Ultrasonic-assisted extraction of antioxidants and antimicrobials from sugarcane bagasse

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Abstract
Sugarcane bagasse, a lignocellulosic waste from the sugar industry, is a potential source of antioxidants and antimicrobials. This study aimed to evaluate the optimal extraction conditions of these compounds from sugarcane bagasse lignin using ultrasonic-assisted solvent extraction. The dilute acid-pretreated sugarcane bagasse was steam-explosed for 10 min at 210°C and then extracted with hydrous ethanol at 40°C for 3, 5 and 7 min, and 45.6, 68.4 and 91.2 micron amplitude. The results showed that the optimal condition was 5 min and 68.4 micron, providing the yield of 92.64 ± 8.59 mg/g, antioxidant activity in terms of half maximal inhibitory concentration of 0.69 ± 0.00 mg/mL, total phenolic contents 26.71 ± 2.21 mg gallic acid equivalents/g, total flavonoid contents of 2.14 ± 0.14 mg quercetin equivalents/g, and total carbohydrate contents of 35.467 ± 1.319 mg glucose equivalents/g. The minimum inhibitory concentrations of the extract against Staphylococcus aureus, Salmonella Enteritidis S003, and Escherichia coli O157:H7 tested by agar dilution were 1.77, 7.10, and 7.10 mg/mL, respectively. These findings suggest that the ultrasonic-assisted solvent extraction is an efficient method. In addition, due to the antioxidant and antimicrobial properties of the extracts, they have potential application in health-related industries.

Key words
Ultrasonic-assisted extraction, antioxidants, antimicrobials, sugarcane bagasse

INTRODUCTION
Sugarcane bagasse has a lignin content of 19.6% (Li et al. 2012) and is mainly composed of p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which all have antioxidant activity (Li et al. 2013) and antimicrobial activity (Zhao et al. 2015). This study aimed to evaluate the optimal extraction condition of these compounds from dilute acid-pretreated and steam-explosed sugarcane bagasse using ultrasonic-assisted solvent (UASE) extraction. Steam explosion is the most commonly used method for pretreatment of lignocellulose (Brodeur et al. 2011). UASE is a novel extraction technique, offering shorter extraction time with higher extraction efficiency compared to conventional techniques.

MATERIALS AND METHODS
UASE of dilute acid-pretreated and steam-explosed sugarcane bagasse
Sugarcane bagasse was provided by Kaset Thai International Sugar Corporation Public Co. Ltd., Thailand. It was pretreated by soaking in 0.85% v/v sulfuric acid for 16 h. Steam explosion was performed by using equipment provided by Japan International Cooperation Agency, Japan. The steam explosion condition was 210°C and 10 min. Then, it was extracted using UAE with hydrous ethanol with the ratio of 1:20 (w/v) at 40°C for 3, 5, or 7 min. The optimal time was selected for a further study of the extraction amplitudes of 45.6, 68.4 and 91.2 micron. The properties of the sugarcane bagasse extracts (SBE) were determined as below.

The solid yield (%) was calculated based on the weight ratio of SBE to sugarcane bagasse.

Antioxidant activity was determined with the ABTS⁺⁺(2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt scavenging assay according to Re et al. (1999). The ABTS⁺⁺ stock solution was diluted with phosphate buffer saline. 20 μL of SBE was added with 2.0 mL of ABTS⁺⁺ solution and the mixture was incubated for 6 min in the dark. The absorbance was then measured at 734 nm and the IC₅₀ value was calculated.

Total phenolic content (TPC) was determined according to Slinkard and Singleton (1977). 0.2 mL of SBE was mixed with 0.8 mL of 0.2 N Folin-Ciocalteu reagent and the mixture was left in the dark for 20 min. 1 mL of 8.5% w/v sodium carbonate...
was then added and the mixture was incubated in the dark for 90 min. The supernatant obtained from centrifugation at 12,000 rpm for 15 min was measured spectrophotometrically at 765 nm. The TPC value was expressed as mg gallic acid equivalents (GAE)/g.

Total flavonoid content (TFC) was determined according to Lin and Tang (2007). 0.25 mL of SBE was mixed with 0.75 mL methanol, and then 50 μL of 10% (w/v) AlCl₃ and 50 μL of 1M CH₃COOK were added. The sample was incubated in the dark for 40 min. The absorbance of the solution was measured at 430 nm. The TPC value was expressed as mg quercetin equivalents (QE)/g.

Total carbohydrate content (TCC) was determined according to Dubois et al. (1956). 0.50 mL of samples was mixed with 0.25 mL of 5% (w/v) phenol and 2.5 mL of 97% (v/v) sulfuric acid. The sample was then incubated at 55°C for 10 min. The absorbance was measured at 485 nm. The TCC value was expressed as mg glucose equivalents (GE)/g.

Minimal inhibitory concentration (MIC) values of SBE against St. aureus TISTR029, S. Enteritidis S003, and E.coli O157:H7 were determined according to Griffin et al. (2000). The sample was diluted with hydrous ethanol and then mixed with nutrient agar containing SBE at various concentrations. The mixture was poured in sterile plates. The bacteria were diluted to 1.25-2.25 x 10⁷ CFU/mL and 10 μL of the cell suspension was dropped on the plate. The plates were incubated at 37°C for 18 h. The MIC value was defined as the lowest concentration without visible bacterial growth.

The results were displayed as mean ± standard deviation. One-way ANOVA followed by Duncan's new multiple range tests were performed to determine any significant differences within 95% using SPSS 15.0 software.

RESULTS AND DISCUSSION

Yield, IC₅₀, TPC, TFC, and TCC of SBE

The effects of the UASE time and amplitude on yield, IC₅₀, TPC, TFC, and TCC of SBE are shown in Tables 1 and 2. The optimal condition providing the highest yield, TPC, TFC, and TCC with the lowest IC₅₀ was 5 min and 68.4 micron. The antioxidants in SBE were phenolic compounds and flavonoids. The phenolic compounds were p-coumaric and ferulic acid (Martín et al. 2007) and the flavonoids could be tricin-4’-o-(erythro)-7-o-glucopyranoside (Colombo et al. 2006).

**Table 1. Effects of UASE times at amplitude of 68.4 microns on yield, IC₅₀, TPC, TFC, and TCC of SBE.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Yield (mg/g)</th>
<th>IC₅₀ (mg/mL)</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg QE/g)</th>
<th>TCC (mg GE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>90.54 ± 5.00a</td>
<td>0.78 ± 0.00a</td>
<td>26.51 ± 1.24a</td>
<td>1.35 ± 0.24a</td>
<td>31.78 ± 1.48a</td>
</tr>
<tr>
<td>5</td>
<td>92.95 ± 7.43a</td>
<td>0.69 ± 0.02a</td>
<td>26.23 ± 1.91a</td>
<td>2.16 ± 0.12a</td>
<td>34.41 ± 2.42a</td>
</tr>
<tr>
<td>7</td>
<td>87.48 ± 3.44a</td>
<td>0.86 ± 0.01a</td>
<td>25.13 ± 0.73a</td>
<td>1.35 ± 0.26a</td>
<td>31.10 ± 0.53a</td>
</tr>
</tbody>
</table>

Means in the same column with different letters are significantly different (p<0.05).

**Table 2. Effects of UASE amplitudes at extraction time of 5 min on yield, IC₅₀, TPC, TFC and TCC of SBE.**

<table>
<thead>
<tr>
<th>Amplitude (microns)</th>
<th>Yield (mg/g)</th>
<th>IC₅₀ (mg/mL)</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg QE/g)</th>
<th>TCC (mg GE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.6</td>
<td>78.47 ± 5.56a</td>
<td>0.88 ± 0.09a</td>
<td>20.45 ± 1.21a</td>
<td>1.14 ± 0.07a</td>
<td>30.73 ± 5.70a</td>
</tr>
<tr>
<td>68.4</td>
<td>92.64 ± 8.59a</td>
<td>0.69 ± 0.00a</td>
<td>26.71 ± 2.21a</td>
<td>2.13 ± 0.14a</td>
<td>35.47 ± 1.32a</td>
</tr>
<tr>
<td>91.2</td>
<td>94.84 ± 9.74a</td>
<td>0.79 ± 0.04a</td>
<td>25.08 ± 1.94ab</td>
<td>1.79 ± 0.22a</td>
<td>33.86 ± 2.74a</td>
</tr>
</tbody>
</table>

Means in the same column with different letters were significantly different (p<0.05).

**Antimicrobial activity**

Table 3 shows the MIC values of SBE obtained from the optimal extraction condition. The results suggest that antimicrobial activity was higher against the Gram-positive bacteria (St. aureus TISTR 029) than the Gram-negative bacteria (S.
Enteritidis S003 and *E. coli* O157:H7). This was probably due to the fact that the lipopolysaccharide layer of the Gram-negative bacteria could reduce the sensitivity of the bacteria against phenolic compounds (Delgado *et al.* 2012). Phenolic compounds in SBE can cause irreversible changes of membranes through altering hydrophobicity and local rupture or pore formation in the cell membranes, resulting in leaking intracellular constituents (Zhao *et al.* 2015).

**Table 3.** Minimal inhibitory concentration (MIC) values.

<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>MIC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. aureus TISTR 029</td>
<td>1.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. Enteritidis S003</td>
<td>7.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>7.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different letters were significantly different (*p*< 0.05).

**CONCLUSION**

The optimal USAE condition for sugarcane bagasses was 5 min and 68.4 micron. SBE showed antioxidant activity and antimicrobial activity.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


Lin JY, Tang CY. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry* 101: 140-147.


**Extraction assistée par ultrasons pour obtenir des antioxydants et des antimicrobiens à partir de la bagasse**

**Résumé.** La bagasse, un déchet lignocellulosique provenant de l’industrie sucrière, est une source potentielle d’antioxydants et d’antimicrobiens. Cette étude évalue les conditions d’extraction optimales de ces composés à partir de lignine de bagasse, à l’aide
d’ultrasons pendant l’extraction par solvant. La bagasse prétraitée avec un acide dilué est explosée par la vapeur pendant 10 min à 210°C et ensuite traitée avec de l’éthanol hydraté à 40°C pendant 3, 5 et 7 min et des amplitudes de 45,6, 68,4 et 91,2 microns. Les résultats montrent que la condition optimale était de 5 min et 68,4 micron, donnant un rendement de 92,64±8,59 mg/g, une activité antioxidante inhibitrice maximale de 0,69 ± 0,00 mg/mL, un contenu phénolique total de 26,71 ± 2,21 mg d’acide gallique équivalents/g, un contenu total de flavonoïdes de 2,14 ± 0,14 mg quercétine équivalents/g et des glucides de 35,467 ±1,319 mg glucose équivalents/g. Les concentrations minimales inhibitrices de l’extrait contre les Staphylococcus aureus, Salmonella enteritidis S003 et Escherichia coli O157:H7 testé par dilution de la gélose étaient 1,77, 7,10 et 7,10 mg/mL, respectivement. Ces résultats suggèrent que l’extraction par solvant assistée par ultrasons est une méthode efficace. En outre, en raison des antioxydants et des propriétés antimicrobiennes des extraits, ils ont un potentiel dans les industries liées à la santé.

Mots-clés: Extraction assistée par ultrasons, antioxydants, agents antimicrobiens, bagasse de canne à sucre

Extraccion ultrasonica asistida de antioxidantes y antimicrobianos del bagazo de caña de azucar

Resumen. El bagazo de caña de azucar, un desperdicio lignocelulósico de la industria azucarera, es una fuente potencial de antioxidantes y antimicrobianos. Este trabajo tiene como objetivo evaluar las condiciones de extracción optima de estos componentes de la lignina del bagazo de caña usando extracción con solvente ultrasonico asistido. El bagazo de caña diluido con acido pretratado fue explotado con vapor durante 10 minutos a 210°C y luego extraído con etanol hidro a 40°C durante 3, 5 y 7 minutos, y 45,6, 68,4 y 91,2 micrones de amplitud. Los resultados muestran que la condicion optima fue 5 minutos y 68,4 micrones, aportando un rendimiento de 92,64 ± 8,59 mg/g, actividad antioxidante en terminos de concentracion inhibitoria a media maxima de 0,68 ± 0,00 mg/mL, contenido total fonolico 26,71 ± 2,21 mg acido galico equivalentes/g, contenido total de flavonoïdes de 2,14 ± 0,14 mg quercetina equivalentes/g, y contenido total de carbohidratos de 35,467 ± 1,319 mg glucose equivalentes/g. Las concentraciones inhibitorias minimas del extracto contra Staphylococcus aureas, Salmonella enteritidis S003, y Escherichia coli O157:H7 probado por la dilucion agar fueron 1,77, 7,10, y 7-10 mg/mL, respectivamente. Estos hallazgos sugieren que la extraccion con solvente ultrasonico asistido es un metodo eficiente. Ademas, debido a las propiedades antioxidante y antimicrobiana de los extractos, estos tienen aplicacion potencial en las industrias relacionadas con la salud.

Palabras clave: Extraccion ultrasonica asistida, antioxidantes, antimicrobianos, bagazo de caña de azucar