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ORIGINAL ARTICLE

Extraction and analysis of the parietal polysaccharides of acorn pericarps from *Quercus* trees

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Abstract

Acorns produced by *Quercus* trees are currently underexploited and undervalued. To evaluate the commercial and health benefits of acorns, we examined the cell wall components of acorn pericarps from *Quercus suber* and *Quercus ilex*, growing in North-Western Algeria. Acorn pericarps were sequentially extracted and the polysaccharide fractions were analyzed by gas liquid chromatography and Fourier-transform infrared spectroscopy. The lignocellulosic fraction was the major component of *Q. suber* and *Q. ilex* cell walls (37.19% and 48.95%, respectively). Lower amounts of pectins and hemicelluloses were also found in both species. Hemicellulose extracts from the two species contained xylose as the major monosaccharide (ranging from 36.7% to 49.4%). Galacturonic acid was the major component of hot water- or ammonium oxalate-extracted pectins from both species (ranging from 20.6% to 46.8%). The results reported in this paper reveal that acorn pericarp cell walls from these two oak could be potential sources of bioactive compounds.

Keywords: *Quercus sp., pericarp, polysaccharides.*

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1. Introduction

The genus *Quercus spp*. is one of the most species-rich genus among forest trees. This genus consists of several hundred species which grow in temperate, as well as in Mediterranean climates, particularly in America, Europe, and Asia[1].

In Algeria, oak trees represent an important forest resource, as they account for nearly 40% of the Algerian forest, and play important ecological, economic roles. In Algeria the local population uses the fruit of *Quercus* as a traditional food resource[2]. Numerous studies have recently explored the health benefits of natural plant compounds including bioactive compounds such as polyphenols, proteins, lipids, vitamins, polysaccharides, and other constituents[3].

Cell walls are important for the growth and the development of plants; they provide a significant barrier to diseases, making them targets for improving the post-harvest storage and processing of the fruits. Thus, studies evaluating how plants synthesize and remodel their cell walls constitute an important and expanding area of research, particularly in the renewable energy field[4] .

Identifying, isolating, and evaluating new sources of bioactive polysaccharides, in order to promote their use in technological applications or food formulations has recently been widely examined[5]. Although a few works have been dedicated to acorns[6], no single study exists which has been specifically devoted to acorn pericarps.

This study was conducted to evaluate the cell wall of acorn pericarps from two *Quercus* species growing in different regions of North-Western Algeria, in the Saida (high plateau region) and Oran (coastal region) areas. To the best of our knowledge, the present study is the first report on cell wall polysaccharides extracted from acorn pericarps.

2. Materials and Methods

2.1 Plant material

The two different oak species, *Q. suber* and *Q. ilex*, were selected because of their high prevalence among North-Western Algerian forests. Acorn samples, from approximately 100 year old trees, were collected in December 2016, *Q. ilex* in the Saida region (34°48'45.5"N 0°09'43.5"E) and *Q. suber* in the Oran region (35°38'20.3"N 0°50'22.6"W) and were identified by Pr. Meriem Kaid Harche. Two voucher specimens (QS 8409 and QI 8410) have been deposited at the Herbarium of the Department of Biotechnology, Mohamed

Boudiaf University of Sciences and Technology, Oran, Algeria. After cleaning, pericarps were manually detached from acorns and dried in a ventilated oven (40 °C). Pericarps were then ground (particle size <200 µm), and the resulting powder was stored in desiccators at room temperature.

2.2 Sequential extraction of parietal components

The sequential and selective extraction of parietal polysaccharides present in acorn pericarps from *Q. suber*, and *Q. ilex*, was carried out according to Hachem et al.[7]. All extraction procedures were carried out using magnetic stirring. All extracts were filtered through a porous glass frit (Porosity number 3) and then transferred to pre-soaked dialysis tubing (Spectra/Por; molecular weight cutoff 6000–8000 Da). After dialysis, polymers were precipitated by addition of 3 volumes of 96% ethanol, centrifuged, and finally freeze-dried. The subsequent fractionation procedure is summarized in Figure 1.

2.3 Colorimetric assay of total sugars

Sugars in the polysaccharide fraction were identified using the sulfuric phenol methodfor neutral sugars with glucose as standard[8], and the meta-hydroxydiphenyl (m-HDP) method for uronic acids with glucuronic acid as standard[9]. Because of the interference of uronic acids in the determination of neutral sugars and vice versa, it was necessary to apply the correction method established by Montreuil et al.[10].

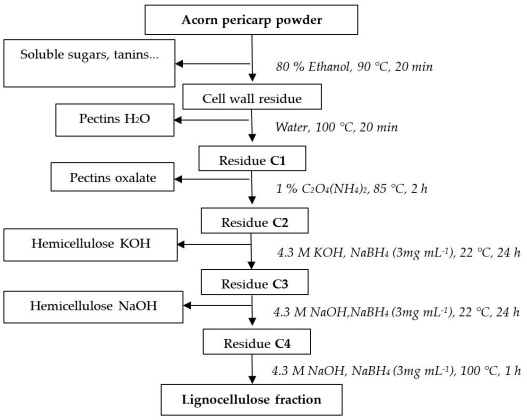

Figure 1. Extraction and isolation of cell wall polysaccharides.

2.4 Qualitative analysis by gas liquid chromatography

The monosaccharide composition of the extracted fractions was determined after methanolysis (MeOH/HCl 1 M, 24 h, 80 °C) by gas-liquid chromatography of pertrimethylsilylated methylglycosides as previously described by Hachem et al.[7].

2.5 Fourier-transform Infrared spectroscopy (FT-IR)

The different fractions from the acorn pericarps of *Q. suber* and *Q. ilex* were compressed into KBr pellets. The FTIR spectra of these pellets were obtained using a Cary 600 FTIR spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) over the 400–4000 cm^{-1} range.

3. Results and Discussions

3.1 Polysaccharide yields at each extraction step

The freeze-dried cell wall residue contained 82.11 ± 2.54% and 88.93 ± 2.30% of the dry mass of the *Q. suber* and *Q. ilex* pericarp, respectively (Table 1). The lignocellulosic fraction comprised the main part, representing 37.19 ± 8.63% and 48.95 ± 8.51%, respectively. The KOH and NaOH hemicellulose extracts represented 10.16±1.24% and 3.40 ± 1.05% of the *Q. suber* cell wall residue, respectively, whereas these same hemicellulose fractions from *Q. ilex* represented 7.38 ± 1.67%, and 4.81±0.64%, respectively. The hot water pectin extract of *Q. suber* and *Q. ilex* represented 6.47 ± 3.04% and 3.88 ± 0.78% of the cell wall residues and was composed of 9.6% and 20% uronic acid respectively. While ammonium oxalate pectin extracts of both species (4.19 ± 1.73%, 2.61 ± 1.61%) contained more uronic acid than hot water extracts (68.5% and 39.3%). This difference may be the result of different environmental conditions and/or genetic factors that can affect the cell wall structure and also affect the levels of its various components[11].

Comparing the composition of the two cell walls, it can be seen that the *Q. ilex* acorn pericarp appears to contain much more lignocellulose, but less pectin and hemicellulose polysaccharides, than *Q. suber*. This difference could be due to different environmental conditions and/or genetic factors, since these two distinct species of *Quercus* have different provenances; Saïda belongs to the high plateau, while Oran is situated on the Northwestern Mediterranean

Table 1. Yields of differentially extracted fractions prepared from *Q. suber* and *Q.ilex* acorn pericarps.

Fractions	*Q. suber*		*Q. ilex*	
	Extraction yield (%)***	Uronic acid (%)	Extraction yield (%)***	Uronic acid (%)
Cell wall residue*	82.11 ± 2.54	Nd	88.93 ± 2.30	Nd
Pectins H_2O**	6.47 ± 3.04	9.6	3.88 ± 0.78	20
Pectins oxalate**	4.19 ± 1.73	68.5	2.61 ± 1.61	39.3
Hemicellulosic KOH**	10.16 ± 1.24	19.6	7.38 ± 1.67	8
Hemicellulosic NaOH**	3.40 ± 1.05	10.6	4.81 ± 0.64	10.2
Lignocellulosic fraction**	37.19 ± 8.63	Nd	48.95 ± 8.51	Nd

*Percentage of 15 g starting dry weight of pericarp powder; **% Weight of cell wall residue, Nd: not detected; ***values are means ± standard deviation of three samples.

coast of Algeria. Numerous studies have shown that many abiotic and biotic factors, such as geographic location, soil salinity, light intensity, levels of water nutrition, plant species, time of harvest, and stage of life cycle can cause changes in cell wall structure and can significantly affect the levels of various cell wall components[11,12] .

3.2 Monosaccharide composition of cell walls

The amounts of pectins extracted with hot water or ammonium oxalate confirmed the high levels of pectin (Table 2), in agreement with the high levels of galacturonic acid (20.6 - 46.8%). Rhamnose levels in the pectin extracts ranged from 5.2% to 8.3%, suggesting that these fractions also contain rhamnogalacturonans that can be substituted with arabinan, galactan, and/or arabinogalactan side chains. The detection of fucose in pectins extracted from *Q. ilex* pericarps suggests the presence of rhamnogalactoronan II. Moreover, high levels of glucuronic acid in the pectins extracted with hot water from *Q. ilex* pericarps (19.1%) suggest differences in the pectic components between the two species. According to Alba and Kontogiorgos[13], the diversity of pectin structures depends on the botanical source, plant ripening stage, and extraction procedure as well. Hemicelluloses extracted with KOH or NaOH were found to be rich in xylose (Table 2), suggesting the presence of xylans. The Ara/Xyl ratio is interesting because it allows to compare the degrees of substitution of the polymer. In the fraction extracted with KOH this ratio is 0.37, and 0.59 in *Q. ilex* and *Q. suber* pericarps respectively, compared to 0.40 and 0.42 for the fraction extracted with NaOH. This suggests that the main xylan chain of *Q. suber* pericarps is more substituted than that of *Q. ilex*. The results obtained also show that the pericarps of both species contain xylans with significant degrees of substitution, compared to the results obtained by Habibi et al.[14,15] with the seed pericarps of *Opuntia ficus-indica* and *Argania spinosa*. In the case of arabinoxylans, a low Ara / Xyl ratio corresponds to a low degree of polymer branching, making it less water soluble, while water soluble arabinoxylans are characterized by a higher Ara / Xyl ratio[16,17]. However, the presence of arabinoglucuronoxylans in the fraction extracted with KOH cannot be excluded given the presence of glucuronic acid (2.4 to 2.7%). Glucose (5.5 to 15.2%) was also found, suggesting the presence of hemicelluloses of the xyloglucan type. According to Hu et al.[18], xyloglucans are the predominant family of hemicelluloses and are mainly found in dicotyledons, but at lower levels in monocotyledons. The results obtained in our study are consistent with previous studies on the same tissues[19].

3.3 FT-IR spectra

FT-IR spectra of pectins and hemicelluloses are presented in Figures 2 and 3. The large intense band between 3200 and 3500 cm^{-1} can be attributed to the elongation vibration of hydroxyl groups (-OH)[20]. Small vibration bands indicating C-H bonds were observed between 2800 and 3000 cm^{-1}. Other signals at 1746 and 1756–1760 cm^{-1} suggest the presence of acetyl groups in pectic residues[21]. Absorption bands around 1600 and 1400 cm^{-1} can be attributed to carboxylate

Table 2. Monosaccharide composition of acorn pericarps from *Q. suber*, and *Q. ilex* assessed by gas liquid chromatography.

	Species	Monosaccharide composition (%mol)								
		Ara	Rha	Fuc	Xyl	Gal A	Man	Gal	Glc	Glc A
Pectins H_2O	***Q.ilex***	16.1	6.9	1.5	4.5	24.3	2.1	11	14.5	19.1
	Q.suber	36.9	8.3	Nd	3.1	20.6	0.8	5.9	21.5	2.9
Pectins Oxalate	***Q.ilex***	13	5.2	0.9	3.5	46.8	5.6	12.1	4.4	8.5
	Q.suber	18.6	7.2	Nd	1.6	23.5	1.7	11	24.3	12
Hemicelluloses KOH	***Q.ilex***	18.5	4.8	Nd	49.4	8.5	Nd	9.8	6.3	2.7
	Q.suber	21.9	5.8	Nd	36.7	8.6	Nd	9.4	15.2	2.4
Hemicelluloses NaOH	***Q.ilex***	18.3	4.7	Nd	45.7	13.8	1.4	9.5	6.5	Nd
	Q.suber	20.5	6.2	Nd	48.5	13	Nd	6.3	5.5	Nd

Ara: arabinose; Rha: rhamnose; Fuc: fucose; Xyl: xylose; Gal A: galacturonic acid; Man: mannose; Gal: galactose; Glc: glucose; Glc A: glucuronic acid; Nd: not detected.

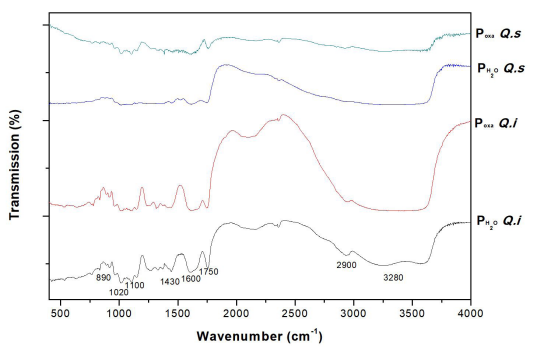

Figure 2. Infrared spectrum of the different pectin extracts; (P_{H2O}): pectins extracted with hot water. (P_{oxa}): pectins extracted with ammonium oxalate.(*Q.s): Q. suber*. (*Q.i):Q. ilex*.

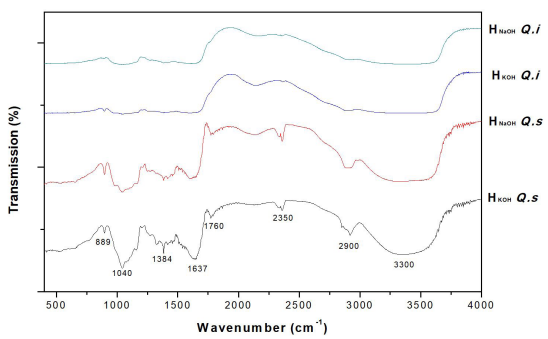

Figure 3. Infrared spectrum of different hemicellulose extracts; H_{NaOH}: hemicelluloses extracted with NaOH. H_{KOH}: hemicelluloses extracted with KOH. (*Q.s): Q. suber*. (*Q.i):Q. ilex*.

groups (COO^-). The large band at 1610 to 1637 cm^{-1} and the band near 1430 cm^{-1} were attributed to asymmetric and symmetric stretching of C=O, respectively[22]. Finally, the bands observed between 890 and 1200 cm^{-1} are specific of the vibrations of the C-O-C and C-O-H bonds present in polysaccharide structures[23].

4. Conclusions

The results reported in this paper reveal that the cell wall of acorn pericarps from two *Quercus* species could be potential sources of bioactive constituents, mainly polysaccharides (pectins, hemicelluloses, celluloses) and lignin. These include xylans, xyloglucan type hemicelluloses, and homogalacturonan and rhamnogalacturonan pectins. These constituents are non-toxic, biocompatible, and biodegradable and hold a high potential for their broad application in food or for their pharmacological effects, which have yet to be exploited.

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