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Effects of ultrasonic-assisted alkaline pretreatment on xylose production from pineapple peel waste

Choosit Hongkulsup✉, Panchalee Pathanibul

Department of Food Science and Technology, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand

✉Corresponding author: *choosit.ho@ssru.ac.th*

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Abstract

The pineapple industry generates large amounts of unusable waste (peel and core) with adverse environmental impacts. This experimental study aims to systemize the potential of ultrasonic-assisted alkaline pretreatment for xylose production from pineapple peel waste. The best condition for single alkaline pretreatment (1 % NaOH w/v, 100 °C, 60 min) has obtained hemicellulose, cellulose, and lignin composition at 34.80 %, 32.16 %, and 8.66 %, respectively, retained in the biomass. Meanwhile, a combination of alkaline (1 % NaOH, w/v) and ultrasonic (frequency 40 kHz, 45 min) pretreatment has obtained the percentage yield of hemicellulose and lignin at 51.15 % and 7.15 %, respectively. Both single alkaline and ultrasonic-assisted alkaline pretreated samples were subsequently hydrolyzed with 2 % H_2SO_4 (w/v). After acid hydrolysis for 30 min, the maximum xylose concentration of 48.85 $g.L^{-1}$ was achieved by using ultrasonic-assisted alkaline pretreatment, while single alkaline pretreatment contributed to the lowest yield of xylose (37.11 $g.L^{-1}$). It is shown that the ultrasonic-assisted alkaline treatment is more favorable than single alkaline pretreatment as it can produce high xylose concentration after the subsequent hydrolysis. These results indicated that ultrasonic-assisted alkaline pretreatment and its subsequent acid hydrolysis were appropriate for producing xylose from pineapple peel waste.

Introduction

Smooth cayenne pineapple (*Ananas comosus* L. Merr.) is one of the most valuable and popular tropical fruits in the world. As stated by the Ministry of Agriculture and Cooperatives of Thailand, Thailand is ranked the first in the production and export of pineapple, with approximately 50 % of the international market share (Wattanakul *et al.* 2020). In Thailand, different pineapple *products are* exported to the global market, including *fresh*, *frozen*, *canned*, and *juice products*. However, according to the Agricultural Informatics Center (2018), the demand and supply of pineapple products are always fluctuated. As a result, production of pineapples is higher than domestic consumption and export demand, resulting in an oversupply of pineapple in Thailand. This consequently leads to a decline in the price of pineapple products. The manufacturing of canned pineapple or pineapple juice usually generates large amounts of pineapple waste including 29 – 40 % (w/w) of peel, 9 – 10 % (w/w) of core, and 2 – 5 % (w/w) of stem, which is mainly used as a supplement in animal feed or discarded in landfills at a very low price (Ketnawa *et al.* 2012). In recent years, Thailand's manufacturing process of pineapple products generated approximately 1.62 million tons of pineapple residues (Ritthisorn 2016; Banerjee *et al.* 2019). The greater expansion of pineapple production and export will subsequently generate more pineapple waste. However, pineapple peel wastes contain a major source of lignocellulosic materials. The chemical composition of pineapple residues contains approximately 38% to 50 % of cellulose, 23 % to 32 % of hemicellulose, and 13% to 30 % of lignin (Sierra *et al.* 2008). Therefore, hemi-cellulosic resources, including agricultural and industrial residues, offer a suitable alternative for the extraction of high-value renewable products (Kunaver *et al.* 2016). Lignocellulosic waste has become an alternative natural resource for the extraction of a valuable sugar, xylose.

The interesting challenges for sustainability in industrial chemistry and biotechnology have encouraged the development of renewable agricultural residues. Apparently, the conversion of agricultural wastes to alternative raw materials requires additional techniques to develop economic and effective procedures (Binder and Raines 2010). The typical conversion of lignocellulosic residue to reducing sugars consists of two main steps: (1) pretreatment and (2) hydrolysis. The removal and depolymerization of unwanted components and other impurities found in pineapple peel waste by various pretreatments have been widely studied. The valuable sugar can be extracted by different hydrolysis methods using chemicals and enzymes (Chongkhong and Tongurai 2017).

The application of ultrasonication in solid-liquid extraction processes is considered as mechanical energy by particles vibrating or moving within a medium. Theoretically, the formation of microbubbles during sonication treatments allows pretreated substrates to be more readily hydrolyzed by increasing accessible surface area, decreasing particle size and crystallinity (Yunus *et al.* 2010). Some studies reported that pretreatment of l*ignocellulosic materials* with ultrasonic irradiation at working frequency higher than 20 kHz could be advised for the intensification of bioconversion with different physical and chemical effects (Asakura *et al.* 2008). The utilization of sonication for the improvement of agricultural by-product seems to be a promising technology that can be readily applied for the extraction of value-added sugar from pineapple waste (Ong *et al.* 2019).

However, the excessively long reaction time and the complicated procedure for enzymatic hydrolysis as well as the higher costs compared to chemical method have limited process applicability on an industrial scale (Niwaswong *et al.* 2014). In addition, the direct usage of lignocellulosic residue from the pineapple industry as a hemicellulose extract for xylose production has been limited. Agriculturalists and local enterprises play an important role in the utilization of agricultural residues. Nevertheless, they have limited professional knowledge and lack the ability to use novel technology for converting agricultural waste into value-added products. Consequently, the government should provide a policy environment and adequate financial support that encourages the sustainable development of agricultural waste utilization (Xia and Ruan 2020). The improvement of pretreatment processes to produce xylose and its derivatives is therefore necessary. The effect of ultrasonication on the pineapple peel prior to acid hydrolysis was evaluated. This experimental study aims to demonstrate the potential of ultrasound-assisted alkaline pretreatment and acid hydrolysis for xylose extraction from pineapple peel waste in a systematic way.

Experimental

Fresh pineapple peels were collected from the fruit market in Prachuap Khiri Khan province, Thailand. Firstly, the pineapple peels were washed with tap water to remove foreign materials and left under the sun to dry. Washed and dried peels were cut into small pieces and further dried at 60 °C in a hot-air oven until the moisture content is less than 10 % of wet basis. After that, the dried peels were ground and sieved to obtain particle sizes of less than 2 mm. The ground dried peels were then packed in sealed plastic bags and stored at room temperature before use. The smaller particle size is expected to increase the accessible surface area, improving the efficiency of ultrasonic treatment (Zheng *et al.* 2009). Proximate analysis of pineapple peel wastes was evaluated in accordance with the standard AOAC (2012).

Chemical pretreatment

Dried pineapple peel waste (20 g) was treated under different solvent conditions: water; 1 %, 2 %, and 4 % H_2SO_4 (w/v); 1 %, 2 %, and 4 % NaOH (w/v); a solid-to-liquid ratio of 1 : 10 (w/v) and stirred at ambient temperature for 15 min. The chemical pretreated sample was heated at 100 °C and atmospheric pressure for 60 min in a sealed 1 L glass beaker placed in a water bath with temperature controller (Sukruansuwan and Napathorn 2018). It was then neutralized and filtered by water washing to remove a solvent on the surface of the sample. The neutralized pineapple peel was dried overnight at 60 °C in a hot air oven. The dried sample was then ground to a mesh size of 0.75 – 1 mm by a laboratory mill machine (CT 293 Cyclotec™, FOSS, Hillerød, Denmark). The chemical composition of pretreated samples was analyzed for hemicellulose, cellulose, and lignin following the detergent fiber analysis according to Van Soest *et al.* (1991). The best conditions for chemical pretreatment of the pineapple peel obtained were used for the next experiment.

Ultrasonic pretreatment

The ultrasonic pretreatment procedure of pineapple peels was performed with 1 % w/v NaOH (the pretreatment condition obtained from the previous section) in a solid-to-liquid ratio of 1 : 10 (w/v). The mixture samples then underwent sonication using 4 different ultrasonication times (15, 30, 45, and 60 min) under the following conditions: temperature 50 °C; working frequency 40 kHz; and ultrasonic power 200 W (LT-200-PRO, TierraTech®, Guarnizo, Spain). The control sample was unsonicated (0 min) but was added to 1 % NaOH (w/v), incubated at 50 °C and stirred for 60 min. After ultrasonication, the pretreated samples were washed using distilled water and filtered using a filter paper. The solid residue was then dried at temperature of 60 °C overnight in a hot air oven until a constant weight was reached. The ultrasonic pretreated samples were kept in vacuum packaging before subsequent chemical hydrolysis (Koutsianitis *et al.* 2015).

Acid hydrolysis

Pretreated pineapple peel (10 g) was completely mixed with 2 % H_2SO_4 (w/v) in a solid-to-liquid ratio of 1 : 10 (w/v). The acid hydrolysis treatments were conducted at 100 °C and atmospheric pressure for 30 min in a sealed 1 L glass beaker placed in a water bath with temperature controller. After hydrolysis, the hydrolysates were neutralized by 6 M NaOH and filtered using a 0.22 µm membrane filter. The filtrates (hemicellulosic fraction) were then kept in vials prior to xylose analysis by HPLC (Yunus *et al.* 2010).

Characterization of pineapple peel

The detergent fiber method (Van Soest *et al.* 1991) for the analysis of fiber-rich feeds, and currently most frequently applied for fiber analysis in forage, provides a more suitable alternative to better characterize the carbohydrates in the plant cell walls. In this technique, the fractions of fiber that are insoluble either in acid detergent or in neutral detergents were measured, and the residue after treatment of acid detergent fiber (ADF) fraction with 12 $mol.L^{-1}$ sulfuric acid was considered to be acid detergent lignin (ADL) and calculated by the following equations (Eq. 1; Eq. 2):

$$\%\ \text{cellulose} = \text{ADF} - \text{ADL} \qquad (1)$$

$$\%\ \text{hemicellulose} = \text{NDF} - \text{ADF} \qquad (2)$$

where NDF is the neutral detergent fiber (%, w/w) and ADF is the acid detergent fiber (%, w/w), while ADL represents the acid detergent lignin (%, w/w).

HPLC analysis

The oligomer composition of sugar supernatant was determined by High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan) equipped with two LC-20AD pumps, a DGU-20A5 degasser, an SIL-20AC auto sampler and a refractive index detector (RID-10A). The column was an Inertsil NH2 column (5 µm particle size, 250 mm x 4.6 mm, GL Sciences Inc., Tokyo, Japan) and its temperature was maintained at 40 °C. The mobile phase was 85 % acetonitrile and 15 % ultra-pure water at a flow rate of 0.50 mL/min and 10 µL of sample was injected through a 0.22 µm filter into the HPLC (Veena *et al*. 2018).

D-(+)-xylose standard purchased from Merck Life Science UK Limited (Gillingham, UK) was used as standard. The peak areas were used to plot the calibration curve for the determination of the amount of oligomer in the sample, which were determined from the calibration curves of the corresponding standards.

Statistical analysis

All data were statistically considered by analysis of variance (ANOVA) followed by post hoc test using Duncan's multiple range test (DMRT) and mean plots treatment. Statistical processing was analysed by SPSS 19.0 software (IBM, Somer NY, USA) for the Window statistical package. The differences between the mean values were carried out at 95 % confidence interval ($P < 0.05$). All data described in figures and tables were the mean values of triplicate determinations.

Results and Discussion

Proximate composition of pineapple peel

The chemical compositions of the pineapple peels were determined following the methods described above. The results obtained are given in Table 1. It is evident that the values given compare favorably with the normal values for fiber and carbohydrate content (37.30 % and 52.33 %, respectively) reported by Zakaria *et al.* (2021). The fiber content of pineapple peels was about 31.31 %, while the total carbohydrate content is approximately 56.94 %. In general, pineapple waste was rich in lignocellulosic material, especially the peels and the leaves of the fruit (Sukruansuwan and Napathorn 2018).

Table 1. Proximate composition on % (w/w) dry basis of pineapple peel.

Sample	**Chemical compositions [%, dry basis]**					
	Moisture	**Ash**	**Protein**	**Fat**	**Fiber**	**Carbohydrate**
Pineapple peel	12.51 ± 0.62	4.82 ± 0.35	6.29 ± 0.55	0.64 ± 0.10	31.31 ± 0.91	56.94 ± 2.03

Effects of different chemical pretreatments on pineapple peels

The major lignocellulosic components, such as cellulose, hemicellulose and lignin, were determined by the detergent fiber analysis based on the gravimetric method. As shown in Table 2, the percentage yields of cellulose, hemicelluloses, and lignin in the raw pineapple peel powder were 31.56 %, 26.35 %, and 10.70 %, respectively. In the current study, the effect of pretreatment using different solvents, such as water, acid, and alkaline, was systematically investigated. It is clear from Table 2 that pineapple peels pretreated with water and acid solution (H_2SO_4) yielded the lower amount of hemicellulose, whereas pretreated with alkaline solution (NaOH) still retained a high hemicellulose content of 31.57 % to 34.80 %. This observation suggests that hemicellulose degraded more easily under acid treatment than under alkaline treatment (Ying *et al.* 2014). Owing to its amorphous structure, hemicellulose is more prone to hydrolysis by mild acid solution compared to crystallized cellulose which likely requires more severe treatment conditions (Zhang *et al.* 2012). Considering the utilization of hemicellulose, its component is usually composed of xylan which can further be converted into xylose monosaccharides. For lignin analysis, the lignin contents from water and acid pretreatment were similar or slightly lower than that of the untreated sample, which demonstrated that a small part of the lignin was removed during pretreatment (Ying *et al.* 2014). Noticeably, the removal of hemicellulose and cellulose may slightly increase the percentage of lignin in fiber. In contrast, alkaline pretreatment was more capable in removing lignin; where up to 33.36 % of lignin content was removed resulting in the availability of cellulose and hemicellulose for chemical hydrolysis (Pandey *et al.* 2000). Considering, both hemicellulose yield and lignin removal, alkaline pretreatment using 1 % NaOH was chosen for obtaining the highest hemicellulose content and deemed to be suitable for further experiments.

Table 2. Effects of different chemical pretreatments on characterization of pineapple peel.

Pretreatment condition	Composition of pineapple peel [%, dry basis]		
	Hemicellulose	Cellulose	Lignin
Untreated	31.56 ± 1.90^{b}	26.35 ± 1.45^{b}	10.70 ± 1.04^{a}
water	28.72 ± 1.79^{c}	23.48 ± 2.33^{c}	11.16 ± 1.02^{a}
1 % NaOH	34.80 ± 1.10^{a}	32.16 ± 2.16^{a}	8.66 ± 0.98^{b}
2 % NaOH	33.36 ± 1.94^{a}	31.36 ± 1.45^{a}	7.56 ± 0.81^{c}
4 % NaOH	31.57 ± 1.35^{b}	28.83 ± 2.11^{b}	7.13 ± 0.86^{c}
1 % H_2SO_4	26.97 ± 1.90^{c}	23.44 ± 1.45^{c}	10.30 ± 0.98^{a}
2 % H_2SO_4	24.43 ± 1.78^{d}	21.53 ± 1.28^{cd}	10.95 ± 1.32^{a}
4 % H_2SO_4	20.28 ± 1.54^{e}	20.51 ± 1.22^{d}	9.46 ± 1.07^{ab}

* Untreated sample is raw pineapple peel.
** Percent composition is percentage of retained lignocellulosic materials in a sample.
*** Values with different superscripts (a-e) in the same column are statistically different ($P \leq 0.05$).

Effect of ultrasonic-assisted alkaline pretreatment on pineapple peel

The ultrasonic-assisted alkaline pretreatment of pineapple peel powder was undertaken under five conditions depending on varying ultrasonication time from 0 to 60 min. Based on the results of the previous experiment; an alkali pretreatment (1 % NaOH) was chosen to determine the effect of ultrasonic-assisted alkaline pretreatment on the composition of pineapple peel. Table 3 demonstrates that the combination of ultrasonic and alkaline pretreatments could significantly decrease the total lignin content with time up to 60 min to reach a minimum value of 6.94 %. A significant percentage of hemicellulose increases gradually with irradiation time up to 45 min, and then it decreases slightly to 50.63 %. The application of excessive ultrasonication time did not further increase hemicellulose yield. During pretreatment, ultrasonication helped in delignification due to cleavage of inter- and intramolecular linkages and the cavitation of microbubbles which increased the available surface area of the cellulose and hemicellulose (Koutsianitis *et al.* 2015).

Table 3. Effects of ultrasonication time and 1% NaOH pretreatment on characterization of pineapple peel.

Ultrasonication time [min]	After pretreatment with 1 % NaOH [%, dry basis]		
	Hemicellulose	Cellulose	Lignin
0	33.22 ± 2.13^{d}	28.31 ± 1.32^{c}	9.96 ± 0.94^{a}
15	40.88 ± 2.45^{c}	26.80 ± 1.90^{bc}	8.67 ± 0.86^{b}
30	47.19 ± 2.16^{b}	27.62 ± 1.10^{bc}	7.46 ± 0.98^{bc}
45	51.15 ± 1.90^{a}	29.82 ± 1.83^{ab}	7.15 ± 0.97^{c}
60	50.63 ± 2.77^{a}	30.01 ± 1.68^{a}	6.94 ± 1.18^{c}

* Percent composition is percentage of retained lignocellulosic materials in a sample.
** Values with different superscripts (a-d) in the same column are statistically different ($P \leq 0.05$).

A similar research was demonstrated by Zhang *et al.* (2018) who stated that there was a reduction in lignin content after ultrasonication on rice straw. This is also in agreement with the report of Loow *et al.* (2016), who stated that the ultrasonic method helped to decrease quantities of lignin in biomass for reducing sugar recovery. In addition, ultrasonic application has been shown to increase the delignification of lignocellulosic materials, as the degradation of lignin-carbohydrate bonds would increase the accessibility for solvent penetration which may improve the extraction of reducing sugar (Ong *et al.* 2019). Therefore, ultrasonication time of 45 min is suitable for obtaining the highest hemicellulose yield from pineapple peel.

Effect of ultrasonic-assisted alkaline pretreatment and acid hydrolysis on xylose production

Basically, xylose is a reducing sugar obtained by hydrolysis of hemicelluloses, as it can be converted to valuable by-products such as xylitol, ethanol, and furfural. As shown in Table 4, the ultrasonication time of 45 min followed by 2 % H_2SO_4 with hydrolysis time of 30 min could yield a maximum xylose concentration around 48.85 $g.L^{-1}$ which is a considerable improvement in comparison with the highest xylose concentration (24.1 ± 0.4 $g.L^{-1}$) obtained in previous studies (Sukruansuwan and Napathorn 2018; Banerjee *et al.* 2019; Ariffin *et al.* 2020). An upward trend in sugar yield by using ultrasonic-assisted alkaline pretreatment was observed with a reduction in lignin content. These results were obtained due to the breakdown of the lignin matrix leading to an increase in the accessible surface of hemicelluloses within the pineapple peel. However, an excessive ultrasonic irradiation time of more than 45 min caused no further increment in xylose yield. This finding suggested that prolonged ultrasound duration would not be necessary for producing higher sugar yield but unfavorably it may cause adverse effects due to collision and aggregation between particles (Baruah *et al.* 2018; Ong *et al.* 2019). Similar findings were revealed by Ong *et al.* (2019) and Yunus *et al.* (2010) who stated that ultrasonic irradiation helps to increase xylose contents extracted from oil palm fronds and empty fruit bunches, respectively. Under the conditions obtained from this study (NaOH concentration of 1 %, ultrasonication time of 45 min, and hydrolysis time of 30 min), the maximum xylose yield was achieved. Thus, the data obtained from the best conditions of xylose production from pineapple peel waste using combination pretreatment would be potentially useful information not only for producing value-added product but also helps solve the environmental pollution.

Table 4. Effect of ultrasonic-assisted alkaline pretreatment and acid hydrolysis on xylose production.

Pretreatment with 1 % NaOH	After 2 % H_2SO_4 hydrolysis 30 min
Ultrasonication time [min]	**Xylose [$g.L^{-1}$]**
0	37.11 ± 2.10^{d}
15	40.13 ± 2.94^{c}
30	44.05 ± 2.76^{b}
45	48.85 ± 2.45^{a}
60	47.20 ± 3.12^{ab}

* Values with different superscripts (a-d) in the same column are statistically different ($P \leq 0.05$).

Conclusions

The feasibility of using pineapple peel waste as a lignocellulosic material for xylose production was evaluated. The present work represents a systematic attempt to investigate the effects of ultrasonic-assisted alkaline pretreatment on the best conditions of xylose production. The results have shown that alkaline pretreatment using simple ultrasonic technology is a practical alternative for production of valuable xylose sugar compared to costly catalysts or expensive enzymes. In this study, the best ultrasonic condition at 40 kHz for 45 min with 1 % NaOH pretreatment and hydrolysis time of 30 min successfully achieved 33.18 % delignification while producing high xylose concentration up to 48.85 $g.L^{-1}$. The ultrasonic-assisted alkaline pretreatment with acid hydrolysis exhibited better xylose yields and lower costs of extraction in comparison with an enzymatic method. Thus, the data obtained from the effect of ultrasonication on alkaline pretreatment and acid hydrolysis would be potentially useful for the application of pineapple peel waste as raw material for large scale xylose production.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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