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Research note

Preparation of octacosanol from filter mud produced after sugarcane juice clarification

Shiyi Ou*, Jian Zhao, Yong Wang, Ye Tian, Jiong Wang

Department of Food Science and Engineering, Jinan University, Guangzhou 510632, PR China

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ABSTRACT

Filter mud from sugarcane juice clarification containing 6.85 g/100 g waxes was used for octacosanol extraction by supercritical CO_2 and hot ethanol reflux method, respectively. Comparing with hot ethanol reflux extraction, supercritical CO_2 extraction provided a similar yield of waxes but a higher content of octacosanol in the waxes (29.65 g/100 g vs. 22.52 g/100 g). However, saponification of the waxes extracted by hot ethanol reflux extraction has significantly increased octacosanol content to 47.8 g/100 g. For high efficient preparation of octacosanol from filter mud, hot ethanol reflux extraction of waxes followed by saponification was the method of choice.

1. Introduction

Octacosanol is a main component of policosanols, the fatty alcohol mixture, found in plant waxes common in fruits, leaves, surface of plants, and whole seeds (Keller, Gimmler, & Jahreis, 2008; Taylor, Rapport, & Lockwood, 2003). Previous researchers have shown that octacosanol possessed cholesterol-lowering, anti-aggregatory, cytoprotective, ergogenic, neurological, antioxidant properties and protective effects on parkinsonism (Oliaro-Bosso et al., 2009; Taylor et al., 2003; Wang et al., 2010). Long-term clinical studies have demonstrated that, octacosanol was well-tolerated and safe (Irmak, Dunford, & Milligan, 2006), and a number of dietary supplements containing octacosanol are commercially available in the US market (Irmak et al., 2006).

Sugarcane is one of the major crops in the world and in China. Which is an ideal source of octacosanol, as its bagasse contains a higher amount of policosanol than sugarcane leaves and other materials, and has a high and stable content of octacosanol (Irmak et al., 2006; Oliaro-Bosso et al., 2009). After cane harvesting and processing, every 1000 kg of cane would produce 33 kg press mud or filter mud (Almazan, Gonzalez, & Galvez, 1998) that contains 7% of crude wax, in which octacosanol amounts to 81% (Nuissier, Bourgeois, Grignon-Dubois, Pardon, & Lescure, 2002). This suggests that the recovery of octacosanol from filter mud has potential commercial value in the health food industry. In this research, the extraction of octacosanol by supercritical CO_2 and by hot ethanol was compared.

2. Material and methods

2.1. Materials and reagents

Sugarcane bagasse and filter mud were provided by Overseas Chinese Sugar Processing Company (Taishan, Guangdong, China). The bagasse and filter mud were oven dried at 105 °C for 6 h and then grounded and sieved in a 45-mesh sieve.

Octacosanol (97.1%) was purchased from Sigma (St. Louis, MO) and was used without further purification. All other chemical reagents were of analytical or HPLC grade.

2.2. Supercritical fluid extraction

Extractions were carried out on a SFE221-50-06 extractor (Nantong Huaxing Oil equipment Co Ltd., Jiangsu, China). 100 g of filter mud was suspended in 500 ml of absolute ethanol in a 1 L stainless extraction vials and extracted with 99.99%CO_2 at a flow rate of 30 L/h.

The waxes were collected in a cooled separator at 25 °C and determined gravimetrically. The content of octacosanol in the extract was analysed according to the procedure described below. Extractions were performed in triplicate. The effects of time, temperature and pressure on the extraction efficiency were investigated.

* Corresponding author.
E-mail address: tosy@jnu.edu.cn (S. Ou).

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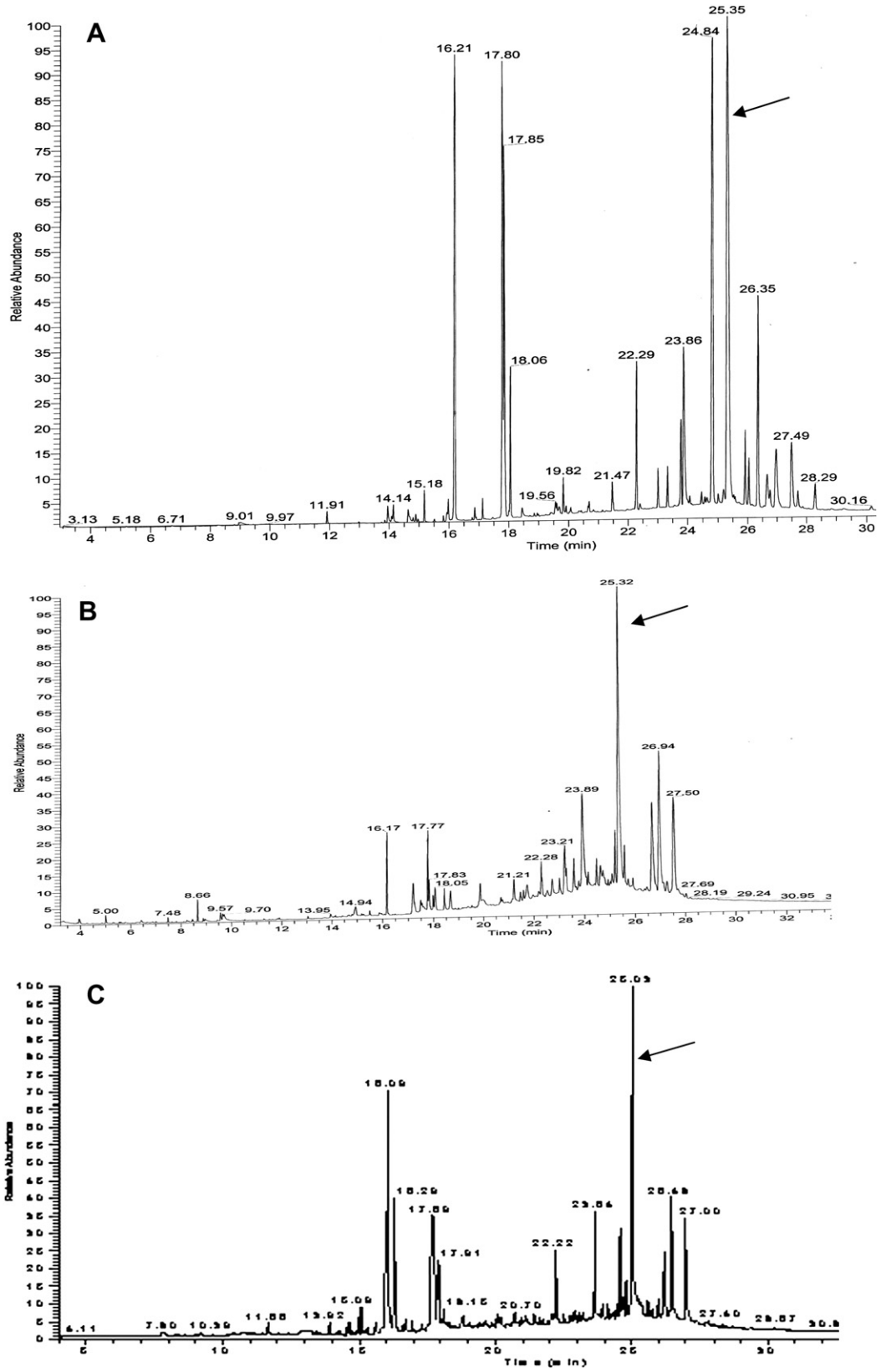

Fig. 1. GC chromatograms of waxes obtained by extraction with hot ethanol reflux before (A) and after saponification (B), and waxes obtained by supercritical CO_2 extraction (C); the arrow points to octacosanol according to the standard.

Table 1
Effect of temperature and time on the waxes yield (g/100 g) by supercritical CO_2 extraction at 30 Mpa.

Temperature(°C)	1 h	2 h	3 h	4 h	5 h
35	2.13 ± 0.02[a]	2.21 ± 0.02	2.44 ± 0.05	2.36 ± 0.02	2.64 ± 0.15
45	3.19 ± 0.03	3.48 ± 0.03	3.56 ± 0.11	3.52 ± 0.08	3.53 ± 0.17
50	3.80 ± 0.07	4.15 ± 0.07	4.32 ± 0.08	4.32 ± 0.04	4.31 ± 0.21

[a] Means ± SD($n = 3$).

2.3. Hot ethanol reflux extraction

One thousand grams of filter mud was suspended in 8 L of absolute ethanol in a 20 L multi-functional reactor (model F20H, Shanghai SENCO Technology Co. Ltd., Shanghai, China) and refluxed at 80 °C, 120 rpm for 4 h.

After extraction, the mixtures were filtered using 300-mesh nylon cloth, the filtrate was cooled to 4 °C , the green flocculates were collected by centrifugation, the sediments stand in the open air for 4 h to evaporate ethanol and dried in an oven at 60 °C. The solid (waxes) was determined gravimetrically, and the content of octacosanol in the waxes was determined by GC/MS.

2.4. Further purification of the waxes

The waxes were further purified by the following procedures. 10.0 g of the waxes was extracted using 200 mL of acetone in a Soxhlet extractor to remove chlorophyll and fat and the residue was air-dried and saponificated. The residue was placed in a 250 ml of flat bottom flask containing 100 mL of 95% ethanol and 4 g powdered sodium hydroxide and was refluxed at 80 °C for 6 h; the mixture was cooled to 50 °C and extracted with 200 mL of petroleum ether three times. The combined petroleum ether phase was cooled to 4 °C and then was filtrated using filter paper, the filtrate cake was air-dried and determined gravimetrically.

2.5. Analysis of octacosanol

2.5.1. Determination of octacosanol contents in sugarcane bagasse and filter mud

Policosanols in sugarcane bagasse and filter mud were extracted, for determination of octacosanol in the raw materials, according to Irmak et al. (2006).

Octacosanol in the extracts was analysed according to the methods developed by Chen et al. (2007) and by Imark et al. (2006). Octacosanol was analysed on a Agilent 5975C GC/MS system, equipped with an HP-5 (30 m × 0.25 mm × 0.25 μm) capillary column. The conditions used for the GC measurement were as follows. Oven temperature programmed from 80 °C to 320 °C, at 10 °C/min, and maintained at 320 °C for 15 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 300 °C. GC/MS operating temperatures were as follows: MS transfer line 280 °C, ion source 230 °C, and MS quadrupole 150 °C. The ionisation energy was 70 eV. The scan range and rate were 50–600 amu and 2 scans/s, respectively. The injection volume was 10 μL.

The calibration curves were obtained by injecting the standard solutions with concentrations ranging from 100 to 900 μg/mL.

Table 2
Effect of pressure and time on the waxes yield (g/100 g) by supercritical CO_2 extraction at 50 °C.

Presuure (Mpa)	1 h	2 h	3 h	4 h	5 h
20	2.96 ± 0.14[a]	3.24 ± 0.09	3.40 ± 0.08	3.55 ± 0.10	3.54 ± 0.08
25	3.99 ± 0.10	4.77 ± 0.14	4.87 ± 0.06	4.99 ± 0.10	5.02 ± 0.09
30	4.91 ± 0.04	5.51 ± 0.05	5.58 ± 0.07	5.66 ± 0.06	5.64 ± 0.10

[a] Means ± SD($n = 3$).

3. Results and discussion

3.1. Octacosanol and other main components in the filter mud waxes

The filter mud and sugarcane bagasse contained 6.85 g/100 g and 2.12 g/100 g crude waxes (based on dry matter), respectively. Testing results showed that the octacosanol content in filter mud (12970 mg/kg) was much higher than that in sugarcane bagasse (467 mg/kg). GC/MS result showed there were two major policosanols in the filter mud, namely, hexacosanol ($m/z = 364$) and octacosanol. No docosanol, tetracosanol, and tricontanol were found in the filter mud as being reported in sugarcane peel and leaves (Irmak et al., 2006). Other identified components (Fig. 1) according to the GC/MS library searching included *n*-hexadecanoic ethyl ester (16.21 min), 9,12-octadecadienoic acid ethyl ester (17.80 min), and campesterol (26.35 min).

3.2. Effects of extraction time, temperature, and pressure on waxes yield in supercritical CO_2 extraction

A single-factor experiment was carried out to determine the optimal parameters for extraction of waxes in filter mud by supercritical CO_2 extraction. Extraction temperature and pressure significantly influenced the extraction efficiency of waxes. Increase of the temperature from 35 °C to 50 °C raised the yield of waxes (as shown in Table 1); a similar trend was observed for pressure (Table 2). Prolonging extraction time slightly increased the yield of waxes (Table 1 and Table 2). Raising extraction temperature increases the vapour pressure of the alcohols but also decreases the CO_2 density and its solvent power. An increase in pressure increases the density of the solvent and the solubility of the target ingredients. Thus, the optimal extraction parameters were determined as following: 100 g of filter mud was suspended in 5 times volume of absolute ethanol and followed by being extracted with supercritical CO_2 at 50 °C, 30 MPa for 2 h.

3.3. Comparison between supercritical CO_2 extraction and hot ethanol reflux

As shown in Table 3, hot ethanol reflux extraction and supercritical CO_2 extraction had similar extraction efficiency for waxes and had extracted 78.1% and 80.4% of the waxes from the raw material respectively (ratio of waxes yield in Table 3 to waxes content of filter mud described in 3.1). However, octacosanol

Table 3
Comparison of the extraction efficiency by supercritical CO_2 and hot ethanol reflux extraction.

	Waxes (g/100 g)	Octacosanol in the waxes(g/100 g)
Hot ethanol reflux	5.35 ± 0.06[b]	22.52 ± 1.24[a]
Super critical fluid extraction	5.51 ± 0.05[b]	29.65 ± 1.46[b]
Hot ethanol reflux after saponication	2.31 ± 0.03[a]	47.8 ± 2.12[c]

Values (means ± SD, $n = 3$) with different letters within a column are significantly different at 5% level.

content in the waxes obtained by supercritical CO_2 extraction was significantly higher than that by hot ethanol reflux extraction (Table 3), mainly due to the removal of two kinds of esters (*n*-hexadecanoic ethyl esterand 9,12-octadecadienoic acid ethyl ester) by supercritical CO_2 extraction (Fig. 1).

When the waxes obtained by hot ethanol reflux extraction was saponificated using sodium hydroxide, 72.2% and 70.% of the two esters were removed (based on the difference of peak area before and after saponification), and the content of octacosanol increased to 47.8 g/100 g (Table 3). Moreover, the colour of the product became much lighter after acetone extraction.

4. Conclusion

Our research showed that supercritical CO_2 extraction had similar yields waxes, compared to ethanol reflux extraction, but resulted in higher content of octacosanol in the waxes from filter mud. However, when the waxes obtained by hot ethanol reflux extraction were subjected to decolouring treatment and saponification, octacosanol content in the waxes significantly increased and was much higher than that by supercritical CO_2 extraction.

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