

Supercritical Carbon Dioxide Extraction of Sorghum Bug (*Agonoscelis pubescens*) Oil Using Response Surface Methodology

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Abstract Supercritical fluid extraction (SFE) of sorghum bug oil (SBO) with carbon dioxide was performed and compared with Soxhlet extraction using hexane. Response surface methodology (RSM) was used to determine the effects of pressure (200–400 bar) and temperature (50–70 °C) on the sorghum bug oil yield in SC-CO₂. The high extraction yield (more than 45.0%) was obtained at 300 bar and 60 °C followed by 400 bar and 70 °C, while the lower yield was obtained at 159 bar and 60 °C. At low pressure levels (159 and 200 bar), the oil yield decreased due to the reduced density of CO₂ at higher temperatures. Gas chromatography was used to characterize the fatty acids of the oils obtained while α -tocopherol was quantified by HPLC. No differences were found in the fatty acid compositions of the various extracts, while the α -tocopherol extracted from sorghum bug oil by the conventional solvent method was

less than that extracted by the SFE process using CO₂. It can be observed that the conventional solvent extraction method exhibited notable DPPH radical-scavenging activity, with an efficacy slightly lower (IC₅₀ 7.45 ± 0.3) than that of the SFE extracts.

Keywords *Agonoscelis pubescens* · Antioxidant activity · 1,1-Diphenyl-picrylhydrazyl · Fatty acid · Oil extraction · Response surface methodology · Supercritical carbon dioxide

Introduction

Carbon dioxide (CO₂) is often used in the development of supercritical fluid extraction (SFE) instead of the organic solvents normally employed in conventional extraction methods such as steam distillation and solvent 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].

The main advantages of using carbon dioxide fluid are: a reduced potential for the oxidation of extracted solutes, higher selectivity, increased sample throughput, shorter extraction time and a low critical temperature. The latter is beneficial in extracting thermally labile compounds, such as natural vegetable products. Supercritical carbon dioxide also has chemical inertness, suitable solvent strength, permits the separation of compounds of widely different polarities and molecular masses, has low cost and, what is more, can be removed from the extracted products without leaving any chemical residue. In addition, carbon dioxide is both non-toxic and non-explosive and its use can reduce the consumption of organic solvents; this is especially

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useful for the production of natural products used in foods and pharmaceuticals [2]. Supercritical carbon dioxide (SC-CO₂) has been successfully used for the extraction of oils from oil seeds including parsley seed [1], cottonseed [3], palm kernel [4] and kenaf seed [5].

Agonoscelis pubescens (Sorghum bug) also known as cluster bug belongs to the order Hemiptera (family Pentatomidae), commonly known in Sudan as Dura andat. The adult bug is shield-shaped, about 11–13 mm long, and 6–7 mm wide, and its upper and underside of the body is covered with a fine silvery pubescence. The bugs attack, in the adult and/or nymph stages, a number of crops such as Lucerne (*Medicago sativa*), sorghum, sunflower, wheat and sesame. Mainly the adults infest sorghum during the milky-stage. The bugs feed on the developing grains, which become atrophied. The adults shelter during the dry season in clusters, which may consist of many thousands or hundred thousands of individuals, for a period of about 9 months on the stems and branches of trees and bushes [6] (Schmutterer, 1969).

The physiological conditions of the internal organs of the insects, especially of fat bodies, depend entirely on the intensity of insect feeding on ripening sorghum grains during October and November. Ample feeding during this period leads to substantial accumulation of metabolic compounds, especially fats. Insufficient feeding, alternatively, results in an atrophied mid-intestine and meager fat deposition. The fat content of resting insect has been found to be highest at the beginning of the resting period when the insects first arrive at the resting sites; females already contain more fat than males. In fact the highest fat content is to be expected at the end of the feeding period in late November, just before migration flight itself [7] (Razig, 1978).

In Sudan many edible insects are consumed and the desert locust is considered the most famous one in many parts of the country beside sorghum and melon bugs [8, 9]. In some areas of the Sudan, oil is extracted from the collected bugs and the oil is used for cooking and some medicinal uses. The oil content of the sorghum bug is 60% with 40.9, 34.5, and 12.1% of oleic, linoleic, and palmitic, respectively, the oil contains 34.0 mg/100 g tocopherols [8]. In Western Sudan, the sorghum bug adults are collected and eaten after frying, while in some areas of Sudan the collected bugs are extracted by pressing and the oil obtained is used for cooking and some medicinal uses. In the Botana area of central Sudan, nomads use tar obtained from bugs which have been heated highly against dermatological infections of their camels [8]. Published works include: Mariod et al. [8] on the fatty acid, tocopherols, sterols and oxidative stability of edible oils from the sorghum bug; Mariod et al. [9] on effects of different processing steps on the quality and stability of edible oils from melon and sorghum bugs; Mariod et al. [10] on the frying

quality and oxidative stability of sorghum bug oil. These authors reported that the oil was suitable for deep-frying of potatoes, only for 6–12 h. After that, the oil and the potatoes fried in it did not meet the requirements with regard to the sensory assessment or chemical parameters.

The minor components are naturally occurring compounds with antioxidative properties that help protect the oils against oxidative deterioration and hence play an important role in their oxidative stability [11]. The minor components of vegetable oils are primarily phospholipids, tocopherols, phenolic compounds, pigments (carotenoids, chlorophylls), sterols, and free fatty acid, as well as mono and diglycerides [12].

Response surface methodology (RSM) uses an experimental design such as the central composite design to fit a model by a least squares technique. If the proposed model is adequate, contour plots can be usefully employed to study the response and locate the optimum, so it can be an effective method for optimizing the conditions of SC-CO₂ extraction [13].

Despite the large number of matrices processed, only some models of SC-CO₂ have been published. To optimize the extraction conditions, a relationship between the composition of vegetable oils and their solubility in SC-CO₂ must be taken in account [14].

The main objective of this study was to obtain and characterize oil extracts from dried powdered sorghum bugs using the SFE method and using CO₂ as the extraction solvent and EtOH as the modifier for recovery of the extracts. The effects of extraction pressure and temperature were studied on total extraction yields, fatty acid and α -tocopherol composition profiles and on the extracts antioxidant activities. The results were compared with the Soxhlet extraction method.

Materials and Methods

Materials and Sample Pre-Treatment

Sorghum bug adults used in the present study was collected from the Rahad agricultural area in Sudan. The initial moisture content of the bug powder determined by oven drying at 105 ± 1 °C for 24 h was 6.61% on a dry basis and the oil content was 60.1%. The chemicals used were of analytical reagent grade and included 1,1-diphenyl-picrylhydrazyl (DPPH–90% purity, Sigma-Aldrich Co., St. Louis, MO, USA), toluene (Merck, Darmstadt, Germany), *n*-hexane (Fisher Scientific, Loughborough, Leicestershire, UK); carbon dioxide (99.8%, Malaysian Oxygen Company, Petaling Jaya, Selangor, Malaysia), (+)- α -tocopherol (Sigma-Aldrich Co., St. Louis, MO, USA). Nitrogen (pure, Malaysian Oxygen Company, Petaling Jaya, Selangor,

Malaysia) and absolute ethanol (Fisher Scientific, Loughborough, Leicestershire, UK).

Methods

Oil Extraction by Solvent (Soxholet Method)

Oil was extracted from 20 g of powdered insects following the Mariod et al. [8] method, in brief: *A. pubescens* bugs were collected from the Rahad agricultural area. The bugs were stored in a tight polyethylene bag, killed by treatment with hot water for few minutes and then sun dried. After crushing using a lab mortar the oil was extracted using hexane following the AOCS method [15]. The residual solvent was evaporated with a rotavapor (Rotavapor R210, Buchi, Flawil, Switzerland), and the oil content was determined. The oil obtained by the extraction was stored at 4 °C until further investigation.

Supercritical Carbon Dioxide (SC-CO₂) Extraction

SC-CO₂ extraction experiments were carried out following Bimakr et al. [16] using a supercritical fluid extractor (ABRP200, Pittsburgh, PA, USA) Fig. 1, with the extractor vessel volume 500 ml. The flow rate of CO₂, extraction temperature and pressure were adjusted by using ICE software, and the extraction time was measured. Liquid CO₂ was supplied from a gas cylinder. Before the liquid CO₂ passed into the extraction vessel, it was pressurized to the desired pressure and heated to the specified temperature by the means of a pump (P-50, Pittsburgh, PA, USA) to reach the supercritical state. Absolute ethanol (EtOH) acting as the modifier was added at 3 ml per min. The

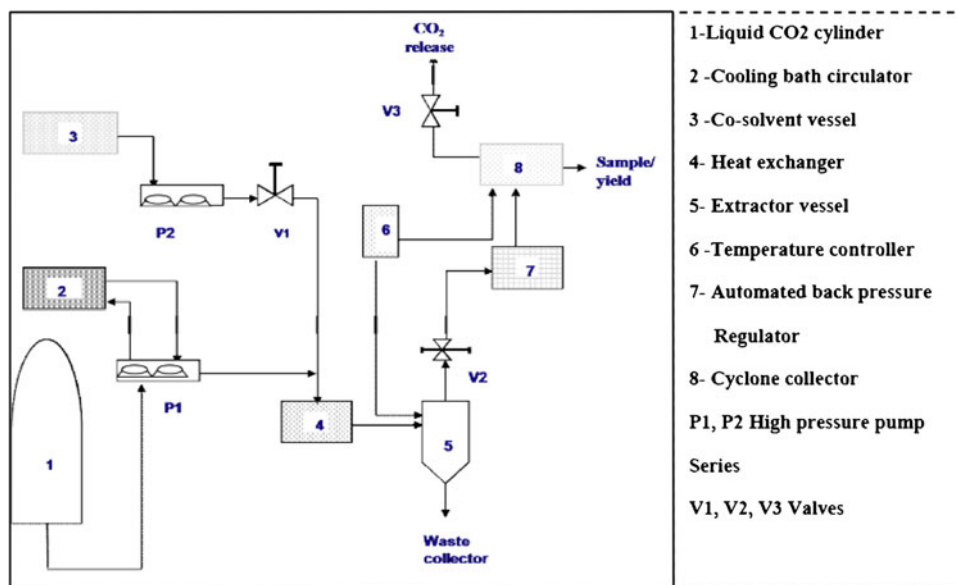
SC-CO₂ flow rate was maintained at 15 g/min and also, the duration of extraction time was fixed at 30 min. The powdered insects (19 g) were placed in the extractor vessel. The SC-CO₂ extraction was initiated after the desired temperature and pressure were achieved. All the extracts obtained were collected with a modifier. In order to remove the ethanol, extracts were vacuum evaporated using a rotary evaporator (Rotavapor R210, Buchi, Flawil, Switzerland) at 39 °C. After the extraction was completed, the extraction vessel was depressurized and the extract was collected. The total oil obtained from the extraction was calculated. Finally, the extracts obtained were transferred to glass bottles and stored in a freezer until GC analysis. The extractions were performed in duplicate.

Analysis of Extracts

FA Composition

The FA compositions of the oil samples were determined using the Bozan and Temelli [17] modified FAME method. The oil extracts obtained were transmethylated to fatty acid methyl esters (FAME) by heating (90 °C for 45 min) with a mixture of 35% (vol/vol) BF₃/MeOH (12% BF₃/MeOH; Supelco, Oakville, Ontario, Canada), 45% (vol/vol) MeOH, and 29% (vol/vol) hexane. The FAME were then analyzed by GC (Varian 3600 GC; Mississauga, Ontario, Canada). The system was equipped with an autosampler (Model 8200; Varian) and an FID. The resulting data were processed by a computer using a Class VP data processor (Shimadzu Corporation, Columbia, MD). Helium was used as the carrier gas. The FAME were separated on a fused-silica capillary column (50 m × 0.32 mm, BPx-70, SGE Column, Pty. Ltd., Victoria, Australia) with the film thickness of 0.25 μm.

Fig. 1 Schematic diagram of a supercritical fluid extractor



The detector temperature was set at 230 °C. The injector temperature was 230 °C. The initial column temperature was 50 °C for 0.1 min and increased to 170 °C at a rate of 25 °C/min, held at 170 °C for 1 min, then increased to 180 °C at a rate of 2 °C/min, and then increased to 230 °C at a rate of 10 °C/min and held for 3 min.

Tocopherol Analysis

Collected oil samples were analysed by HPLC following the method of Balz et al. [18]. For the determination of tocopherols, a solution of 250 mg oil in 25 mL *n*-heptane was used directly for the high-performance liquid chromatography (HPLC) analysis. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a 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. The samples (20 µL) were injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column (25 cm × 4.6 mm ID; Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v). All determinations were carried out in triplicate. Quantification was performed by comparing the sample peak areas to those of known amounts of standard compounds. The tocopherol content was expressed as mg/g sorghum bug oil.

DPPH Radical Scavenging Activity

The anti-radical scavenging activity of sorghum bug oil extracts was measured according to the method described by Ramadan et al. [19] with some modifications. Ascorbic acid was used as the standard lipophilic antioxidant in this test. In brief, 0.1 mL of a toluenic sample solution at different concentrations was added with 0.39 mL of fresh toluenic DPPH solution (0.1 mM). Then, the mixture was shaken vigorously and left in darkness for 60 min. Finally, the absorbance of the mixture was measured against pure toluene (blank) at 515 nm by using a UV-Visible spectrophotometer (Pharmaspec uv-1700, Shimadzu, Kyoto, Japan). The antiradical activity of the sorghum bug oil was calculated from the following equation:

$$\% \text{ Inhibition} = ([A_{\text{control}} - A_{\text{sample}}] / A_{\text{control}}) \times 100$$

A_{blank} Absorbance of the control reaction (containing all reagents except samples),

A_{sample} Absorbance of the test compound.

IC₅₀ in this test was defined as the concentration of the oil that was able to inhibit 50% of the total DPPH radicals.

IC₅₀ of the sample was expressed in µL/mL and calculated through the interpolation of linear regression analysis.

Measurement of Pigments

Pigments present in the extracted oil samples were determined qualitatively by measuring the absorbance at 430–460 nm for carotenoids and 550–710 for chlorophylls and their derivatives [20]. The oil sample was mixed with hexane 1:4 (w/v) and transferred into glass cells; the absorbance was measured against the blank at 750 nm by a U-2000 Spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The absorption spectra were recorded between 430 and 710 nm.

Chromatic Measurements

A photometric color index PCI was used to measure the color of the fats and oils with an automatic instrument in triplicate at wavelengths of 430, 460, 550, 620, and 670 nm against 100% hexane. The color parameter was calculated from the edible oil visible spectrum using the following equation: $PCI = 1.29 (A_{460}) + 69.7 (A_{550}) + 41.2 (A_{620}) - 56.4 (A_{670})$ [21], where *A* is the absorbance measured at 460, 550, 620, and 670 nm, respectively.

Statistical Analysis

The analysis of variance (ANOVA) and RSM were used to compare the means or averages. All the analyses were carried out using the statistical software, MINITAB release 14. A probability value of <0.05 was considered significant and <0.01 was considered very significant.

Results and Discussion

Response Surface Methodology (RSM)

The ranges of each parameter (temperature and pressure) that influence yield, antioxidant activity, fatty acid, and tocopherol composition were selected. In this study, the lower and upper values for the variables were set at + α - (+ α =1.41421) and - α - (- α = 1.41421) and so all the factor levels were chosen within the limits that were practical and desirable. In RSM, natural variables are transformed into coded variables that have been defined as dimensionless with mean zero and same standard deviation [20]. The experimental and predicted values for response (yield) under different combinations of extraction conditions via SC-CO₂ are illustrated in Table 1. The results indicate that the yield of sorghum bug oil, obtained at different combinations of extraction conditions via SC-CO₂, ranged from 18.24 to 45.11(%). By using multiple regression analysis, the relationship between the tested

Table 1 Effect of extraction pressure and temperature on the extraction yields, fatty acid and tocopherol of insect oil from sorghum bug using SC-CO₂ and solvent extraction methods

Run	Temperature °C		Pressure (bar)	Yield (%)	DDPH as IC ₅₀ (µl/ml)	α-Tocopherol (mg/g oil)	Fatty acid (%)				
	50	70					16:0	16:1	18:0	18:1	18:2
SC-CO ₂ Extraction ^a	Ex1	50	400	39.87 ± 0.1.5	12.69 ± 0.0	0.030 ± 0.0	15.30 ± 0.2	3.92 ± 0.2	5.72 ± 0.2	40.04 ± 0.5	29.33 ± 0.4
	Ex2	70	200	24.22 ± 0.9	22.81 ± 0.4	0.033 ± 0.0	15.66 ± 0.1	4.39 ± 0.1	5.22 ± 0.2	40.82 ± 0.6	29.52 ± 0.3
	Ex3	70	400	45.09 ± 1.4	7.31 ± 0.3	0.030 ± 0.1	14.59 ± 0.1	4.16 ± 0.2	5.23 ± 0.2	40.06 ± 0.5	29.27 ± 0.3
	Ex4	60	300	45.16 ± 1.3	16.28 ± 0.7	0.030 ± 0.0	16.38 ± 0.2	3.92 ± 0.1	6.01 ± 0.3	39.23 ± 0.4	27.57 ± 0.3
	Ex5	60	300	45.10 ± 1.2	17.45 ± 0.8	0.040 ± 0.1	15.41 ± 0.2	4.05 ± 0.2	5.41 ± 0.3	38.80 ± 0.4	27.75 ± 0.3
	Ex6	60	300	45.11 ± 1.4	25.20 ± 0.8	0.030 ± 0.0	15.47 ± 0.2	4.19 ± 0.2	5.35 ± 0.3	39.99 ± 0.4	28.79 ± 0.3
	Ex7	50	200	40.86 ± 0.8	6.84 ± 0.5	0.030 ± 0.0	15.02 ± 0.1	3.84 ± 0.1	5.89 ± 0.3	40.54 ± 0.5	28.92 ± 0.4
	Ex8	60	300	40.77 ± 0.9	12.62 ± 0.6	0.030 ± 0.0	15.42 ± 0.1	3.57 ± 0.1	5.35 ± 0.3	37.95 ± 0.4	31.98 ± 0.3
	Ex9	74	300	39.83 ± 1.1	19.51 ± 0.7	0.020 ± 0.0	15.97 ± 0.2	4.15 ± 0.1	5.61 ± 0.3	39.62 ± 0.4	28.14 ± 0.3
	Ex10	60	300	38.50 ± 1.0	13.20 ± 0.6	0.030 ± 0.0	15.65 ± 0.2	4.26 ± 0.2	5.28 ± 0.3	40.18 ± 0.5	29.18 ± 0.2
	Ex11	60	300	37.99 ± 1.0	10.35 ± 0.3	0.025 ± 0.0	16.64 ± 0.3	4.09 ± 0.2	5.90 ± 0.3	40.23 ± 0.5	28.21 ± 0.2
	Ex12	60	159	18.23 ± 0.7	14.80 ± 0.5	0.030 ± 0.0	16.64 ± 0.3	4.30 ± 0.2	5.73 ± 0.3	39.87 ± 0.4	27.97 ± 0.2
	Ex13	46	300	40.53 ± 0.9	26.1 ± 0.7	0.022 ± 0.0	16.94 ± 0.3	4.14 ± 0.2	5.80 ± 0.3	40y7 ± 0.5	28.57 ± 0.2
	Ex14	60	441	36.31 ± 0.7	4.52 ± 0.6	0.020 ± 0.0	16.29 ± 0.3	4.26 ± 0.2	5.44 ± 0.3	40.39 ± 0.5	28.79 ± 0.2
Solvent extraction			60.12 ± 1.5	7.45 ± 0.3	0.009 ± 0.1	12.22 ± 0.2	1.04 ± 0.1	7.27 ± 0.4	40.97 ± 0.5	34.53 ± 0.3	

Means of duplicate values ± standard deviations

^a The extracts (Ex. 1–14) were collected at constant intervals at 30 min

independent variables and dependencies are explained in the following equation.

$$Y = 16.8 - 0.413X_1 + 0.0185X_2 + 0.00060 X_1^2 - 0.000117X_2^2 + 0.00104X_1 X_2$$

where:

- Y The yield of sorghum bug oil,
- X₁ Temperature,
- X₂ Pressure.

The high coefficient of determination ($R^2 = 0.957$) as well as the high adjusted R^2 (0.92) are good indicators of the data validity. The closer the R^2 value to unity, the better the empirical model fits the actual data [22]. In some cases, R^2 increases with the increase of factors even though some residuals exist in the data. In such cases, R^2 adjusted, which removes unnecessary terms from the system, will not increase; on the contrary, it will decrease [23]. As result, the simultaneous increase of both R^2 and adjusted R^2 in our data, reveals the accuracy and are a good estimation of it.

Oil Extraction

The sorghum bug oil obtained from the various SFE extracts (Ex1–Ex14) and solvent extract, extracted with either SC-CO₂ or hexane, was intensely yellow in colour. From Table 1 the solvent extraction gave a higher yield (60.12%) than supercritical CO₂ extraction. Hexane extraction results are in good agreement with Mariod et al. [8].

Influence of Pressure and Temperature On SC-CO₂ Oil Extraction

The functional relationships between pressure and temperature were investigated. The influence of pressure and temperature on the SC-CO₂ oil extraction from sorghum bug was determined at pressures of 200 and 400 bar, and at temperatures of 50 and 70 °C, respectively, and at a constant flow rate (15 g/min). These results are given in Table 1 where the oil yield is reported as the percentage of oil per kilogram. The high extraction yield (more than 45.0%) was obtained at 300 bar and 60 °C followed by 400 bar and 70 °C, while the lower yield was obtained at 159 bar and 60 °C. In view of these results it can be stated that the effect of extraction pressure and temperature was significant ($p > 0.05$). At low pressure levels (159 and 200 bar), the oil yield decreased due to the reduced density of CO₂ at higher temperatures [13]. However, at higher pressures (400 and 441 bar), the oil yield decreased with the rise in temperature.

Figure 2 shows the effect of the extraction pressure and temperature on the yield of sorghum bug oil at a constant CO₂ flow rate 15 g/min, it was clear that there was a significant ($p > 0.05$), effect of the extraction temperature on

oil yield. At low pressure levels (less than 300 bar), the oil yield decreased, this is most likely due to the reduced density of CO₂ at higher temperatures [13]. However, at higher pressures (400 and 441 bar), the oil yield decreased with the rise of temperature. Figure 3 shows the contour plots for the response of extraction pressure and extraction temperature to the yield of sorghum bug oil. The contours indicated that enhanced extraction pressure can increase the yield of sorghum bug oil. However, when the extraction pressure exceeded 400 bars, the yield of sorghum bug oil started to decrease. In other words, it was not advisable to use very high extraction pressure (more than 400 bars) when extracting sorghum bug oil from sorghum bug.

Essentially there is a question as to whether it is necessary to extract completely all components from sorghum bug raw materials, or prefer a selective method and extract

Surface Plot of Ex efficiency(yield) vs Pressure, Temperature

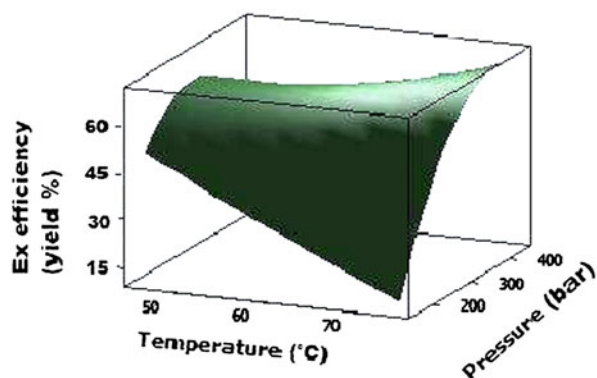
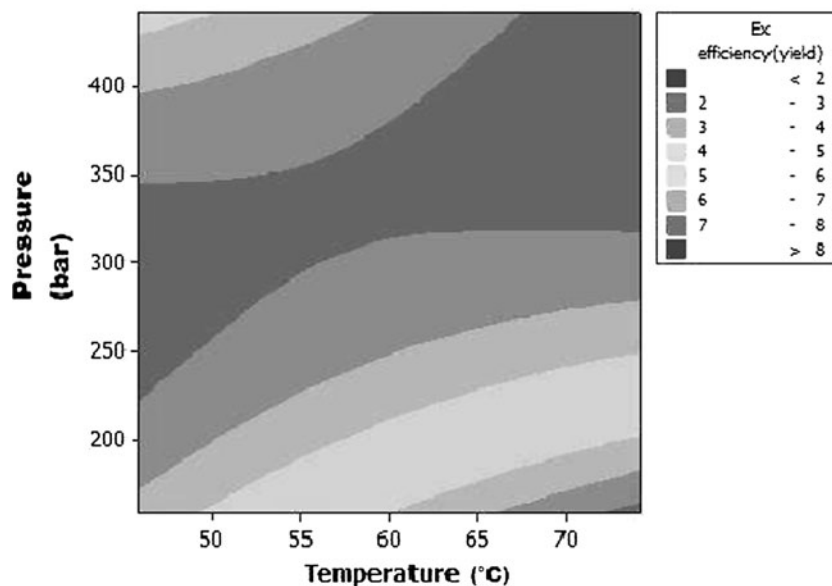


Fig. 2 Effect of the extraction pressure and temperature on the yield of sorghum bug oil at a constant CO₂ flow rate

Fig. 3 Contour plot showing the effect of extraction pressure and extraction temperature on the yield of sorghum bug oil

Contour Plot of Ex efficiency(yield) vs Pressure, Temperature



only those groups of substances, which are able to improve the qualitative characteristics of the product. The use of such an expensive technology such as SC-CO₂ extraction can be justified for the extraction of selective substances from sorghum bug that have important uses.

Fatty Acid and Tocopherol Composition

Lipids from dried powdered sorghum bug extracted by hexane and SC-CO₂ were analyzed in order to find the relationship between changes in oil antioxidant activity and its fatty acid and tocopherol composition. The methyl ester fatty acid analysis in both the hexane and the SC-CO₂ extracted oils is presented in Table 1. No differences in the fatty acid compositions of the various extracts are apparent. From the analysis, the main components of sorghum bug oil were as follows: oleic, linoleic, palmitic and stearic acids. The composition of the fatty acid (%) in the oil extracted by SC-CO₂ was varied when compared with conventional method, based on the extraction conditions. Alpha-tocopherol extracted from sorghum bug oil by the conventional solvent method was found to be 0.009 mg/g oil which was lower than that extracted by SFE process using CO₂ (Table 1). Alpha-tocopherol extracted from sorghum bug oil by SC-CO₂ ranged from 0.02 to 0.04 mg/g oil and it was not affected significantly neither by pressure nor temperature.

Antioxidant Activity by DPPH

The antioxidant capacity of sorghum bug oil extracts from sorghum bug was assessed with the DPPH radical-scavenging assay, in comparison with the known antioxidant

Table 2 Effect of extraction pressure and temperature on the color of sorghum bug oil

Extraction method	Absorbance	430 nm	460 nm	550 nm	620 nm	670 nm	PCI
SC-CO ₂ Extraction ^a	Ex1	0.6884	0.5049	0.1063	0.0801	0.1006	5.69
	Ex2	0.6350	0.5054	0.1298	0.0718	0.0664	8.91
	Ex3	0.5876	0.5037	0.1340	0.0878	0.0802	9.08
	Ex4	0.5364	0.4362	0.1446	0.1208	0.1249	8.57
	Ex5	0.5043	0.4361	0.1221	0.0826	0.0824	7.83
	Ex6	0.4493	0.3663	0.0720	0.0494	0.0536	4.50
	Ex7	0.4413	0.3807	0.0731	0.0539	0.0791	3.35
	Ex8	0.4771	0.4131	0.1205	0.1165	0.1259	6.63
	Ex9	0.5174	0.3745	0.1349	0.1005	0.1108	7.78
	Ex10	0.4923	0.4185	0.2760	0.3055	0.3411	13.14
	Ex11	0.6513	0.5319	0.1742	0.1341	0.1300	10.55
	Ex12	0.7113	0.6204	0.2233	0.1757	0.1692	43.70
	Ex13	0.4750	0.3739	0.0732	0.0508	0.0542	4.61
	Ex14	0.5518	0.4376	0.1411	0.1084	0.1055	8.50
Solvent extraction		0.3639	0.2994	0.0676	0.0531	0.0513	4.34

Means of duplicate values \pm standard deviations

^a The extracts (Ex. 1–14) were collected at constant intervals of 30 min

ascorbic acid. The DPPH radical-scavenging activity of SBOE is shown in Table 1. The DPPH radical scavenging capacity assay was used to examine the antioxidant activity of SBO extracts. The SBO extracts were assayed over a range of dilutions to establish the concentration of each oil extract required to scavenge 50% of the DPPH radical present in the assay medium, referred to as the IC₅₀ defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Lower IC₅₀ value reflects better DPPH radical scavenging activity [24]. Under the assay conditions employed here, the IC₅₀ of ascorbic acid as standard was 0.24 μ l/ml, between the SBO extracts Ext 14, 3, and 7 possessed greater DPPH radical scavenging activities with the lowest IC₅₀ values of 4.52, 6.84, and 7.31 μ l/ml, respectively. It can be observed that conventional solvent extraction method exhibited notable DPPH radical-scavenging activity, with an efficacy (IC₅₀ 7.45 \pm 0.3) slightly lower than that of the SC-CO₂ extracts. The IC₅₀ values for SBO extracts extracted by SC-CO₂ ranged from 4.52 to 26.1 depending on pressure and temperature, where an increase in the pressure resulted in an increase in its antioxidant ability.

Measurement of Oil Pigments

The color of sorghum bug oils was measured using visible absorbance together with the photometric color index PCI (Table 2). Photometric color values were determined as a measure of comparing the changes in the color of extracted oils to express them as a single number. The values for oil extracted by SC-CO₂ differed in their behavior as PCI of the oils. According to their pigment content as absorbance at 400 and 550 nm corresponds to the absorption of

carotenoid and chlorophyll pigments, and absorption at 620–670 nm corresponds to the absorption of chlorophyll pigments. When compared with sorghum bug oil extracted using the conventional solvent method the PCI, the values for oil extracted by SC-CO₂ seem to be more intensive and contain more pigments such as carotenoids, which absorb at 430–460 nm, and chlorophylls, which absorb light at 550–710 nm [25], these pigments were present at a significantly ($p < 0.05$) higher quantity, than that found in oil extracted by the solvent.

Conclusion

In conclusion, the use of supercritical CO₂ in sorghum bug oil extraction, under the specific conditions reported above, resulted in a lower oil yield, with high α -tocopherol content when compared with that obtained by solvent extraction. Solvent extracted oils exhibited notable DPPH radical-scavenging activity, with an efficacy (IC₅₀ 7.45 \pm 0.3) slightly lower than that of the SC-CO₂ extracted oils.

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