Carbohydrate Research, 207 (1990) 126–130
Elsevier Science Publishers B.V., Amsterdam

Note

Steam pretreatment of almond shells for xylose production

Juana Pou-Ilinas, Jaime Canellas,
Departemento de Ingenieria Química, Universitat de les Illes Baleares, E-07071 Palma de Mallorca (Spain)
Hughes Driguez, Gérard Excoffier, and Michel R. Vignon.
Centre de Recherches sur les Macromolécules Végétales, CNRS, B.P. 53 X, F-38041 Grenoble (France)

(Received November 22nd, 1989; accepted for publication, January 17th, 1990)

The world production of almonds is around 10^6 tons per year. In Spain alone, up to 3×10^5 tons are produced per year, and 2×10^5 tons of almond shells (endocarp) are burnt[1]. The almond shells have been reported to contain up to 60–70% of their weight as carbohydrates (cellulose and hemicelluloses) which constitute a potential source of low-molecular-weight sugars[2]. The almond shells need to be treated by physical or chemical methods prior to the saccharification of hemicelluloses and cellulose. Steam explosion is a pretreatment which combines physical and chemical methods, and allows fractionation of lignocellulosic materials into its three major components: hemicelluloses (water-soluble), lignin (soluble in organic solvent or aqueous sodium hydroxide), and cellulose (insoluble)[3].

During steaming of the shells at high temperature, some acetyl groups present in the hemicelluloses are hydrolyzed, and the resulting acetic acid decreases the pH of the steam, and catalyzes the hydrolysis of the hemicelluloses[4]. When the hemicelluloses are mainly composed of xylan, the steam explosion pretreatment yields xylose and water-soluble xylooligosaccharides. We report, herein, the use of steam explosion as a pretreatment method of dry almond shells from the Balearic Islands for D-xylose production. This treatment was compared to conventional acid hydrolysis.

The chemical composition of almond shells (endocarp) from Balearic Islands used in this work is presented in Table I. These data are slightly different from those of

TABLE I

Properties and chemical composition of the endocarp of the almond shell[a].

Fiber neutral detergent index	*Fiber acid detergent index*	*Content (% of dry material)* *Hemicelluloses*	*Cellulose*	*Lignin*
91	62	29	34	28

[a] These results are the average of five measurements.

0008-6215/90/$ 03.50

Sinner *et al*[2], which could be explained by the different origins of the starting materials. In a study of the acid hydrolysis, the effect of sulfuric acid concentration and of reaction time were examined. The best yield (22% of reducing sugars) was obtained by use of a 3% sulfuric acid solution at 121° during 30 min. Under these conditions, the sugar fraction analyzed by h.p.l.c. showed that xylose was the major constituent with arabinose, glucose, galactose, and rhamnose as minor constituents.

In the study of the steam-explosion treatment, the influence of temperature and time parameters were examined. The experiments were carried out between 224 and 250° for two different steaming times (60 and 135 s). The results showed that temperature and steaming time have a significant effect on the yield of hemicellulose recovery. The best yields (22–24% of water-soluble hemicelluloses) were obtained at 227° for 135 s (Table II, *a*) or at 250° for 60 s (Table II, *b*). In order to improve the fractionation, the almond shells were first impregnated with a 0.1% H_2SO_4 solution. The results reported in Table II showed that the sulfuric acid-impregnated almond shells gave the best result for the water extract (28.5%) by steaming for 135 s at 227° (Table II, *c*). When the steam treatment was performed for 60 s at 227° (Table II, *d*), the proportion of water-soluble hemicelluloses obtained was similar to that obtained from nonimpregnated materials, but the proportion of 1,4-dioxane–water-soluble lignin was very low (9%), probably owing to the incomplete depolymerization of the lignin under these conditions.

The gel-permeation chromatograms of the water-soluble hemicelluloses showed (Fig. 1) a wide distribution of the molecular weights, from monosaccharides up to excluded oligosaccharides, for a treatment for 135 s at 227° (*a*) and for 60 s at 250° (*b*); however, for the 135-s treatment (*c*), the monomers were the main components and there were only low-molecular-weight xylooligosaccharides along with a small proportion of excluded acidic or acetylated oligosaccharides. After soaking the shells with 0.05M H_2SO_4, treatment for 60 s at 227° (*d*) still produced a large proportion of excluded oligosaccharides (d.p. > 12).

The optimization of D-xylose production was carried out on the water-soluble extracts (hemicelluloses). The results of acidic and enzymic saccharification after steam

TABLE II

Optimal conditions for the steam treatment of almond shells[a]

Exp.	*Conditions*		*Components* (%)			
	Temp. of steaming (degrees)	*Time of treatment (s)*	*Cellulose*	*Hemicelluloses*	*Lignin*	*Total*
(*a*)	227	135	46.0	22.0	19.5	87.5
(*b*)	250	60	40.5	23.5	25.0	89.0
(*c*)	227[b]	135	41.0	28.5	21.5	91.0
(*d*)	227[b]	60	63.5	22.5	9.0	95.0

[a] Average values of three different experiments. [b] These samples were soaked with 0.05M H_2SO_4 for 4 h before steaming.

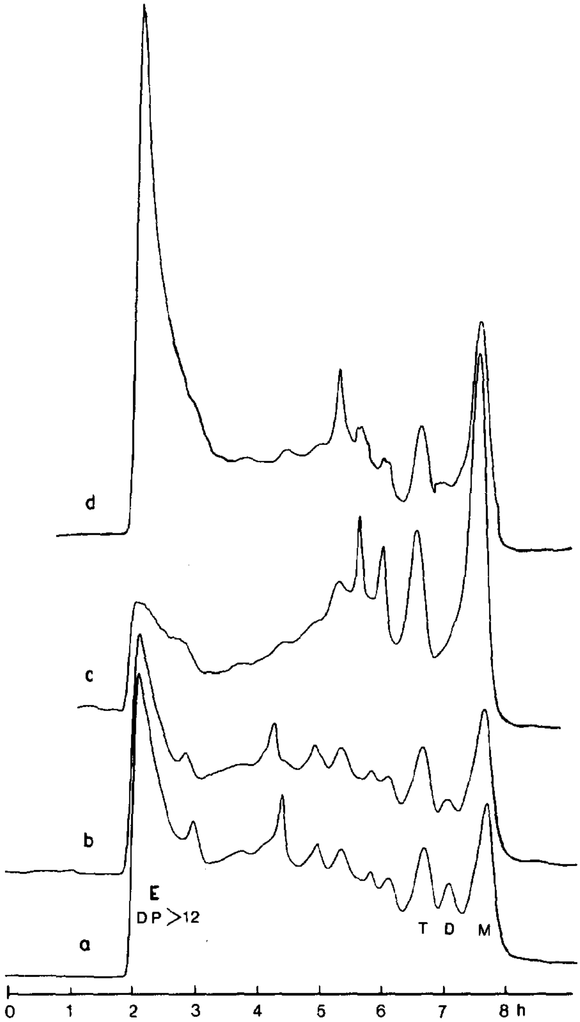

Fig. 1. Gel-permeation chromatograms of water-soluble hemicelluloses on a Bio-Gel P-2 column as described in the Experimental section: (M) monosaccharides, (D) disaccharides, (T) trisaccharides, and (E) acidic oligosaccharides or excluded oligosaccharides with d.p. > 12. Treatment without impregnation: (a): 227°, 135 s, and (b) 250°, 60 s. Treatment after soaking for 4 h with 0.1% H_2SO_4, (c) 227°, 135 s, and (d) 227°, 60 s.

explosion are shown in Table III. Unexpectedly, very low yields of xylose (4%) were obtained after enzymic hydrolysis. This could arise because of the presence of remaining acetyl or 4-*O*-methyl-D-glucuronic acid groups (or both) along the xylan chain, which hinder the enzymic hydrolysis. By use of acid hydrolysis, as described in the Experimental section, up to 16.5% of xylose was obtained.

In conclusion, the effectiveness of steam explosion in terms of hemicellulose solubilization and xylose production was determined. The best yield (30% of dry

TABLE III

Yields of D-xylose from dry almond shells obtained after various treatments[a]

Exp.	*Temp. of steaming (degrees)*	*Time of treatment (s)*	*Yield (%)*	
			Enzymic hydrolysis	*Acid hydrolysis*
(*b*)	250	60	4.0	14.0
(*c*)	227[b]	135	5.0	16.5
(*d*)	227[b]	60	4.0	12.5

[a] Average values of three different experiments. [b] These samples were soaked with 0.01% H_2SO_4 for 4 h before steaming.

matter) was obtained by soaking the material during 4 h with 0.01% sulfuric acid and steaming it for 135 s at 227°. Enzymic or acid hydrolysis after this treatment led tot 5 or 16.5% of xylose, respectively. This method, which combines sulfuric acid impregnation and steam explosion, followed by a post acid hydrolysis is superior to the classical acid treatment for the production of xylose from almond shells (10%) yield. Thus, xylose was isolated with about the same efficiency from white birch wood and from almond shells, an agricultural waste from Southern European countries.

EXPERIMENTAL

Materials. — A mixture of several varieties of almond shells (endocarp) from the Balearic Islands were used. This material had been broken into small pieces (1–2 cm) during industrial processing to remove the almond seed. The chemical composition was determined on almond shells which were ground in a Willey mill and screened (51 mesh), whereas the acid hydrolysis was carried out on almond shells ground and screened (5–25 mesh). Commercial cellulase preparations were a gift from Novo/NordiskA/S.

Analytical methods. — Reducing sugar content was measured by the 3,5-dinitrosalicylic acid method[5] with D-xylose used as a standard. The fiber neutral detergent (FND) and the fiber acid detergent (FAD) indeces were measured according to the methods of Van Soest and assoc.[6,7]. Gel-permeation chromatography (g.p.c.) of the hemicelluloses fraction was performed on poly(acrylamide)gel (Bio-Gel P-2 from Bio-Rad Laboratories, 200–400 mesh). The column was heated at 50° and the refractive index measured with a differential refractometer (Waters R 401 or 403). Excluded oligosaccharides (E) and monomers (M) were eluted within 2 and 8 h, respectively. H.p.l.c. was performed with a Waters 6000A μ-Bondapak-NH_2 column eluted with acetonitrile–water. Xylose was quantitatively determined by integration of the peak which was compared to a standard. In one experiment, the eluate was collected and the sugar was identified as xylose by ^{13}C-n.m.r. spectroscopy and mass spectrometry.

Acid hydrolysis. — Dry almond shells (10 g, 5–25 mesh) were hydrolyzed with H_2SO_4 solutions (10 mL) at different concentrations (0.5–3%) at 121° during 15–90 min. The acid was neutralized with $CaCO_3$ and the suspension filtered. The reducing sugars were determined by the 3,5-dinitrosalicylic acid method and the sugar fractions characterized by h.p.l.c. or g.p.c. analysis.

Steam treatment. — All the steam-explosion experiments were performed with 100 g of dry almond shells in a 1-L reactor especially built in our laboratory* in order to study the mechanism of hydrolysis and its optimization. The principle of operation and the description of the Pilot unit EC 300 are described in a leaflet directly available from Deltalab. In some experiments prior to steam explosion, the almond shells were soaked in 0.01% H_2SO_4 for 4 h under diminished pressure.

Fractionation. — The fractionation was performed as previously described[8]. Water extraction of the exploded material (3 times, 500 mL), at room temperature, solubilized the oligosaccharides and low-molecular-weight phenols. The phenols were removed by the ethyl acetate extraction of the aqueous solution. The insoluble material remaining after the water extraction was extracted continuously in a Soxhlet apparatus with a 1,4-dioxan–water azeotrope for 12 h in order to remove the depolymerized lignin from the cellulosic residue. Four fractions were obtained.

Enzymic and acidic saccharification. — After the steam explosion, each water extract (20 mg) was incubated with 500 μg of enzymes (250 μg of Celluclast™ and 250 μg of Novozyme™) at 50° during 24, 48, 72, and 96 h in a shaking water-bath. A drop of toluene was added as a microbial growth-inhibitor. The acid treatment was carried out on water extracts obtained after steam explosion (20 mg each) with 3% H_2SO_4 (1 mL) during 30 min at 121°. The acid was then neutralized with $CaCO_3$ and the suspension filtered. In both treatments, the reducing sugars and the sugar fractions were measured as previously described.

ACKNOWLEDGMENT

The authors acknowledge the CNRS for an Action Initative Europe grant for the partial support of this research.

REFERENCES

1 F. Saura-Calixto, J. Canellas, and L. Soler, in *La Almendra*, Instituto Nacional de Investigationes Agrarias, Ministerio de Agricultura (Ed.), 1988, pp. 21–23.

2 M. Sinner, J. Puls, and H. Dietrichs, *Staerke*, 31 (1979) 267–269.

3 R. F. H. Dekker and A. F. A. Wallis, *Biotechnol. Lett.*, 5 (1983) 311–316.

4 K. Sudo, K. Shimizu, T. Ishii, T. Fujii, and S. Nagasawa, *Holzforschung*, 40 (1986) 339–345.

5 G. L. Miller, *Anal. Chem.*, 31 (1959) 426–428.

6 P. J. Van Soest and R. H. Wine, *J. Assoc. Off. Agri. Chem.*, 50 (1976) 50–53.

7 P. J. Van Soest and J. B. Robertson, *Proc. Stand. Anal. Methodol. Feeds, Ottawa*, (1979) pp. 49–60.

8 G. Excoffier, M. Rinaudo, and M. R. Vignon, *Proc. Intl. Dissol Pulp Conf., Geneva*, (1987), pp. 53–56.

---

* This flash-hydrolysis, laboratory-pilot unit is entirely automated and is produced commercially by Deltalab, F-38340 Voreppe, France.