Chemical Characterization of Non-Saccharidic and Saccharidic Components of Rapeseed Hulls and Sunflower Shells

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The main compositional characteristics of rapeseed hulls (RH) and sunflower shells (SS) were examined in terms of non-cellulosic components. The non-sugar fractions were analyzed by solid nuclear magnetic resonance (NMR) and pyrolysis-gas-chromatography/mass spectrometry (Pyr-GC-MS). Unlike SS, RH is a non-lignified biomass. The presence of large amounts of catechol and cresol suggested the presence of phytomelanin in both materials. Sugars accounted for 60% of RH and 45% of SS. Pectic compounds were extracted from the holocellulose of RH with ammonium oxalate or with citric acid, with 17% and 31% yield, respectively. A glucuronoxylan was isolated from the holocellulose of SS in basic conditions with 16% yield.

Keywords: Sunflower shell; Rapeseed hull; Pectin; Hemicellulose; Phytomelanin

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INTRODUCTION

Rapeseed and sunflower oils represent 10% to 15% and 7% to 9%, respectively, of world oil crop production (Oil world data base, 2014). Since the early 2000s, the production of rapeseed oil has increased, especially in Europe, because of the development of biodiesel plants. In addition to oil, organic residues, such as hulls, shells, and meal, are produced in large quantities after oil extraction. The meals have high protein content and are traditionally used as feed for farm animals. However, compared to soybean meal, the use of rapeseed meal for animal feed is limited because of its lower digestibility (higher levels of cellulose and carbohydrates). In Europe, 59% of the oilseed meal used in animal feed originates from imported soybean meal, compared to only 24% from rapeseed meal (Heuzé *et al.* 2016). Most of the industrial lines for rapeseed processing do not involve a de-hulling stage, so the rapeseed meal contains about 30% hulls, which are rich in polysaccharides with low protein content. Sunflower shells and rapeseed hulls have little economical value, but they can be used for animal feed and as an energy source. There are few reports regarding the composition and potential uses of these industrial co-products. Most of the reports deal with thermal applications like pyrolysis and combustion (Demirbas 2003; Bilgic *et al.* 2016), bioethanol production (Sharma *et al.* 2004; Brahim *et al.* 2016), and phenolics extraction (Naczk *et al.* 1998; Amarowicz *et al.* 2000; Liu *et al.* 2012).

There is a growing interest in the exploitation of agricultural wastes as sources of new, value-added polysaccharides. Additionally, environmental concerns have made the

development of green alternatives to oil-based synthetic polymers and aluminum for films and coating necessary. The utilization of non-cellulosic polysaccharides for material applications is a rapidly expanding research field (Hansen and Plackett 2008). Pectin can also be used in polymeric/oligomeric/monomeric forms in the food industry (Ciriminna *et al.* 2015). Pectins and hemicelluloses can be extracted from biomass using traditional solid-liquid extraction in aqueous acid or basic media, respectively (May 1990; Moine 2007).

The fractionation of rapeseed hulls for a better recovery of biopolymers using unconventional pretreatments was recently studied. The results showed that coupling treatments constituted a promising alternative for adding value to a highly recalcitrant biomass for bioethanol production. However, the chemical composition of rapeseed hulls and sunflower shells is still unclear. Rapeseed hulls are described as a pectin-rich feedstock (Aspinall and Jiang 1974). In terms of the non-saccharidic fraction, it seems that in contrast to sunflower shells, rapeseed hulls are a non-lignified biomass (Brahim *et al.* 2016). Nevertheless, the literature on this topic is scarce.

Thus, a better knowledge of the composition of RH and SS should stimulate their industrial use and the extension of the de-hulling stage in rapeseed industrial processing. This additional stage would improve the quality of the recovered rapeseed meal for animal feed and for protein extraction by removing most of the fibrous material and phenolic compounds.

In the present study, the chemical composition of RH and SS was studied. The non-saccharidic fractions were analyzed for the first time by solid nuclear magnetic resonance (NMR) and pyrolysis-gas-chromatography/mass spectrometry (Pyr GC-MS). The polysaccharides (hemicelluloses and pectins) were extracted and analyzed in terms of monosaccharides by size exclusion chromatography and 2D NMR.

EXPERIMENTAL

Materials and General Methods

Rapeseed hulls and sunflower shells (collected from CREOL, Pessac, France) were ground with a laboratory mill equipped with a 1-mm sieve and oven dried at 80 °C overnight. Soxhlet extraction was used with hexane for oil extraction from the raw materials. Ash was determined by incineration in a furnace at 550 °C, followed by weighing.

Determination of acid-insoluble residue and monosaccharide quantification.

Hexane-extracted, dry biomass (175 mg) was hydrolyzed with 2 mL of sulfuric acid (72%) for 1 h at 30 °C, diluted to 3% sulfuric acid with the addition of water, and autoclaved (Tuttnauer, Breda, The Netherlands) for 1 h at 121 °C. The autoclaved samples were filtered, and the dried residue was weighed to obtain the acid-insoluble residue content. The sugar-rich liquid was analyzed by ionic chromatography using a Dionex ICS-3000 system (Sunnyvale, USA) as previously described (Brahim *et al.* 2016).

Delignification

The dried material (10 g) was transferred into a flask with 5 g of sodium chlorite and 5 mL of acetic acid in 1 L of water. The mixture was heated and stirred at 70 °C for 2 h. The process was repeated until the mixture turned white. The solid residue (holocellulose) was subsequently washed with abundant water and dried overnight at 60 °C.

Extraction of Hemicelluloses and Pectins

Sodium hydroxide extraction

The holocellulose (2 g) was placed into 100 mL of 2% NaOH and stirred at 80 °C for 2 h. The liquid phase was recovered using a Buchner funnel, and the solid phase was placed in the flask with a further 100 mL of 2% NaOH, and then stirred at 80 °C for 2 h. The solid phase was washed with distilled water, and the aqueous phase was collected by centrifugation, and then neutralized to a pH of 5.5 with 6 M HCl. The hemicellulose was precipitated with 3 volumes of ethanol added into the aqueous phase, and then it was kept in the refrigerator overnight. It was filtered and dialyzed for three days with a 1000 g/mol molecular weight cutoff membrane before freeze-drying

Ammonium oxalate extraction

The holocellulose (10 g) was stirred with ammonium oxalate (0.8 g) in 80 mL of distilled water at 90 °C for 6 h. The supernatant were added into 3 volumes of ethanol without setting the pH, and then it was kept in the refrigerator overnight. The precipitated pectin was filtered and dialyzed for three days with a 1000 g/mol molecular weight cutoff membrane before freeze-drying.

Citric acid extraction

The 80-mL citric acid solution was prepared with distilled water at a pH of 2 in a flask, and then 10 g of holocellulose were added. The mixture was stirred at 90 °C for 6 h. The supernatant were treated in the same way as previously described.

Monosaccharide Quantification

The quantification of sugars was performed using a Dionex ICS-3000 system consisting of a gradient pump, an autosampler, an electrochemical detector with a gold working electrode, an Ag/AgCl reference electrode, and Chromeleon version 6.8 (Dionex Corp., Sunnyvale, USA) as previously described (Brahim *et al.* 2016). The sugar content of the polysaccharides was determined based on the monomer content measured after a two-step acid hydrolysis procedure. The 0.1 g of sample were treated with 1 mL of 72% wt H_2SO_4 at 30 °C for 1 h in the first step. The reaction mixture was then diluted to 4% wt H_2SO_4 and autoclaved at 121 °C for 1 h.

Other Characterizations

The solid-state nuclear magnetic resonance (NMR) spectroscopy experiments were performed on a Bruker Advance-400 spectrometer (Billerica, USA). Thermal treatment was performed in a Turbomatrix 300 Thermal Desorber system from Perkin Elmer, Waltham, USA, and GC–MS analysis was performed on a Perkin-Elmer Clarus 500 GC gas chromatograph (conditions given in Brahim *et al.* 2016).

RESULTS AND DISCUSSION

Composition of the Raw Materials

Table 1 gives the composition of the RH and SS used in this study. Rapeseed hulls had a higher proportion of acid-insoluble residue (AIR). The term "Klason lignin" was not used here because of the absence of lignin in the starting materials. The term "acid-insoluble residue" does not necessarily indicate the presence of lignin. Table 2 shows the

sugars accounted for about 60% and 45% of the RH and SS, respectively. The pectin content of the RH was about 7% (oxalate extraction). The pectic composition of the oxalate extract was confirmed by a MALDI TOF experiment by the detection of galactose/acetylated galactose and methylgalacturonate acid (data not shown). Sunflower shells had a high proportion of glucose and xylose (54.2% and 28.8% of the whole sugars, respectively), similar to that of other major sources of annual lignocellulosic crops. On the other hand, RH had lower glucose content, but higher galacturonic acid and arabinose contents. This result was in accordance with relatively high pectin content previously described in RH (Aspinall and Jiang 1974).

In a previous paper the authors demonstrated the absence of lignin in RH (Brahim *et al*. 2016). In the present study, the comparison of the AIR fractions from RH and SS was established by ^{13}C NMR and Pyr-GC-MS.

Table 1. Composition of Rapeseed Hulls (RH) and Sunflower Shells (RS) Expressed as % of Dry Raw Material

Raw Materials	RH	SS
Ash	3.75	4.29
Hexane Extraction	6.9	7.0
Acid-Insoluble Residue	31.44	25.88
Protein	13.38	-
Holocellulose (Chlorite)	59.8	44.5
Tannins	0.61	0.74
Pectins (Oxalate)	6.9	0

Table 2. Sugars in Rapeseed Hulls (RH) and Sunflower Shells (RS) Expressed as % Using Hydrolysis

Sugars	RH	SS
Fucose	0.81	0.16
Rhamnose	1.88	1.65
Arabinose	18.32	4.05
Galactose	9.68	2.04
Glucose	39.69	54.21
Xylose	5.23	28.81
Mannose	1.79	1.57
Galactu.ac.	22.10	6.52
Glucu. ac	0.45	0.95

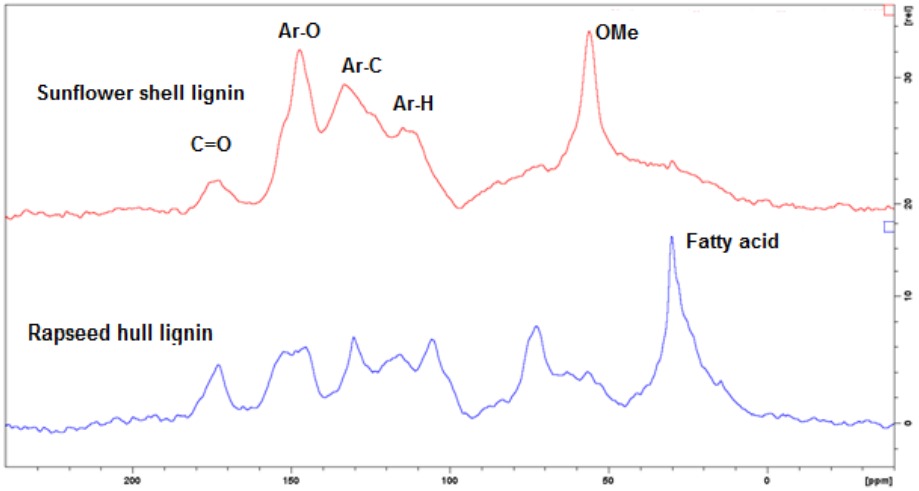

Fig. 1 Solid ^{13}C NMR of AIR isolated from RH and SS

The solid-state ^{13}C NMR spectra are given in Fig. 1. The acid-insoluble residue extracted from SS showed characteristic peaks consistent with that of lignin. Specifically, an intense signal was detected at 56 ppm, and the region of aromatic carbons was observed from 100 ppm to 160 ppm. The signal pattern for the residue from RH was very different. A large amount of fatty acids were detected as their aliphatic chains at 20 ppm to 35 ppm and the carbonyl peaks around 175 ppm. The intensity of the aromatic carbons cluster (100 ppm to 160 ppm) was lower, and the absence of the methoxy peak was clearly observed.

Acid-insoluble residues were also analyzed by thermodesorption coupled to GC-MS (Pyr-GC-MS). The major compounds identified from the AIR of SS and RH are given in Table 3. Examination of SS Pyr-GC-MS data clearly showed the presence of methoxylated aromatic compounds, in accordance with the NMR study. A significant amount of G-type compounds was detected (2-methoxyphenol, 2-methoxy-4-methylphenol, 2-methoxyvinylphenol, vanillin, vanillic acid, and acetoguaiacone), and the presence of S-type lignin units was attested by a small amount of acetosyringone. Compared to lignins previously described by Pyr-GC-MS studies, a relatively high proportion of cresol and catechol derivatives was observed. Del Rio *et al.* (2007) studied soda lignins extracted from different non-woody plants (hemp, flax, jute, sisal, and abaca) by Py-GC/MS. The lignins showed a predominance of G- and S-type unit fragments (around 90%), with catechol and cresol accounting for 1% to 4% of total pyrolysis products. In the AIR of SS, catechol, and to a lower extent, cresol derivatives accounted for approximately 40% of the total phenolics detected by Py-GC/MS. The catechol-rich composition of the AIR could be in accordance with the description of the phytomelanin layer previously described (Costa *et al.* 2012). Phytomelanin is a resistant black layer in the pericarb (hull) of the sunflower family that acts as a protective barrier of the seed. Phytomelanine has no physical structure of its own, and its chemical composition has not been clearly established, but has been the subject of much speculation (Pandey and Dhakal 2001; De-Paula *et al.* 2013; Kumar and Kumar 2013).

For rapeseed hulls, the absence of methoxylated aromatic compounds (associated to lignin polymer) and the high content of fatty acids (mainly oleic acid) confirmed the NMR data. The phenolic compounds of the AIR of RH were composed of cresols and catechols, suggesting the presence of a phytomelanine layer in the rapeseed pericarb as previously proposed (Brahim *et al.* 2016). The absence of lignin in RH was confirmed.

Table 3. Retention Times (RT) and Relative Concentration (Rel. Conc.) of Identified Compounds in RH and SS Acid-Insoluble Residues by Pyr-GC-MS Analysis

	RT[a]	RH Rel. Abund.[b]	SS Rel. Abund.[b]
Phenol	10.7	0.6	3.1
O-Cresol	13.3		1.8
P-Cresol	14.1	0.8	2.2
Phenol, O-Methoxy	14.3		3.1
Dimethylphenol	16.4	0.2	
Benzoic Acid	17.0		0.7
Phenol-2-Methoxy-4-Methyl	17.6		2.5
Catechol	17.9	1.34	10.8
3-Methylcatechol	19.6		2.2
4-Methylcatechol	20.5	0.2	5.2
2-Methoxyvinylphenol	21.1		1.55
4-Ethylcatechol	23.1		1.2
Vanillin	23.5		0.7
Phenol-Methoxy-Methoxymethyl	24.6		1.0
Guaiacylacetone	26.7		0.8
Vanillic Acid	27.6		1.1
Acetoguaiacon	28.1		2.0
8-Heptadecen	30.3	0.6	
Acetosyringone	33.3		0.7
Hexadecenoic Acid	35.9	1.1	3.4
Oleic Acid	40.0	5.1	3.0
Campesterol	52.9	0.8	
Stigmastan-3,5-Diene	54.5	2.0	

[a] Retention time in mn. [b] Relative abundance.

Extraction and Characterization of Hemicelluloses and Pectins

Starting from raw RH and SS, the hemicellulose polysaccharides were extracted from their holocellulose by extraction with alkaline solutions. The hemicelluloses were recovered after acidification, precipitation in EtOH, and dialysis (1kDa Cutoff). The extraction yields, the sugar compositions, and the molecular masses are given in Table 4.

The results indicated that the hemicellulose extracts accounted for approximately 24% and approximately 16% of the holocellulose of RH and SS, respectively.

Table 4. Analysis of Polysaccharide Compounds Extracted from Rapeseed Hulls and Sunflower Shells (g/100 g Raw Material).

		From RH			From SS
		Hemicell. (NaOH)	**Pectin (Oxalate)**	**Pectin (Citric Ac.)**	**Hemicell. (NaOH)**
Yield %		24.07	16.75	30.75	15.91
DM[a] %		<1	16.5	25.1	<1
Ac[b] %		<1	7.7	12.9	<1
MM[c]	Mn	13176	19530	13472	12377
	Mw	53393	86469	52239	58162
	PD	4.05	4.42	3.87	4.69
Fucose		0.79	0.44	0.62	0.19
Rhamnose		4.07	4.83	8.67	2.42
Arabinose		15.61	20.61	15.72	4.71
Galactose		7.46	5.78	11.50	3.52
Glucose		36.78	1.73	2.73	6.71
Xylose		7.60	6.50	12.41	70.90
Mannose		1.46	0.09	0.20	0.23
Ac Galactu.		25.78	59.70	47.70	8.92
Ac Glucu.		0.4	0.32	0.42	2.37

[a]Degree of methylation (NMR), [b]Degree of acetylation (NMR), [c]Molecular mass (SEC), Number average (Mn), Weight average (Mw), Polydispersity (PD).

The average molecular weight (M_w) and polydispersities (PD) of RH and SS hemicelluloses were quite close ($M_w \approx 53{,}000$ g.mol^{-1}, PD = 4.0 for RH; $M_w \approx 58{,}000$ g.mol^{-1}, PD = 4.7 for SS), corresponding to the degree of polymerization values of approximately 350 and 400, respectively. From the monosaccharide composition, it can be concluded that SS hemicelluloses were mainly xylan-type sugars with a high proportion of xylose. The presence of small amounts of galacturonic acid, rhamnose, arabinose, and galactose may indicate a contamination with pectin compounds.

The composition of this fraction was confirmed by a 2D NMR study ^{1}H-^{13}C HSQC (see Fig. 2A and 2B). The detection of signals assigned to 4-O-methylglucuronic acid residues not detected by ionic chromatography, in addition to xylan signals, reveals that glucuronoxylan was the principal hemicellulose of SS. The chemical shifts of both residues were in accordance with those described for glucuronoxylans from other materials (Moine

2007). The presence of a small amount of pectic galacturonans was also detected. This observation was in accordance with the sugar composition previously discussed. The hemicellulose extracts from RH displayed a very different sugar composition, with a low proportion of xylose (approximately 8%), and a high content of glucose (approximately 37%), galacturonic acid (approximately 26%), and arabinose (approximately 16%). The presence of high quantities of galacturonic acid can be explained by the extraction in basic conditions of a part of the pectic components of RH. Interestingly, the detection of relatively high proportions of glucose (approximately 37%), galactose (approximately 7%), and to a lower extent, fructose (1%) could be in accordance with previous results. Aspinall *et al.* (1977) extracted in basic conditions and characterized by gas chromatography a (fucogalacto)-xyloglucan from RH.

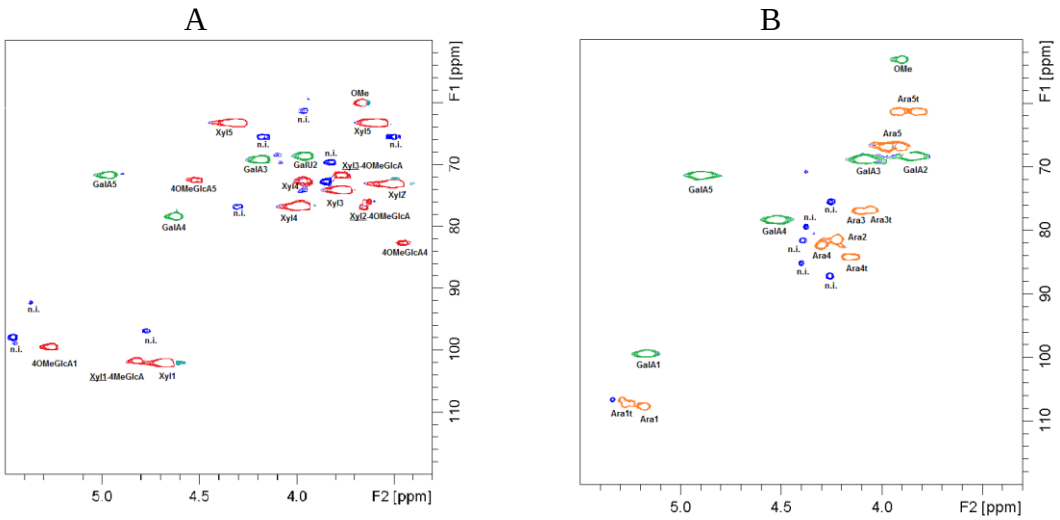

Fig. 2. HSQC spectrum of (A) NaOH extract from SS, and (B) oxalate pectine extract from RH. Ara t = terminal Ara, n.i. = not identified

Pectin extraction from RH holocellulose was performed using an aqueous solution of citric acid at pH 2 using conditions described for industrial extraction of pectins from citrus peels or apple pomace (May 1990). In this study, a second extraction process was employed using ammonium oxalate. The characterizations of the pectin fractions given in Table 4 were performed after ethanol precipitation and dialysis. A much higher yield was obtained in acidic conditions (approximately 31%) compared to oxalate extraction (approximately 17%). The sugar composition in both fractions indicated a high pectic content with high proportions of galacturonic acid and arabinose. With pectic acid extraction, the higher proportions of xylose (approximately 12%) and also glucose (approximately 3 %), galactose (approximately 6%), and fructose (approximately 1%) suggested the co-extraction of xyloglucan in acidic conditions. The lower molecular mass of the pectins extracted with citric acid (M_w = 52,000 g.mol^{-1}) compared to oxalate (M_w = 86,000 g.mol^{-1}) can be explained by an acid-catalyzed hydrolysis of the polysaccharide during the extraction. The values of the arabinose to galactose ratio for the pectins extracted with citric acid (1.36/1 compared to 3.56/1 with oxalate) indicated an extensive degradation of arabino-galatane type polysaccharides during the acid extraction.

The esterification degree of both extracts was also evaluated using a described procedure. Methylation and acetylation degree were determined by ^{1}H NMR after saponification using an internal standard (trimethylsilyl) propionic-2,2,3,3-d$_4$ acid sodium

salt). The results given in Table 3 showed that the isolated pectins contained low amounts of ester groups. This observation could be explained by a hydrolysis reaction of methyl ester and acetate groups that occurred during the previous delignification step, performed in acidic conditions. The oxalate pectin was characterized by HSQC NMR (Fig. 2B and Table 3) (Muller-Maatsch *et al.* 2014). The dominant signals were in accordance with the sugar composition and corresponded to non-methylated galacturonic acid and arabinose residues (Kostalova *et al.* 2013). Signals belonging to terminal and to 5-linked arabinofuranose units have been assigned according to previous studies (Fissore *et al.* 2010).

CONCLUSIONS

1. The fact that rapeseed hull (RH) is a non lignified biomass was confirmed.
2. RH and sunflower shell (SS) contained large amounts of cortical and crucial compounds, suggesting the presence of phytomelanin in both materials.
3. Regarding future developments, RH appears to be an interesting source of pectins, extracted in high yield in acidic conditions (up to 30%).
4. A glucuronoxylan can be easily extracted from SS in basic conditions.

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REFERENCES CITED

Amarowicz, R., Naczk, M., and Shahidi, F. (2000). "Antioxidant activity of crude tannins of canola and rapeseed hulls," *J. Am. Oil Chem. Soc.* 77(9), 957-961. DOI: 10.1007/s11746-000-0151-0

Aspinall, G. O., and Jiang, K.-S. (1974). "Rapeseed hull pectin," *Carbohyd. Res.* 38, 247-255. DOI: 10.1016/S0008-6215(00)82355-1

Aspinall, G. O., Krishnamurthy, T. N., and Rosell, K. G. (1977). "A fucogalactoxyloglucan from rapeseed hulls," *Carbohyd. Res.* 55(1), 11-19. DOI: 10.1016/S0008-6215(00)84439-0

Bilgic, E., Yaman, S., Haykiri-Acma, H, and Kucukbayrak, S. (2016). "Is torrefaction of polysaccharides-rich biomass equivalent to carbonization of lignin rich biomass," *Bioresource Technol.* 200, 201-207. DOI: 10.1016/j.biortech.2015.10.032

Brahim, M., Boussetta, N., Grimi, N., Vorobiev, E., and Brosse, N. (2016). "Innovative physically-assisted soda fractionation of rapeseed hulls for better recovery of biopolymers," *RCS Adv.* 6, 19833-19842. DOI: 10.1039/C5RA27548B

Ciriminna, R., Chavarria-Hernandez, N., Hernandez, A. I. R., and Pagliaro, M. (2015). "Pectin: A new perspective from the biorefinery standpoint," *Biofuels Bioprod. Biorefin.* 9(4), 368-377. DOI: 10.1002/bbb.1551

Costa, T. G., Younger, R., Poe, C., Farmer, P. J., and Szpoganicz, B. (2012). "Studies on synthetic and natural melanin and its affinity for Fe(III) ion," *Bioinorg. Chem. App.* 2012, 712840. DOI: 10.1155/2012/712840.

De-Paula, O. C., Marzinek, J., Oliveira, D. M. T., and Machado, S. R. (2013). "The role of fibres and hypodermis in Compositae melanin secretion," *Micron* 44, 312-316. DOI: 10.1016/j.micron.2012.08.003

Del Rio, J. C., Gutierrez, A., Rodriguez, I. M., Ibarra, D., and Martinez, A. T. (2007). "Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS, Py/TMAH and FT-IR," *J. Anal. Appl. Pyrol.* 79, 39-46. DOI: 10.1016/j.jaap.2006.09.003

Demirbas, A. (2003). "Relationships between heating value and lignin, fixed carbon and volatile material contents of shells from biomass products," *Energ. Source.* 25(7), 629-655. DOI: 10.1080/00908310390212336

Fissore, E. N., Ponce, N. M. A., de Escalada Pla, M., Stortz, C. A., Rojas, A. M., and Gerschenson, L. N. (2010). "Characterization of acid-extracted pectin-enriched products obtained from red beet and butternut," *J. Agr. Food Chem.* 58(6), 3793-3800. DOI: 10.1021/jf903844b

Hansen, N. M. L., and Plackett, D. (2008). "Sustainable films and coatings from hemicelluloses: A review," *Biomacromolecules* 9(6), 1493-1505. DOI: 10.1021/bm800053z.

Heuzé, V., Tran G., Sauvant D., Lessire M., and Lebas F. (2016). "Rapeseed meal," Feedipedia, a programme by INRA, CIRAD and FAO. http://www.feedipedia.org/node/52

Kostalova, Z., Hromadkova, Z., and Ebringerova, A. (2013). "Structural diversity of pectins isolated from the styrian oil-pumpkin (*Cucurbita pepo* var. *styriaca*) fruit," *Carbohyd. Polym.* 93(1), 163-171. DOI: 10.1016/j.carbpol.2012.05.017

Kumar, J. B., and Kumar, M. S. (2013). "A general idea about phytomelamin layer in some Compositae," *Indian J. Plant Sci.* 2(4) 44-48.

Liu, Q., Wu, L., Pu, H., Li, C., and Hu, Q. (2012). "Profile distribution of soluble and insoluble phenolics in Chinese rapeseed (*Brassica napus*)," *Food Chem.* 135(2), 616-622. DOI: 10.1016/j.foodchem.2012.04.142.

May, C. D. (1990). "Industrial pectins: Sources, production and applications," *Carbohydr. Polym.* 12, 79-99. DOI: 10.1016/0144-8617(90)90105-2.

Moine, C., Krausz, P., Chaleix, V., Sainte-Catherine, O., Kraemer, M., and Gloaguen, V. (2007). "Structural characterization and cytotoxic properties of a 4-O-methylglucuronoxylane from *Castanae sativa*," *J. Nat. Prod.* 70(1), 60-66. DOI: 10.1021/np060354p

Muller-Maatsch, J., Caligiani, A., Tedeschi, T., Elst, K., and Sforza, S. (2014). "Simple and validated quantitative 1H NMR method for the determination of methylation, acylation and feruloylation degree of pectin," *J. Agr. Food Chem.* 62(37), 9081-9087. DOI: 10.1021/jf502679s

Naczk, M., Amarowicz, R., Sullivan, A., and Shahidi, F. (1998). "Current research developments on polyphenolics of rapeseed/canola: A review," *Food Chem.* 62(4), 489-502. DOI: 10.1016/S0308-8146(97)00198-2

Oil World Database (2014), http://www.oilworld.biz/database.

Pandey, A. K., and Dhakal, M. R. (2001). "Phytomelanin in Compositae," *Curr. Sci. India* 80(8), 933-940.

Sharma, S. K., Kalra, K. L., and Kocher, G. S. (2004). "Fermentation of enzymatic hydrolysate of sunflower hulls for ethanol production and its scale-up," *Biomass Bioenerg.* 27, 399-402. DOI: 10.1016/j.biombioe.2004.03.005

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