Steam-Explosion Pretreatment of Olive Cake

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ABSTRACT: Olive cake was processed by steam-explosion under different steam conditions, followed by fractionation to separate the main components. In the water-soluble fraction, the main compounds were carbohydrates. Glucose represented a significant part of the total monosaccharide content, especially under conditions of mild severity, followed by arabinose, but the solubilization of sugars occurred predominantly in the oligomeric fraction. Mannitol was also found in significant amounts (1.5%), similar to that in the initial material. In the ethyl acetate extract, low molecular weight phenols were identified, the most abundant being hydroxytyrosol, which is present in the olive pulp. Hydroxytyrosol is abundant and has great antioxidant activity, reaching 149 mg/100 g of dry olive cake. The procedure used in this study obtained all the hydroxytyrosol residual present in the by-product. The constitutive polymers were quantified in the insoluble fraction, and the sugar composition showed that cellulose was associated with a high proportion of xylans and other polysaccharides rich in arabinose and galactose. This cellulose was nearly amorphous, as it was highly susceptible to hydrolytic enzymes. The extractables in dilute alkali (not true lignins) increased as steaming became more severe; the residual "lignin" in this fraction decreased. Enzymatic hydrolysis of the insoluble fraction using a cellulolytic complex was also studied. The slight increase in the extent of saccharification was not proportional to the high alkaline delignification. However, when the residues were efficiently delignified with chlorite treatment, the susceptibility to enzymatic hydrolysis greatly increased.

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KEY WORDS: By-product, cellulose, olive cake, phenols, saccharification, steam-explosion, sugars.

Olive cake is a by-product derived from the olive oil extraction industry. It is obtained by pressing the fruit, leaving a residue of seed husks (fragmented olive stones), seed, pulp and peel (olive cake). Olive-mill wastewater and/or vegetation water is also obtained.

Olive cake has been utilized as an energy source, fertilizer, and animal feed (1). In spite of its high fiber and protein contents, this by-product has low nutritional value owing to phenolic compounds inhibiting digestive enzymes. During oil extraction, polymers are formed among the phenolic substances themselves and/or protein and cellulose so that they are unavailable for ruminant digestion. Consequently, these polymers constitute an integral part of the cell wall component and show physical and chemical properties very similar to lignin (2). By way of certain pretreatments, it is possible to reduce the degree of lignification of the olive cake, thus enhancing its nutritional value.

High-pressure steaming followed by rapid decompression is called steam-explosion. Recently, steam-explosion has been considered an effective pretreament (3) of waste cellulosic materials for further processing, including olive stones (4,5). The resultant material is finely divided; and the main components—cellulose, hemicelluloses, and lignin—are separated. Furthermore, the enzymatic hydrolysis of cellulose is enhanced, which could create alternative uses for the olive cake.

The aim of this study was to apply steam-explosion to olive cake under various conditions of severity to enhance the effective utilization of such by-products. The solubilization of the carbohydrates and the isolation of different organic soluble substances, as well as the effect of additional treatments on the enzymatic saccharification of steam-exploded olive cake, were also determined.

EXPERIMENTAL PROCEDURES

Materials. Nondefatted and destoned olive cake was supplied as pellets (average size 0.5–2.5 cm) by the oil extraction factory "Oleícola El Tejar" (Córdoba, Spain). The olive cake, which included the residual olive stone was subjected to vibratory ball milling for the purpose of determining the original chemical composition.

Steam explosion. Steam explosion was carried out using a 2-L reactor, with a maximal operating pressure of 42 kg/cm^2, equipped with a ball valve opening. All experiments were carried out on samples corresponding to 100 g of dry weight.

The olive cake was steamed for different periods of time and temperatures, prior to rapid decompression. The severity of the treatment was designated by a single factor called R_o which links the effects of time (t, min) and temperature (T, °C) (6).

$$R_o = t \exp(T - 100)/14.75) \qquad [1]$$

Fractionation. After steam explosion, the samples were filtered through Albet filter paper (weight, 73 g/cm^2; Albet, Barcelona, Spain) in a Buchner funnel using vacuum. The residue was washed with distilled water (3 × 150 mL) for 30

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min at 60°C, shaken, and then filtered. The filtrate was concentrated to 250–300 mL by rotary evaporation at 40°C. The aqueous concentrate was continuously extracted for 5–6 h with ethyl acetate (refluxed at 77°C). The aqueous and organic phases were separated, and the organic phase was rotary evaporated under vacuum at 40°C for several hours to remove all traces of ethyl acetate. A viscous dark brown extract was obtained. The aqueous phase was freeze-dried. The water-insoluble material was extracted with 0.5 N NaOH (250 mL) for 15 min at room temperature, and the aqueous alkali extract was purged with nitrogen as necessary until the extract was relatively colorless. The dissolved lignin was acidified by drop-wise addition of 5 N H_2SO_4 to pH 2–3. The precipitate was centrifuged, washed to neutral pH, and freeze-dried.

Analytical methods. Moisture, fat, and ash contents were determined according to AOAC methods (7). Protein was determined by the micro-Kjeldahl method using the N × 6.25 conversion factor. Free sugars and uronic acids were quantified by colorimetric methods (8,9). Mannitol content was determined by high-pressure liquid chromatography (HPLC) using an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA), refractive index detection, and 0.01 N H_2SO_4 as an eluent. Total polyphenols were colorimetrically determined with Folin-Denis reagent and caffeic acid as standard (10). Cellulose, lignin, and hemicelluloses were determined according to Goering and Van Soest (11).

Detection and quantification of phenolic compounds in ethyl acetate extracts were carried out by HPLC in a Waters 600 apparatus (Milford, MA) with an ultraviolet-visible photodiode array detector (Waters 996). The separation was performed using a Spherisorb ODS-2 column (5 µm, 250 × 4.6 mm, Tecnokroma, Barcelona, Spain), and the flow rate was 1 mL/min. The detection wavelength was 280 nm. The mobile phase consisted of orthophosphoric acid in water, pH 2.5, and acetonitrile, with a gradient from 5 to 25% of acetonitrile in 30 min, maintained for 10 min, and increased to 50% after 5 min. Phenolic compounds were identified by their retention times and absoption spectra in the 200–380 nm range. Phenolic standards were purchased from Sigma Chemical Co. (St. Louis, MO) except for the oleuropein, which was obtained from Extrasynthese (Genay, France). Hydroxytyrosol (3,4-dihydroxyphenyl ethanol) was obtained from oleuropein by acid hydrolysis.

The content of water-soluble low-molecular-weight sugars was determined by analysis with or without trifluoracetic acid (TFA) hydrolysis (2 N TFA at 121°C for 1 h) prior to derivatization to alditol acetates and then gas chromatography (GC) (12).

The composition of the noncellulosic neutral sugars of the water-and-alkali insoluble fraction was determined by acid hydrolysis with 2 N TFA (see above). Total sugars (cellulosic and noncellulosic) were determined by two-stage acid hydrolysis using 72% H_2SO_4 at 40°C for the first stage and posthydrolysis with 1 M H_2SO_4 at 100°C for 4 h for the second stage. Neutral sugar released by the acid hydrolysis was measured by GC as alditol acetates.

Klason lignin was determined gravimetrically (13). The α-cellulose determination was carried out from bleached cellulose, which was extracted with 17.5% NaOH, and the residue was measured gravimetrically. The hemicelluloses were also determined as the difference between bleached cellulose and α-cellulose. Bleached cellulose was prepared by chlorite delignification (14).

Crystallinity of bleached cellulose preparations was determined by X-ray diffraction on a X-ray diffractometer (Siemens D-5000) with a position-sensitive proportional counter. The relative crystallinity indices (RCI) were calculated by the empirical method described by Segal *et al*. (15).

Cellulase activity was determined by the filter-paper assay on Whatman number 1 paper to determine the total cellulolytic activity after incubation in 0.05 M sodium acetate buffer, pH 4.8 at 50°C for 1 h (16). The released reducing sugars (RS) were determined by the dinitrosalicylic acid (DNS) method using D-glucose as standard (17). One unit of filter-paper activity (FPU) was the amount of enzyme that released 1 µmol of glucose/min.

Enzymatic hydrolysis. Hydrolysis was carried out in 50-mL glass flasks in a shaker at pH 4.8 (0.05 M sodium acetate buffer) and 50°C using a commercial cellulase (Cellubrix). Cellubrix (Novo Nordisk Ferment AL, Dittingen, Switzerland) is a mixture of cellulase originating from *Trichoderma reesei* and β-glucosidase originating from *Aspergillus niger*. A weighed amount of water-and-alkali-insoluble fibers and/or additional treated sodium chlorite fiber was placed in a flask, and an enzyme solution supplemented with 0.01% Thimerosal as a biocide was then added to a final volume of 5 mL. The enzyme activity was 70 FPU/g substrate, and the concentration of the insoluble material was 8% (wt/vol). To follow hydrolysis, 0.1-mL aliquots of the hydrolysate were separated from the solid residue by filtration (through glass wool placed in Pasteur pipettes) and after 1, 8, 24, 48, and 72 h were analyzed for RS. The overall sugar yield, or percentage of polysaccharides utilization, was calculated in relation to the polysaccharide content of the untreated original material (% saccharification).

RESULTS AND DISCUSSION

Composition, treatment, and fractionation. The chemical composition (g/100 g of dry weight ± SD, $n = 3$) of milled olive cake was as follows: Moisture, 5.42 ± 0.45; fat, 3.28 ± 0.15; protein, 9.80 ± 1.30; free sugars, 1.88 ± 0.17; uronic acids, 1.15 ± 0.01; fiber detergent-acid, 47.0 ± 0.22; fiber detergent-neutral, 61.6 ± 4.58; cellulose, 27.9 ± 1.49; hemicelluloses, 14.6 ± 4.80; lignin, 16.8 ± 1.38; α-cellulose, 17.7 ± 0.90; klason lignin, 19.5 ± 1.40; polyphenols, 1.21 ± 0.01; ash, 7.72 ± 0.61; mannitol, 1.45 ± 0.21.

Olive cake contains a large amount of fiber, approximately 60% of the dry matter, the cell walls being mainly polysaccharides (cellulose 18–28%, hemicellulose 14%) and lignin (17–20%). Since the type of parenchymal cell of the olive fruit pulp is scarcely lignified (18), the lignin data seem to be

TABLE 1
Experimental Conditions for Stream-Treated Olive Cake and Yield (g/100 g of dry initial olive cake) of Both the Water Soluble Fraction and the Insoluble Residue Recovered

	Severity index (log R_o)[a]					
	3.04	3.36	3.81	3.95	4.10	4.25
Time (s)	135	135	135	135	135	135
Temperature (°C)	193	204	219	224	229	234
Water-soluble substances	23.3	24.0	28.0	28.6	26.5	27.6
Ethyl acetate-extracted	1.60	2.00	2.20	2.60	2.80	2.00
Water-insoluble substances	56.9	51.7	54.7	54.5	60.9	60.0
Alkaline-extracted	9.60	10.7	12.5	21.6	25.8	33.5
Olive stone fragments	12.9	7.05	4.60	4.70	4.30	2.10

[a] $R_o = t \exp(T - 100/14.75)$, where t is time (min) and T is temperature (°C).

overestimated. It is composed mostly of condensed tannin and other polymers. The protein (10%) and ash (8%) contents were high. The fat contents (3%) of the samples studied were high enough to continue with oil extraction. Free sugars constituted about 2% of the dry matter, a low value compared to the free sugar content of the olive pulp (13–14%) (19). This indicated that a large part of the free sugars was lost during the olive oil extraction process and/or during the storage of this by-product. Since mannitol (1.5% of the dry weight) is an unfermentable substance, the losses regarding the mannitol content in the pulp (about 5%) are minor.

The low uronic acid content (1.1%), compared to cellulose and hemicellulose contents, indicates that only a small amount of pectic substances was present, when in fact they were the main polysaccharides of the olive cell wall. The reason for this loss is similar to the loss of free sugar.

Olive cake was processed by steam-explosion under different steam temperatures for 135 s prior to rapid decompression (explosion). Table 1 shows the experimental conditions of this study, as well as yields of both the water-soluble fraction recovered (in g/100 g of dry initial olive cake) and the insoluble residue recovered by filtration after washing.

The content of olive stone fragments (>0.2 mm) that remained in the insoluble residue decreased as the steam conditions became more severe, declining from 13 to 2%. These data confirm that the pretreatments were effective in autohydrolysis of the heavily lignified material and the hard olive seed husk (5).

Table 1 shows the yield of ethyl acetate extracts obtained from their soluble fractions as a function of the severity of the pretreatment. A considerable portion of the insoluble fraction was extractable with dilute alkali and increased severity. Although the lignin recovered by alkaline extraction, followed by acid precipitation from steam-exploded olive stones, was similar to exploded hardwood lignin (4), the "lignin" obtained by alkaline delignification from steam-exploded olive cake was not a true lignin. This was confirmed by ultraviolet and infrared spectroscopy (data not shown), and supports the idea regarding the composition of the "lignin" present in the olive cake.

Characterization of the soluble fraction. The water-soluble fraction was characterized after phenol and other products formed from the thermal degradation with ethyl acetate had been removed (Table 2). The main compounds were carbohydrates (23–27%), representing from 5.6 to 6.8% of the dry weight of the initial olive cake.

The effect of steam-explosion pretreatment on hemicellulose solubilization was low compared to other lignocellulosic materials rich in glucuronoxylans, where the hemicelluloses became almost completely water soluble (20). This can be explained by the characteristics of the major polysaccharides of olive pulp, which is rich in pectic polysaccharides, xylan, xyloglucan, arabinan, and galactoglucomannan.

The amounts of neutral sugars, released either in monomeric or oligomeric form, are given in Table 3. Glucose, together with arabinose, represented a significant part of the total monosaccharide content. Although part of these sugars may originate from hemicellulose, a certain amount of the glucose can be due to free sugars, which decreased in quantity because of thermal degradation occurring at high severities.

Solubilization of sugars occurred predominantly in the oligomeric fraction (Table 3), showing a large difference in sugar composition when steam explosion was applied at lower or higher severity. While the content of depolymerized hemicelluloses decreased with severity, more xylose-rich oligomers were solubilized. These differences were more pronounced between the points of treatment of log R_o 3.36 and 3.81. Since the response of olive cake to the steam-explosion process was different from that of other plant tissues, even olive stones (which contain much more lignin and xylan than olive cake with a large solubilization of oligosaccharides of

TABLE 2
Chemical Characterization of the Water-Soluble Fraction After Ethyl Acetate Extraction (g/100 g water-soluble fraction freeze-dried)

	Severity index (log R_o)					
	3.04	3.36	3.81	3.95	4.10	4.25
Total sugars	26.3	25.6	24.9	22.7	24.3	26.6
Protein	8.21	11.0	10.5	10.9	11.1	11.0
Mannitol	7.05	5.37	5.00	5.45	4.99	5.41
Ash	16.1	17.1	16.1	15.4	15.2	14.6
Polyphenols	4.39	4.69	4.00	4.75	4.42	5.30
Uronic acids	2.56	2.7	2.18	2.16	2.10	2.01
Other compounds[a]	35.4	33.6	37.3	38.6	37.9	35.1

[a] Quantified by difference: 100 – % known compounds.

TABLE 3
Composition of Neutral Sugars in Monomeric and Oligomeric Soluble Fraction (g/100 g water-soluble fraction freeze-dried)[a]

		Severity index (log R_o)					
		3.04	3.36	3.81	3.95	4.10	4.25
Rhamnose	M	0.36	0.47	0.41	0.48	0.42	0.45
	O	2.75	2.62	1.64	1.4	1.71	1.54
Arabinose	M	1.12	1.30	1.23	1.47	1.23	1.31
	O	8.48	6.95	4.16	3.37	3.98	4.78
Xylose	M	0.15	0.17	0.22	0.29	0.30	0.42
	O	1.47	4.15	9.37	7.70	8.08	9.44
Mannose[b]	M	0.53	0.24	0.38	0.83	0.76	0.34
	O	0.39	0.56	0.61	0.60	0.59	0.68
Galactose	M	0.17	0.13	0.15	0.18	0.22	0.17
	O	2.10	2.28	1.84	1.70	1.76	1.80
Glucose	M	3.06	2.26	1.14	1.13	1.44	1.13
	O	5.74	4.42	3.80	3.56	3.77	4.57
Total sugars	M	5.39	4.57	3.53	4.38	4.37	3.82
	O	20.9	21.0	21.4	18.3	19.9	22.8

[a]Determined without (M) and with (O) trifluoroacetic acid hydrolysis prior to gas chromatographic derivatization.
[b]Data corrected with mannitol content obtained by high-performance liquid chromatography.

xylose) (5,20), one can suppose that at least a certain amount of the xylose recovered from the pretreated olive cake could be part of residual olive stones present in this material that decreased with severity (Table 1).

In the soluble fraction, mannitol also stands out (5–7%) (Table 2). The amount of this compound after the milder treatment (7%) was similar to the initial material before treatment, decreasing only slightly owing to thermal decomposition. These quantities are relatively important and could represent an economically interesting product from olive cake.

Table 2 also shows that a substantial portion of the water-soluble material produced during pretreatment (34–39%) was not identified and was quantified only by its difference from known compounds. This could be due to the high percentage of chemical transformations and to condensation reactions between carbohydrates, proteins, and polyphenols.

In the ethyl-acetate extracted phase, HPLC analysis showed the presence of low molecular weight products that were identified by comparing their retention times and absorption spectra in the ultraviolet region (200–400 nm) with those of known compounds. The quantitative analyses of all identified compounds are shown in Figure 1.

The amounts of identified phenolic acids (vanillic and syringic acids) and aldehydes (vanillin and syringaldheyde) were low compared to those obtained from true lignocellulosic material (i.e., steam-exploded olive stones) (5). The reasons are (i) that these substances originated from lignin degradation and (ii) that the content of true lignin in olive cake was low, because of a parenchymal skin with a primary wall. Only vanillic acid, which has been reported as a phenol present in olive pulp, reached values of 8.5 mg/100 g of the dry matter, double the amount of the other phenols. Also present were 3,4-dihydroxybenzoic acid and pyrocatechin, which has also been detected in the olive-mill wastewater or "alpechin" (21).

Of all the low-molecular-weight phenols solubilized by the steaming pretreatment, hydroxytyrosol and tyrosol were the most abundant (Fig. 1). These compounds were present in the olive pulp (21) and seed (22), respectively, where they were part of the phenolic glucosides. Hydroxytyrosol stands out for its abundance and great antioxidant power. The amount solubilized increased as the steaming conditions increased in severity, reaching 149 mg/100 g of the dry olive cake. Starting at log R_o of 4.10, it began to decrease. This substance is a commercially unavailable natural antioxidant and is responsible for the stability of oils that contain it (23). Its recovery could be of interest.

These water-soluble, noncarbohydrate compounds released or formed during steam explosion show strong inhibitory effects against enzymes and microorganisms (24). Therefore, these compounds must be removed before the other solubilized sugars or cellulose can be efficiently converted by microorganisms to a wide variety of products.

Characterization of the residual insoluble material. Table 4 shows the chemical composition of the insoluble fraction obtained from olive cake after steam explosion, water washing, and alkali extraction. The constitutive polymers were quantified from the standard methods used for quantification of wood polymers. These results were similar when hemicelluloses and cellulose were determined by hydrolysis with TFA and H_2SO_4, respectively, followed by quantification of monosaccharides by GC. The residual lignin was insoluble in H_2SO_4, the so-called Klason lignin (13).

The composition of sugars (Table 4) shows that cellulose was associated with a high proportion of xylans and other polysaccharides rich in arabinose and galactose. The relatively high amount of hemicellulosic material that remained in the insoluble residue (almost in their totality by milder treatments log R_o 3.04 or 3.36) contrasts with the almost com-

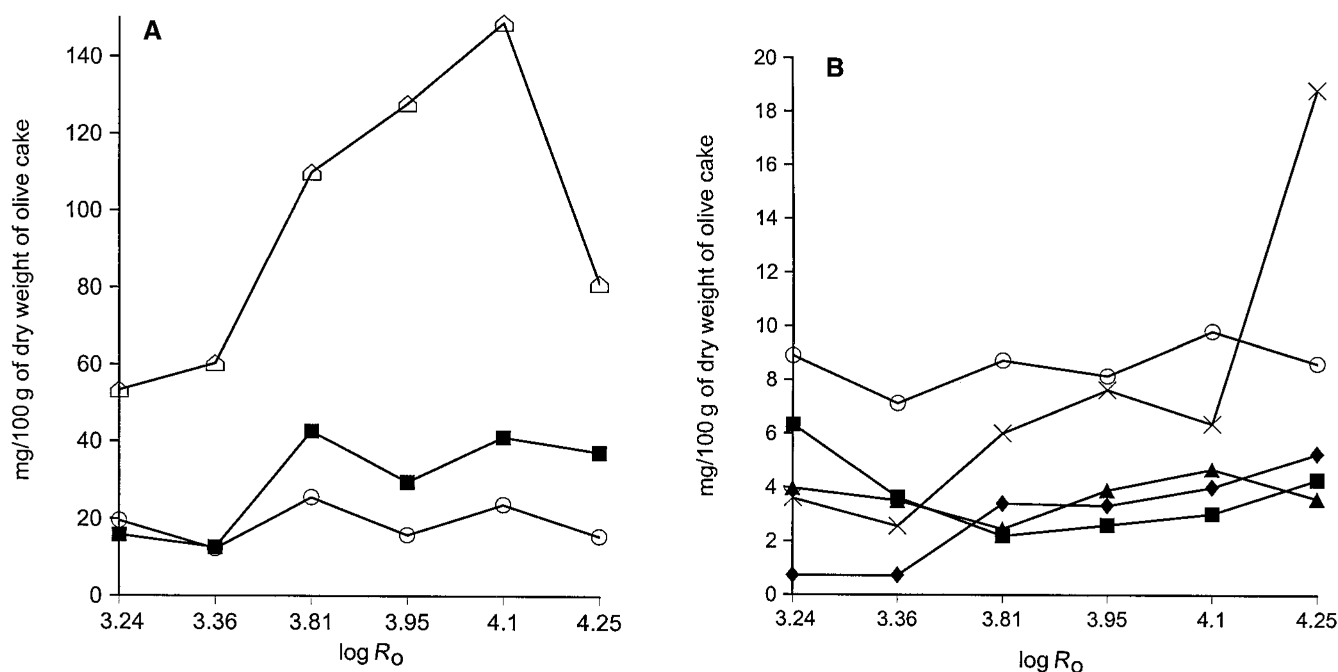

FIG. 1. Evolution of the content (mg/100 g dry matter) of low-molecular-weight phenols obtained from steam-exploded olive cake as a function of treatment severity (log R_o), where R_o = t exp (T – 100)/14.75, and t is time (min) and T is temperature (°C). (A) ○, 3,4-Dihydroxybenzoic acid; ⌂, hydroxytyrosol; ■, tyrosol. (B) ×, Pyrocatechin; ○, vanillic acid; ▲, syringic acid; ◆, vanillin; ■, syringaldehyde.

plete solubilization of the hemicelluloses in other heavily lignified tissue rich in glucuronoxylans, where the autohydrolysis reaction was effective.

Since the total amount that could be extracted with dilute alkali increased as the steaming became more severe (Table 1), the residual "lignin" in this fraction decreased notably, while the protein and ash content remained virtually unchanged (data not shown).

The cellulose recovered from treated olive cake was nearly amorphous, as the relative crystallinity index (RCI) was low (Fig. 2). This characteristic of the cellulose had a profound effect on its reactivity, for the hydroxyl groups located in the amorphous regions were highly accessible, reacted readily, and made the cellulose susceptible to hydrolytic enzymes (25).

The enzymatic hydrolysis of the insoluble fraction using a cellulolytic complex Cellubrix (an endo/exo cellulase prepa-

TABLE 4
Chemical Characterization and Sugar Composition of the Water-and-Alkali-Insoluble Fraction (g/100 g insoluble fraction)

		Severity index (log R_o)					
		3.04	3.36	3.81	3.95	4.10	4.25
α-Cellulose		27.4	32.4	43.0	47.5	56.7	59.0
Hemicelluloses[a]		29.8	27.6	19.4	16.6	18.4	21.8
Klason lignin		36.6	34.9	35.1	32.5	23.6	21.7
Sugars[b]							
Arabinose	TFA	1.59	—[c]	2.29	—	—	1.33
	H_2SO_4	2.03	—	1.00	—	—	1.06
Xylose	TFA	18.3	—	7.08	—	—	6.54
	H_2SO_4	17.5	—	4.28	—	—	7.20
Mannose	TFA	0.13	—	0.35	—	—	0.37
	H_2SO_4	0.27	—	1.29	—	—	0.90
Galactose	TFA	0.73	—	0.71	—	—	0.71
	H_2SO_4	0.87	—	0.98	—	—	0.71
Glucose	TFA	0.67	—	1.33	—	—	1.87
	H_2SO_4	26.0	—	32.6	—	—	45.1

[a]Hemicellulose was determined by difference between bleached cellulose and α-cellulose.
[b]Sugars determined by 2 N trifluoroacetic acid (TFA) and H_2SO_4 hydrolysis prior to gas chromatographic derivatization.
[c]Not determined.

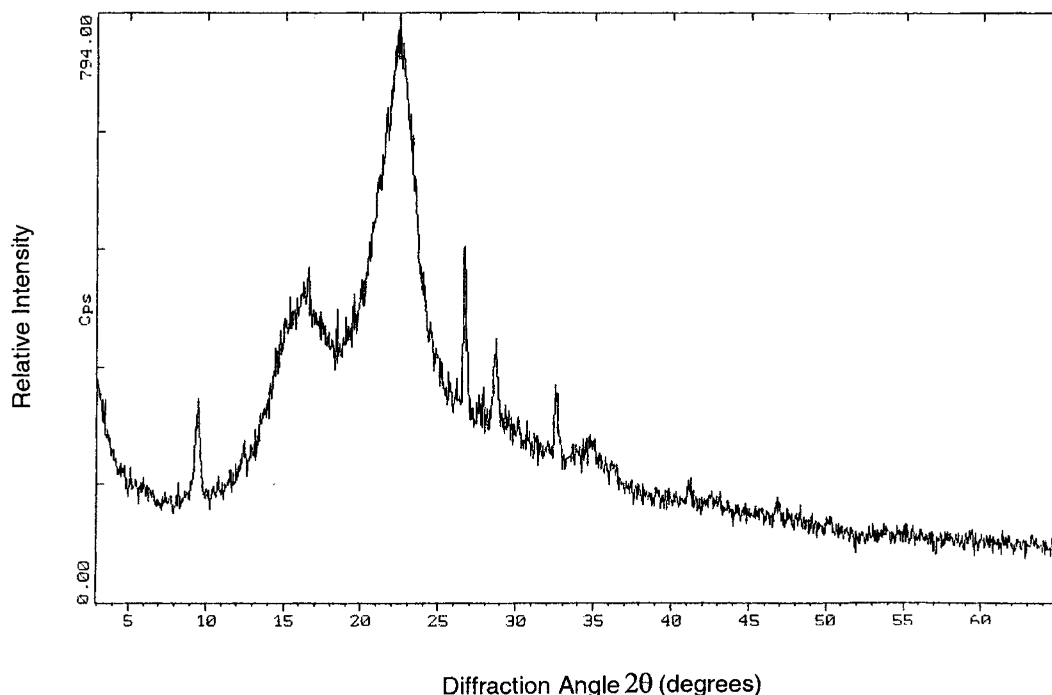

FIG. 2. X-ray diagram of cellulose from steam-exploded olive cake (log R_0 = 3.36). The relative crystallinity index (45.2) was calculated from X-ray diffractograms by the method of Segal *et al.* (15) as $[(I_{(002)} - I_{am})/I_{(002)}] \times 100$, where $I_{(002)}$ is the maximum intensity of 2 θ = 22.5° (reflection attributed to the crystalline region) and I_{am} is the intensity at 2 θ = 18.5° (reflection attributed to amorphous region).

ration without pectinase activity) was also studied. At high enzymatic concentrations, a maximum of 41.8 and 57.7% of the total polysaccharides present in the insoluble residue of pretreated material at log R_0 3.36 and 4.10, respectively, were solubilized after 72 h (Fig. 3A). The slight increase in the extent of saccharification was not proportional to the large alkaline delignification (Table 4), which occurred when the severity of the steaming treatment was increased. When the residues were efficiently delignificed with chlorite treatment, however, the susceptibility to enzymatic hydrolysis increased notably, reaching 80–92% saccharification after only 24 h of incubation (Fig. 3B). These results indicate that the accesibility of substrate may be hindered by certain residual phenolic compounds or "lignin"-forming linkages with the polysaccharides, so that drastic treatment of delignification for improving the enzymatic action is necessary.

The amount of sugars released by enzymatic hydrolysis was on the order of 15 g of fermentable sugar per 100 g of dry olive cake after 72 h of incubation for a water-insoluble fraction extracted with dilute alkali, and the yield increased to about 20 g for combining alkali and chlorite bleaching pretreatment after only 24 h of incubation. Therefore, in addition to the sugars solubilized during steam explosion, up to 7 g/100 g of dry matter such as monosaccharides or oligosaccharides was left (Tables 1 and 3). The sugar yield obtained by hydrolysis with cellulase from insoluble material of the olive cake, steamed and alkali/chlorite pretreated, could be considered a good source of fermentable carbohydrate.

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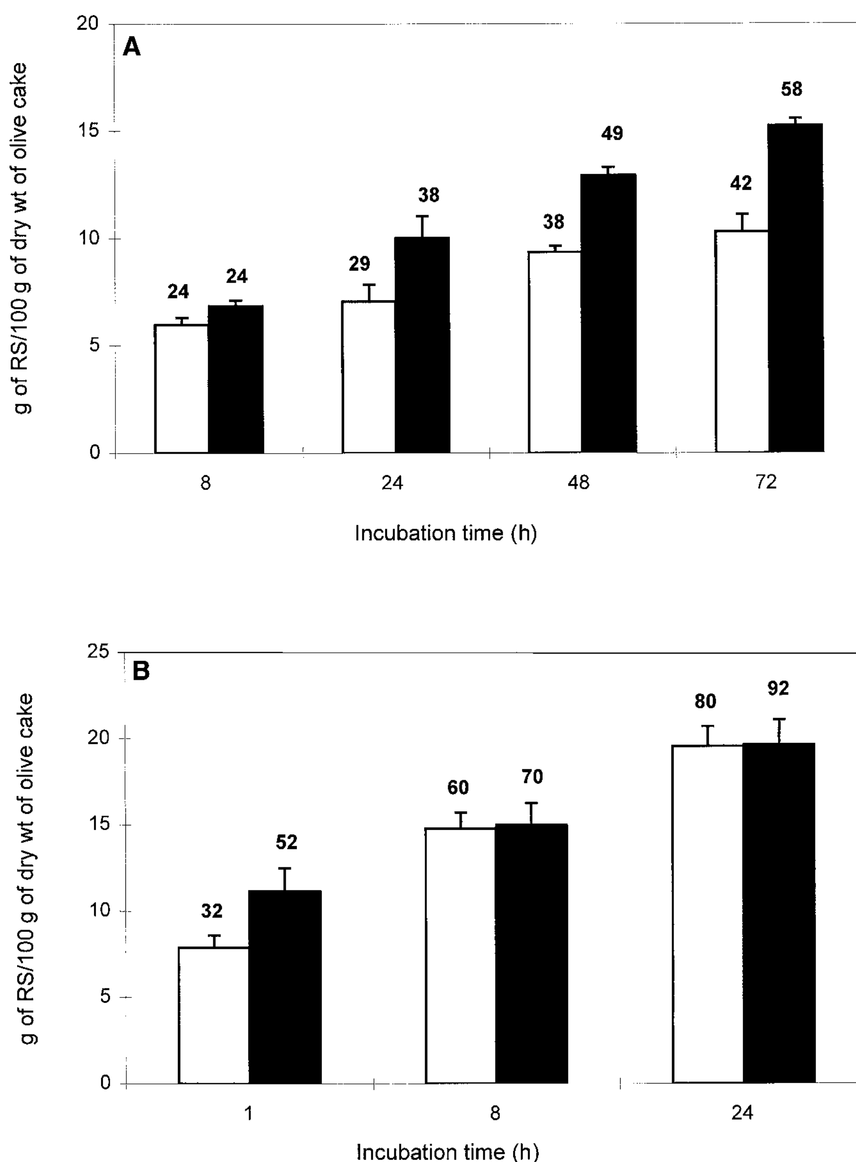

FIG. 3. Enzymatic hydrolysis of (A) the water-and-alkali insoluble fraction, and (B) the water-and-alkali insoluble fraction posttreated with sodium chlorite, at severities of log R_0 3.36 (open bars) and of log R_0 4.10 (solid bars). Experiments were done in triplicate at an enzyme concentration of 70 FPU/g cellulose. The values for the reducing sugars (RS) are the averages ± SD. Values above each bar represent saccharification ratios (%) referred to as a percentage of the theoretical yield of the maximal total sugars. The hydrolysis conditions are given in the Experimental Procedures section. FPU, filter paper activity units.

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