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Preliminary characterization of residual biomass from *Hibiscus sabdariffa* Calyces

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Hibiscus sabdariffa **calyces are mainly used for different agro-food and beverages applications. The residual biomass generated contains various useful substances that were extracted and characterized. It contained 23% (w/w) soluble pectic material, a food additive, extracted with hot acidified water (80°C, pH = 1.5) and precipitated with ethanol. The molecular weight (28.5 and 109.7 kDa), the degree of methylation (70.6 and 44.3%) and the degree of acetylation (19.0 and 4.9%) were determined for two Senegalese cultivars (koor and vimto, respectively). The effect of the extraction method on these parameters was highlighted. The residual lignocellulosic material (LCM) was chemically degraded to monosaccharides and the amount of glucose and xylose (39% of dry LCM) determined to estimate its potential as feedstock for biofuels production. However, an enzymatic degradation test revealed a recalcitrant LCM, as only 50 to 55% of its polymeric glucose content was degraded to monosaccharides without pretreatment. Xylo-oligosaccharides (XOS) are functional foods with a real market potential as prebiotics, characterized by their degree of polymerization (DP). The production of XOS synthetized by the enzymatic degradation of LCM was monitored. The results of analyses performed showed that XOS produced had mainly DP3 and DP4 values.**

Key words: Pectin, lignocellulosic material, enzymatic degradation, xylo-oligosaccharides.

INTRODUCTION

Hibiscus sabdariffa var. sabdariffa is a member of the malvaceae family. This plant is an erect, annual shrub characterized by large and succulent sepals particularly rich in anthocyanins (Pouget et al., 1990; Tsai et al.,

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Abbreviations: LCM, Lignocellulosic material; **XOS,** xylo-oligosaccharides; **DP,** degree of polymerization; **CWE,** crude water-soluble extract; **MM,** molecular mass; **DM,** degree of methylation; **DA,** degree of acetylation; **GalA,** galacturonic acid content.

2002). Once considered a secondary crop, it is gaining increasing interest for the food industry as a source of anthocyanins for neutraceuticals (Da-Costa-Rocha et al., 2014; Patel, 2014) or food color production (Giusti and Wrolstad, 2003). In Senegal it was traditionally used to manufacture drinks and only a small proportion of calyces were processed locally. The national production of red *H. sabdariffa* was of 2885 tons in 2012, 200 tons of which were consumed locally (Cissé et al., 2008). However newly developed industrial products (spray dried instant drinks, energy drinks, bottled drinks etc.) made from dry calyces are manufactured by local small and medium facilities. The biggest amounts processed by a single company are estimated at 100 tons for the current year. The residual biomass produced is sometimes used as animal feed after a thorough washing designed to eliminate residual acidity, otherwise it is discarded as waste material. Due to environmental and economic considerations agro-industrial byproducts are processed to recover and/or produce valuable products. To date *H. sabdariffa's* residual biomass has not been considered with the objective of increasing its value.

H. sabdariffa calyces contain pectic polysaccharides whose chemical structure and biological activity have been studied (Muller and Franz, 1992; Brunold et al., 2004; Badreldin et al., 2005). Generally, industrial juice extraction processes are designed to avoid the extraction of pectins (Taylor, 2007); therefore, considerable amounts are still present in the residues. Apple pomace and citrus peels are the industrial byproducts traditionally used for extraction of commercial pectins, additives commonly used in food, cosmetic and pharmaceutical industries as gelling agent, thickener or emulsion stabilizer (Sila et al., 2009; Smith and Hong-Shum, 2011). However, alternative sources with new interesting characteristics are constantly sought (Happi Emaga et al., 2008; Fissore et al., 2013). Lignocellulosic material (LCM), primarily made of cellulose, hemicellulose and lignin, have the potential to be used as feedstock for the production of bioenergy, neutraceuticals, chemicals, biodegradable composites (Moure et al., 2006; Satyanarayana et al., 2009; Faruk et al., 2012; Menon and Rao, 2012). The products considered depend on the characteristics and composition of the biomass and on the profitability of the process selected (Uçkun Kiran et al., 2014).

The objective of the present study was to characterize the waste material from the processing of *H. sabdariffa* calyces in order to determine the potential of this by-product as a source of new derived products. Pectic polysaccharides from the two main Senegalese cultivars (Koor and Vimto) were extracted and characterized. The determination of cellulose, hemicellulose and lignin content together with an enzymatic degradation assay were used to estimate the potential as feedstock for biofuels production. Finally, the enzymatic release of xylooligosaccharides (XOS), neutraceuticals with a real market potential, was studied (Aachary and Prapulla, 2011; Finegold et al., 2014; Singh et al., 2015).

MATERIALS AND METHODS

Equipment, solvents and reagents

The centrifuge used throughout this work was a Jouan B4i centrifuge. Unless specified, centrifugation was performed for 15 min at 4000 rpm. All solvents and chemicals (acetone, formic acid, dibasic sodium phosphate, chloroform, and acetonitrile) were analytical grade and purchased from VWR International BVBA (Leuven, Belgium).

Residual biomass samples preparation

The experiments were made on calyces of Vimto and Koor cultivars from the Louga region (Northwest of Senegal). Dry whole calyces were extracted at 70°C in water with a 1/20 (w/vol.) ratio for 90 min under constant agitation. After filtration the calyces residues (CR) were dried at 55°C. CR was used in the following experiments.

Characterization of soluble polysaccharides

Extraction and purification of soluble polysaccharides

10 g of CR sample was submitted to extraction with distilled water (150 ml) at room temperature for 24 h. The supernatant, called crude water-soluble extract (CWE) hereafter, was collected by centrifugation and stored for further analysis. A second extraction was performed on the sediment with water acidified to pH 1.5 with hydrochloric acid (HCl). Temperature was kept, at 80°C for 2 h under constant stirring. The slurry was centrifuged and the supernatant, called crude acid-soluble extract (CAE) hereafter, was collected by centrifugation and stored for further analysis. The residual insoluble fraction, called *H. sabdariffa* LCM hereafter, was collected and dried to constant weight at 55°C before being ground in a blender (Waring, Marne-la-Vallée, France) and stored for further characterization. Crude extracts (CWE and CAE) were purified as follows (Lama-Muñoz et al., 2012): 4 volumes of 96% ethanol were added to an aliquot of the extract. After 24 h at 4°C, the ethanol-insoluble fraction was recovered by centrifugation, dispersed in ethanol, allowed to settle for 60 min. The liquid fraction was removed; the ethanol insoluble fraction was dissolved in pH 2 water (0.01 M HCl), precipitated again with one volume of ethanol (96%), allowed to settle for 60 min then centrifuged (15 min at 4000 rpm) to recover the precipitates. The sediment was recovered and dissolved in acidified water (0.01 M HCl), to give purified water extract and purified acid extracts (PWE and PAE) stored at 4°C before analysis.

Molecular distribution of H. sabdariffa soluble polysaccharides

Molecular mass (*MM*) distribution of polysaccharides contained in samples of CWE, CAE, PWE and PAE was determined by High-Performance Size Exclusion Chromatography (HPSEC). The apparatus used was a Waters 2690- High Performance Liquid Chromatography (HPLC) system (Waters Inc., Milford, MA, USA) equipped with a TSKgel GPWXL column (300 mm × 7.8 mm) (Tosoh Co. Ltd., Tokyo, Japan) and coupled on-line with a Waters 2410 differential refractometer that measures the refractive index (RI). Elution was performed at room temperature with 50 mM sodium nitrate solution containing 0.05% sodium azide at a flow

rate of 0.7 ml/min. The system was calibrated using dextran standards (Sigma-Aldrich NV / SA, Bornem, Belgium).

Galacturonic acid content

Enzymatic hydrolysis was performed with the commercial enzyme Frimapec W70 (Beldem, Groot Bijgaarden, Belgium), generally used to extract anthocyanins from red grape mashes. A 10 mg sample was mixed with 20 ml of enzyme dissolved in 20 mM of pH 5 sodium acetate buffer. The mixture was incubated at 40°C for 24 h and then heated at 100°C for 5 min to inactivate the enzymes. The final hydrolysate was filtered through a 0.2 µm filter membrane before analysis. Galacturonic acid (GalA) and glucuronic acid contents were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as previously described (Combo et al., 2013).

Degree of methylation and acetylation

The samples were submitted to de-esterification by alkaline treatment, and the amount of methanol and acetic acid released were determined by HPLC as described by Happi Emaga et al. (2008).

Characterization of *H. sabdariffa* LCM

Composition in xylose and glucose

Total monosaccharides concentration of *H. sabdariffa* LCM was determined as follows: 3 ml of 72% sulfuric acid was added in a 100 ml flask to a 300 mg sample. After 60 min of incubation at 30°C, 84 ml of distilled water were added to the flask that was put in an autoclave at 121°C for 60 min. Subsequently the supernatant was collected, neutralized with $Ba(OH)_2$, then centrifuged. An aliquot of the supernatant was taken for HPLC analysis. Total lignin content was determined by weighing accurately the solid residue previously dried to constant weight at 105°C.

Enzymatic hydrolysis

LCM samples were submitted to enzymatic degradation with a previously optimized (Rodriguez et al., 2005) mixture of commercial cellulases and hemicellulases in order to estimate the amount of LCM that could be degraded to monosaccharides (called enzymatic glucose or enzymatic xylose hereafter). This value was reported to the total concentration of monosaccharides previously determined. Biodegradability was defined here as the ratio (w/w) of enzymatic glucose or xylose to total LCM dry matter. Recovery was defined as the ratio (w/w) of enzymatic glucose or xylose to their total respective content. The enzymes used were: Viscozyme L (an endo β-glucanase that hydrolyzes (1,3)- and (1,4)-linkages in β-D-glucans, and had enzymatic side-activities such as cellulase, hemicellulase and xylanase), Celluclast 1.5 L (a cellulase with an endoglucanase activity) and Celluclean Classic 700T (a cellulase). All the enzymes used were from Novozymes A/S (Bagsværd, Denmark). Celluclean Classic 700T was dissolved in phosphate buffer (0.1 N, pH 5.5). $NaNO_3$ was added (0.05% w/v) to prevent microbial growth. The solutions were then filtered on a GF/C membrane (Whatman, Maidstone, England). Celluclast 1.5 L and Viscozyme L were dialyzed overnight in phosphate buffer (0.1 N, pH 5.5 with 0.05% $NaNO_3$) using nitrocellulose membranes with a cutoff of 10 kDa (Sigma-Aldrich, Bornem, Belgium). One liter of enzymes preparation was obtained by mixing 500 ml of 20 g/l Celluloclean Classic 700T, 100 ml of dialyzed Viscozyme L and 50 ml of dialyzed Celluclast 1.5 L. *H. sabdariffa* LCM hydrolysis was performed as follows: 30 ml of enzyme preparation was added to 500 mg sample in a 100 ml flask incubated in a water bath at 40°C. After 48 h an aliquot was centrifuged, the supernatant was analyzed by HPLC as described hereafter. Enzymatic activity was tested as follows: 1.5 ml of enzyme preparation was incubated in test tubes with 50 mg of cellulose (Whatman filter paper grade 1) for 60 min at 40°C under constant agitation. The test tubes were then put for 5 min in a 100°C water bath to deactivate the enzymes. The supernatant's monosaccharides content was determined by HPLC analysis as described hereafter.

HPLC analysis

HPLC analysis was performed on an Agilent 1100 series apparatus (Agilent Technologies, Diegem, Belgium) equipped with a RI detector. Separation was made with a C-610-H ion exchange column (300, 7.8 mm, Supelco, Bellefonte, PA, USA) and quantified using standards. All samples were filtered through 0.2 µm Minisart Syringe filter (Vivascience, Hannover, Germany).

Enzymatic production of xylo-oligosaccharides (XOS)

XOS production rate by enzymatic hydrolysis of *H. sabdariffa* LCM was monitored. The enzyme preparation and the enzymatic hydrolysis procedure were identical to those previously used. A sample was removed at increasing time periods and after deactivation of enzymes at 100°C for 5 min, the samples were analyzed by HPAEC-PAD as described above to determine the amount of XOS produced.

RESULTS AND DISCUSSIONS

Main characteristics of residual biomass from *H. sabdariffa* calyces

Residual biomass (CR) accounted for more than 50% of calyces' dry mass (Table 1). It presumably contained residual amounts of anthocyanins, soluble and insoluble polysaccharides, and other compounds whose extraction was minimized with the aqueous extraction method previously used. In the present study focus was put on the polysaccharide composition. CR were submitted to a sequential extraction procedure. A first extraction was performed at room temperature with distilled water (CWE) followed by a second extraction with acidified water (CAE). CWE accounted for 10.9 and 16.5% weight of soluble dry matter from koor and vimto cultivars total CR, respectively, while CAE represented nearly 30% of total CR. Combined crude extracts accounted for around 40% of CR dry mass (Table 1). This was in accordance with the study of Chan and Choo (2013) who found no significant effect of water temperature (50 to 95°C) on the extraction yield of cocoa husk pectin, whereas hot acidified water helped increase pectin solubility leading to an improved extraction yield. Similarly, Yapo et al. (2007) showed that pectin extraction yield was increased significantly with increasing acid strength but also with increasing extraction time. Extraction temperature on the other hand did not have any significant effect on the

Table 1. Composition of residues from *H. sabdariffa* calyces.

Cultivar	CR (% of dry calyces (w/w))	Crude pectic material extracts(% of dry CR (w/w))		LCM (% of dry CR)
		CWE	CAE	
Vimto	54.8±1.3	16.5±1.7	29.4±4.9	52.3 ± 3.0
Koor	55.6±2.1	10.9±0.3	28.6±2.0	58.4 ± 1.8

Results are expressed as average of 3 determinations ± standard deviation (sd); Vimto and koor are the local names of two *H. sabdariffa* cultivars.

Table 2. Composition of precipitated water soluble (PWE) and acid soluble (PAE) polysaccharides of *H. sabdariffa* calyces.

	PWE (% of dry CR)	PAE (% of dry CR)
Vimto	2.7±0.8	21.0±3.3
Koor	6.2±1.6	17.0±1.2

Results are expressed as average of 3 determinations ± sd; Vimto and koor are the local names of two H. *sabdariffa* cultivars.

extraction yield. Both crude extracts, CWE and CAE, were submitted to precipitation aiming at removing low molecular mass (*MM*) and ethanol-soluble compounds from the extracts. Results in Table 2 show that the amount of ethanol-precipitated extracts (PWE and PAE) represent 23.7 and 23.2% of CR (dry mass basis). CR can thus, be favorably compared to citrus peel and apple pomace, the main raw materials used for industrial extraction of commercial pectin, that contain 25 and 12%, respectively, pectin (Kalapathy and Proctor, 2001; Sila et al., 2009). In order to further characterize *H. sabdariffa* polysaccharides, some characteristics influencing their functional properties such as molecular mass (*MM*), degree of methylation (DM), degree of acetylation (DA) and galacturonic acid content (GalA) were determined.

Characterization of soluble polysaccharides

Molecular mass distribution

In order to determine *MM* distribution of *H. sabdariffa* soluble polysaccharides, PWE and PAE were analyzed by HPSEC. Figure 1 is an overlay of CWE, CAE and PWE, PAE HPSEC chromatograms for Vimto and Koor cultivars. From those, the different populations of compounds could be evaluated. The results (Figure 1 and Table 3) show that two populations of molecular sizes were found in the extracts: low *MM* oligosaccharides (100 to 1,000 Da) and higher *MM* polysaccharides (>1,000 Da). In the crude extracts (CWE and CAE) low *MM* compounds represented 60 to 80% of total solubilized matter (Table 3). Following the ethanol precipitation procedure, the mass percent of high *MM* polysaccharides, initially ranging from 18.5 to 41.8% (Table 3) in CWE and CAE, was increased to more than 70% in PWE and almost 50% mass in PAE (Table 4). The purification procedure can be thus considered efficient. It allowed the removal of interfering compounds and a more precise determination of *MM* of the soluble polysaccharides. Comparison of *MM* values for PWE and PAE showed that polysaccharides extracted with water at room temperature had a *MM* twice that of polysaccharides extracted with hot acidified water (Table 4). This suggests that the hot acidified water extraction procedure may be too harsh, breaking down high *MM* polysaccharides (28.5 and 109.7 kDa fractions) to lower *MM* polysaccharides (13.3 and 50.3 kDa). Likewise, Koubala et al. (2008) showed that the average *MM* of HCl-extracted mango pectins is lower than water-extracted pectins. Furthermore, Garna et al. (2007) highlighted the influence of extraction and purification methods on the *MM* of polysaccharides. Indeed, apple pectins were partially degraded (degradation of side sugar chains) after 60 min of acidified water extraction, whatever the temperature.

Biochemical characterization

Results of characterizations performed on PWE and PAE samples are summarized in Table 5. PWE samples had DM values of 70.6 and 44.3% for vimto and koor cultivars respectively. These values decreased in the samples extracted with hot acidified water (Table 5). Yapo (2009) observed similar effects on yellow passion fruit pectins and pointed out the least deesterifying action of citric acid compared to mineral acids. The DA was 19.0 and 4.9% for the vimto and koor cultivars pectic polysaccharides respectively. These values dropped to 3.2% and null for the pectic polysaccharides extracted with hot acidified water. Such a decrease in DA was also reported by Garna et al. (2007) and it was probably due to the hydrolysis of acetyl groups from the GalA during the extraction step with acidified water. Levigne et al. (2002) evidenced the more marked effect of pH on DA compared to extraction time and temperature. Furthermore, the use of water as an extractant did not have a significant effect on DA values of extracted pectins even at 95°C (Chan and Choo, 2013).

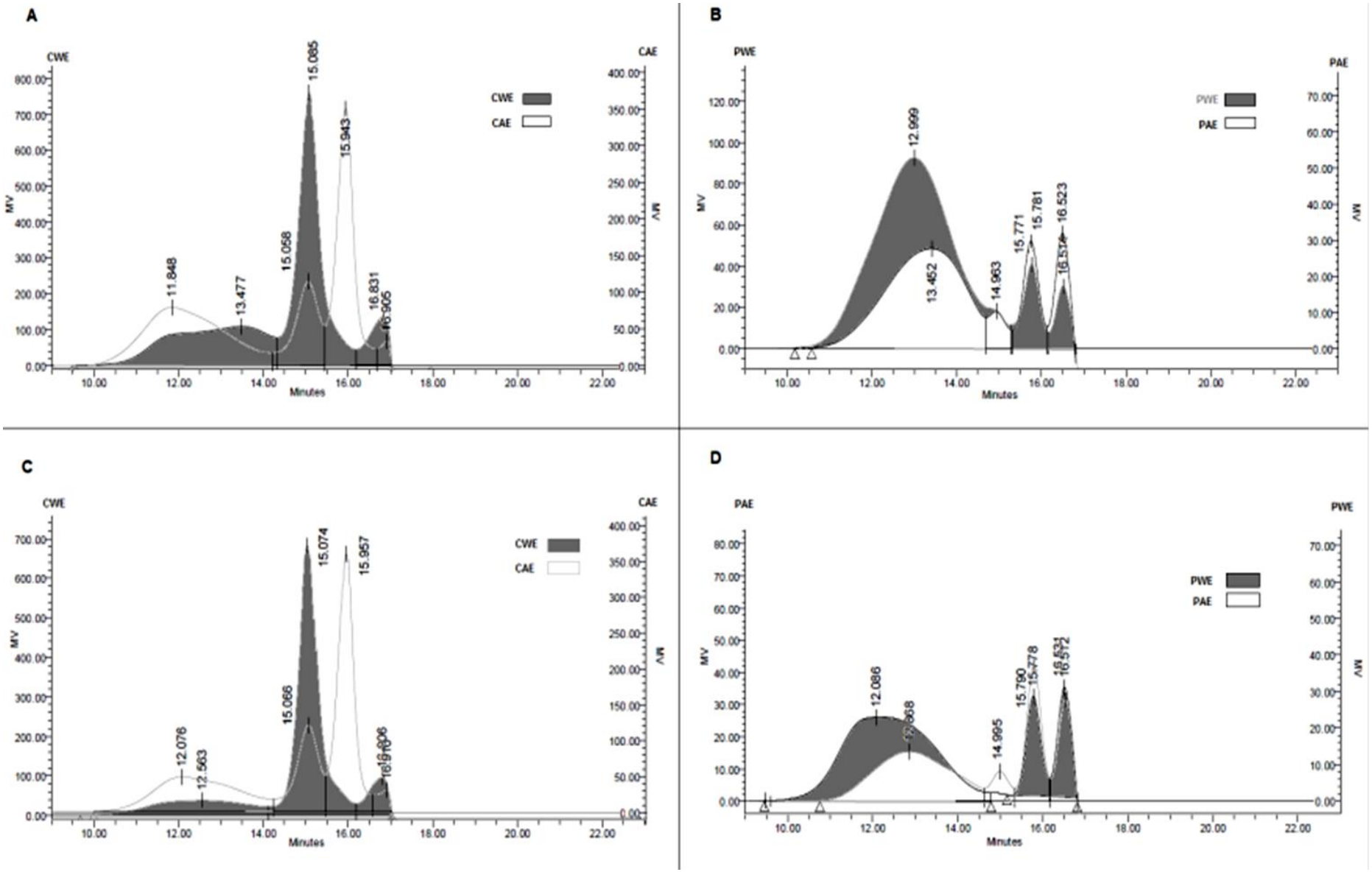

Figure 1. Overlay of HPSEC chromatograms of crude (A and C) and precipitated (B and D) extracts of residual biomass from of *H. sabdariffa* calyces. Cultivars studied were Vimto (top) and Koor (bottom). Precipitated water extracts (PWE) and crude water extracts (CWE) in grey, precipitated acid extracts (PAE) and crudes acid extracts (CAE) in white.

Table 3. HPSEC analysis of crude extracts of *H. sabdariffa* calyces residues: apparent MW and relative abundance of polysaccharides.

	Cultivar	MW (Da)	Percentage of total area* (%)
CWE	Vimto	12.429	34.1
		731	57.0
	Koor	62.226	18.5
		745	72.1
CAE	Vimto	219.377	41.8
		766	17.3
		161	38.8
	Koor	146.789	30.6
		756	20.8
		157	45.6

*calculation based on the total HPSEC determined surface. Results are expressed as average of two determinations; Vimto and koor are the local names of two *H. sabdariffa* cultivars.

Table 4. Molecular Weight distribution and main characteristics of polysaccharides from extracts of two cultivars of *H. sabdariffa* calyces.

Cultivar	Fraction	Major polysaccharide	
		MW (Da)	Percentage of total area (%)
Vimto	PWE	28.500	86.3
Koor		109.700	72.1
Vimto	PAE	13.300	66.2
Koor		50.300	49.3

Vimto and koor are the local names of two H. *sabdariffa* cultivars – PWE and PAE are pectins obtained by ethanol precipitation of water extracts (PWE) and acidified water extracts (PAE). Results are expressed as average of two determinations.

Table 5. Biochemical properties of pectin extracts precipitated with ethanol after aqueous extraction (PWE) or after acidified water extraction (PAE).

Cultivar	Fraction	Biochemical properties		
		DM (%)	DA (%)	GalA (%)
Vimto	PWE	70.6±27.4	19.0±0.02	7.4±0.98
Koor		44.3±28.1	4.9±0.15	10.7±0.59
Vimto	PAE	41.8±10.2	3.2±1.33	11.2±0.34
Koor		13.0±8.5	0.0±0	6.6±0.23

DM (Degree of methylation): % moles methanol/moles Galacturonic acid (GalA) - DA (Degree of acetylation): % moles acetic acid/moles of galacturonic acid. Vimto and koor are the local names of two *H. sabdariffa* cultivars. Results are expressed as average of 3 determinations ± SD.

GalA content determined by HPAEC-PAD after enzymatic hydrolysis of PWE and PAE extracts ranged from 6.6 to 11.2% (w/w) of soluble extract (Table 5). The pectins extracted with acidified water contained more GalA than pectins extracted with water for both cultivars. This confirms the findings by Yapo et al. (2007) who showed that the extraction pH was the parameter that mostly influenced GalA content. Previous studies by Muller and Franz (1992) on chemical structure of *H. sabdariffa* polysaccharides extracted with water at room temperature, revealed the presence of two neutral polysaccharides and a pectin-like polysaccharide (84% GalA) whose average *MM* were respectively 20.0, 6.3 and 100.0 kDa. The main characteristics of polysaccharides contained in CR are shown in Table 5. According to American Chemical Society classification, these are considered pectinic acids (Marshall and Joseph, 1986). In the present work, the more soluble polysaccharides were presumably extracted during the aqueous extraction procedure (90 min at 70°C) previously used on *H. sabdariffa* calyces to produce CR samples.

Table 6. Composition of Lignocellulosic material (LCM) from residual biomass of *H. sabdariffa* calyces.

Cultivar	Glucose* (%)	Xylose* (%)	Lignin (%)
Vimto	29.7±3.7	9.5±0.7	15.8±2.4
Koor	25.3±1.9	9.3±0.7	16.2±4.8

Results are expressed as average of 4 determinations ± standard deviation and on a dry mass bases as percentage of total LCM. Vimto and koor are the local names of two *H. sabdariffa* cultivars. *Expressed as polymeric form.

Table 7. Biodegradation of Lignocellulosic Material (LCM) from residual biomass of H. sabdariffa calyces cultivars.

Cultivar	Glucose		Xylose	
	LCM Bioavailability* (%)	Recovery** (%)	LCM Bioavailability* (%)	Recovery** (%)
Vimto	15.0±0.8	50.8±4.0	3.0±0.1	31.5±1.5
Koor	13.8±1.8	54.4±7.5	3.4±0.5	37.5±8.6

Results are expressed as average of 4 determinations ± sd. Vimto and koor are the local names of two *H. sabdariffa* cultivars.*ratio (w/w) of enzymatic glucose or xylose to total LCM dry matter. **Ratio (w/w) of enzymatic glucose or xylose to total glucose or xylose content.

Lignocellulosic residues from *H. sabdariffa* calyces

Potential as bioenergy resource

After the previously described pectin extraction procedure, 40 to 45% of CR mass was extracted as pectic material. The remaining insoluble plant material, representing 55 to 60% of CR dry mass, was characterized by determination of total glucose, xylose and lignin content. Results summarized in Table 6 show that the total amount of glucose and xylose under polymeric form represented 34.6 and 39.2% (w/w) of koor and vimto cultivars dry LCM, respectively. These results were used to estimate the potential of *H. sabdariffa* residual biomass as feedstock for biofuels production. Considering total glucose content, a potential of 25.3 to 29.7% of *H. sabdariffa* LCM dry mass could be enzymatically hydrolyzed then fermented to ethanol by *Saccharomyces cerevisiae*. Based on these results and on the assumption that 100 tons of *H. sabdariffa* calyces are processed annually by the same plant, considering the theoretical ethanol yield of 0.51 kg ethanol/kg glucose (Drapcho et al., 2008b), the amount of ethanol that could be produced is estimated at 4500 L. This amount is in our opinion too small to justify the investment for a bioethanol production plant that are profitable only for production capacities of several millions liters of ethanol (Hamelinck et al., 2005; Drapcho et al. 2008b). To further characterize *H. sabdariffa* LCM a biodegradation test was performed. The amount of monosaccharides released by a containing cellulase and hemicellulase was determined in order to evaluate the biodegradability of the waste material (Table 7). The fraction of material degraded to glucose represented 15.0 and 13.8% (w/w) of total LCM dry mass, whereas the amount of xylose was 3.0 and 3.4% for vimto and koor cultivars, respectively. These results suggest that a further degradation was hindered by intrinsic characteristics of *H. sabdariffa* LCM. Such recalcitrance was described in literature as being directly proportional to lignin content (Fang and Dixon, 2007). This issue is usually overcome with a pretreatment preceding the enzymatic treatment designed to increase the depolymerization yield and to minimize the amount of enzyme used (Öhgren et al., 2007; Mussatto et al., 2008). Lignin content that varies with the plant species was estimated at 16.0% of LCM dry mass for both *H. sabdariffa* cultivars.

Results of the biodegradability test showed that *H. sabdariffa* LCM could also be used as feedstock for biomethane production by anaerobic fermentation. Indeed, biodegradability is correlated to the amount of biomethane produced as documented by various authors (Rodriguez et al. 2005; Fang and Dixon, 2007; Khalid et al., 2011; Monlau et al., 2012). It is generally assumed that vegetable wastes 1 kg of organic wastes could generate 1.0 m^3 of biogas that contains 6.5 kWh of energy (Drapcho et al., 2008a). Based on these assumptions we calculated that 195 MWh of energy could be theoretically produced each year by the biomethanation of LCM from 100 t of *H. sabdariffa* calyces. However, the biodegradation test showed that only 50.8% of glucose and 31.5% of xylose under polymeric form was degraded to fermentable monosaccharides (Table 7). The results show a lower

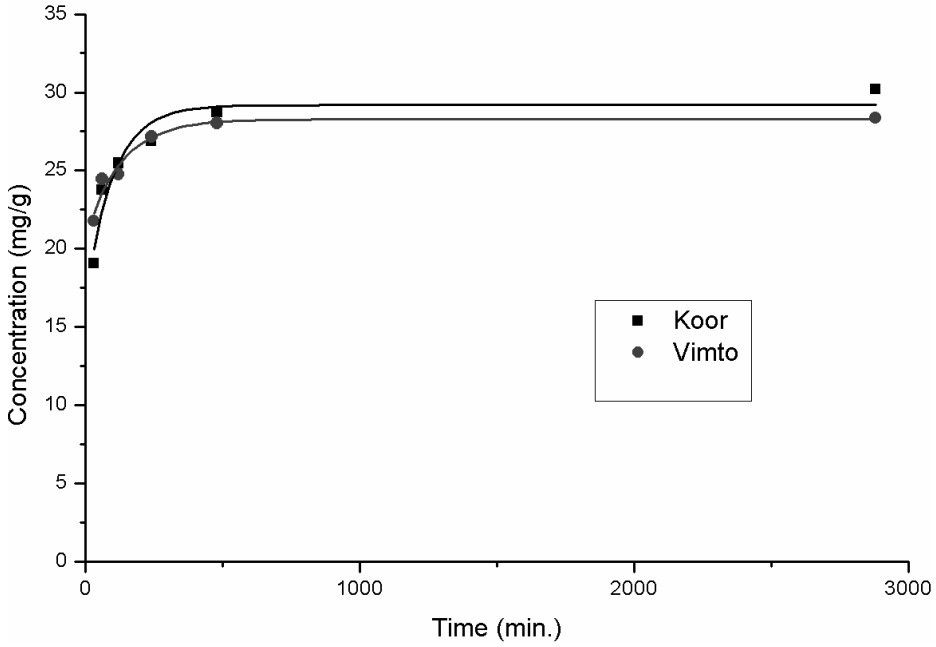

Figure 2. Time dependence of xylose production by enzymatic hydrolysis of lignocellulosic material (LCM) from residual biomass of *H. sabdariffa* calyces. Cultivars studied were Vimto and Koor (Concentration in mg xylose/ g LCM).

biodegradation of hemicellulose that has been described elsewhere as being caused by its more complex structure. The energy recovered from the biomethanation could be reinjected in the production system and help reduce the environmental impact of the processing plant. Unlike bioethanol production, biomethanation plants that allow the processing of small amounts of wastes in a profitable way are available (Cheng et al., 2014; Surendra et al., 2014). The removal of lignin should be considered in order to exploit the full potential of these residues (Adl et al., 2012; Shafiei et al., 2013).

Potential as a source of xylooligosaccharides

Alternatively, *H. sabdariffa* LCM could be used for the production of xylooligosaccharides (XOS). These oligosaccharides mainly made of xylose are characterized by their molecular size, defined by the degree of polymerization (DP) which represents the number of monosaccharide residues (2 to 10). *H. sabdariffa* LCM enzymatic hydrolysis was monitored by determination of the types and amounts of XOS generated at increasing time periods. The analysis revealed that xylose and mainly oligosaccharides with DP3 and DP4 were produced during the enzymatic hydrolysis of *H. sabdariffa* LCM. Figure 2 shows the kinetics of xylose production: *H. sabdariffa* LCM is rapidly degraded into xylose that reached 20 mg/g (dry mass) after 30 min and a maximum of 30 mg/g that remained constant after 4 h. DP3 and DP4 were detected at much lower concentrations but with production kinetics similar to xylose, as illustrated by Figure 3 for Vimto cultivar (shown for illustration). DP3 reached a maximum concentration of 2.0 mg/g (dry mass bases) and DP4 a maximum of 5.2 mg/g after 48 h. The overall XOS production was low throughout the reaction. It was due to the fact that the degradation of LCM into monosaccharides was favored, indicating that the commercial enzyme mixture contained a high exoxylanase activity. The enzymes hydrolyzed (1,3)- and (1,4)-linkages in β-D-glucans and also had enzymatic side-activities such as cellulase, hemicellulase and xylanase. The types and amounts of oligosaccharides generated by LCM enzymatic hydrolysis are dependent on the substrates and enzymes employed (Akpinar et al., 2009), therefore, other available enzymes should be tested to improve the production of monosaccharide-free XOS. An additional step could also be considered, where an extraction of hemicellulose using alkali (sodium or potassium hydroxide) would precede the enzymatic treatment in order to increase hemicellulose recovery (Akpinar et al., 2009; Samanta et al., 2012).

Conclusion

The work presented here studied the various fractions extracted from the processing byproducts from calyces of two *H. sabdariffa* cultivars. Besides, pectins, the total

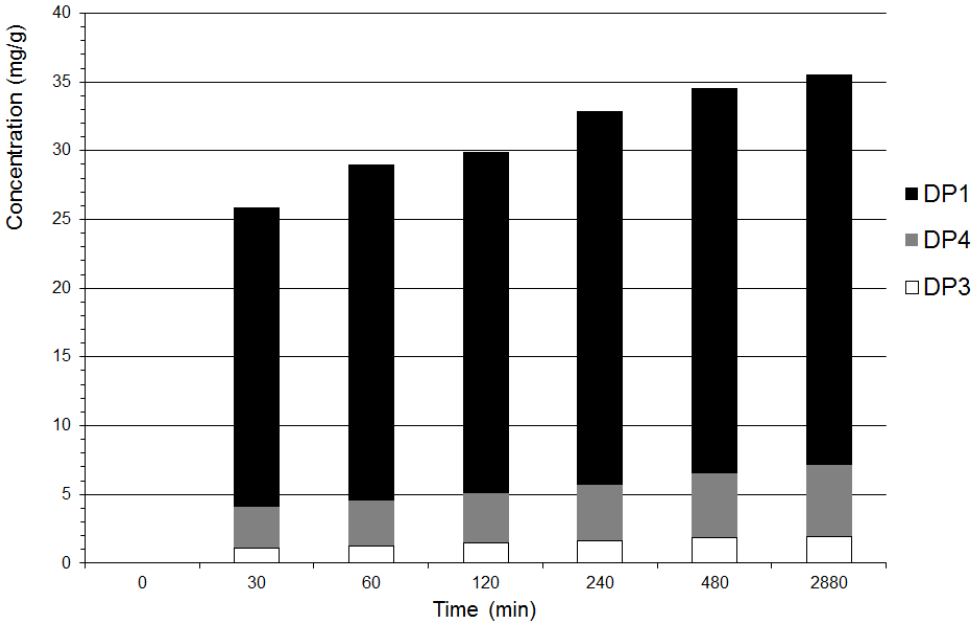

Figure 3. Time-dependence of xylooligosaccharides production by enzymatic degradation of lignocellulosic material (LCM) from residual biomass of Vimto calyces (Concentration in mg xylose/ g LCM).

content of monosaccharides and lignin of the pectin-free biomass were evaluated. This residue was then treated by enzymes and the release of mono- and oligosaccharides was followed. The whole constitutes an original approach for a possible integrated valorization of this agro-waste. More precisely, extraction of polysaccharides using a procedure similar to industrial extraction of pectin from apple pomace was performed. The two cultivars appeared to originate different types of pectins in terms of *MM* and degree of esterification. Considering the amounts extracted (23%, dry mass basis), this byproduct has the potential to be used as a low price feedstock for pectin production. Further studies should be made for a complete characterization of these polysaccharides including a study of their functional properties. LCM represented over 50% of the byproduct studied, 39% of which were made of xylose and glucose. This makes it a potentially highly biodegradable material that could be used to make biogas to inject in the processing plant. However, its enzymatic degradation was limited (50.8 to 54.4% of total glucose) presumably by the high lignin content (16% w/w of LCM dry mass). Otherwise, enzymatic production of xylooligosaccharides with DP3 and DP4 as neutraceuticals would be an alternative to bring added value to the residual biomass from *H. sabdariffa* calyces.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Aachary AA, Prapulla SG (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Compr. Rev. Food Sci. Food Saf. 10(1):2-16.

Adl M, Sheng K, Gharibi A (2012). Technical assessment of bioenergy recovery from cotton stalks through anaerobic digestion process and the effects of inexpensive pre-treatments. Appl. Energy 93: 251-260.

Akpinar O, Erdogan K, Bostanci S (2009). Enzymatic production of Xylooligosaccharide from selected agricultural wastes. Food Bioprod. Process. 87(2):145-151.

Badreldin HA, Naser Al W, Gerald B (2005). Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. Phytother. Res. 19(5): 369-375.

Brunold C, Deters A, Knoepfel-Sidler F, Hafner J, Müller B, Hensel A (2004). Polysaccharides from Hibiscus sabdariffa Flowers Stimulate Proliferation and Differentiation of Human Keratinocytes. Planta Med. 70(4):370-373.

Chan S-Y, Choo W-S (2013). Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. Food Chem. 141(4):3752-3758.

Cheng S, Li Z, Mang H-P, Huba E-M, Gao R, Wang X (2014). Development and application of prefabricated biogas digesters in developing countries. Renew. Sustain. Energy Rev. 34:387-400.

Cissé M, Dornier M, Sakho M, Diop CM, Reynes M, Sock O (2008). La production du bissap (*Hibiscus sabdariffa* L.) au Sénégal. Fruits 64(2):111-124.
Combo AMM, Aguedo M, Quiévy N, Danthine S, Goffin D, Jacquet N, Blecker C, Devaux J, Paquot M (2013). Characterization of sugar beet pectic-derived oligosaccharides obtained by enzymatic hydrolysis. Int. J. Biol. Macromol. 52:148-156.
Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M (2014). *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food Chem. 165: 424-443.
Drapcho CM, Nghiem NP, Walker T (2008a). Methane. Biofuels Engineering Process Technology. McGraw Hill Professional, New York, USA.
Drapcho CM, Nhuan NP, Walker T (2008b). Ethanol Production. Biofuels Engineering Process Technology. McGraw Hill Professional, New York, USA.
Fang C, Dixon RA (2007). Lignin modification improves fermentable sugar yields for biofuel production. Nat. Biotechnol. 25(7):759-761.
Faruk O, Bledzki AK, Fink H-P, Sain M (2012). Biocomposites reinforced with natural fibers: 2000–2010. Prog. Polym. Sci. 37(11):1552-1596.
Finegold SM, Li Z, Summanen PH, Downes J, Thames G, Corbett K, Dowd S, Krak M, Heber D (2014). Xylooligosaccharide increases bifidobacteria but not lactobacilli in human gut microbiota. Food Funct. 5(3): 436-445.
Fissore EN, Rojas AM, Gerschenson LN, Williams PA (2013). Butternut and beetroot pectins: Characterization and functional properties. Food Hydrocoll. 31(2): 172-182.
Garna H, Mabon N, Robert C, Cornet C, Nott K, Legros H, Wathelet B, Paquot M (2007). Effect of Extraction Conditions on the Yield and Purity of Apple Pomace Pectin Precipitated but Not Washed by Alcohol. J. Food Sci. 72(1):C001-C009.
Giusti MM, Wrolstad RE (2003). Acylated anthocyanins from edible sources and their applications in food systems. Biochem. Eng. J. 14(3):217-225.
Hamelinck CN, Hooijdonk Gv, Faaij APC (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass Bioenergy 28(4):384-410.
Happi Emaga T, Ronkart SN, Robert C, Wathelet B, Paquot M (2008). Characterisation of pectins extracted from banana peels (Musa AAA) under different conditions using an experimental design. Food Chem. 108(2):463-471.
Kalapathy U, Proctor A (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. Food Chem. 73(4): 393-396.
Khalid A, Arshad M, Anjum M, Mahmood T, Dawson L (2011). The anaerobic digestion of solid organic waste. Waste Manage. 31(8):1737-1744.
Koubala BB, Kansci G, Mbome LI, Crépeau MJ, Thibault JF, Ralet MC (2008). Effect of extraction conditions on some physicochemical characteristics of pectins from “Améliorée” and “Mango” mango peels. Food Hydrocoll. 22(7):1345-1351.
Lama-Muñoz A, Rodríguez-Gutiérrez G, Rubio-Senent F, Fernández-Bolaños J (2012). Production, characterization and isolation of neutral and pectic oligosaccharides with low molecular weights from olive by-products thermally treated. Food Hydrocoll. 28(1):92-104.
Levigne S, Ralet M-C, Thibault J-F (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. Carbohydr. Polym. 49(2):145-153.
Marshall LF, Joseph JJ (1986). Chemistry and Function of Pectins, American Chemical Society.
Menon V, Rao M (2012). Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. Prog. Energy Combust. Sci. 38(4):522-550.
Monlau F, Sambusiti C, Barakat A, Guo XM, Latrille E, Trably E, Steyer J-P, Carrere H (2012). Predictive Models of Biohydrogen and Biomethane Production Based on the Compositional and Structural Features of Lignocellulosic Materials. Environ. Sci. Technol. 46(21):12217-12225.
Moure A, Gullón P, Domínguez H, Parajó JC (2006). Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. Process Biochem. 41(9):1913-1923.
Muller BM, Franz G (1992). Chemical structure and biological activity of polysaccharides from Hibiscus sabdariffa. Planta Med. 58(1): 60-67.
Mussatto SI, Fernandes M, Milagres AMF, Roberto IC (2008). Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. Enzyme Microb. Technol. 43(2):124-129.
Öhgren K, Bura R, Saddler J, Zacchi G (2007). Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. Bioresour. Technol. 98(13): 2503-2510.
Patel S (2014). *Hibiscus sabdariffa*: An ideal yet under-exploited candidate for nutraceutical applications. Biomed. Prev. Nutr. 4(1):23-27.
Pouget MP, Vennat B, Lejeune B, Pourrat A (1990). Identification of anthocyanins of *Hibiscus sabdariffa, L*. Lebensmittel Wissenschaft und Technologie 23(2):101-102.
Rodriguez C, Hiligsmann S, Ongena M, Charlier R, Thonart P (2005). Development of an enzymatic assay for the determination of cellulose bioavailability in municipal solid waste. Biodegradation 16(5):415-422.
Samanta AK, Jayapal N, Kolte AP, Senani S, Sridhar M, Suresh KP, Sampath KT (2012). Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (Sehima nervosum). Bioresour. Technol. 112:199-205.
Satyanarayana KG, Arizaga GGC, Wypych F (2009). Biodegradable composites based on lignocellulosic fibers—An overview. Prog. Polym. Sci. 34(9):982-1021.
Shafiei M, Kabir MM, Zilouei H, Sárvári Horváth I, Karimi K (2013). Techno-economical study of biogas production improved by steam explosion pretreatment. Bioresour. Technol. 148: 53-60.
Sila DN, Van Buggenhout S, Duvetter T, Fraeye I, De Roeck A, Van Loey A, Hendrickx M (2009). Pectins in Processed Fruits and Vegetables: Part II—Structure–Function Relationships. Compr. Rev. Food Sci. Food Saf. 8(2):86-104.
Singh RD, Banerjee J, Arora A (2015). Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. Bioactive Carbohydr. Dietary Fibre 5(1):19-30.
Smith J, Hong-Shum L (2011). Food Additives Data Book, Wiley.
Surendra KC, Takara D, Hashimoto AG, Khanal SK (2014). Biogas as a sustainable energy source for developing countries: Opportunities and challenges. Renew. Sustain. Energy Rev. 31: 846-859.
Taylor B (2007). Fruit and juice processing Chemistry and Technology of Soft Drinks and Fruit Juices. Blackwell Publishing Ltd.
Tsai P-J, McIntosh J, Pearce P, Camden B, Jordan BR (2002). Anthocyanin and antioxidant capacity in Roselle (Hibiscus Sabdariffa L.) extract. Food Res. Int. 35(4): 351-356.
Uçkun Kiran E, Trzcinski AP, Ng WJ, Liu Y (2014). Bioconversion of food waste to energy: A review. Fuel 134: 389-399.
Yapo BM (2009). Biochemical Characteristics and Gelling Capacity of Pectin from Yellow Passion Fruit Rind as Affected by Acid Extractant Nature. J. Agric. Food Chem. 57(4):1572-1578.
Yapo BM, Robert C, Etienne I, Wathelet B, Paquot M (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chem. 100(4):1356-1364.