Ciencia y Tecnología Alimentaria
ISSN: 1135-8122
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Sociedad Mexicana de Nutrición y Tecnología de Alimentos
México

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Ciencia y Tecnología Alimentaria, vol. 5, núm. 4, julio, 2007, pp. 271-277

Sociedad Mexicana de Nutrición y Tecnología de Alimentos

Reynosa, México

SOMENTA
Sociedad Mexicana de Nutrición
y Tecnología de los Alimentos

Cienc. Tecnol. Aliment. 5(4) 271-277 (2007)
www.somenta.org/journal ISSN 1135-8122

CIENCIA Y TECNOLOGÍA ALIMENTARIA

SOLUBILIZATION OF LIGNIN COMPONENTS OF FOOD CONCERN FROM SUGARCANE BAGASSE BY ALKALINE HYDROLYSIS

DISOLUCIÓN DE COMPONENTES LIGNÍNICOS DE INTERÉS ALIMENTARIO A PARTIR DE BAGAZO DE CAÑA DE AZÚCAR POR HIDRÓLISIS ALCALINA

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Recibido/Received 01-12-2006; aceptado/accepted 25-01-2007

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Abstract

Solubilization of sugarcane bagasse was performed in autoclave by alkaline treatment at 121 °C for one hour to follow the release of its main components. The major products extracted were *p*-coumaric acid, vanillin, ferulic acid, and syringic acid, whose concentrations increased significantly with increasing the concentration of the NaOH solution up to 2.0 M or decreasing the liquid/solid ratio of the raw material to be treated to 50 g/g, respectively. These aromatic compounds could be usefully exploited in food industry because of their antioxidant power; however, a lot of experimental work is needed to optimize this process.

Resumen

Ha sido efectuada la disolución del bagazo de caña de azúcar por tratamiento alcalino en autoclave a 121 °C durante una hora para estudiar la extracción de sus componentes principales. Los principales productos extraídos fueron el ácido *p*-cumárico, la vainillina, el ácido ferúlico y el ácido siríngico, cuyos niveles se incrementaron significativamente con un aumento de la concentración de la disolución de NaOH hasta 2.0 M o con la disminución de la relación líquido/sólido de la materia prima hasta 50 g/g, respectivamente. Los compuestos aromáticos podrían ser empleados en la industria alimentaria debido a su poder antioxidante; aunque es necesaria una gran cuantidad de trabajo experimental para la optimización de este proceso.

Keywords: Alkaline hydrolysis, lignin, ferulic acid, *p*-coumaric acid, sugarcane bagasse
Palabras clave: Hidrólisis alcalina, lignina, ácido ferúlico, ácido *p*-cumárico, bagazo de caña de azúcar

INTRODUCTION

The use of renewable resources, such as sugarcane bagasse, as starting materials for the production of various chemicals, has increased in recent years. Sugarcane bagasse is a residue nowadays produced in huge amounts, mainly in Brazil, by the sugar and alcohol industries, which has found wide applications as fuel for boilers to recover energy (Sene *et al.*, 2002).

Alternative promising applications include its use as low-cost animal feedstock, as raw material for biological production of fuels, chemicals, and food additives, such as vanillin (Mathew and Abraham, 2005) and xylitol (Carvalho *et al.*, 2003, 2005; Santos *et al.*, 2003, 2005), and even as cell support in different bioprocesses (Pandey *et al.*, 2000; Sene *et al.*, 2002).

There are several processes that can be used to release and/or purify lignin components from biomass. However, each process uses several chemical agents to extract materials from lignocellulosic biomass and produces other materials with different composition and properties. There are two main chemical processes of biomass hydrolysis, which use acids or bases, whose choice mainly depends on the material structure and characteristics desired for the products to be recovered. Alkaline-based methods are generally more effective because they are able to solubilize a greater fraction of lignin (Knauf and Moniruzzaman, 2004).

The mechanism of alkaline hydrolysis is similar to that occurring in the Kraft paper pulping technology, which consists in the hydrolysis (saponification) of the intermolecular ester bonds cross-linking lignin to other components as hemicelluloses. Lignin is a complex biopolymer mainly constituted by cross-linked phenylpropanoid subunits, primarily *p*-coumaryl, coniferyl, and sinapyl alcohols (Gidh *et al.*, 2006). The phenolic compounds such as vanillin, syringic, ferulic, and *p*-coumaric acids and others are commonly found in agro-industrial wastes as associated with lignin through ether linkages (Pan *et al.*, 1998; Xu *et al.*, 2005). They are abundant, valuable, renewable aromatic compounds with great potential as antioxidants in food industry (Xu *et al.*, 2005). Among these, the *p*-coumaric acid is also recognized as being an important chemical for pharmaceutical industry as well as for human health as a drug against stomach cancer (Abdel-Wahab *et al.*, 2003).

The aim of this work was to investigate the release of the main lignin-soluble compounds from sugarcane bagasse, under different conditions of hydrolysis with NaOH, with particular concern to vanillin, syringic, ferulic, and *p*-coumaric acids.

MATERIALS AND METHODS

Pretreatment of sugarcane bagasse

The sugarcane bagasse used in this work was kindly supplied by the Department of Biotechnology of the Engineering School of Lorena, University of São Paulo, Lorena (Brazil). It was first dried in sunlight, then in oven at 105 °C, cut into small pieces and stored in desiccator at room temperature. The bagasse sample analyzed did not have a homogeneous structure, because it was a mixture of raw and ground sugarcane bagasse. The dried biomass of bagasse (ranging from 1 to 5 g) was ground using a laboratory mill and screened to prepare a 40 mesh powder.

Characterization of sugarcane bagasse

Sulphuric acid (72 %) was used for quantitative determination of components in sugarcane bagasse lignin. The reaction mixture (0.5 g of biomass plus 5 ml of H_2SO_4) was stirred for 1 h at 30 ºC in glass tubes with 2 cm diameter and 5 cm length, diluted with water until 4 % (using Pyrex glass bottles, 250 ml, with plastic caps) and then autoclaved at 121 °C for 1 h. The liquid phase was separated by filtration and analyzed by HPLC. The solid residue was washed to remove the excess acid, dried in oven at 105 °C for 24 h and weighted.

Alkaline sugarcane bagasse hydrolysis

Pretreated sugarcane bagasse powder was suspended in 100 ml of NaOH solution (with concentration ranging from 0.5 to 4.0 M) and autoclaved at 121 °C for 1 h, using 250 ml Pyrex glass bottles with plastic caps. Liquid/solid ratio of the raw material and concentration of the NaOH solution were selected among those reported in the literature for other raw materials (Parajó *et al.*, 1998). Bagasse extract solutions obtained from alkaline hydrolysis were filtered through filters with 0.45 μm pore diameter and stored at 4 °C until analyzed. Prior to the analysis, all liquid samples were acidified with H_3PO_4 up to pH 3.0 and filtered through membranes with 0.20 μm pore diameter. A schematic diagram of the extraction process is shown in Figure 1.

Table 1. Time variations of the relative proportions of solutions used as mobile phases for the separation of phenolic acids components obtained by sugarcane bagasse alkaline hydrolysis.

Tabla 1. Variaciones en función del tiempo de las proporciones relativas de disoluciones empleadas como fase móvil para la separación de ácidos fenólicos obtenidos por hidrólisis alcalina de bagazo de caña de azúcar.

Time (min)	A%	B%
0	100	0
30	60	40
40	100	0
50	100	0

All the tests were performed in quadruplicate and the results expressed as mean values. Variability of the experimental data was expressed as standard deviations from the mean values.

Analytical procedures

Liquid samples were analyzed by HPLC, model 1100 (Hewlett-Packard, Palo Alto, CA), equipped with a refractive index detector, model HP 1047A, and a 300 mm x 7.8 mm column, model Supelcogel C610H (Supelco, Bellefonte, PA). Two different solutions were used as mobile phases, namely 1% v/v acetic acid in distilled water (solution A) and 9% v/v distilled water plus 1% v/v acetic acid in methanol (solution B), under the following elution conditions: 1 ml/min flow rate, time 55 min, injection volume 5 μl. A complete separation of components required variations of the relative proportions of these solutions, according to the protocol listed in Table 1.

Chemical compounds used as reference standards for HPLC analysis, namely D-glucose, D-xylose, D-arabinose, acetic acid, 5-hydroxymethylfurfural, 2-furaldehyde, vanillic acid, vanillin, syringic acid, *p*-coumaric acid, syringaldehyde, ferulic acid, and cinnamic acid, were purchased from Sigma-Aldrich (Steinheim, Germany).

RESULTS AND DISCUSSION

In this study, the solubilization of major components from sugarcane bagasse released by alkali treatments with

Table 2. Percent dry weight composition of sugarcane bagasse. Because minor components are not taken into consideration, these data do not sum to 100 %.

Tabla 2. Composición porcentual del peso seco del bagazo de caña de azúcar. Debido a que los componentes menores no se tomaron en cuenta, los datos no suman el 100 %.

Cellulose	Hemicellulose	Lignin	Reference
40	32	20	Varhegyi *et al*., 1988
33.3	22.6	6.15	Aiello *et al*., 1996
35.8	43.1	21.1	García-Pérez *et al*., 2002
43.6	33.5	18.1	Sun *et al*., 2004 ; Xu *et al*., 2006

Table 3. Percentage composition of dry sugarcane bagasse obtained after acid hydrolysis with H_2SO_4.

Tabla 3. Composición porcentual del peso seco del bagazo de caña de azúcar obtenido por hidrólisis ácida con H_2SO_4.

Component	Percentage (%)
D-Glucose	36
D-Xylose	20
D-Arabinose	3.5
Acetic Acid	5.7
5-(hydroxymethyl)-Furfural	0.3
2-Furaldehyde	1.6
Klason Lignin	27
Moisture	5.9

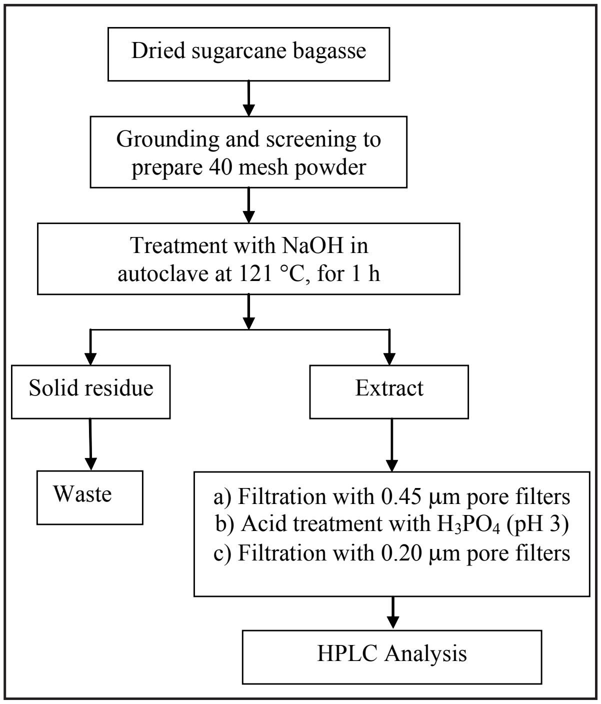

Figure 1. Schematic flowchart of the extraction of sugarcane bagasse soluble components by alkaline hydrolysis.

Figura 1. Esquema de flujo de extracción de los componentes del bagazo de caña de azúcar a través de hidrólisis alcalina.

NaOH, namely vanillin, syringic, ferulic, and *p*-coumaric acids was investigated. These phenolic compounds are present in agro-industrial wastes being associated to lignin; for example, *p*-coumaric acid is linked to lignin by ester bonds, and ferulic acid by both ether and ester bonds (Sun *et al*., 2003). The distribution of these components depends on biomass origin. Bagasse is a waste produced when sugar is extracted from sugarcane, typically in a moisture and ash free condition; it generally contains 33-48% cellulose, 19-43% hemicelluloses and 6-32% lignin, its composition normally depending on the region and growth conditions. Table 2 lists the most representative compositions founds in the literature for different batches of sugarcane bagasse (Varhegyi and Antal, 1988; Aiello *et al*., 1996; García-Pérez *et al*., 2002; Sun *et al*., 2004; Xu *et al*., 2006). Table 3 shows the percent composition of sugarcane bagasse extract obtained by acid hydrolysis with H_2SO_4. It should be noticed that the major products of acid hydrolysis were glucose (36%), lignin (27%) and xylose (20%) and that the Klason Lignin value is in agreement with the corresponding values reported in the literature (Table 2).

The simultaneous effects of different concentrations of alkali (0.5 to 4.0 M) and bagasse liquid/solid ratios (20-100 g/g) in the reacting mixture on the extraction of chemical compounds from sugarcane bagasse was explored. As shown in Table 4, the major products of biomass hydrolysis were *p*-coumaric acid, vanillin, ferulic acid, syringic acid and some traces of others compounds such as vanillic acid and syringaldehyde (results not shown).

Notwithstanding the relatively high standard deviations of these results (errors in the range 10–15 %), the concentrations of components extracted with alkali treatment appeared to increase significantly with the concentrations of NaOH solution within the concentration range investigated. It is also evident that at higher concentration of the raw material lower concentrations of phenolic compounds were obtained. Probably, when the concentrations of material were higher than 20 g/l, the liquid/solid ratio was too low (< 50 g/g) to achieve an effective delignification process, even using the highest NaOH concentration. This result suggests investigating in future work higher concentrations of NaOH, even if Nardini *et al.* (2002) demonstrated that high alkali concentrations could lead to the degradation of a portion of phenolic acids released by alkaline hydrolysis, likely owing to redox reactions.

Figure 2 illustrates the simultaneous effects of the levels of biomass and NaOH on the concentrations of the main components released. It is noteworthy that a reduction of biomass liquid/solid ratio led to a rise in the concentrations of the released products and that the *p*-coumaric acid was always the major product, with the highest yield being obtained with 4.0 M NaOH and 50 g/g liquid/solid ratio.

Taking into account only syringic acid, vanillin, *p*-coumaric acid and ferulic acid as the predominant components, their percentages in the reaction mixture obtained with 2.0 M NaOH and 50 g/g liquid/solid ratio were 3.2, 8.0, 70.2, and 18.6% respectively. The chromatographic analysis of Figure 3 shows that *p*-coumaric acid was always present in the extract in much higher concentration than the other components, followed by ferulic acid. This result agrees with those reported by Xu *et al.* (2005) for the same raw material.

Figure 4 shows the concentration of *p*-coumaric acid released by alkali treatment at several concentrations of NaOH (0.5 to 4.0 M) and sugarcane bagasse liquid/solid ratios (20 to 100 g/g). These results confirm that a liquid/solid ratio of 50 g/g assured the largest release of this component, as the likely consequence of an insufficient availability of alkali in the presence of too high biomass levels. Besides, the results confirm the need to investigate the effect of further increase in the alkali concentration on the hydrolysis yield as well as to optimize this process.

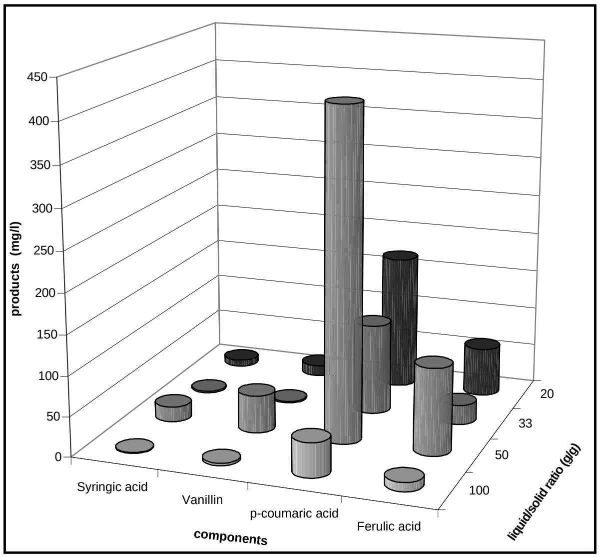

Figure 2. Results of the alkaline treatment of sugarcane bagasse with 2.0 M NaOH under different conditions. Biomass liquid/solid ratio (g/g): (■) 20; (■) 33; (■) 50; (■) 100.

Figura 2. Resultados del tratamiento alcalino del bagazo de cana de azúcar con 2.0 M NaOH bajo diferentes condiciones. Relación líquido/sólido de biomasa (g/g): (■) 20; (■) 33; (■) 50; (■) 100.

CONCLUSIONS

During this study dealing with the effect of alkaline treatment of sugarcane bagasse, it was noticed that increasing the amount of biomass rised the concentrations of all the released products and that the *p*-coumaric acid

Table 4. Concentrations of sugarcane bagasse components released by alkaline treatment. Values in bracket are mean standard deviations of four independent experiments.

Tabla 4. Concentraciones de los componentes del bagazo de caña de azúcar extraídos por tratamiento alcalino. Los valores entre paréntesis indican la desviación estándar de 4 experimentos independientes.

NaOH conc. (M)	**0.5**				**1.0**				**2.0**				**3.0**				**4.0**			
Bagasse liquid/solid ratio (g/g)	100	50	33	20	100	50	33	20	100	50	33	20	100	50	33	20	100	50	33	20
Syringic acid (mg/l)	0.6 (0.06)	18 (2.4)	1.1 (0.04)	4.4 (0.57)	1.0 (0.14)	24 (0.80)	1.4 (0.07)	5.9 (0.19)	1.1 (0.15)	19 (2.4)	1.9 (0.10)	8.4 (1.0)	1.1 (0.10)	16 (2.0)	2.3 (0.23)	7.7 (1.1)	1.2 (0.13)	20 (2.2)	3.1 (0.43)	11 (1.5)
Vanillin (mg/l)	1.8 (0.20)	26 (3.6)	1.1 (0.15)	7.6 (1.1)	3.0 (0.37)	59 (2.0)	1.2 (0.08)	8.1 (0.76)	3.0 (0.39)	47 (5.6)	2.0 (0.21)	13 (0.95)	2.6 (0.34)	32 (4.4)	0.9 (0.11)	12 (1.7)	2.3 (0.32)	23 (3.1)	1.8 (0.24)	18 (2.6)
p-Coumaric acid (mg/l)	46 (5.3)	420 (61)	110 (7.7)	188 (22)	50 (4.7)	410 (57)	116 (9.6)	184 (11)	43 (2.7)	412 (56)	114 (4.1)	170 (17)	48 (5.5)	450 (64)	124 (11)	194 (23)	53 (5.3)	550 (78)	135 (17)	245 (32)
Ferulic acid (mg/l)	9.3 (1.1)	89 (12)	20 (1.4)	47 (6.1)	11 (1.6)	115 (15)	23 (2.1)	51 (1.9)	11 (1.6)	109 (16)	25 (0.64)	62 (8.9)	12 (1.6)	89 (11)	27 (2.8)	57 (6.1)	13 (1.5)	128 (19)	32 (4.2)	69 (10)

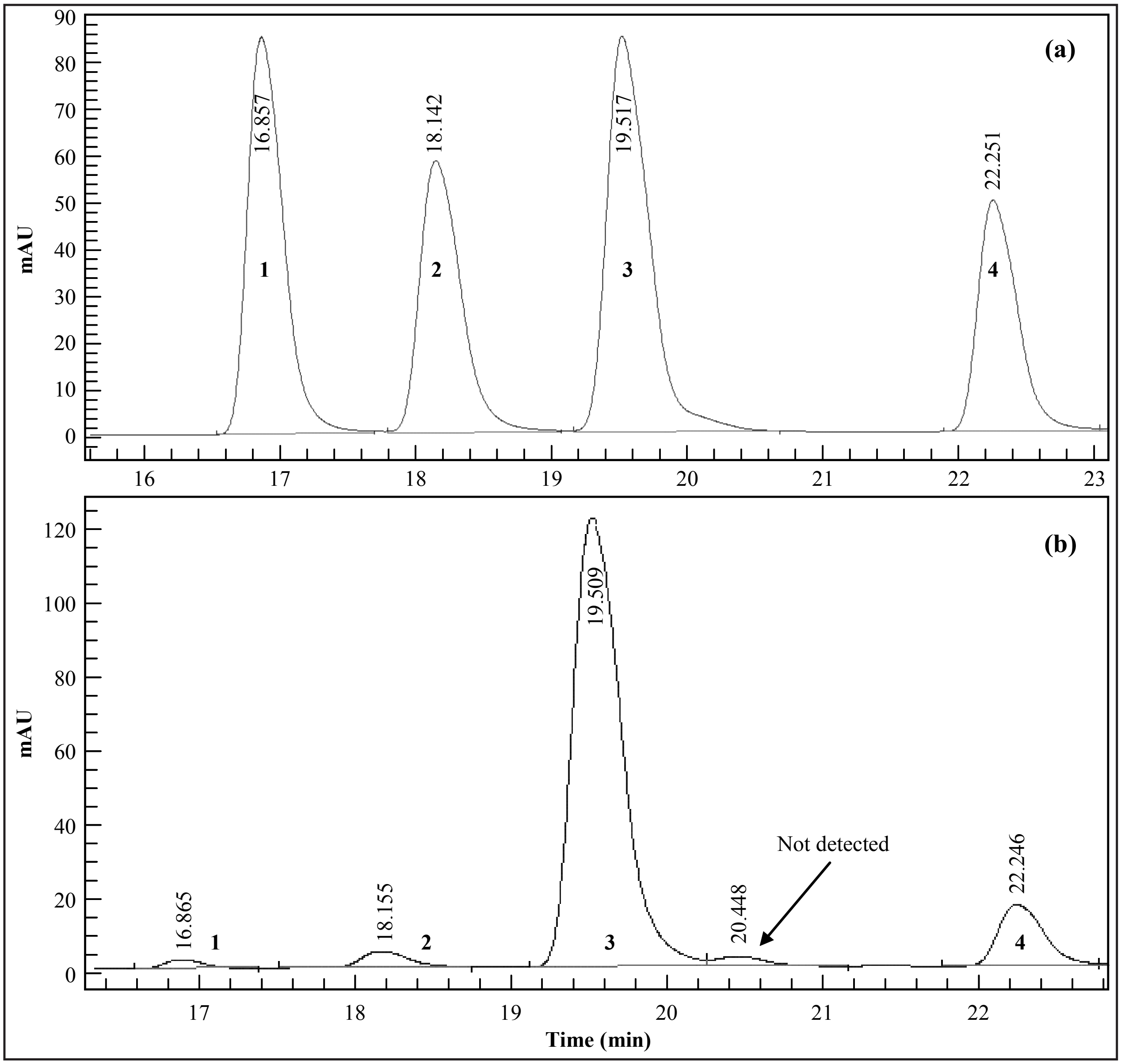

Figure 3. High-performance liquid chromatogram (HPLC) of the a) standard solution and b) sample obtained by alkaline hydrolysis of sugarcane bagasse. Biomass liquid/solid ratio = 100 g/g; NaOH concentration = 3.0 M. Operating conditions reported in the Materials and Methods section. Peaks identification: 1- syringic acid; 2- vanillin; 3- *p*-coumaric acid; 4- ferulic acid.

Figura 3. Cromatograma de HPLC de a) disolución padrón y b) muestra obtenida por hidrólisis alcalina de bagazo de cana de azúcar. Razón líquido/sólido de biomasa = 100 g/g; concentración de NaOH = 3.0 M. Condiciones operativas mencionadas en la sección de Materiales y Métodos. Picos de identificación: 1- ácido siríngico; 2- vainillina; 3- ácido *p*-cumárico; 4- ácido ferúlico.

was always the major product, with the highest yield being obtained with 4.0 M NaOH and 50 g/g biomass liquid/solid ratio. These results suggest the possibility of effectively exploiting sugarcane bagasse as raw material to recover chemical products with antioxidant power, because such an agroindustrial residue is an abundant, valuable, and renewable source of aromatic compounds. Moreover, they confirmed that the alkaline treatment could be an effective way to release phenolics; however, it is necessary to develop a highly quantitative method for the purification of *p*-coumaric acid and other compounds from plant cell walls.

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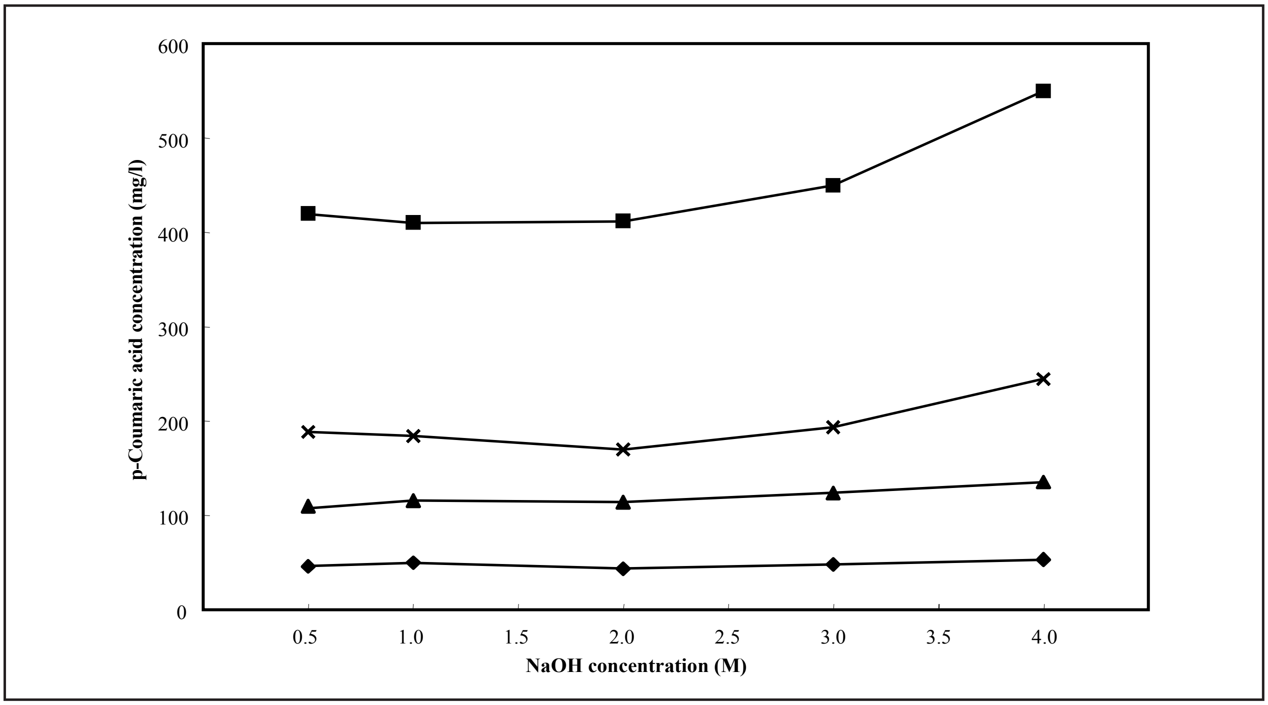

Figure 4. Concentration of *p*-coumaric acid released by alkali treatment of sugarcane bagasse using various concentrations of NaOH and various liquid/solid ratios (g/g): (x) 20; (▲) 33; (■) 50; (◆) 100.

Figura 4. Concentración del ácido *p*-cumárico extraído por tratamiento alcalino del bagazo de caña de azúcar empleando diferentes concentraciones de NaOH y varias relaciones líquido/sólido (g/g): (✕) 20; (▲) 33; (■) 50; (◆) 100.

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