts. The major non-cellulosic polysaccharides from the lignified tissues are glucuronoxylans. Concerning the use of enzymes for oil extraction, microscopy and cell wall polysaccharide analysis showed that the use of pectic enzymes followed by a xylanase or a cellulase should be explored.

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Keywords: Rosa Mosqueta; light microscopy; cell wall polysaccharides; seed structure; staining methods

INTRODUCTION

The recovery and use of vegetable oils from seeds have played an important role in the manufacture of a large number of industrial products and human food items.

In recent years there has been a growing interest in the oil extracted from the seeds of Rosa Mosqueta. Despite the small amount of oil extracted from these seeds (approximately 8% according to Malec *et al*¹), the annual seed production is roughly 20 000 tons,² increasing the economic interest in this product owing to its use for cosmetic and medicinal purposes.^{1,3,4} 'Rosa Mosqueta' is the common name used for at least three species widely distributed in Chile: *Rosa aff rubiginosa*, the main representative (a good morphological description of this species, both plant and fruit, can be found in Ref 3), *Rosa canina* and *Rosa moschata*.³ Information about the *Rosa* species is rather scarce.

The process for extraction of vegetable oils is based on the observation that the oil is found inside the plant cells, linked together with protein and various complex carbohydrate molecules.⁵ The permeability of the cells to oil can be increased by mechanical or thermal conditioning.⁶ A more recent technology involves the

utilisation of enzymes to degrade the cell walls. However, the enzyme preparations must have a broad spectrum of activity owing to the complex structure of the cell walls.⁵ In order to define more precisely the type(s) of enzyme(s) to degrade the cell wall polysaccharides, and consequently increase the efficiency of degradation of Rosa Mosqueta cell walls, polysaccharides were extracted and characterised. In a parallel study the seed structure and localisation of the embryo's food reserves were determined by light microscopy.

MATERIALS AND METHODS

Plant material

Rosa Mosqueta seeds were obtained from Chile, with an average length of 5.2 mm, an average diameter of 4.2 mm and 2% moisture content. They were stored inside a metallic container at room temperature and analysed within 1 year after harvest.

Optical microscopy

Sample preparation

The seeds were placed in boiling distilled water for

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5 min in order to inhibit endogenous enzymatic activity and to soften the thick seed coat. The seeds were then washed with distilled water and conserved in aqueous medium for 4 days to further soften the seed coat. Afterwards the extremities of the oval-shaped seeds were cut to allow better penetration of the fixing agents. Sectioned seeds were fixed for 18 h at 4 °C with 10% formalin in phosphate buffer (0.1 M, pH 7.0). Fixation was followed by washing with phosphate buffer at 4 °C for 18 h with several batches of buffer solution, followed by dehydration in a graded ethanol series (50, 60, 70, 80, 90% and absolute ethanol) at 4 °C. Each dehydration step lasted for 20 min. The seeds were then transferred to a monomer mixture (two steps, 3 days each, at room temperature) containing 94% glycol methacrylate, 3% 2-butoxyethanol, 1.5% polyethylene glycol 400 (PEG 400), 0.75% divinylbenzene, 0.75% methyl methacrylate and 0.72 g benzoyl peroxide for each 80 ml of monomer mixture (modified from Ref 7). Embedding was carried out in the same mixture without PEG but with 25 µl 2,2'-azobis(2-methylpropionitrile) for each 3 ml of mixture, allowing the polymerisation to occur at room temperature. Sections of 4–6 µm were obtained using glass knives and an ultramicrotome model 1401 (Leitz).

Staining

The sections were examined with the following methods: Safranin and Picro Aniline Blue; PAS (Periodic Acid Schiff's reagent) –Amido Black;⁸ Coomassie Brilliant Blue R250;⁹ Sudan Black¹⁰ (sections were stained for 30 min; the saturated staining solutions were also made in 70% propanol). Sections were also stained with Premixed Iron Hematoxylin, Safranin and Fast Green⁸ (results not shown). For some of the stained sections the original mounting medium referred to in Ref⁸ was Canada balsam. In this work, Entellan was used to prevent colour fading.

Preparation of alcohol-insoluble residue (AIR)

The seeds (74.5 g) were frozen in liquid nitrogen and immediately ground in a coffee mill to a final particle size of approximately 0.5 mm after sieving. The material was then refluxed in 300 ml ethanol for 15 min. The mixture was cooled and filtered through a size 1 sintered glass funnel (P160/ISO 4793, 100–160 µm pore size). In order to remove the oil, the residue was treated twice with 300 ml of a chloroform/methanol (2:1 v/v) solution for 30 min. The residue was washed with absolute ethanol and diethyl ether before being placed in an oven at 40 °C until no weight variation was detected.¹¹

Sequential extraction of AIR

AIR (20 g) was treated with distilled water (400 ml) at 40 °C for 75 min (W-1) and sequentially extracted with dimethyl sulfoxide (DMSO, 400 ml) at 20 °C for 20 h (DMSO-1), then washed with distilled water (5 × 150 ml) to complete removal of DMSO (W-2),

0.5 M KOH (500 ml) at 4 °C for 2 h, 1 M KOH (500 ml) at 4 °C for 2 h, 1 M KOH (500 ml) at 20 °C for 2 h and 4 M KOH (500 ml) at 20 °C for 2 h. After each extraction step the mixture was filtered, concentrated under reduced pressure and dialysed for 3 days, with several changes of distilled water. The alkali extracts were previously acidified to pH 5.0 with glacial acetic acid, concentrated and dialysed. After dialysis, all the extracts were centrifuged and the precipitates (ppt) were separated from the soluble material (supernatant (sn)). Finally, they were concentrated and freeze-dried. For convenience of presentation and discussion of the results, the KOH extracts were combined in fractions KOH-1 sn and KOH-1 ppt. The residue obtained (CR-1) was washed with distilled water (250 ml), neutralised, dialysed and freeze-dried; the supernatant was collected separately (sn-CR-1). To prevent β -elimination, peeling reactions and alkaline oxidation of the polysaccharides, the KOH extractions were carried out under an inert atmosphere (Ar) with O₂-free solutions containing 20 mM NaBH₄. KOH solutions were prepared according to the following procedure: the distilled water used for the preparation of the solutions was boiled for 20 min and allowed to cool under Ar; KOH (for each required concentration) and NaBH₄ were then added and dissolved.

Mild chlorite treatment

CR-1 (12.5 g) was treated with 1 l of 30 mg l⁻¹ NaClO₂ and 1.2 ml l⁻¹ glacial acetic acid at 70 °C for 4 h. During the reaction, 30 mg l⁻¹ NaClO₂ and 1.2 ml l⁻¹ glacial acetic acid were added every hour. The chlorite-soluble material was concentrated and dialysed. The supernatant (Ch-1 sn) was removed from the precipitate (Ch-1 ppt) and they were both freeze-dried. The residue was extracted with DMSO (600 ml) at 20 °C for 90 h to give DMSO-2. To completely remove the DMSO, the residue was washed with distilled water (6 × 150 ml), which was collected for analysis (W-3).

Delignification treatment

The washed residue was placed in 1 l of reaction mixture containing 3 g l⁻¹ NaClO₂ and 1.2 ml l⁻¹ glacial acetic acid at 70 °C for 4 h, according to the procedure of Jermyn and Isherwood.¹² During the reaction, 3 g l⁻¹ NaClO₂ and 1.2 ml l⁻¹ glacial acetic acid were added every hour. The obtained yellow solution was filtered and the residue was washed thoroughly with distilled water (6 × 150 ml). All filtrates were combined and flushed with N₂ until a pale yellow solution was obtained, indicating that the dissolved chlorine dioxide was removed. The solution was then concentrated in a rotary evaporator at 40 °C, dialysed and freeze-dried (Ch-2). The residue obtained after the chlorite treatment is usually referred to as holocellulose.¹³ Chlorine dioxide can inhibit the free radical chain reaction between chlorine and polysaccharides during delignification by acting as free radical scavenger. It acts without affecting the

chlorination of lignin, which is not a free radical reaction. Chlorine dioxide by itself reacts only very slowly with polysaccharides.^{14,15}

Sequential extraction of holocellulose

The holocellulose was sequentially extracted with DMSO (600ml, 20 °C, 20h) (DMSO-3), then washed with distilled water (5 × 150ml) to complete removal of DMSO (W-4), 0.5M KOH+20mM NaBH₄ (500ml, 20 °C, 2h), 1M KOH+20mM NaBH₄ (500ml, 20 °C, 2h) and 4M KOH+20mM NaBH₄ (500ml, 20 °C, 2h). The extracts were processed using the same procedure as described for the AIR sequential extraction, and the aqueous suspension containing the final residue was neutralised, dialysed and, before freeze-drying, the supernatant solution (sn-CR-2) was separated from the residue (CR-2) by centrifugation.

Carbohydrate analysis

Neutral sugars were released by Saeman hydrolysis¹⁶ and analysed as their alditol acetates by gas-liquid chromatography^{17,18} in a Carlo Erba GC 6000 series 2 with an FID detector and DB 225 column. Hexuronic acids (HexA) were determined colorimetrically by a modification of the method of Blumenkrantz and Asboe-Hansen¹⁹ according to Coimbra *et al.*¹¹

RESULTS AND DISCUSSION

Optical microscopy

Plate 1 shows the light micrographs of transverse sections of *Rosa Mosqueta* mature embryo. It is possible to observe the different parts of the mature embryo stained by the PAS-Amido Black method (Plates 1A, 1C, 1E and 1G), where proteins stain blue and carbohydrates stain red (from personal experience we can affirm that the intensity of the colour varies from light straw to red according to the polysaccharide composition). Coomassie Blue, a stain for proteins (which stain blue), was also used (Plates 1D, 1F and 1H). The transverse section (Plate 1A) shows that this angiospermic seed has two voluminous cotyledons. These cells (Plates 1E and 1F) have a quite homogeneous internal organisation, with big protein bodies, or aleurone grains,²⁰ as food reserves. Some of these protein bodies are structurally simple and apparently consist only of a proteinaceous matrix, whereas others have inclusions (in) such as globoid crystals, protein crystalloids or calcium oxalate crystals. Mineral nutrient reserves are usually concentrated as globoid crystals and are a constituent of phytin.²¹

The provascular tissue (Plates 1C and 1D) is oriented in small bundles perpendicular to the transverse embryonic axis (Plate 1A). The protein bodies of these cells are the smallest in size and number, and apparently the simplest in terms of structure, when compared to those of the cotyledons and endosperm (Plates 1G and 1H), indicating that these cells have fewer nutrient (protein) reserves. Also, the higher density of the cytoplasm (as compared to

the cotyledon and endosperm cells) suggests that the provascular cells have a higher number of cellular organelles (Plate 1D).

The endosperm tissue (en) can be observed in Plates 1G and 1H. These cells are smaller, more elongated and have a higher number of protein bodies. These bodies are smaller than those of the cotyledon cells but appear to have a higher number of inclusions. The endosperm tissue is restricted to two layers of cells that surround the mature embryo (Plates 1A, 1G and 1H). This tissue is an important food reserve for the young developing embryo, but in many dicotyledonous seeds the development of the embryo prior to seed germination destroys all or most of the endosperm. Seeds of this type are designated as exalbuminous and, at maturity, consist of a large embryo enclosed within the seed coat.²²

The embryo's epidermis (ep), derived from its protoderm during maturation, is shown in Plate 1H.

Plate 1B shows the cotyledon cells stained with Sudan Black, where lipids stain black. Lipids are present in both the embryo and endosperm. The cytoplasm of these cells is completely stained, suggesting that small lipid droplets, also known as oleosomes and spherosomes,²⁰ are dispersed throughout the cytoplasm. However, in our experiments we verified that the plastic embedding medium (GMA) was also stained, thus rendering the above observation inconclusive. Moreover, as mentioned in the Introduction, data from Ref 1 show that the oil content of these seeds is approximately 8%, which is not consistent with the staining results shown here. The protein bodies were also stained. Since the amount of protein content of the seeds, following complete removal of the oil, is nearly 10%,¹ it is possible that the protein bodies consist of a complex matrix composed of lipids and proteins.

Plate 2 shows the light micrographs of transverse sections of *Rosa Mosqueta* seed coat. An overall observation of the cell walls of the seed coat (Plates 2I–2P) shows that they are relatively thick when compared to the different embryonic structures (Plates 1A–1H). This suggests that the reserve carbohydrates in this seed are rather scarce. Using the Safranin and Picro Aniline Blue method (where cellulose stains blue and lignin stains red), the embryo and endosperm tissue cell walls stained blue (data not shown), indicating that it is probable that they are mainly composed of cellulose.

Plate 2I shows an overall view of the very thick embryonic seed coat (or testa), which made GMA sections very difficult to obtain for this type of seed. In this micrograph it is possible to distinguish the outer tegument or integument (oi) from the inner tegument (ii). However, no visible separation exists between these two structures. Probably this is the result of a degeneration or compression of the separation layer, as both integuments achieved their maximum thickness when mature. A proper ontogenic study would clarify this hypothesis and would also clarify the development

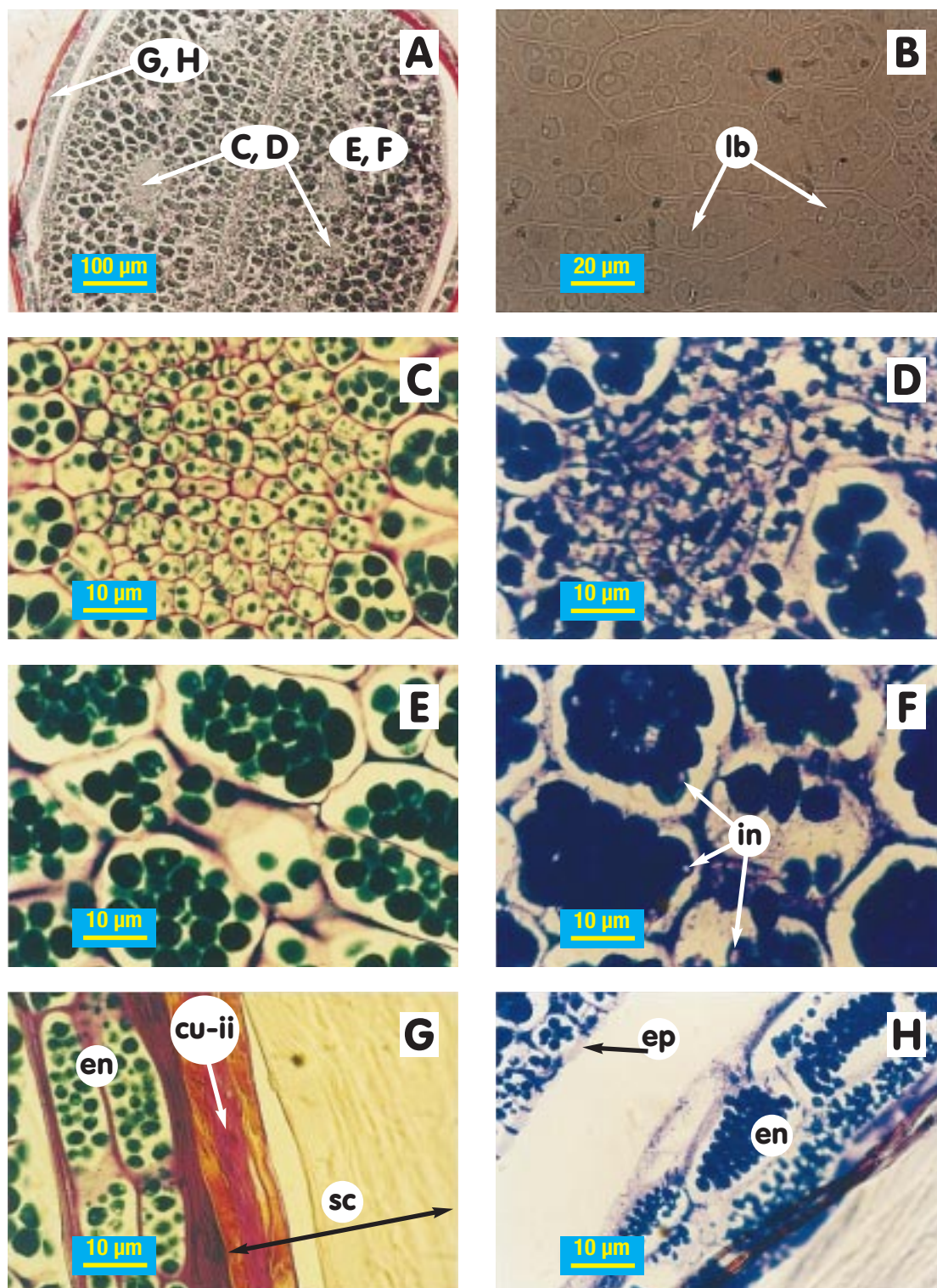


Plate 1. Light micrographs of transverse sections from the mature embryo of *R Mosqueta*
A: Mature embryo, 100 X; **C, D:** Provascular tissue, 1000 X; **B:** Cotyledon cells, 630 X; **E, F:** Cotyledon cells, 1000 X; **G, H:** Endosperm (**en**), 1000 X
lb - Lipid bodies; **en** - endosperm; **ep** - epidermis; **sc** - seed coat; **in** - inclusions; **cu-ii** - cuticle from the inner integument
Staining: **A, C, E, G:** PAS - Amido Black; **D, F, H:** Coomassie Blue; **B:** Sudan Black

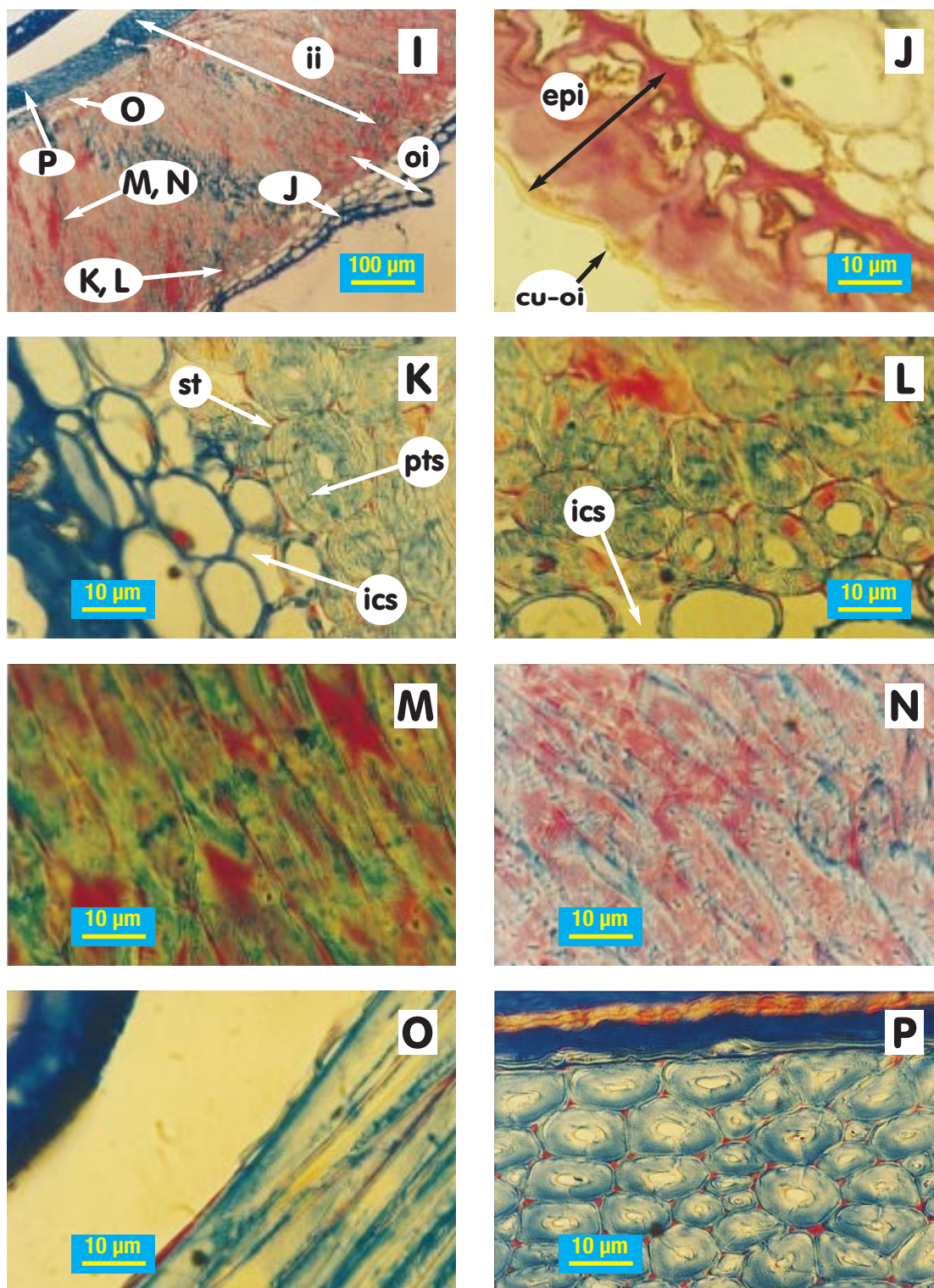


Plate 2. Light micrographs of transverse sections from the seed coat of R Mosqueta seeds

I: Overview of testa (seed coat), 100 X; **J:** Epidermis (**epi**) of the seed coat; **K, L:** Stone cells (**st**); some cells contain conspicuous simple with pits (**pts**) with many branches, 1000 X; **M, N:** Sclerenchyma tissue, 1000 X; **O:** zone of very long fibers, disposed longitudinally, 1000 X; **P:** Zone of fiber disposed transversally, 1000 X
oi - outer integument; **ii** - inner integument; **cu-oi** - cuticle covering the epidermis (**epi**); **cu-ii** - cuticle from the inner integument; **ics** - intercellular spaces

Staining: **J:** Pas - Amido Black; all others: Safranin and Picro Aniline Blue

of the cuticle of the inner integument (cu-ii) (Plates 1G and 2P).

The epidermis (epi) (Plate 2J) is the outermost layer of cells surrounding the outer seed coat. In this type of seed, this tissue consists of a monolayer of cells covered by a protective tissue named the cuticle (cu-oi) (Plate 2J). As shown by the Safranin and Picro Aniline Blue stains (cellulose stains blue and lignin stains red), the epidermis cell wall is non-lignified, probably mainly composed of cellulose.

There is a double (and, in some regions, triple) layer of cells next to the epidermis, displaying a small degree of thickening (Plates 2J and 2K). These cells exhibit a small degree of orientation and packing, and inter-cellular spaces (ics) can be observed (Plates 2K and 2L). Some deposition of lignin is evident. These figures also show stone cells or sclereids (st). The secondary walls of these cells are very thick and contain conspicuous simple pits (pts) with many branches. This tissue is heavily lignified.

An observation of the morphological structure of the seed coat closer to the embryo shows a thick layer of sclerenchyma cells (Plates 2M and 2N) that also stained strongly for lignin. These cells are tightly packed. A zone of fibres is evident in Plates 2O and 2P. The outer layer (Plate 2O) of this cellulosic tissue shows that these fibres are very long and also well packed. In the inner layer, close to the embryo's endosperm (Plate 2P), the same fibres are disposed perpendicularly.

The complex structure of this seed coat provides high protection against adverse environmental conditions and diseases. For the sake of clarity, a schematic representation of the embryo's seed coat is shown in Fig 1.

AIR preparation and sequential extraction prior to chlorite treatment

The strategy used for the sequential extraction of cell wall polysaccharides was defined to remove first those polymers present in the embryo endosperm and then, after the delignification step, those polymers held in the cell walls by lignin.

The preparation of AIR retained 85% of the initial seed weight. AIR is rich in xylose and glucose residues, and the total AIR carbohydrate content accounted for only 52% of its dry weight (Table 1). CR-1, the residue obtained after the sequential extraction of AIR with water, DMSO and KOH solutions, was richer in carbohydrates than AIR (63%). This residue contains 53 mol% Glc and 37 mol% Xyl. The high amount of Xyl in CR-1 indicates that the reagents used were not efficient for the extraction of a significant amount of Rosa Mosqueta hemicellulosic material. In this first extraction sequence the majority of the material was solubilised with KOH solutions (KOH-1), and 45% of this extract became insoluble upon neutralisation. These two fractions were very rich in Xyl and also contain HexA. The absence of significant amounts of sugars other than Xyl and HexA suggests that this extract contains mainly glucuronoxylan, a polymer that occurs in the cell walls of lignified dicotyledonous plants.^{23–25} The ratio Xyl/HexA gives the mean degree of branching of the glucuronoxylans. The polymers present in KOH-1 ppt had a ratio of 8.8 Xyl residues to 1 HexA residue; KOH-1 sn had a proportion of 4.3:1. The higher solubility of the more branched glucuronoxylans in water, after dialysis, was in accordance with the observations reported previously for olive seed hull glucuronoxylans.¹⁵ In order to further solubilise the hemicellulosic material from Rosa

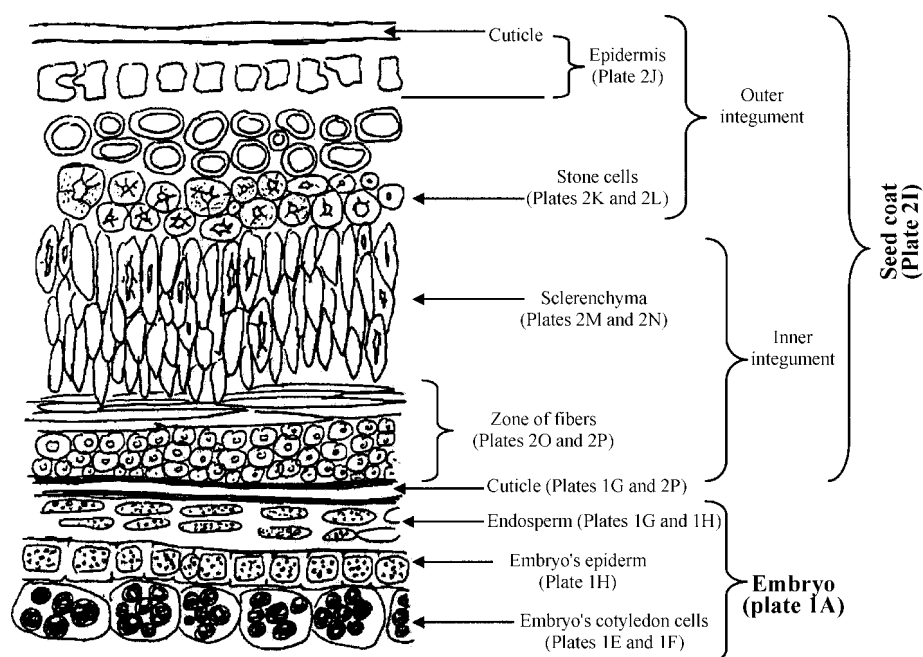


Figure 1. Schematic representation of the Rosa Mosqueta seed coat.

Table 1. Sugars composition of the cell wall fractions from *Rosa Mosqueta* seeds obtained by sequential extractions

Fraction	Recovery (% w/w)	Cell wall sugars (mol%)								Total sugars ^a (mg g ⁻¹)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
AIR	85.33	tr		2	43	tr	1	40	14	524
H ₂ O 40°C (W-1)	0.62 ^b	7		27	4	tr	12	9	41	324
DMSO-1	0.87 ^b	6		10	59	3	2	20	tr	127
W-2	0.37 ^b	6		19	20	2	8	22	23	163
KOH-1										
sn ^d	3.27 ^b	5		4	73		1	tr	17	266
ppt ^d	2.72 ^b	tr		1	88			1	10	546
Residue										
sn-CR-1	0.53 ^b	3		10	39		3	18	27	206
CR-1	62.62 ^b	tr		2	37	tr	tr	53	8	627
Ch-1										
sn	1.27 ^c			15	20	tr	5	tr	60	857
ppt	0.72 ^c	tr		1	15	tr	tr	16	68	920
DMSO-2										
sn	1.30 ^c	1	tr	tr	78		1	2	18	568
ppt	1.16 ^c	tr	2	tr	51	tr	1	33	13	350
W-3										
sn	0.23 ^c	tr	tr	tr	69	tr	1	10	20	327
ppt	0.04 ^c	tr	1	tr	30	1	1	54	13	374
Ch-2										
sn	6.39 ^c	8	8	6	49		6	1	22	149
ppt	0.20 ^c	tr		tr	17	tr	1	37	45	435
<i>Holocellulose</i>										
DMSO-3										
sn	3.62 ^c	tr	2	tr	81	tr	1	tr	16	488
ppt	0.27 ^c	4	tr	tr	74	1	tr	9	12	519
W-4										
sn	0.49 ^c	tr		tr	80	tr	1	4	15	396
ppt	0.06 ^c	tr	1	tr	25	1	1	62	10	565
0.5M KOH										
sn	6.02 ^c	1		2	67	2	2	tr	26	484
ppt	1.61 ^c	1		2	81				16	799
1M KOH										
sn	2.14 ^c	1	1	1	78	tr	2	1	16	852
ppt	6.79 ^c	tr		tr	86			1	13	877
4M KOH										
sn	3.41 ^c	tr	1	tr	90		1	1	7	288
ppt	0.47 ^c	1	tr	1	83	tr		2	13	796
Cellulosic residue 2										
sn-CR-2	0.68 ^c	10		2	5		1	52	30	432
CR-2	30.69 ^c	2	tr	tr	6	1	tr	76	15	629

HexA, hexuronic acid; sn, supernatant on dialysis; ppt, precipitate on dialysis; tr, trace; blank spaces, not detected

^a Values are expressed as mg anhydrosugar g⁻¹.^b Values are expressed as% AIR.^c Values are expressed as% CR-1.^d Values are a weighted average of KOH sn and ppt extracts in the first sequential extraction.

Mosqueta cell walls using the less aggressive possible agents, a mild chlorite treatment at 70 °C was assayed.

Mild delignification

Mild chlorite treatment provided two fractions very rich in sugars. The fraction that remained soluble after dialysis (Ch-1 sn) represented 1.3% of CR-1. It was rich in HexA, Xyl, Ara and Gal, a sugar composition characteristic of pectic polysaccharides (HexA, Ara and Gal) and glucuronoxylans. The fraction that precipitated was rich in HexA, Glc and Xyl, which

are characteristic of unbranched pectic polysaccharides, xylans and, possibly, glucans. The FT-IR spectra of these fractions^{26,27} confirmed the presence of pectic polysaccharides (data not shown). The occurrence of pectic substances in highly lignified tissues was previously reported by Coimbra *et al*¹⁵ in olive seed hull, and recently it was also shown to occur in cork middle lamella.²⁸

DMSO is known to be a good solvent for xylans.¹³ DMSO-2 soluble material accounts for 2.5% of CR-1 and mainly consists of glucuronoxylans, although the

material insoluble in water also contains some glucans. The water used to wash out DMSO (W-3), although in small amounts, contained polymers rich in Glc that tended to become insoluble upon complete removal of DMSO by dialysis.

Because the amount of xylans solubilised in DMSO-2 was not significant when compared with the amount of Xyl of CR-1, a delignification treatment was performed.

Delignification treatment

The delignification of the residue obtained after DMSO-2 extraction gave the fraction Ch-2, which accounted for 6.6% of CR-1. The polymeric material present in this extract is composed mainly of degraded lignin (as suggested by the strong UV absorption—data not shown), since the total sugars accounted for only 16% of this fraction. The fraction that remained soluble after dialysis is rich in Xyl and contains significant amounts of HexA; the insoluble fraction is richer in HexA and contains Glc and Xyl. These polymers are probably low-molecular-weight xylan and pectic polysaccharides, as reported to occur in pine²⁹ and olive¹⁵ seed hull.

Sequential extraction of delignified residue (holocellulose)

The DMSO treatment of holocellulose (DMSO-3) extracted 3.9% of the material. This extract is very rich in glucuronoxylans. The analysis of the DMSO-3 washing water (W-4) showed the presence of a glucan that could be separated from xylans by centrifugation. This glucan, although in small amounts, tends to appear in the majority of aqueous or DMSO extracts.

KOH extracts accounted for 20.4% of CR-1. The polysaccharides present in these extracts were glucuronoxylans. The precipitates are very rich in sugars and present Xyl/HexA ratios of 5.1:1, 6.6:1 and 6.4:1 respectively for the 0.5, 1 and 4M KOH extractions. The glucuronoxylans present in the supernatants tend to have lower ratios (except for 4M KOH sn). The occurrence of this type of polymer was confirmed by the FT-IR spectra.^{26,27} The small amount of pectic material that was still associated with CR-2 residue was released upon neutralisation. CR-2 was very rich in sugars and is mainly composed of Glc (from cellulose), although small amounts of HexA and Xyl are still present.

The amount of material other than polysaccharides (lignin, other phenolic compounds, protein) present in AIR is 47.6%. This result is comparable to the amount of sugars obtained by the sum of all extracts in relation to the material extracted (44.2%). As observed from the microscopic analysis, this material is mainly deposited in the embryo's seed coat. The extraction of polysaccharides from the seed coat of Rosa Mosqueta was only possible after the delignification treatment. Although the types of polymers present before and after the delignification treatment were similar, the sugar composition obtained showed that

the non-lignified tissues are richer in pectic polysaccharides and the seed coat is richer in glucuronoxylans. Using the results of the yields and sugar composition shown in Table 1, it can be estimated that Rosa Mosqueta cell wall polysaccharides comprise mainly glucuronoxylans (47%), cellulose (34%), pectic polysaccharides (17%) and non-cellulosic glucans (2%). Pectic polysaccharides may play an important role in the maintenance of the tissue and cell wall integrity. Rocha *et al*²⁸ reported that a pectinase treatment of cork cubes containing 1.5% pectic polysaccharides was responsible for the occurrence of cellular separation and tissue collapse. To increase the extraction of oil from Rosa Mosqueta seeds, its polysaccharide composition allows us to suggest that the pectinase should be used prior to the use of xylanases and/or cellulases.

CONCLUSION

The mature seed of Rosa Mosqueta has a very thick and structurally complex seed coat with heavily lignified tissues. The embryo has two layers of remaining endosperm tissue (indicating that this is an exalbuminous seed), two voluminous cotyledons that contain the oil, and bundles of provascular tissues distributed perpendicularly to the transverse axis of the embryo.

The extraction of the majority of the polysaccharides from the cell walls of the seeds of Rosa Mosqueta is greatly improved after a delignification treatment. However, 24% of the material was extracted before delignification. The major non-cellulosic polysaccharides present in the extracts obtained before delignification are pectic polysaccharides, glucuronoxylans and traces of a glucan-type polysaccharide soluble in DMSO but insoluble in water. These polymers seem to arise from the non-lignified tissues of the embryo endosperm. The major non-cellulosic polysaccharides obtained from the lignified tissues are glucuronoxylans, extracted mainly with DMSO and KOH solutions. This type of polymer seem to arise from the lignified tissues of the seed coat.

Concerning the use of enzymes for oil extraction from the embryo endosperm, microscopy and cell wall polysaccharide analysis showed that the use of pectic enzymes followed by a xylanase or even a cellulase should be explored.

ACKNOWLEDGEMENTS

This work was partially funded by the European Union (EU Contract INCO-DC: 96-2205). The authors thank Dr Clara Cramez and Dr Jovita Oliveira (Departamento de Engenharia Polímeros, Universidade do Minho, Guimarães) for their help in the sectioning of the GMA, and Dr Rubim A Silva (Departamento de Botânica, Faculdade de Ciências, Porto) for his help in the preparation of the GMA mixture. We are also grateful to Alexandra Nunes and

Dr António Barros (Departamento de Química, Universidade de Aveiro) for the FT-IR experiments and data analyses.

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