J Am Oil Chem Soc (2011) 88:931–935
DOI 10.1007/s11746-010-1754-z

ORIGINAL PAPER

Comparison of Supercritical Fluid and Hexane Extraction Methods in Extracting Kenaf (*Hibiscus cannabinus*) Seed Oil Lipids

Abdalbasit Adam Mariod · Bertrand Matthäus · Maznah Ismail

Received: 7 September 2010 / Revised: 30 October 2010 / Accepted: 11 December 2010 / Published online: 20 January 2011

Abstract The objective of this study was to investigate and compare fatty acids, tocopherols and sterols of kenaf seed oil extracted by supercritical carbon dioxide and traditional solvent methods. Fatty acids, tocopherols and sterols were determined in the extracted oils as functions of the pressure (400 bar, 600 bar), temperature (40 °C, 80 °C) and CO_2 flow rate (25 g/min) using a 1-L extraction vessel. Gas chromatography was used to characterize fatty acids and sterols of the obtained oils while tocopherols were quantified by HPLC. No differences were found in the fatty acid compositions of the various oil extracts and the main components were found to be linoleic (38%), oleic (35%), palmitic (20%) and stearic acid (3%). Extraction of tocopherols using high pressure (600 bar/40 °C, 600 bar/80 °C) gave higher total tocopherols (88.20 and 85.57 mg/100 g oil, respectively) when compared with hexane extraction which gave yield of 62.38 mg/100 g oil. Extraction of kenaf seed oil using supercritical fluid extraction at high temperature (80 °C) gave higher amounts of sterols when compared with hexane extraction.

A. A. Mariod
Department of Food Science & Technology,
Sudan University of Science & Technology,
PO Box 71, Khartoum North, Sudan
URL: http://www.sustech.edu

B. Matthäus
Department for Lipid Research, Max Rubner-Institute,
Federal Research Institute for Nutrition and Food,
48147 Münster, Germany

A. A. Mariod · M. Ismail (✉)
Laboratory of Molecular Biomedicine, Institute of Bioscience,
Universiti Putra Malaysia, 43400UPM Serdang,
Selangor, Malaysia
e-mail: maznah@medic.upm.edu.my; myhome.e@gmail.com

Keywords Fatty acid · Kenaf seed · Sterols · Supercritical fluid extraction · Hexane extraction · Tocopherol

Introduction

Carbon dioxide (CO_2) is often used in the development of supercritical fluid extraction (SFE) instead of the organic solvents normally used in conventional extraction methods, e.g. hexane extraction, since thermal degradation and solvent contamination of the extract are avoided. Thus, over the last few years SFE has been applied to the extraction of oil from a large number of plant sources [1]. SFE of lipids has received attention as an alternative method to organic solvent extraction and has been shown to be useful for extracting certain lipids [2]. Indeed, SFE has immediate advantages over traditional extraction techniques: it is a flexible process due to the possibility of continuous modulation of the solvent power/selectivity of the SCF, it allows the elimination of polluting organic solvents and of the expensive post-processing of the extracts for solvent elimination [3]. There are several excellent articles on the use of supercritical carbon dioxide techniques in various analytical areas, including the extraction of vegetable oil and tocopherols [4, 5].

Kenaf (*Hibiscus cannabinus*), Malvaceae, is a tall herbaceous annual woody tropical plant, the leaves are consumed as a vegetable in certain parts of the world, and possess an erythrocyte protective activity against drug-induced oxidative stress [6]. In Malaysia recently, kenaf was introduced as a fiber crop to substitute tobacco. Kenaf seed oil (extracted by Soxhlet extraction) is high in unsaturated fatty acids, phospholipids and sterols (81.5, 6.0 and 0.9% of the oil, respectively). Owing to it is unique

composition kenaf seed oil may present as an alternative and economical source of edible oil for human consumption [7]. Kenaf seeds are the waste part of the plant. Recently our group reported that kenaf seed oil extracted using SFE possess better antioxidant activity when compared with commercial edible oils and it can potentially serve as high antioxidative edible oil [4].

The objective of our study was to study the effect of high pressure (400, 600 bar) and temperature (40, 80 °C) on fatty acids, tocopherol and sterols present in kenaf seed extracts. The results of the SFE extraction were also compared with those obtained by using the conventional (Soxhlet) method.

Materials and Methods

Samples, Solvents, and Reagents

Kenaf seeds (*Hibiscus cannabinus L.*) were obtained from Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia.

The chemicals used were of analytical grade, carbon dioxide (99.8%) (Malaysian Oxygen Berhad, Petaling Jaya, Selangor, Malaysia), and nitrogen (pure) (Malaysian Oxygen Berhad, Petaling Jaya, Selangor, Malaysia). Hexane, *n*-heptane, sodium methanolate, methanol, HCL, butyl methyl ether, sodium hydroxide, betulin aluminium oxide and sodium hydrogen sulphate (Merck, Darmstadt, Germany).

Methods

Preparation of Dried Kenaf Seeds

Kenaf seeds were cleaned under running tap water for 10 min, rinsed twice with distilled water and air-dried to constant weight at 50 °C in an oven (FD 115, Fisher Scientific, Loughborough, Leicestershire, UK). The seeds were ground to a powder using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 10 min and then passed through a 35 mm (42 mesh) sieve. The oil was extracted from the ground seeds by extraction with *n*-hexane (b.p. 50–60 °C) using a Soxhlet apparatus for 6 h. following the AOCS [8].

Supercritical Fluid Extraction (SFE) with Carbon Dioxide

Kenaf seeds were extracted by using a Supercritical Carbon Dioxide Extractor (Thar 1000 F, Thar Technologies, Inc., Pittsburgh, PA, USA) using four different extraction parameters (pressure (bar)/temperature (°C): 600/40; 600/80; 400/40; 400/80). Following Chan and Ismail [4]. Briefly, 100 g of kenaf seed were ground using a stainless steel blender (National, Model MX-915, Kadoma, Osaka, Japan) for 1 min and placed into a 1 L extraction vessel. After the extraction vessel was tightly sealed, the desired extraction temperature was set. Pressure within the extraction vessel was built up with a constant carbon dioxide flow rate (25 g/min) and regulated by automated back pressure regulator. The SFE extraction was initiated after the desirable temperature and pressure were achieved. The entire extraction process lasted for 150 min. After the extraction was completed, the extraction vessel was depressurized and the oil was collected. The extracted oil obtained from SFE was collected and stored at 4 °C for fatty acid, tocopherol and sterol analysis.

Oil Extraction by Soxhlet

Fifty grams of kenaf seeds were ground using a stainless steel blender (National, Model MX-915, Kadoma, Osaka, Japan) for 1 min and put into extraction thimble, then transferred into a Soxhlet extractor (Witeg-Labortechnik GmbH, Wertheim, Germany). The oil was extracted using hexane following AOCS method [8]. After extraction was completed, *n*-hexane was removed at 50 °C under reduced pressure using a rotary evaporator (Rotavapor R210, Buchi, Flawil, Switzerland). The obtained oil was stored at 4 °C until further analysis.

Fatty Acid Analysis

The fatty acid composition of kenaf seed oil (KSO) was determined following ISO method [9]. In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 µl 2 M sodium methanolate in methanol were added, and the closed tube was agitated vigorously for 1 min. After addition of 100 µl of water, the tube was centrifuged at 4,500 g for 10 min and the lower aqueous phase was removed. After adding 50 µl 1 M HCL to the heptane phase, the two phases were mixed for a short time and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulfate (monohydrate, extra pure, Merck, Darmstadt, Germany) was added and after centrifuged at 4,500 g for 10 min. The top *n*-heptane phase was transferred into a vial and injected into a Varian 5,890 gas chromatograph equipped with a capillary column Cp-Sil 88 (100 m long, 0.25 mm i.d., film thickness 0.2 µm). The temperature was from 155 °C heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 µl. The peak areas were computed by integration software and percentages of the fatty acid methyl esters (FAME) were obtained

as weight percent by direct internal normalization. All analyses were done in duplicate.

Tocopherols

For the determination of tocopherols (TOC) a solution of 250 mg of the studied oil in 25 mL *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low–pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system; 20 µl of the samples was injected by a Merck 655- A40 Auto-sampler onto a Diol phase μ1 3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v) [10].

Sterols

The sterol composition of the studied oils was determined following ISO\FIDS 12228 [11]. In brief, 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid phase extraction on an aluminium oxide column (10 g Al_2O_3 [Merck, Darmstadt, Germany] in a 1 × 25 cm column), on which fatty acid anions were retained and sterols passed through. The sterol fraction from the unsaponifiable matter was separated by thin-layer chromatography (TLC) on silica gel 20 × 20 cm; layer thickness of 0.25 mm (Merck, Darmstadt, Germany), using hexane/diethyl ether (1/1 [v/v]) as the developing solvent, and was re-extracted from the TLC material and afterwards the composition of the sterol fraction was determined by gas chromatography (GC) using betulin as an internal standard. The compounds were separated on an SE 54 CB (Macherey–Nagel, Düren, Germany) (50 m long, 0.32 mm i.d., 0.25 mm film thickness). Other parameters were: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program: 245–260 °C at 5 °C/min.

Statistical Analysis

The analyses were performed in duplicates. The mean values and standard deviation (mean ± SD) were calculated and tested using the Student's *t* test ($P < 0.05$). Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statgrafics Statistical Graphics System version 4.0 [12].

Results and Discussion

Oil Extraction

The kenaf seed oil extracted with either hexane or supercritical CO_2 was intensely yellow in color having a variable content of tocopherols and sterols. After oil extraction, the residual defatted kenaf seed was a gray flour with variable grain size, more or less fine, depending on the degree of grinding of the fresh matrix and on the effect of the operating conditions (pressure, temperature) used to remove the oil.

Fatty Acid Composition

The methyl ester fatty acid analysis in both *n*-hexane and CO_2 extracted oils is presented in Table 1. From the analysis, the main components of kenaf seed oil were linoleic acid (38%), oleic acid (35%), palmitic acid (20) and stearic acid (3%).

Table 1 Fatty acid composition (%) of kenaf seed oil (KSO) extracted by Soxhlet and supercritical fluid extraction using carbon dioxide

Fatty acid sample *P/T* °C	KSO 400/40	KSO 600/40	KSO 400/80	KSO 600/80	KSO SOX
14:0	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02
16:0	20.2 ± 0.5	20.0 ± 0.5	20.2 ± 0.5	19.3 ± 0.4	20.0 ± 0.5
16:1n-7	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
16:1n-9	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
18:0	3.2 ± 0.4	3.2 ± 0.4	3.3 ± 0.4	3.4 ± 0.4	3.3 ± 0.4
18:1n-9	35.6 ± 1.3	35.4 ± 1.3	35.2 ± 1.2	35.7 ± 1.4	35.6 ± 1.3
18:1n-7	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	0.8 ± 0.3
18:2n-6	38.2 ± 1.5	38.1 ± 1.5	38.2 ± 1.5	38.3 ± 1.5	38.0 ± 1.5
20:1n-9	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
18:3	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Others	1.1 ± 0.02	0.1 ± 0.02	0.4 ± 0.01	0.7 ± 0.2	0.5 ± 0. 2

Means of duplicate values, *P* pressure in bar, *T* temperature in °C

Table 2 Tocopherol (TOC) composition (mg/100 g) of kenaf seed oil (KSO) extracted by Soxhlet and supercritical fluid extraction using carbon dioxide

TOC oil	α-T	β-T	γ-T	P8	δ-T	Total
KSO 400/40	0.69[a]	0.43[a]	9.55[a]	0.00[a]	2.13[a]	12.79[a]
KSO 600/40	40.07[b]	0.64[b]	45.73[b]	0.59[b]	1.17[b]	88.20[b]
KSO 400/80	1.32[c]	0.35[c]	19.08[c]	0.32[c]	10.94[c]	32.00[c]
KSO 600/80	13.05[d]	0.52[a]	34.17[d]	1.77[d]	36.06[d]	85.57[d]
KSO Soxhlet	19.76[e]	0.49[a]	39.53[e]	1.28[e]	1.32[b]	62.38[e]

Means of duplicate values in the same column with the same letter are not significantly different at $P < 0.05$

These results are totally different than those reported early by Mohamed et al. [7] who reported high linoleic acid (45.9%) and low oleic (29.2%), but similar palmitic acid content in nine American kenaf genotypes, using solvent for oil extraction. From Table 1 as a general statement, the fatty acid does not change with pressure and temperature of SFE, and no significant differences were observed between fatty acid profiles. Similarity in the fatty acid profile of SFE and Soxhlet-extracted oils had already been observed [13, 14], although the fatty acid composition of SFE extracted oil had been reported to vary slightly with temperature and pressure [15].

Tocopherol Composition

The tocopherols are mainly present in oil seeds, oils, meats and the green parts of higher plants. The most abundant natural antioxidants in vegetable oils are the α- and γ-tocopherols [16]. Data comparing the tocopherol concentrations obtained by SFE and traditional organic solvent methods are reported in Table 2. In all the analyses of solvent and CO_2 extracts, γ-tocopherol was the main isomer, same results in different oils were already observed [17], α, β, δ- tochopherols were also detected. Generally, SFE results gave high tocopherols yield comparable to the traditional method and even higher when high pressure was used. From Table 2 extraction of tocopherol using high pressure (600/40, 600/80 bar/°C) gave higher tocopherol yield (88.20 and 85.57 mg/100 g oil, respectively) when compared with low pressure (400/40, 400/80 bar/°C), which gave total amount of 12.79 and 32.00 mg/100 g oil, respectively. The use of SFE provides the advantage of fractioning the components of kenaf seed oil and obtaining oil enriched in tocopherol. Similar results for the extraction and enriched tocopherol fractions have been obtained using supercritical CO_2 by several authors [18, 19].

Sterols Composition

Sterols, or plant sterols, are minor components of vegetable origin. They function as structural components in membrane lipids and as precursors to steroid hormones. There is increasing interest in isolating these biologically active components for nutraceutical applications and as ingredients for functional foods [20]. Table 3 shows sterols composition of kenaf seed oil extracted by Soxhlet and supercritical fluid extraction using carbon dioxide. From this table the total sterols were 6366.89, 5870.26, 7421.95, 6954.07 and 6570.19 mg/100 g oil for KSO 400/40, KSO 600/40, KS7 400/80, KSO 600/80 and KSO Soxhlet, respectively. Extraction of kenaf seed oil using SFE at high temperature (80 °C) gave higher amounts of sterols when compared with hexane extraction, this results were in good agreement with previous ones from literature, Snyder et al. [21] reported an increase in sterol concentration after the

Table 3 Phytosterol composition (%) of kenaf seed oil (KSO) extracted by Soxhlet and supercritical fluid extraction using carbon dioxide

Oil sterol	KSO 400/40	KSO 600/40	KSO 400/80	KSO 600/80	KSO Soxhlet
Cholesterol	0.24[a]	0.25[a]	0.26[a]	0.29[a]	0.23[a]
Brassicasterol	0.10[a]	0.11[a]	0.08[b]	0.08[b]	0.08[b]
24-Methylene cholesterol	0.21[a]	0.17[b]	0.17[b]	0.25[a]	0.30[c]
Campesterol	9.91[a]	9.72[a]	9.79[a]	10.45[b]	10.62[b]
Campestanol	0.20[a]	0.16[a]	0.19[a]	0.21[a]	0.33[b]
Stigmasterol	6.30[a]	6.13[a]	6.54[b]	5.82[c]	6.01[c]
Δ7-Campesterol	0. 25[a]	0.23[a]	0.24[a]	0.29[a]	0.26[a]
Clerosterol	1.31[a]	1.43[a]	1.40[a]	1.43[a]	1.39[a]
β-Sitosterol	76.52[a]	76.94[a]	76.61[a]	75.68[b]	75.23[b]
Sitostanol	0.81[a]	0.74[a]	0.75[a]	0.76[a]	0.85[a]
Δ5-Avenasterol	2.03[a]	2.18[a]	2.01[a]	2.64[b]	2.60[b]
5,24-Stigmastadienol	0.44[a]	0.43[a]	0.43[a]	0.49[a]	0.49[a]
Δ7-Stigmasterol	1.30[a]	1.24[a]	1.24[a]	1.26[a]	1.23[a]
Δ7-Avenasterol	0.27[a]	0.28[a]	0.28[a]	0.36[b]	0.39[b]
Total sterols mg/kg oil	6366.89	5870.26	7421.95	6954.07	6570.19

Means of duplicate values in the same row with the same letter are not significantly different at $P < 0.05$)

SFE-based fractionation of soybean and corn fibre oil samples. Mohamed et al. [7] reported total sterols ranged from 0.6 to 1.2% of kenaf seed oil extracted by solvent. From Table 3 the major component in all extracts was β-sitosterol, which represents 75.23–76.94% of the total sterols, followed by campesterol (9.72–10.62%) and stigmasterol (5.82–6.54%). In KSO extracted by solvent the major sterol fractions were β-sitosterol which represents 75.23%, followed by campesterol 10.62% and stigmasterol 6.01% of the total oil which is higher than that reported by Mohamed et al. [7] who studied sterol composition in nine American kenaf genotypes where they reported 72.3% for β-sitosterol, 9.9% for campesterol and 6.07% for stigmasterol. In all the studied oil extracts a remarkable amount of Δ5-avenasterol was found at 2.01–2.64% of the total sterols. The Δ5-avenasterol is known to act as an antioxidant and as an antipolymerization agent [22–24].

In conclusion, applying supercritical technology in the extraction of kenaf seed oil resulted in high amounts of tocopherol as well as sterol compared with the hexane extraction. The use of SFE provides the advantage of fractioning the components of kenaf seed oil and obtaining oil enriched in tocopherols and sterols. The fatty acid composition did not change in both extraction methods.

References

1. Louli V, Folas G, Voutsas E, Magoulas K (2004) Extraction of parsley seed oil by supercritical CO_2. J Supercritical Fluids 30:163–174
2. Patel RN, Ganesh A (2006) Extraction of cashew (Anacardium occidentale) nut shell liquid using supercritical carbon dioxide. Biores Technol 97:847–853
3. Reverchon E, De Marco I (2006) Review: supercritical fluid extraction and fractionation of natural matter. J Supercritical Fluids 38:146–166
4. Chan KW, Ismail M (2009) Supercritical carbon dioxide fluid extraction of *Hibiscus cannabinus* L. seed oil: a potential solvent-free and high antioxidative edible oil. Food Chem 114:970–975
5. Mariod AA, Abdalwahab SI, Gedi MA, Solati Z (2010) Supercritical carbon dioxide extraction of sorghum bug (*Agonoscelis pubescens*) oil using response surface methodology. J Am Oil Chem Soc 87:849–856
6. http://foodafrica.nri.org/index.html. (Retrieved 01.09.2010)
7. Mohamed A, Bhardwaj H, Hamama A, Webber C (1995) Chemical composition of kenaf (*Hibiscus cannabinus* L.) seed oil. Ind Crop Prod 4:157–165
8. AOCS (1998) Official methods and recommended practices of the American Oil Chemists' Society, 5th edn, In: Firestone D, AOCS Press, Champaign, method Aa 4–38
9. ISO (2000) International Standard 5509 animal and vegetable fats and oils–preparation of methyl esters of fatty acids. ISO, Geneva
10. Balz M, Shulte E, Thier HP (1992) Trennung von Tocopherol und Tocotrienolen durch HPLC. Fat Sci Technol 94:209–213
11. ISO (1999) International standard 12228 animal and vegetable fats and oils—determination of individual and total sterols contents—gas chromatographic method. ISO, Geneva
12. Statgraphics®. Statgraphics Statistical Graphics Systems, Version 4.0, STSC Inc. & Statistical Graphics Cooperation, Rockville, MD 1985–1989
13. Devittori C, Gumy D, Kusy A, Colarow L, Bertoli C, Lambelet P (2000) Supercritical fluid extraction of oil from millet bran. J Am Oil Chem Soc 77(6):573–579
14. Fors S, Eriksson CE (1990) Characterization of oils extracted from oats by supercritical carbon dioxide. Lebensm Wiss Technol 23:390–395
15. Cheung PCK, Leung AYH, Ang PO (1998) Comparison of supercritical carbon dioxide and soxhlet extraction of brown seaweed *Sargassum hemiphyllum* (Turn.) C. Ag. J Agric Food Chem 46:4228–4232
16. Christine MS, Song Q, Csallany AS (2010) The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. J Am Oil Chem Soc 87:469–481
17. Gunstone FD, Hardwood JL, Padley FD (1992) The Lipids Handbook, 2nd edn. Chapman and Hall, London, p 130
18. Leo L, Rescio L, Ciurlia L, Zacheo G (2005) Supercritical carbon dioxide extraction of oil and α-tocopherol from almond seeds. J Sci Food Agric 85:2167–2174
19. Turner C, King J, Mathiasson L (2001) Supercritical fluid extraction and chromatography for fat-soluble vitamin analysis. J Chromatogr A 936:215–237
20. Holser RA, Bost G, Van Boven M (2004) Phytosterol composition of hybrid Hibiscus seed oils. J Agric Food Chem 52:2546–2548
21. Snyder JM, King JW, Taylor SL, Neese AL (1999) Concentration of phytosterols for analysis by supercritical fluid extraction. J Am Oil Chem Soc 76:717–721
22. White PJ, Armstrong LSA (1986) Effect of selected oat sterols on the determination of heated soybean oil. J Am Oil Chem Soc 63:525–529
23. Savage GP, Mcneil DL, Dutta PC (1997) Lipid composition and oxidative stability of oils in hazelnuts (*Corylus avellana* L.) grown in New Zealand. J Am Oil Chem Soc 74:755–759
24. Mariod AA, Matthaus B, Eichner K (2004) Fatty acid, tocopherol and sterol composition as well as oxidative stability of three unusual Sudanese oils. J Food Lipids 11:179–189