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Sugar production from durian (*Durio zibethinus* Murray) peel by acid hydrolysis

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Agricultural waste is mainly composed of cellulose and hemicelluloses which can be converted to sugars. The inexpensive sugar from durian peel was hydrolyzed with H₂SO₄, HCl and H₃PO₄ at a concentration of 0.5 to 2.0% (v/v). The hydrolysis times ranging from 15 to 60 min were investigated when the mixture was autoclaved at 121°C. The result shows that acid hydrolysis efficiency (AHE) with H₂SO₄, HCl and H₃PO₄ had values of 72.15 to 77.55, 70.78 to 80.99 and 73.33 to 77.34%, respectively. Hydrolysis with H₂SO₄ and HCl led to increased concentration of AHE, reducing sugar and total sugar, while hydrolysis with H₃PO₄ had no effect on all values. Hydrolysis time had no effect on AHE, reducing sugar and total sugar in hydrolysis with H₂SO₄ and HCl; while in hydrolysis with H₃PO₄, the increasing time increased AHE and reducing sugar but had no effect on total sugar. Sugar in the hydrolysates was determined by high performance liquid chromatography (HPLC). In hydrolysis with H₂SO₄ and HCl, glucose, fructose and xylose were found while in hydrolysis with H₃PO₄, glucose and fructose were found.

Key words: Acid hydrolysis efficiency (AHE), hydrolysis, durian peel, reducing sugar, total sugar.

INTRODUCTION

Thailand is known as the agricultural country where great agricultural products are produced. As a result, agricultural waste has caused significant problems. However, most waste has been transformed into other products particularly as animal food and fertilizer. This increases the value of the waste as well as decreases its amount. The major components of agricultural wastes are lignin, cellulose and hemicelluloses. Hemicellulose accounts for 10 to 40% of natural cellulosic biomass. Hemicellulose is primarily a polymer of pentoses and hexoses. These polymers can be easily reduced to monomeric sugars, xylose and glucose by using dilute acids under mild acid condition. In recent years, it was demonstrated (Ferrari et al., 1992; Eken-Saracoglu et al., 1998; Aguilar et al., 2002; Jargalsaikhan and Saracoglu, 2009) that D-xylose containing hemicellulosic hydrolysates is a potential

substrate for the production of chemicals like ethanol and xylitol through biological means.

Durian is an important fruit in South east Asia. The common name 'king of fruit' (Subhadrabandhu and Ketsa, 2002) *Durio zibethinus* Murray, commonly known as durian, is one of the most important seasonal fruits in Tropical Asia. It is a climacteric fruit (Booncherm and Siriphanich, 1991). Thailand is one of the primary producers and a world exporter of fresh and frozen durian (Hokputsa et al., 2004).

Peel of durian is a major agricultural waste in Thailand. Production of durians in Thailand reached 537,277 tons per year in 2011. The amount of durian peel generates approximately 358,000 tons per year, which is likely to be increased during the next few years as durian can be harvested several times in a year. Furthermore, increa-

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sing rate of durian consumption is evident as most people like it. The massive amount of the peel is disposed as waste which could lead to environmental problems. Durian peel could be further utilized as a source of valuable materials of commercial importance; such as particle board component of construction panels for energy conservation in building (Khedari et al., 2004), biosorbent for the removal of acid dry (Hameed and Hakimi, 2008) and removal of heavy toxic metals (Wong et al., 2010), activated carbon (Nuithitikul et al., 2010), carboxy methyl cellulose (Siralertmukul et al., 2005) and polysaccharide gel (PG), which were isolated, purified and characterized (Hokputsa et al., 2004).

The main objective of this research was to study the production of sugar from durian peel by hydrolysis with sulfuric acid, hydrochloric acid and phosphoric acid of varying concentrations and hydrolysis time at 121°C. This is done by converting durian biomass to sugar for potential production of ethanol by *Saccharomyces cerevisiae* and for reducing environmental problem.

MATERIALS AND METHODS

The raw material used in the experiment was durian (*D. zibethinus* Murray), which was collected from fruit markets in Chanthaburi Province of Thailand, during May to June 2010. The collected material was washed with distilled water several times to remove all dirt particles. The washed material was cut into small pieces (1 to 2 cm) and then dried in a hot oven at 60°C until constant weight was shown.

The sample was ground with a blender, passed through a 500 micron screen, packed into a sealable plastic bag and stored in desiccators for further use. Later, it was cut into a uniform size; the sample was then analyzed for cellulose, hemicellulose, lignin, moisture, protein, fat, ash, crude fiber and carbohydrate using AOAC method (1995).

Durian peel of 10% (w/v) was hydrolyzed in autoclave at 121°C for 15-60 min, with 0.5 to 2% (v/v) of H₂SO₄, HCl and H₃PO₄. The hydrolysis samples were centrifuged at 3,000 rpm for 20 min. The solid fraction was washed with distilled water and dried in hot air oven at 70°C until constant weight was obtained. Then, weight of percentage of acid hydrolysis efficiency (AHE) was calculated using the following equation. The supernatant was collected and determined for reducing sugar, using dinitrosalicylic acid (Bernfeld, 1955) and total sugar, using phenol sulfuric acid (Hansen and Phillips, 1981). The maximum amount of total sugar from hydrolysate was determined for morphology by SEM and sugar polymers through high performance liquid chromatography (HPLC), using a YMC-Pack Polyamine II column (conditions: 1.0 ml / min, 25°C).

$$\% \text{ AHE} = \frac{\text{TS of initial hydrolysis} - \text{TS after hydrolysis} \times 100}{\text{TS after hydrolysis}} \quad (1)$$

Where, TS refers to total solid.

Statistical analysis

Type 4² factorial research design was carried out to study the effect of two factors of concentration (X₁) and hydrolysis time (X₂) on the two responses mentioned above. This experiment was carried out

randomly to minimize the effects of unexpected variability in the observed responses. Forty-eight (48) runs were required to cover all possible combinations of levels of factors with three replicates. Experimental data were analyzed to fit the following regression model with interaction terms.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_1 + \beta_4 X_1 X_2 + \beta_5 X_2 X_2 \quad (2)$$

Where, β_0 , β_1 , β_2 , β_3 , β_4 and β_5 are regression coefficients.

RESULTS AND DISCUSSION

Durian peel consisted of 30.92% cellulose, 17.99% hemicellulose, 7.69% lignin, 6.92% moisture content, 3.15% protein, 4.01% ash, 0.26% fat, 27.81% crude fiber and 57.85% total carbohydrate. The results obtained from durian peel hydrolysis with 2% H₂SO₄ for 30 min was 77.55% AHE. This was not significant ($p < 0.05$) under the condition of 2.0% concentration for 45 and 60 min 1.5% concentration for 15, 30 and 45 min. The highest reducing sugar was 50.71 g/L under the condition of 2.0% concentration for 30 min, which was not significant ($p < 0.05$) under the condition of 1.5% concentration for 45 min. The highest total sugar was 53.18 g/L under the condition of 2.0% concentration for 30 min, which was significant ($p < 0.05$).

Similar results of acid hydrolysis of cassava waste showed that acid hydrolysis time at 0.6 M H₂SO₄ and 120°C had effect on reducing sugar content. The reducing sugar contents in the early phase of hydrolysis rapidly increased, but after 30 min, the reducing sugar contents were almost constant (Srinorakutara et al., 2006). This suggests that less reducing sugar in the solution may be derived from dehydration or oxidation by sulfuric acid on hydrolytic products. The solution obtained in this study showed various colors (Delegenes et al, 1990).

Sun and Chenge (2005) studied the dilute H₂SO₄ (0.6-2.2% w/w) hydrolysis of rye straw and Bermuda grass and found that the release of fermentable sugars was over 30 g/Ldry biomass when treated with 1.5% H₂SO₄ for 90 min at 121°C and 40 g/Ldry biomass with 1.5% H₂SO₄ for 60 min at 121°C, respectively. Tellez-Luis et al. (2002) investigated the dilute H₂SO₄ hydrolysis of sorghum straw and found that the release of fermentable sugar was 24.9 g/Ldry biomass when treated with 2.0% H₂SO₄ for 71 min at 122°C

Hydrolysis with HCl showed that the highest AHE was 80.99% under the condition of 2.0% concentration for 15 min. The highest reducing sugar was 56.07 g/Lat 2.0% concentration for 45 min. The highest total sugar was 59.83 g/Lat 2.0% concentration for 45 min, which was not significant ($p < 0.05$) at 2.0% concentration for 30 min and 1.5% concentration for 45 and 60 min. Hydrolysis with H₃PO₄ showed that the highest AHE was 77.34% at 0.5% concentration for 60 min, which was not significant ($p < 0.05$) at 1.0% concentration for 60 min. The highest reducing sugar was 50.51 g/L at 1.0% concentration for 45 min. The highest total sugar was 55.85 g/L at 1.0% concentration for 60 min, which was significant ($p < 0.05$). The

result of the hydrolysis is shown in Table 1.

Dawei et al. (2011) studied optimum dilute acid hydrolysis of *Enteromorpha* with H₂SO₄, HCl and H₃PO₄ at 0.6 to 2.2% concentrations and hydrolysis time of 30, 60 and 90 min at 121°C. They found that hydrolysis with H₂SO₄ was more effective than HCl and H₃PO₄ at 60 or 90 min. Treatment with H₂SO₄ at concentrations of 1.4 and 1.8% for 60 min gave reducing sugars 47.57 and 47.41 g/L, respectively, much higher than those of other conditions used in this study.

Hydrolysis with increased concentration of H₂SO₄ and HCl increased AHE, reducing sugar and total sugar, which is similar to that reported by Asli and Qatibi (2009) who studied hydrolysis of olive cake with concentration of 0 - 4% (w/v) at 180°C for 10 min. They found that sugar concentration increased when concentration of acid increased. Hydrolysis time does not affect reducing sugar and total sugar as shown in Table 2. Analysis of optimum condition by comparing means was employed using one-way ANOVA and Duncan with SPSS statistics 17.0. It was found that optimum condition released highest total sugar of H₂SO₄, HCl and H₃PO₄ hydrolysates at 2% concentration for 30 min, 2.0% concentration for 45 min and 1.0% concentration for 60 min, respectively. The experimental data were analyzed by the factorial design to fit the regression models of Statistica 7 (Trial version) for AHE hydrolyzed with H₂SO₄, HCl and H₃PO₄, given by Equations 3, 4 and 5, respectively:

$$Y_1 = 70.891 + 5.561X_1 + 0.078X_2 - 2.06 X_1X_1 + 0.047 X_1X_2 - 0.002 X_2X_2 \quad (R^2 = 0.918) \quad (3)$$

$$Y_2 = 73.027 + 10.081X_1 - 0.043X_2 - 3.48 X_1X_1 + 0.045X_1X_2 - 0.0008 X_2X_2 \quad (R^2 = 0.829) \quad (4)$$

$$Y_3 = 72.553 + 1.423X_1 + 0.113X_2 - 0.858 X_1X_1 + 0.009 X_1X_2 - 0.0008X_2X_2 \quad (R^2 = 0.905) \quad (5)$$

The response surface plots for hydrolysis of AHE with H₂SO₄, HCl and H₃PO₄ using Equations 3, 4 and 5 is given in Figure 1. The maximum total sugars from the hydrolysate were selected for morphology by SEM and sugar hydrolysis using HPLC as shown in Figures 2 and 3, respectively. The figures from SEM showed that the structure of durian peel was destroyed by hydrolysis with H₂SO₄, HCl and H₃PO₄. Dilute acid hydrolysis is an effective biomass pretreatment. This process modifies the structure of the biomass by breaking/loosening the lignin, solubilizing hemicelluloses and disrupting the crystalline structure of cellulose (Mosier et al., 2005).

Hydrolysis reactions of sugar polymers in a dilute-acid medium are very complex. The substrate is in a solid phase and the catalyst, in a liquid phase. The mechanism of the hydrolysis reaction includes (Harris, 1952; Fengel and Wegener, 1984; Aguilar et al., 2002): (i) diffusion of protons through the wet lignocellulosic matrix; (ii) protonation of the oxygen of heterocyclic ether bond between the sugar monomers; (iii) breaking of the ether bond; (iv)

generation of the carbocation as intermediate; (v) solvation of the carbocation with water; (vi) regeneration of the proton with cogeneration of the sugar monomer, oligomer or polymer depending on the position of the ether bond; (vii) diffusion of the reaction products in the liquid phase if it permits their form and size; (viii) restarting of the second step.

Analysis of sugar by HPLC showed that hydrolysis with H₂SO₄ produced components identical to glucose, fructose and xylose, which were 20.40, 7.16 and 5.17 g/L, respectively; hydrolysis with HCl produced components identical to glucose, fructose and xylose, which were 19.39, 7.99 and 5.34 g/L respectively; hydrolysis with H₃PO₄ produced components identical to glucose and fructose, which were 9.00 and 9.95 g/L. These differ from the hydrolysis of agricultural residues such as corn hull, rice straw, corn cob, sugarcane bagasse and rice husk, where glucose and cellobiose were found (Chimtung et al., 2009). This showed that the structure and component of the cell walls of plants are significantly different from those of most species, which influence hydrolysis of biomass.

Du Toit et al. (2004) hydrolyzed sugar cane bagasses with 5% (m/v) HCl and found that xylose was the main component in the hydrolyzates; while glucose, arabinose and galactose present in the side chains of the pentosans were initially released at a fast rate. This treatment resulted in obtaining 229 mg/g xylose and 44 mg/g glucose from bagasse. Hydrolysis with H₃PO₄, glucose and fructose is valuable and similar to non-removal of xylose as shown in Figure 2c. This shows the decomposition of xylose to furfural; like in the study of hydrolysis of sugar cane bagasse, it was found that, decomposition of pentoses to furfural is low and confirms the selectivity of this treatment using phosphoric acid (Gamez et al., 2006).

The acid hydrolysis of lignocellulosics releases xylose as the main sugar constituent in hydrolysates along with small fractions of arabinose, mannose, galactose and glucose. Unfortunately, these hydrolysates also contain several fermentation inhibitors, such as furan derivatives from degradation of sugars, aliphatic acids released from hemicellulosic acetyl groups, phenolics from lignin. The compositional profile of hemicellulose hydrolysates depends upon the cell wall composition and the method employed for cell wall digestion (Chandel et al., 2007, 2010; Hahn-Hagerdal et al., 2007).

From the results of the effect of sulfuric and phosphoric acid pretreatment (0 to 2.0% concentrations of H₂SO₄ and H₃PO₄ at 121°C for 30-60 min) of corn stover in batch reactor on enzymatic hydrolysis, it was found that digestibility increased with higher acid concentration; and H₂SO₄ pretreatment method showed higher digestibility. H₂SO₄ effectively solubilized the hemicellulosic portion of the corn stover and increased the digestibility of the cellulose that remained in the solid residues. H₃PO₄ treatment had considerably lower hemicelluloses degradation (Byong-Hwan et al., 2003). In the study of the effect of phosphoric acid concentration on properties of peanut shell adsor-

Table 1. Effects of concentration of acid and hydrolysis time to AHE, reducing sugar and total sugar.

Parameter	Concentration (%)	Time (min)	Hydrolysis durian peel in autoclave at 121°C		
			H ₂ SO ₄	HCl	H ₃ PO ₄
AHE (%)	0.5	15	74.29 ^{ef}	76.41 ^c	74.83 ^e
		30	73.90 ^f	76.23 ^c	75.26 ^{de}
		45	74.40 ^{ef}	76.12 ^c	76.91 ^{ab}
		60	72.15 ^g	70.78 ^d	77.34 ^a
	1.0	15	75.76 ^{cd}	79.20 ^b	74.51 ^e
		30	75.73 ^{cd}	79.16 ^b	76.06 ^{bcd}
		45	76.01 ^{cd}	78.59 ^b	77.05 ^{ab}
		60	74.26 ^{ef}	78.41 ^b	77.33 ^a
	1.5	15	77.33 ^a	79.91 ^{ab}	75.29 ^{de}
		30	77.41 ^a	79.59 ^{ab}	75.47 ^{cde}
		45	77.26 ^a	78.59 ^b	77.09 ^{ab}
		60	76.26 ^b	79.23 ^b	76.87 ^{ab}
	2.0	15	75.15 ^{de}	80.99 ^a	73.33 ^f
		30	77.55 ^a	79.88 ^{ab}	75.01 ^{de}
		45	77.25 ^a	79.02 ^b	76.59 ^{abc}
		60	77.09 ^{ab}	79.33 ^b	76.97 ^{ab}
Reducing sugar (g/L)	0.5	15	22.40 ⁱ	23.39 ^k	15.32 ^j
		30	26.85 ^h	24.03 ^{jk}	24.04 ^{gh}
		45	27.84 ^h	27.08 ^j	37.75 ^c
		60	31.24 ^g	31.81 ⁱ	31.13 ^{ef}
	1.0	15	32.24 ^g	33.24 ⁱ	24.73 ^{gh}
		30	43.62 ^c	40.83 ^{gh}	26.36 ^{gh}
		45	45.56 ^b	48.01 ^{dc}	50.51 ^a
		60	36.23 ^f	47.36 ^{dc}	45.40 ^b
	1.5	15	37.77 ^c	38.77 ^h	25.14 ^{gh}
		30	46.54 ^b	45.17 ^{ef}	26.60 ^g
		45	49.79 ^a	53.63 ^{ab}	42.55 ^b
		60	39.30 ^d	52.84 ^{abc}	34.95 ^{cde}
	2.0	15	42.49 ^c	42.55 ^{fg}	22.13 ^h
		30	50.71 ^a	50.21 ^{cd}	27.99 ^{fg}
		45	46.76 ^b	56.07 ^a	36.04 ^{cd}
		60	37.26 ^e	52.51 ^{bc}	32.71 ^{de}
Total sugar (g/L)	0.5	15	30.67 ^m	27.14 ^j	42.77 ^f
		30	30.95 ^m	27.60 ^j	44.48 ^{def}
		45	36.95 ^j	30.35 ⁱ	45.78 ^{de}
		60	33.83 ^l	31.86 ^h	46.66 ^d
	1.0	15	36.14 ^k	40.47 ^g	44.82 ^{def}
		30	47.29 ^f	41.30 ^g	45.27 ^{de}
		45	48.13 ^{ef}	49.52 ^d	53.04 ^b
		60	43.83 ^h	49.64 ^d	55.85 ^a
	1.5	15	38.26 ⁱ	42.81 ^f	45.55 ^{de}
		30	49.50 ^d	56.58 ^b	53.23 ^b
		45	51.51 ^b	59.56 ^a	43.68 ^{ef}
		60	48.32 ^e	59.36 ^a	49.61 ^c
	2.0	15	43.39 ^h	44.64 ^e	45.89 ^{de}
		30	53.18 ^a	59.51 ^a	49.74 ^c
		45	50.51 ^c	59.83 ^a	39.59 ^g
		60	45.72 ^g	55.38 ^b	40.23 ^g

Different letters in the same row indicate significant differences, $P < 0.05$.

bents, it was found that the acid concentration has been increased upon vaporization of water from the solution. This led to the increase in the acid concentration. Thus,

the rate of reaction between phosphoric acid and organic constituents increased as well (Lochananon and Chatsiriwech, 2008). The results suggested that the con-

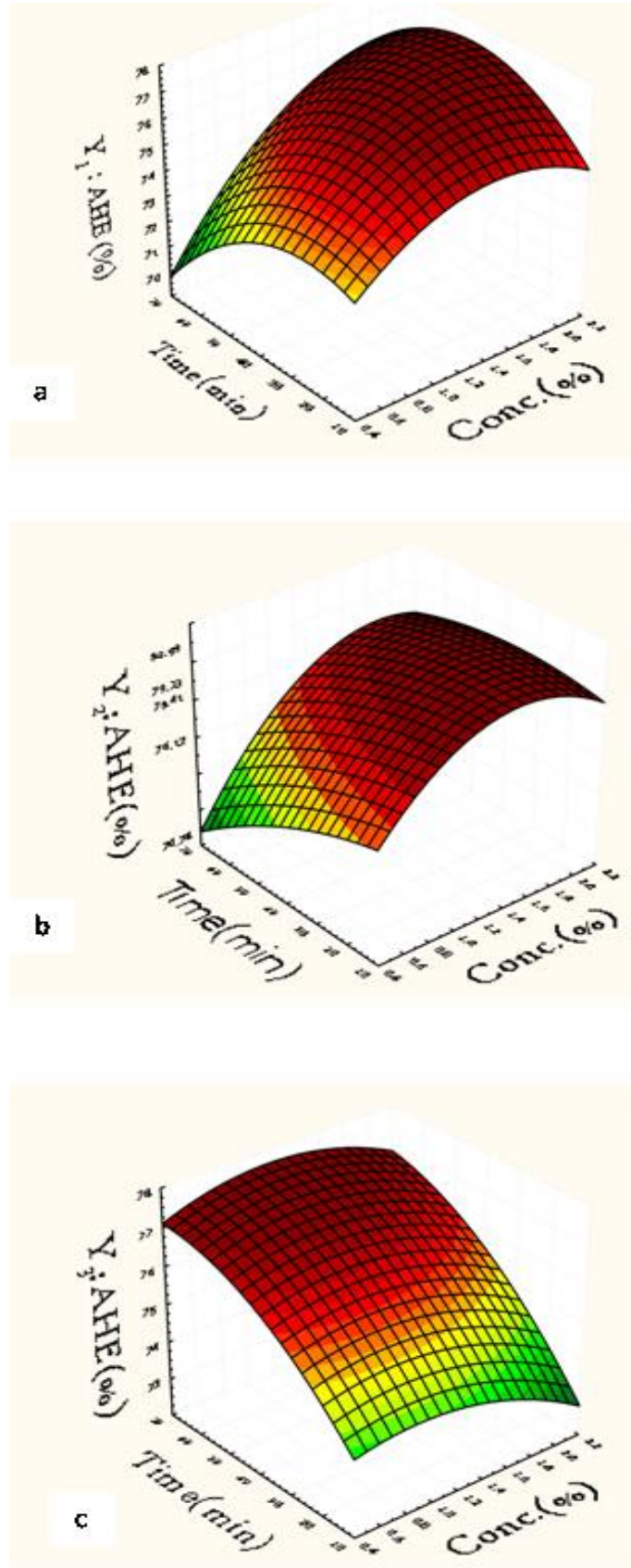


Figure 1. Response surface plots for AHE of hydrolysis with H₂SO₄ (a), HCl (b) and H₃PO₄ (c).

Table 2. Correlation coefficient of durian peel hydrolysis by acid.

Factor	AHE	Reducing sugar	Total sugar
Concentration			
H ₂ SO ₄	0.787 **	0.748 **	0.752 **
HCl	0.730**	0.809**	0.828**
H ₃ PO ₄	-0.281	0.044	- 0.124
Time			
H ₂ SO ₄	0.145	0.089	0.292
HCl	-0.345	0.435	0.331
H ₃ PO ₄	0.898 **	0.720 **	0.185

** , Correlation is significant at p< 0.01; *, correlation is significant at p < 0.05.

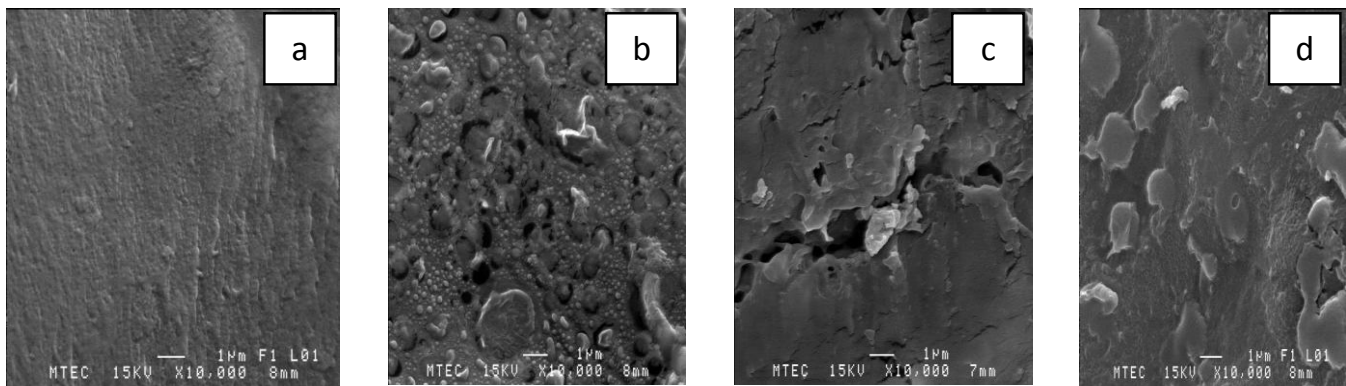


Figure 2. SEM of powders durian peel: (a), hydrolysis by H₂SO₄ at 2.0% concentration for 30 min (b),hydrolysis by HCl at 2.0% concentration for 45 min (c) and hydrolysis by H₃PO₄ at 1.0% concentration for 60 min (d).

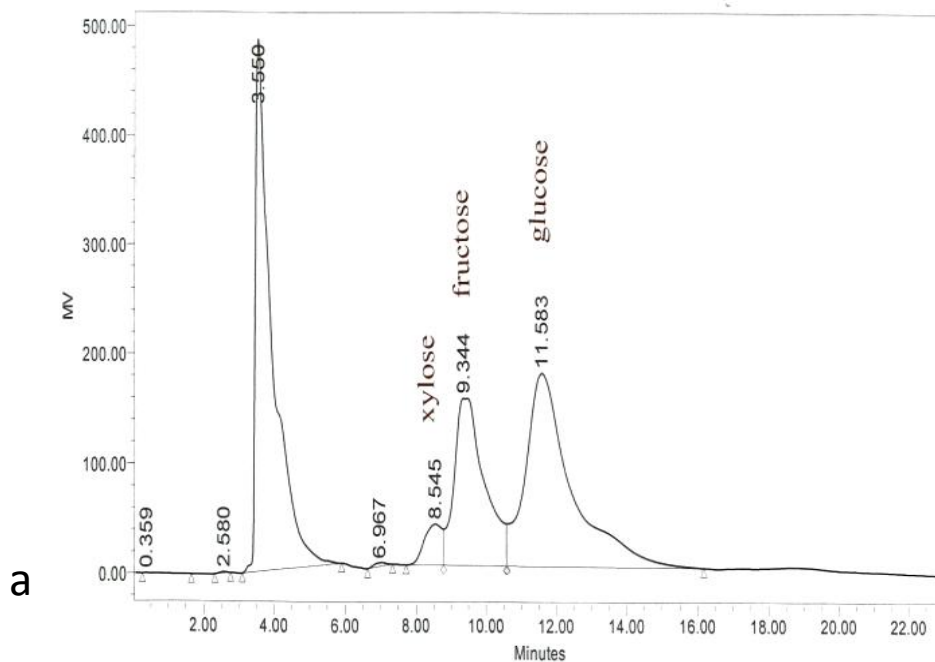


Figure 3. HPLC chromatograms showing peaks of sugars in the durian peel hydrolysis by H₂SO₄ at 2.0% concentration for 30 min (a), hydrolysis by HCl at 2.0% concentration for 45 min (b) and hydrolysis by H₃PO₄ at 1.0% concentration for 60 min (c).

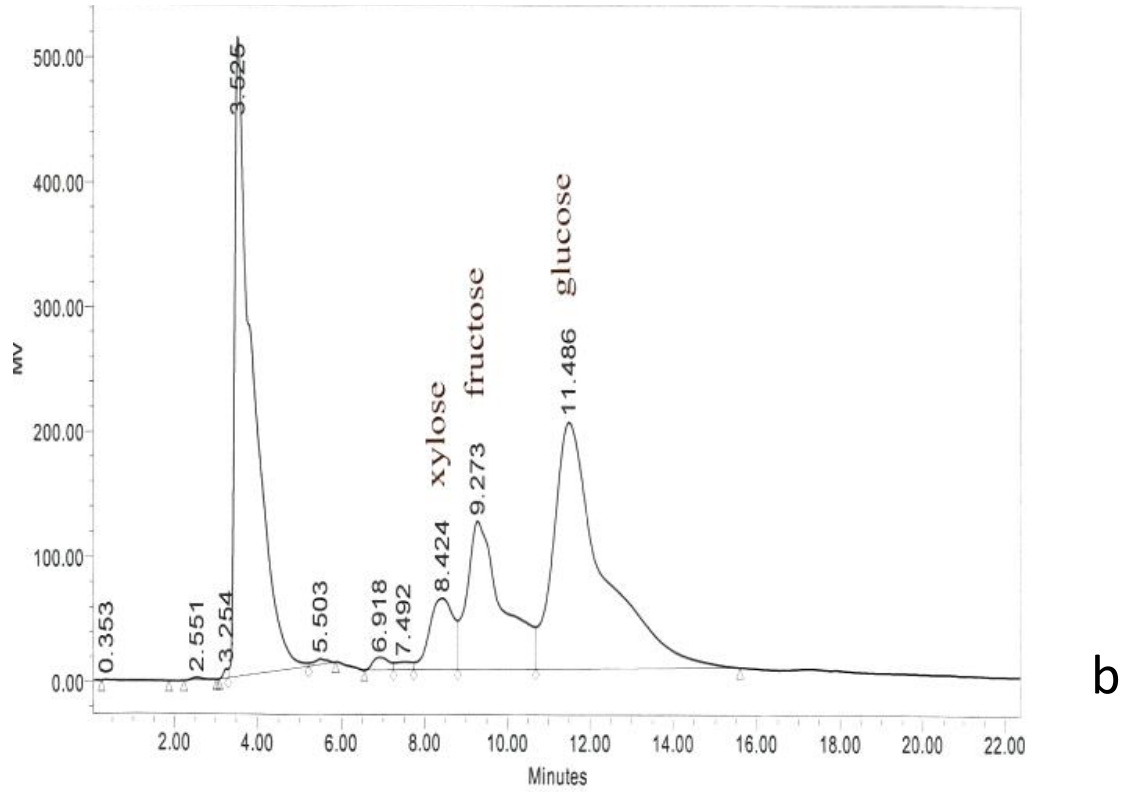


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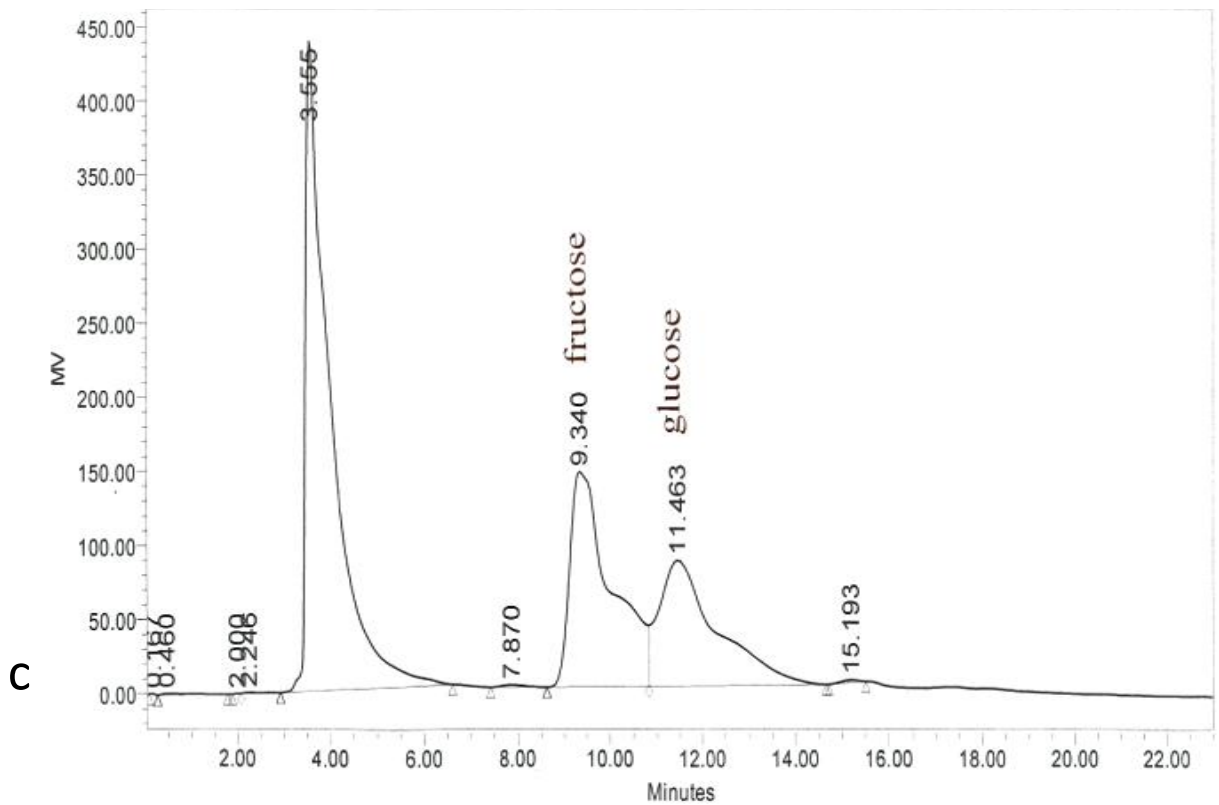


Figure 3. Contd.

stituents in peanut shell might have been dehydrated much effectively with increase in the acid concentration upon vaporization. The original mass reduced correspondingly to the fraction of oxygen and hydrogen in the peanut shell, which is about 52.7 wt% (Gomez et al., 2005); due to the limitation of amount of water and hygroscopic property of phosphoric acid (Patnak, 2003).

The effect of acid concentration and hydrolysis time on sugar produced from durian peel at 2.0% concentration for 30 min, 2.0% concentration for 45 min and 1.0% concentration for 60 min when hydrolysed by H₂SO₄, HCl and H₃PO₄, respectively was shown. Glucose is the main product obtained in the acid hydrolysis of durian peel. The glucose released in the hydrolysis can proceed from both hemicellulosic heteropolymers and cellulose like the acid hydrolysis of sugar cane bagasse (Aguilar et al., 2002). H₂SO₄, HCl and H₃PO₄ hydrolysis can be used to convert durian peel into sugar as substrate for ethanol production. It is an alternative to decrease environment problems. The proposed models were found for the predictions of AHE, reducing sugar and total sugar.

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