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Hydrolysis of Ginger Bagasse Starch in Subcritical Water and Carbon Dioxide

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Ginger bagasse from supercritical extraction was hydrolyzed using subcritical water and CO_2 to produce reducing sugars and other low molecular mass substances. Response surface methodology was used to find the best hydrolysis conditions; the degree of hydrolysis and the yield were the two response variables selected for maximization. The kinetic studies of the hydrolysis were performed at 150 bar and temperatures of 176, 188, and 200 °C. The higher degree of hydrolysis (97.1% after 15 min of reaction) and higher reducing sugars yield (18.1% after 11 min of reaction) were established for the higher process temperature (200 °C). Different mixtures of oligosaccharides with different molecular mass distributions were obtained, depending on the temperature and on the reaction time. The ginger bagasse hydrolysis was treated as a heterogeneous reaction with a first-order global chemical kinetic, in relation to the starch concentration, which resulted in an activation energy of 180.2 kJ/mol and a preexponential factor of 5.79×10^{17}/s.

KEYWORDS: Ginger bagasse; hydrolysis; starch; subcritical water; supercritical fluids

INTRODUCTION

Extracts from ginger and turmeric are widely used in food processing to impart flavor to a variety of foods because of their volatile oil and/or oleoresin (*1*). Ginger and turmeric extracts obtained by supercritical fluid extraction (SFE) showed important functional properties such as antioxidant (*2, 3*), anticancer (*3, 4*), and antimycobacterial (*3*) activities. Because of the content of starch in ginger and turmeric bagasses, these residues or biomass can be used as a source of special starches as well as a substrate for hydrolysis reaction to obtain oligosaccharides, glucose, or even smaller molecules, providing hydrolyzed substances with special aroma and flavor characteristics.

The hydrolytic production of glucose is an important step for the conversion of these residues, because glucose, besides being a substrate for ethanol production, is also a raw material for important products such as sorbitol, vitamin C, and furfural, which in turn is a raw material for production of polyamides, polyesters, and epoxies.

The starch macromolecule is formed by two polysaccharides: amylose and amylopectin. The majority of the starches consist of approximately 75% of semicrystalline amylopectin and 25% of the amorphous amylose. Amylose is a linear chain polymer consisting of several hundred glucose units connected by α-D-(1→ 4) linkages. Amylopectin contains, on the other hand, many oligomeric units of D-glucose joined by α-D-(1→ 4) and α-D-(1→ 6) linkages. The degree of starch acid hydrolysis depends on (i) the effect of the acid to rupture the starch granule; (ii) the degree of hydrolysis of each starch component (amylose and amylopectin) that varies with the starch nature; and (iii) the degree of hydrolysis of the polysaccharide itself, which is a function of the physical distribution of the amylose and amylopectin structures. Acid hydrolysis of starch produces D-glucose and D-glucose degradation products such as 5-hydroxymethyl furfural, levulinic acid, and formic acid; 5-hydroxymethyl furfural is the precursor of the last two substances (*5*). Partial starch hydrolysis produces maltdextrins [dextrose equivalence (DE) < 20], nutritive saccharide polymers, nonsweet substances used as thickening agents, texturizers, auxiliary substances for dry powders, fat substitutes, imitation cheese, sauces, freezing and film production control agents, substances to prevent crystallization, and nutritional additives.

Many processes have been studied to produce these mixture and monomeric sugars. The enzymatic process yields many specific products, but it can be slow and last from 3 to 120 h (*6*−*8*), besides involving the costs of obtaining the enzymes and purifying the product. Because of the presence of the starch and of multiple substrates in the cell walls of the vegetables, the enzymatic process for the biomass hydrolysis requires mixtures of enzymes, thus increasing its cost (*9*). Nonetheless, the enzymatic process is widely used in industry today. On the other hand, the hydrolysis with inorganic acids has a shorter reaction time, but it causes corrosion, and the recovery of the

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Table 1. Identification of the Ginger Bagasse Used in the Hydrolysis Studies[a]

ginger bagasse code	SFE operating conditions	SFE solvent (% cosolvent, v/v)	frozen storage time from SFE tests to the hydrolysis tests (months)
KB	250 bar and 25 °C	CO_2 + ethanol (1.17%)	16
KC	250 bar and 25 °C	CO_2 + ethanol (1.5%)	22
KD	250 bar and 35 °C	CO_2 + ethanol (1.5%)	31
	250 bar and 35 °C	CO_2 + isopropyl alcohol (1.17%)	31
	250 bar and 25 °C	CO_2 + isopropyl alcohol (1.17%)	31

[a] SFE performed by Zancan et al. (*2*) using a CO_2 flow rate of 10^{-5} kg/s with equal amounts of particles of mesh sizes 16, 22, 32, and 48.

acids is difficult. The hydrolysis with organic solvents is as slow as the enzymatic process, requiring huge amounts of solvents and raising pollution issues.

For cellulosic biomass, one of the most promising treatments is the hydrothermal treatment, also known as hydrothermolysis, which uses subcritical and supercritical water as the reaction medium (*10*). Using water satisfies today's pursuit for "green" transformation processes, i.e., beneficial to the environment. The biomass conversion using water near its critical point is faster, if compared with other methods such as biodegradation with microorganisms (*11*). As for the product distribution, a high level of selectivity can be achieved, by adjusting the temperature and the pressure of the process. Different operating conditions cause changes in the water properties (density, ionic product, and dielectric constant), providing different decomposition pathways. Water, near its critical point (374 °C and 221 bar), has properties such as density and dielectric constants that are similar to those of nonpolar organic solvents at room temperature. This makes it a feasible solvent in reactions, with the additional advantage of dispensing with the steps of neutralization and solvent recovery. The temperature increase causes an increase in the dissociation constant, making water a strong acid or a strong base. In such chemical reactions, water plays catalyzing, reagent, and solvent roles (*12*).

The approximate composition of ginger is 30−60% starch, 6−14% raw protein, 3−10% fibers, and 1.7−3% essential oil (*1*). Thus, after the extraction of the oleoresin from ginger, the ginger bagasse contains 40−55% starch (dry mass) and cellulose. As mentioned before, this bagasse is a source of a special starch and can be used as a substrate for hydrolysis reaction to obtain hydrolyzed substances with special aroma and flavor characteristics. Furthermore, if the ginger residue came from a SFE process, then, the hydrolysis process can benefit from the modifications imparted to the starchy and cellulosic structures by the SFE step (*13*−*16*). In the present work, subcritical water in the presence of CO_2 was used to hydrolyze ginger bagasse after the solid matrix was subjected to SFE.

MATERIALS AND METHODS

Raw Material Identification and Characterization. The ginger bagasses used on this work came from previous work done in our laboratory by Zancan et al. (*2*); the ginger bagasses were stored in plastic bags at −10 °C immediately after the SFE process. The hydrolysis experiments were performed using five lots of ginger bagasse from different SFE assays, and the bagasse designated as KD resulted from the mixture of three distinct lots, as indicated in **Table 1**. The bagasses were characterized with respect to humidity (*17*) and reducing sugars (*18*), while other constituents were characterized by standard AOAC methods (*19*): starch content (method no. 32.2.05), lipids (method no. 32.1.25), ashes (method no. 4.1.10), total protein (method no. 32.1.22), and fiber (method no. 4.6.01).

Experimental Planning. The surface response methodology was used to establish the temperature and pressure conditions to maximize the starch hydrolysis without producing excessive degradation products. On the basis of the preliminary assays, the first set of experiments was made using a factorial design with two levels of pressure (100 and 200 bar), two levels of temperature (140 and 180 °C), and a central point (150 bar and 160 °C); the experiments were made in triplicate and quintuplicate at the central point. The assays at the axial points were made at 132 and 188 °C and at 80 and 220 bar in triplicate. These experiments were made using bagasses KB and KC (**Table 1**).

Experimental Procedure. *Search for the Best Operational Conditions*. The hydrolysis tests were made using a model 7071 Speed SFE-NP unit (Applied Separations, Inc., Allentown, PA) and a 5 mL extractor vessel (reactor) (Thar Designs, Inc., Pittsburgh, PA). The SFE unit was operated using the same procedure used for any standard extraction assay (*3*). The reactor was filled with a mixture of ginger bagasse and distilled water (3:7) and assembled in the SFE unit oven. The hydrolysis assays were done using three steps as follows: (i) Keeping the inlet and outlet CO_2 valves closed, the oven heating system was turned on. The time to heat up the system varied accordingly with the assay temperature: at the lower temperature (132 °C) it took 36 min, while at the higher temperature (188 °C) 45 min was required; thus, the average heating time was 39 ± 2 min. (ii) After the desired temperature was reached, simultaneously the CO_2 pump was turned on, the inlet CO_2 valve was opened, and 99.0% purity CO_2 (Gama, S. S. ONU 1013, Campinas, Brazil) was admitted into the system. (iii) Once the operating pressure was reached, the reactor was kept still at the desired temperature and pressure for 15 min (static period or reaction time). Afterward, the outlet CO_2 valve was opened and the reaction products were withdrawn from the reactor using a CO_2 flow rate of $7.48 \pm 0.05 \times 10^{-5}$ kg/s. To speed up the cooling process, that is, to avoid further uncontrolled hydrolysis, the oven door was opened and an external fan helped cool the reactor vessel. The process losses, that is, the mass loss of the hydrolysis process due to leakages and dragging of liquid and gaseous products by the CO_2 stream, were kept below 10%. In this case, if the mass balance indicated losses above 10%, the experiment was rejected.

Kinetic Tests. The hydrolysis kinetic tests were performed with bagasse KD (**Table 1**) at 150 bar and at 176, 188, and 200 °C, using the experimental procedure previously described and keeping constant the ratios between bagasse and water in the reactor, as well as the CO_2 flow. The following static periods or reaction times were evaluated: 1, 5, 7, 9, 11, and 15 min. The starch hydrolysis kinetics for times below 1 min was not studied, due to experimental difficulties.

Characterization of the Reaction Products and Residues. The reaction residues were characterized with respect to the amount of starch and of reducing sugars (*18*) and with respect to the humidity (AOAC method no. 4.1.03) (*19*). The reaction products were characterized with respect to the quantities of reducing sugars (*18*), pH (AOAC method no. 32.1.20) (*19*), proportion of total solids (AOAC method no. 33.2.09) (*19*), and molecular mass distribution, using gel permeation chromatography (GPC). The hydrolysis products at 188 °C were analyzed using high performance liquid chromatography (HPLC). The yields of acids and other sugar degradation products (furfural and hydroxymethylfurfural) were not evaluated. In addition, the content of total sugars was not evaluated because the sample size was insufficient.

GPC. To determine the molecular mass of the reaction products, as well as its molecular mass distribution, a model 9095 high-performance liquid chromatographer was used (Varian Associates Inc., Sunnyvale,

CA), with a model R14 refraction index detector (Varian Associates Inc.) and three columns (G3000 PW, 10 μm, 30 cm × 0.75 cm ID; G4000 PW, 17 μm, 30 cm × 0.75 cm ID; and G6000 PW, 17 μm, 30 cm × 0.75 cm ID; Varian Associates Inc., Micropak series) connected in series and one precolumn (12 μm, 7.5 cm × 0.75 cm ID, Tosoh Corporation, Tokyo, Japan). Ultrapure water (Millipore Corporation, Milli-Q Plus, Bedford, MA), degasified at 1.0 mL/min, was used as the mobile phase. Dextran standards (DXT11 K, DXT 43 K, DXT 79 K, DXT 165 K, DXT 685 K, DXT 1750 K, and DXT 5000 K) were used to construct the standard curve for molecular masses in the range of 12−5000 kDa. The data were processed using the software Millennium Chromatography Manager v 2.1 (Waters Corporation, Milford, MA). The sample molecular mass distribution was obtained using the calibration curve. The dextran standards were dissolved in ultrapure water at the concentration of 0.4 (w/v) and were left for 16−20 h before being injected. This period is necessary to allow the polymer chain to extend to its solvation conformation, particularly for standards with a molecular mass above 200 000 Da.

HPLC. The reaction products for the assays at 188 °C were analyzed using a model LC-10 high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan), with a column 30 cm × 0.79 cm ID Shim-pack SCR-101P (Shimadzu Corporation) at 80 °C, using ultrapure water at 0.6 mL/min and using a RID-6A refractive index detector (Shimadzu Corporation).

Standards for glucose, fructose, saccharose, raffinose, galactose, xylose, and erythrose were prepared as possible hydrolysis products from the substrate. The standards were prepared at 0.2, 0.5, 1.0, 1.5, and 2% (w/v) with ultrapure water and filtered through membranes 0.22 μm (Millipore Corporation).

Calculation Procedure. The dry ginger bagasse was the basis of the mass balance for the process. The process losses were evaluated using the following equation:

$$\%\, L = \left[1 - \frac{(m_P + m_W)}{(m_B + m_A)}\right] \times 100 \quad (1)$$

where m_A is the mass of water in the feed, m_B is the mass of bagasse in the feed, m_P is the mass of products, and m_w is the mass of reaction residue or unreacted material.

The reaction yield (y, wt %) or amount of reducing sugar was calculated as:

$$y = \frac{RS_W + RS_P}{St_B} \times 100 \quad (2)$$

where RS_P is the mass of reducing sugar in the product stream, RS_W is the mass of reducing sugar in the reaction residue, and St_B is the initial mass of starch in the ginger bagasse.

The degree of hydrolysis (X, wt) or the starch conversion was defined as:

$$X = \frac{St_B - St_W}{St_B} \quad (3)$$

where St_W is the mass of starch in the unreacted material.

The residual starch (RSt, wt) was calculated as:

$$RSt = \frac{St_W}{St_B} \quad (4)$$

Statistical Analysis. An analysis of variance (ANOVA) was done, and a response surface was fitted in order to analyze the influence of temperature and pressure on the hydrolyzed starch content and on the reducing sugar yield; Minitab v 13.1 was used.

RESULTS AND DISCUSSIONS

The compositions of ginger bagasse used in the hydrolysis reactions are given in **Table 2**. The differences in the proportions of starch, reducing sugars, and humidity in bagasses KB, KC, and KD may be because these bagasses are SFE residues obtained with different cosolvents, which resulted in differences in the composition of the extracted oleoresins (*2*). As discussed by Rodrigues et al. (*20*), the starchy and cellulosic structures that form ginger do not interact with CO_2 during the SFE process; nonetheless, the two cosolvents used by Zancan et al. (*2*) interact with these structures, and consequently, differences in the bagasse composition were observed.

Table 2. Composition of Ginger Bagasse Used in the Hydrolysis Reactions[a]

	ginger bagasse			
dry basis (%)	KB	KC	KD	ginger before SFE
starch	56.5	48.5	40.1	44
reducing augar	6.1	5.2	5.1	5
humidity	19	16	14.6	12
fiber	3	NQ	NQ	5
ash	5	NQ	NQ	6
lipid	1	NQ	NQ	2
protein	12	NQ	NQ	10

[a] NQ, not quantified.

Table 3. Degree of Hydrolysis (X) and Hydrolysis Yields (y) for a Reaction Time of 15 min

	T (°C)	P (bar)	X (%)	y (%)
corner or factorial points	140	100	55 ± 12	4.6 ± 0.4
		200	67 ± 5	5.1 ± 0.1
	180	100	66 ± 2	4.6 ± 0.1
		200	70.8 ± 0.1	4.3 ± 0.5
central point	160	150	47 ± 7	3.4 ± 0.3
axial or star points	132	150	40 ± 2	4.9 ± 0.4
	160	80	41 ± 6	2.9 ± 0.1
	160	220	54 ± 5	3.1 ± 0.2
	188	150	61 ± 10	8 ± 2

The reaction products obtained for the static period or reaction time of 15 min had a yellow−brown color with a mixed caramel and coffee aroma, which may characterize the Maillard reaction. Color and aroma intensified as the temperature increased. The ANOVA obtained from the data on **Table 3** showed that for the degree of hydrolysis (X) the R-square for the fitted model was 50.1%. Both factors (temperature and pressure) were statistically significant. As for the reducing sugar amount (y), only the temperature was statistically significant (for both coefficients, linear and quadratic, the p-value was less than 0.05), and the R-square for the fitted model was 68.8%. Higher values of the degree of hydrolysis can be obtained at any pressure for temperatures up to 188 °C or at any temperature for pressures up to 220 bar. The best values of the yield were obtained at temperatures above 188 °C. The pressure was not relevant in the tested interval. On the basis of these results, the kinetic assays were done at 150 bar and 176, 188, and 200 °C.

The kinetic studies showed that the degree of hydrolysis increased with temperature (**Figure 1**). This behavior results from the decrease in the density and in the dielectric constant of water as the temperature increases, thus facilitating the water dissociation and accelerating the hydrolysis process (*21*−*26*). The water dissociation as the temperature increases can be verified by noting that the reaction medium pH (**Figure 2**) decreased as the temperature increased. The pH of the product solutions indicated that large amounts of acids were formed at 200 °C. At 188 and 200 °C, the starch hydrolysis was fast during the first minute of reaction and then proceeded at a slower rate, and the reaction was not complete even after 15 min. Assuming a two step reaction sequence, in the first step (reaction times of

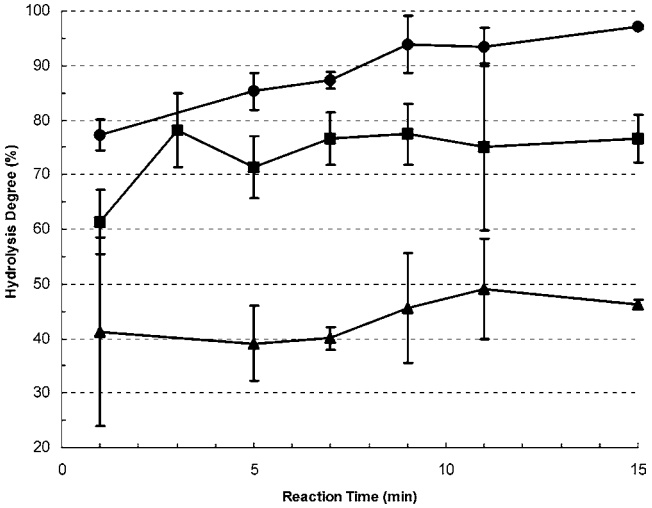

Figure 1. Degree of hydrolysis of the ginger bagasse starch at 150 bar and 176 (▲), 188 (■), and 200 (●) °C.

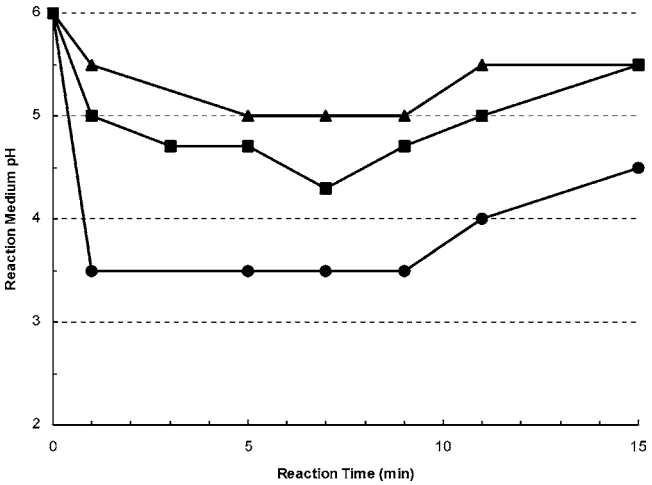

Figure 2. Reaction medium pH variation on ginger bagasse hydrolysis at 150 bar and 176 (▲), 188 (■), and 200 (●) °C.

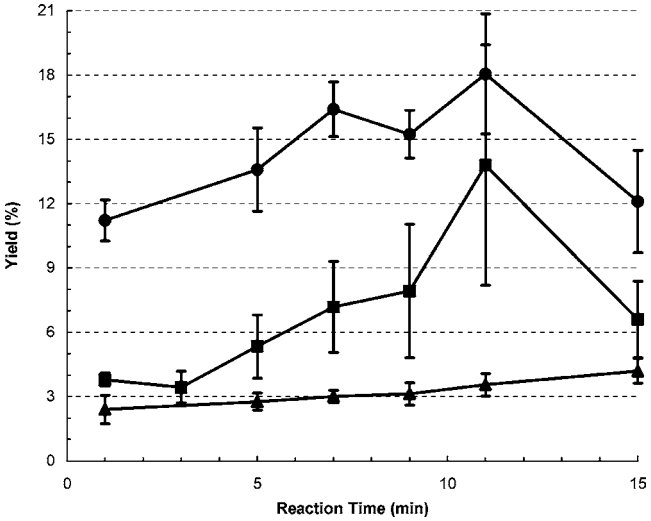

Figure 3. Yield on ginger bagasse hydrolysis at 150 bar and 176 (▲), 188 (■), and 200 (●) °C.

Table 4. Reaction Products Characterization by HPLC at 188 °C and 150 bar[a]

reaction time (min)	1	3	5	7	9	11	15
compound	concentration (wt/v %)						
raffinose	2.58	2.18	2.99				
saccharose				0.20	0.17	0.15	0.10
glucose				0.03			
xylose	0.57	1.29					
galactose	ND	ND	ND	ND	ND	ND	ND
fructose	0.70	0.62	0.69	0.60	0.65	0.57	0.64

[a] ND, not determined.

less than 1 min), intermediate size compounds that are difficult to hydrolyze (*27*) are formed, and reversion reactions can occur, since the product remains in the reaction medium and the amount of water becomes a limiting factor for the occurrence of hydrolysis. Larger liquid-to-solid ratios could be favorable to the increase in reducing sugar yields because a low concentration of sugars in the reaction medium reduces the rate of decomposition (*28*). In the second reaction step, after the first minute of reaction, the intermediate products were hydrolyzed to lower molecular weight at a slower degree of hydrolysis. At 176 °C, the starch hydrolysis was much slower, when compared with the reaction at 188 and 200 °C. The maximum degree of hydrolysis obtained at 176 °C was 46.1% in 11 min, and at 188 and 200 °C, the maximum degrees of hydrolysis obtained were 76.6 and 97.1%, respectively, but for 15 min of reaction.

The process yield for reducing sugars increased with temperature (**Figure 3**), and the DE of the process ranged from 3 to 7% at 176 °C (maximum value at 7 min of reaction), from 5 to 7% at 188 °C (maximum value at 5 min of reaction time), and from 17 to 29% at 200 °C (maximum value at 15 min). The reaction rate of reducing sugars was low at 176 °C. At 188 and 200 °C, the maximum yields of reducing sugars were 14 and 18%, respectively, in 11 min. The decrease in the reducing sugars yield at higher reaction times can be explained due to the degradation of these sugars. An et al. (*21*) concluded that high temperatures are necessary for breaking the glycoside bonds, but monosaccharides and oligosaccharides are more susceptible to decomposition at milder conditions. The temperature increase favored both the starch hydrolysis and the reducing sugars production. Even higher sugar yields could be obtained if the sugars formed were withdrawn from the reaction medium as soon as they were produced, thus reducing their degradation.

Characterization of the Reaction Products by Chromatography. Products with a molecular mass in the range of 10 000−100 000 Da represented 30−40% of the total at 176 and 188 °C, respectively, whereas at 200 °C they represented only 2.5−10%. Products with a molecular mass ranging from 160 to 8400 Da represented 60−75% of the total mass at 176 and 188 °C, respectively. At 200 °C, the molecular mass ranged from 50 to 53% of the total mass. At 200 °C, there was an increase in the generation of smaller oligomers, which reached 100% of the product after 15 min of reaction.

Reaction products at 188 °C and 150 bar were characterized using liquid chromatography (**Table 4**). When galactose was injected together with the other standards, there was a peak merge with that of xylose, so the quantification using this standard was not possible. We noted the presence of raffinose (a trisaccharide) in up to 5 min of reaction time; thereafter, it appeared to be converted into sucrose and glucose. Fructose appeared throughout this kinetic study, which may be the result of the glucose epimerization by the opening of the glucose ring (*29*). The absence of glucose confirms its transformation into fructose.

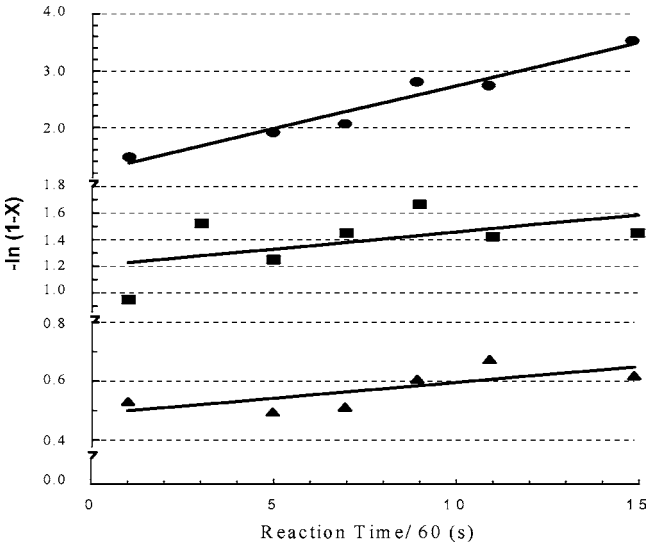

Figure 4. Kinetics of ginger bagasse starch decomposition: $-\ln(1 - X)$ vs time at 150 bar and 176 (▲), 188 (■), and 200 (●) °C.

The decomposition of ginger bagasse starch in subcritical water is an effective method and potentially attractive. The starch hydrolysis and the production of reducing sugars increased with temperature, but the temperature increase also favored the degradation of the reducing sugars while they were being formed. The decomposition happened quickly and in two stages. At 200 °C, the starch was almost completely decomposed after 15 min of reaction, yielding 18% of reducing sugars after 11 min.

Characterization of the Reaction Kinetics. Considering that a hydrolysis mechanism was the major reaction for the starch decomposition at the conditions of this study, the plot of $-\ln(1 - X)$ vs time (**Figure 4**) for the second reaction step (times larger than 1 min) at 200 °C gives a first-order reaction kinetics ($R^2 = 0.9525$). However, at 176 and 188 °C, a first-order equation did not fit the curve well ($R^2 = 0.5297$ and $R^2 = 0.2883$).

Considering starch hydrolysis as having a first-order kinetic dependence, the global expression for the starch decomposition reaction can be written as:

$$\frac{dX_H}{dt} = k_H(1 - X_H) \quad (5)$$

The Arrhenius equation was applied to determine the reaction constant as:

$$k_H = A_H \exp\left(-\frac{(E_a)_H}{RT}\right) \quad (6)$$

where E_a is the apparent activation energy (kJ/mol); A_H is the preexponential factor (s^{-1}); R is the gas constant, 8.314 (J/mol K); and T is the temperature (K). Fitting the data of **Figure 4** to eq 5 and using eq 6, one obtains $(E_a)_H = 185.1$ kJ/mol and $A_H = 5.79 \times 10^{17}$/s. The value of the apparent activation energy obtained in the present work agrees well with values reported in the literature (*30*−*35*). On the other hand, the value of the preexponential factor of this work is close to the values obtained for hydrothermolysis of cellulose (*33*) and thermal decomposition of cellulose (*35*).

This work allowed a first exploratory study of the hydrolysis of ginger bagasse using supercritical fluids in a standard SFE unit. Operating the SFE unit in a continuous fashion, the hydrolysis of bagasse starch would most certainly give higher yields in reducing sugars, because, in this situation, the hydrolysis products would be withdrawn from the reaction vessel as soon as they are formed, thus minimizing their degradation. A lower solid-to-water ratio (wt %) might favor an increase in the degree of hydrolysis. Ginger bagasse starch hydrolysis could be considered as a first-order reaction with respect to the starch concentration at 200 °C.

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