DEVELOPMENT OF SUGARCANE WAX EXTRACTION METHODS FROM SUGARCANE FILTER CAKE FOR VALUE CREATION

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Abstract

Sugarcane filter cake is a by-product from clarification step in sugar production process. Policosanol, a high value added compound is naturally presented in sugarcane wax. To improve value creation of sugarcane filter cake, policosanol was extracted from filter cake in a form of crude wax. The more environmental friendly extraction methods, accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) were studied to extract crude wax from filter cake in comparison with Soxhlet extraction. The result showed that ASE and SFE with co-solvent could separate wax from dried filter cake with % yield of 13.32% and 5.46% while Soxhlet extraction gave 9.03%. Chemical characterization from FT-IR and TLC-FID indicated a similarity in composition and it was not different from the wax extracted directly from sugarcane stem K84-200 a Thai commercial sugarcane variety. Thermal characteristic of crude wax was also analyzed by differential scanning calorimetry (DSC).

Keywords : sugarcane filter cake, sugarcane wax, extraction

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Introduction

In production year of 2014/15, the amount of harvested sugarcane and sugar produced from 50 Thai sugar factories were 105 and 11.3 million tons, respectively. Estimation of bagasse and filter cake production was about 73.5 million tons and 3.15 million tons or equally to 70% and 3% respectively (Office of Cane and Sugar Board [OCSB], 2015). Bagasse, one of a main by-product from sugar industry is generally used to generate steam and electricity for sugar mill process and the excess electricity is sold to Electricity Generating Authority of Thailand (EGAT). In case of filter cake, it is separated as a waste from rotary vacuum during clarification step. It consisted of 8-12% fiber, 32% carbon, 2.4% phosphorus and 14.5% ash content with up to 50-70% moisture content (Pedro et al., 2010). Until now, a large amount of filter cake annually produced is a critical pollution for sugar factories as there is no value and the amount is kept increasing (Alonso et al., 2007). In several sugar mills, filter cake is disposed to plantation area as fertilizer. However, during its decomposition, it generates an acid leachate and produced amounts of greenhouse gases. Therefore, a solution for value creation of filter cake is required from sugar factories. From several works dealt with sugarcane filter cake chemical composition analysis indicated that filter cake is a source of high value-added compound such as, policosanol, long chain fatty acids and phytosterols (Phukan and Boruah (1999), Irmak et al., (2006) and Ledón et al. (2007). To enhance the economic value of filter cake and consequently overall sugar industry, various extraction techniques including health benefits of policosanol from sugarcane wax has been numerously investigated.

Sugarcane wax consisted of many of compounds such as long-chain alkanes, alkenes, aromatic hydrocarbons, fatty acids, ketones, aldehydes, esters and long chain fatty alcohols (Martínez et al., 2002; Asikin et al., 2012). These long chain fatty alcohols (policosanol) has been reported to reduce platelet aggregation, endothelial damage, foam cell formation and lowering low-density lipoproteins (LDL) and increasing high- density lipoproteins (HDL) (Varady et al., 2003). However, there is still debate continuing about the nutraceutical effect of policosanol. For instance, recent publications indicated that policosanol was ineffective in reducing blood cholesterol (Francini-Pesenti et al, 2008) while others associated policosanol to mild-to-moderate lowering of LDL cholesterol among patients intolerant of statin therapy (Wright et al,. 2005).

From previous studies, solvent extraction is mainly used for wax extraction from sugarcane filter cake. Though extraction yield is rather high, this method presents several drawbacks such as toxicity from organic solvent, high cost operation, solvent recovery requirement, as well as being energy-intensive operation (Lucus et al, 2006). Consequently, there is a demand for new extraction techniques that is more environmental friendly. Accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) are the extraction methods that the solvents are used near their supercritical region where they have high extraction properties. ASE is a solid–liquid extraction process performed at elevated temperatures, usually between 50 and 200 0C and at pressure between 10 and 15 MPa. Recently, supercritical fluid extraction (SFE) has been reported to be a novel extraction technique for nutraceutical compounds (Wang and Weller, 2006) due to its high extraction efficiency and easy to remove from the final products. As most of products from green extraction process are potentially recognized by general public as highly desirable, this work aims to study the extraction efficiency from two extraction methods (ASE and SFE) with 95% ethanol as co-extracting solvent compared with a conventional Soxhlet extraction method. Chemical composition of the obtained crude wax from filter cake was also analyzed.

Objective

To study the extraction efficiency from two extraction methods (ASE and SFE) with 95% ethanol as co-extracting solvent compared with a conventional Soxhlet extraction method.

Materials and Methods

Sugarcane filter cake

Sugarcane filter cake sample was collected from sugar mill in Chaiyaphum, Thailand. It was dried at 50oC for 24 hours before grinding with blender to obtain fine particle and keep at -20^{o}C.

Soxhlet extraction

Ten grams of dry filter cake was filled in Soxhlet apparatus. N-hexane (150 ml) was used as solvent and extraction was performed at 80^{o}C for 8 hours. The extract was evaporated to dryness and the obtained crude wax was weighed and kept at -20^{o}C.

Accelerated solvent extraction (ASE)

Extractions were performed with 2 grams of filter cake using accelerated solvent extractor (model 200, Dionex, Corp., Germany). Sample was loaded into extraction cell - stainless steel extraction chambers. The extractions of samples were performed using 95% ethanol at 2 different temperatures (60oC and 100oC) and 2 levels of solvent flushing volume (50% and 100% volume of extraction cell). The volume of obtained extracts from filter cake was adjusted to 2 folds of extract volume with distillation water and kept in cold chamber (3-5 oC) for 5-8 hours in order to solidify crude wax. It was then separated from extracts by filtration as a white insoluble crystal due to its low solubility in ethanol at low temperature. Crude wax dried at 50oC overnight in the oven. The dried weight was recorded and crude wax was stored at -20oC for further analysis.

Supercritical Fluid Extraction (SFE) with Co-solvent

The extractions was performed using small scale supercritical fluid extractor (SFE) (Thar Process, Inc. Pittsburgh, United States,). Extraction temperatures tested were 60 oC and 80 oC with the different pressure of 180 bars and 200 bars. Dried sugarcane filter cake (30 g) were placed into stainless steel extraction vials which connected to the extraction system. It was extracted with 99.99% purity carbon dioxide (Praxair (Thailand) Co.,Ltd., Bangkok, Thailand) with addition of 10% (v/v) commercial ethanol as a co-solvent. The extraction was carried out for 1 hour. The crude wax was collected and dried using rotary evaporator. The obtained crude wax was weighed and the extraction yield (%) was calculated.

Thin Layer Chromatography with flame ionization detector (TLC-FID) Analysis

Standard and sample solutions were analyzed in an IATROSCAN (MK6S, Tokyo, Japan), equipped with a flame ionization detector (FID) and interfaced with a computerized acquisition systalk (I-ChromStar Light 6.2, Software). Pure hydrogen (160 ml/min) and pure air (2.0 l/min) were used for detector. The S-III chromarod (MK1, Tokyo, Japan) used in this study was 15.2 cm long and 1.0 mm in diameter and was coated with silica gel. During experiments the chromarod was spotted with 1 μl of sample, and subsequently was developed with 2 step developing. First developing was used mixture solution of Hexane: Diethyl Ether: Acetic acid (90: 10:1, v/v/v) with 7 cm migration distance. Second developing was used mixture solution of Hexane: Toluene (50: 50, v/v) with 10 cm migration distance. The chromarod was dried at 150OC for 2 min after each development.

Fourier Transform Infrared Analysis

FT-IR spectra of the wax extracts were recorded at 30°C using a FT-IR spectrometers (Thermo Scientifics TM). The FT-IR spectrometer was scanned over the frequency range of 4000–450 cm-1 at a resolution of 8 cm-1. The spectrum was collected by using OMNIC™ Series software.

Differential scanning calorimetry (DSC)

The thermal characteristics of the wax samples were analyzed according to the method modified from Inarkar and Lele (2012) using differential scanning calorimeter (DSC1 METTLER TOLEDO, Mettler-Toledo Inc. United States). In determining the melting point, a flow of nitrogen gas (40 $mL.min^{-1}$) was purged in the cell and cooled by liquid nitrogen in a refrigerated cooling system. The sample (∼2mg) was weighed in an aluminum pan and placed in the test cell along with a similar empty reference pan. The sample was heated from 25 to 105 ^{o}C at a rate of 10°C.min-1, held at 105^{o}C for 1 min, and cooled down to 25 ^{o}C at -10 $oC.min^{-1}$. DSC curves of the last heating cycle were used to determine the melting point range.

Results and discussion

Wax extraction

Soxhlet extraction, accelerated solvent extraction and supercritical fluid extraction were applied for wax extraction from sugar cane filter cake. Four conditions of ASE and SFE were also tested and the extraction yields from all extraction conditions were compared (Figure 1). The results indicated the highest extraction yield of ASE (13.32%) when extraction temperature of 100 °C and 100% flushing volume was applied.

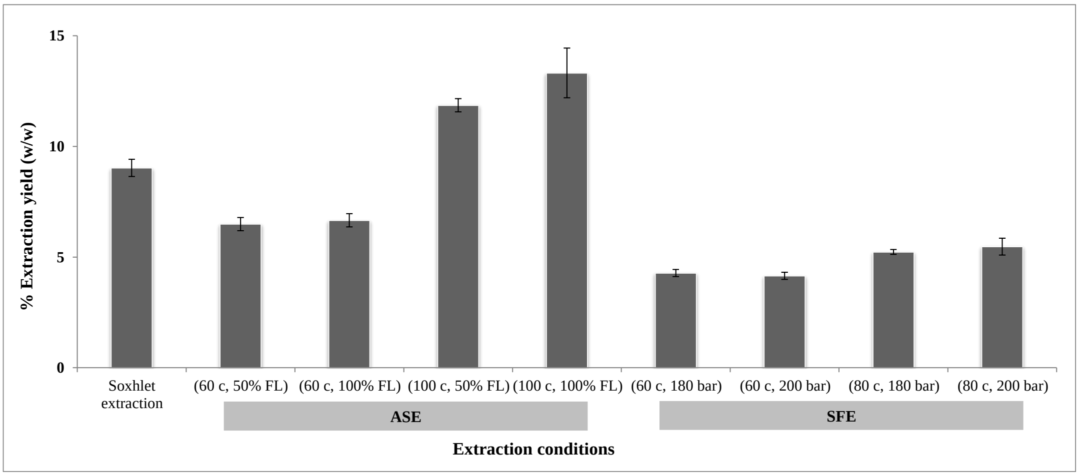

Figure 1 Crude wax extraction yield (w/w dried sugarcane filter cake) obtained from Soxhlet extraction, four condition of ASE (60 °C and 100 °C extraction temperature and 50% and 100% flushing volume (FL)) and four conditions od SFE (60 °C and 80 °C extraction temperature and 180 and 200 bar).

Among the tested conditions of ASE, it was found that at 60 °C of extraction temperature flushing volume of ethanol could not increase crude wax extract yield (6.49 to 6.66%) and its yield was even lower than that of Soxhlet extraction (9.03%). Meanwhile, increasing of extraction temperature from 60 °C to 100 °C could increase yield from around 6.49 – 6.66% to 11.9 – 13.3%. At this temperature, doubled flushing volume of solvent slightly increased the extraction yield. This may be due to the fact that increasing of extraction temperature enhanced the solubility of crude wax from filter cake and thus ethanol could dissolve higher wax content compared to that of 60 °C. This result was similar to that observed by Holser and Akin (2008) as they found that using ethanol with 90 °C extraction temperature was the most effective to separate wax from short chain compounds as compared to 50 °C, 80 °C and 100 °C. In case of supercritical fluid extraction, increasing of pressure from 180 bar to 200 bar in both extraction temperatures had no effect in increasing extraction yield (4.28% for 180 bar and 4.15% for 200 bar). Nevertheless, increasing of temperature from 60 °C to 80 °C increased around 1% extraction yield in both pressures tested. According to Wang et.al (2008), it was mentioned that an increase temperature would increase a solubility of solutes in CO2.

To foresee the potential of each extraction method, it can be seen that ASE provided the best extraction yield compared to other extraction methods. Even SFE is recently recognized as efficient and safe extraction method for various neutracuetical and bioactive compounds from plants, the main concern to adapt this technique to pilot scale is the safety to control pressure in the process. As summarized in Table 1, ASE seems to be the best choice for wax extraction from sugarcane filter cake as it took only 5 -15 minutes/ extraction cycle. Moreover, it provided around 1.5 and 2.4 times higher than Soxhlet and SFE methods, respectively.

Table 1 Extraction condition of Soxhlet extraction, accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) with co-solvent

Element	**Soxhlet extraction**	**ASE**	**SFE with co-solvent**
Dried sugarcane filter cake	10 g	2 g	30 g
Extraction Time	8 h	5-15 min	1 h
Extraction Pressure (bar)	1 bar	100 bar	180 and 200 bar
Solvent type	Hexane	95% Ethanol	95% Ethanol
Solvent volume	150 ml	10-20 ml	30 ml
Recovery time			
- Solvent removed	20 - 30 min	4 - 6 h	5 - 10 min
- Drying time	-	4 - 6 h at 50^{o}C	2 h at 50^{o}C
% Yield (w/w dried filter cake)	9.03%	13.32%	5.46%

Characterization of chemical composition of sugarcane filter cake wax

In order to compare the quality of extracted crude wax from Soxhlet and the best condition of ASE and SFE, samples from these three extraction condition was checked by FT-IR analysis. Crude wax from K84-200 was obtained from scrapping wax from sugarcane stem and then crude wax was extracted with Soxhlet method. This aims to compare if the composition of wax originally found in plants changed during sugar production process.

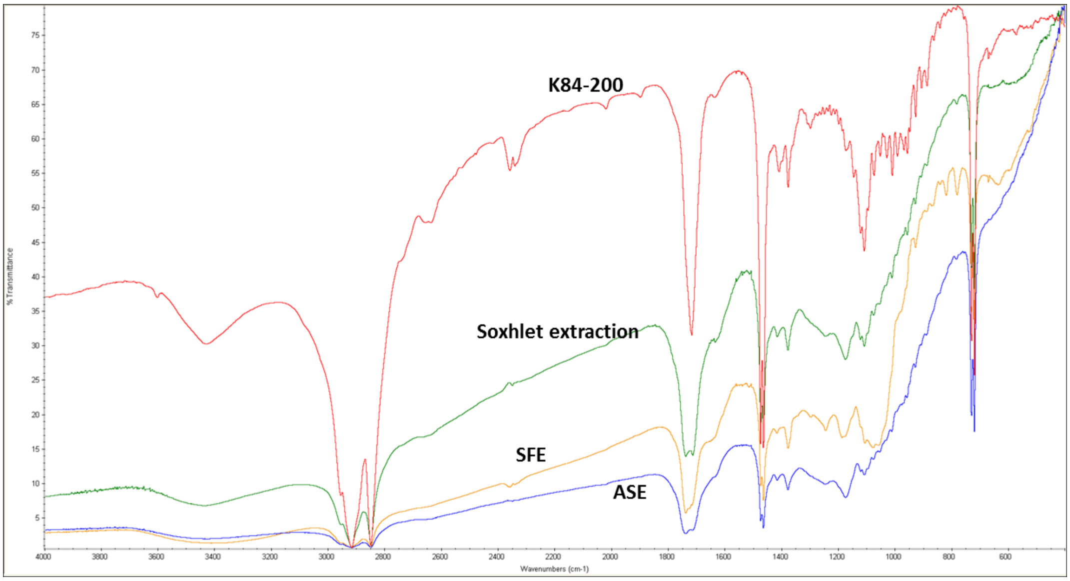

Figure 2 FT-IR spectra of sugarcane stem wax from K84-200 and filter cake wax extracted with Soxhlet extraction, ASE and SFE

FT-IR analysis revealed the presence of many organic functional groups present in crude wax indicating their respective compounds (Figure 2). The samples showed many similarities in the 3000 - 600 cm^{-1} region. According to Inarkar and Lele (2012), the bands at 2921.73 and 2851.64 cm-1 indicated the characteristic of –CH stretching and bending whereas bands at 1463.44 and 1376.96 cm^{-1} indicated –CH2 and –CH3 bending. Bands at 3395.60, 1108.4 and 1170.16 cm^{-1} showed the presence of –OH and –C–O stretching, respectively. These peaks represented the alcoholic groups in the wax samples. Similarly, bands at 1710.25 and 1736.63 cm^{-1} showed the presence of –C=O stretching (aldehyde). Other plant wax was also similarly reported to have peaks at 2918 (-C-H), 2849 (-C-H), 1745 (-C=O), 1462 (-C-H), 1169 (-C-O) and 719 cm^{-1} (-(CH2)n-) (Athukorala et al., 2009). From FT-IR results, it could be seen that profile of crude wax extracted from filter cake using the different extraction techniques were similar. Therefore, with the highest extraction yield, ASE seems to be the suitable condition.

From literature, long chain fatty alcohol or policosanol supplement can usually be found over the counter in drug market and has been reported to have beneficial physiological activities (Hwang et al., 2005). Thus, crude wax composition of filter cake obtained from ASE and Soxhlet extraction was analyzed in order to determine policosanol content. The analysis was performed using TLC-FID and the results are presented in Table 2 compared with K84-200 wax.

Table 2 Chemical composition of sugarcane filter cake wax extracted from ASE and Soxhlet extraction and extracted wax from stem of K84-200 sugarcane variety determined by TLC-FID

Samples	Content (mg/g crude wax)*			
	Wax ester	Long chain fatty aldehyde	Long chain fatty acid	Long chain fatty alcohol
ASE	140 ± 0.01^{b}	92.2 ± 0.01^{a}	74.2 ± 0.08^{b}	97.3 ± 0.05^{a}
Soxhlet	140 ± 0.00^{b}	96.5 ± 0.03^{a}	75.2 ± 0.01^{b}	97.9 ± 0.01^{a}
K84-200	139 ± 0.01^{a}	164 ± 0.01^{b}	64.2 ± 0.01^{a}	91.3 ± 0.01^{a}

The same letter in the same row is not significantly different (P<0.05)

The results showed that detected compounds in sugar cane wax from filter cake extracted with ASE and Soxhlet extractions were quite similar. The highest detected compound from both methods was wax ester (140 mg/g crude wax). The other detected compounds which are long chain fatty aldehydes and long chain fatty alcohols (policosanol) in crude wax were found in the same range of 92 – 97 mg/g crude wax. Long chain fatty acids were found in the smallest quantity compared to the others (75 mg/g crude wax). Compared to crude wax obtained from stalk, the main difference was the higher content of long chain fatty aldehydes (164 mg/ g) than that of crude wax from filter cake. From this information, filter cake could be a simple source of policosanol to access due to its large quantity produced annually.

To determine the quality of wax for application design, thermal characteristic is important. In addition, plant wax is a wide varies of melting temperature, that are important in cosmetic industry, especially in lipstick, soft cream and soap production and should have a melting point between 48 and 56oC (Bennett H., 1975). Therefore, K84-200 wax and crude filter cake wax from Soxhlet and ASE extraction were analyzed by DSC as this technic is always used for quality control technique in food, cosmetic, and pharmaceutical industries (Athukorala and Mazza, 2010). The result showed the different curve with a narrow peak of melting point at around 79.8 oC. Similarly, the melting point of crude sugarcane wax from Soxhlet extraction and ASE were 80.9 oC and 78.4 oC, respectively (Table 3).

Table 3 Thermal characteristics of sugarcane wax from K84-200 (Thai's commercial sugarcane variety, extracted by solvent extraction in hexane), Soxhlet extraction and accelerated solvent extraction (ASE; 95%ethanol, 100 bar, 10 min, 100 ^{o}C, 100% flushing volume)

Sample	Heating cycle		
	Onset (^{o}C)	Melting point (^{o}C)	Endset (^{o}C)
K84-200	76.9	79.7	81.7
Soxhlet	76.1	80.9	83.3
ASE	72.5	78.4	82.2

Conclusion

From this present work, sugarcane filter cake which is one of the main by-products from sugar production was extracted to obtained crude wax. Different extraction techniques were applied and it was found that accelerated solvent extraction (ASE) provided the highest extraction as compared to that of a conventional Soxhlet method and supercritical fluid extraction (SFE). Chemical composition analysis by FT-IR and TLC-FID indicated that extraction condition did not alter the structure of crude wax and the quantity of high value added compound, policosanol was not different. DSC results also provided the fact that this wax would be applicable for cosmetic products such as lipstick. Though the yield of policosanol seemed not to be outstanding compared to other plants but its large quantity should be taken into account. Moreover, ASE is considered as non-toxic, safe and low environment impact. To be able to scale up the extraction process, the study on economical aspect should be considered as well as the potential products that could be made from sugarcane filter cake wax.

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