Supercritical Fluid Extraction of Oil from Millet Bran

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ABSTRACT: Proso millet bran [*Panicum miliaceum* (L.)], variety Dakota White, was extracted with supercritical carbon dioxide (SC-CO_2) to yield crude oil. The effects of operating parameters (pressure, temperature, and specific solvent flow) and of features of the raw material (moisture content and particle size) on oil extraction were investigated. Complete de-oiling of ground millet bran pellets was achieved under 300 bar at 40°C with a specific solvent flow of 2–10 h^{-1} within 200 to 500 min. Solvent requirements were 20–30 kg CO_2/kg raw material. Composition of crude SC-CO_2 oil extracted under optimal conditions, i.e., fatty acid profile, amount of unsaponifiables, tocopherols, free fatty acids, sterols, sterol esters, waxes, hydrocarbons, and phospholipids, was compared to that of crude oil obtained by petroleum ether extraction. These two oils were similar in terms of fatty acid profile and amount of free fatty acids, unsaponifiables, peroxides, and tocopherols. They differed in respect to phospholipids (present in petroleum ether-extracted oil and absent in SC-CO_2 extracted oil), metals, and waxes (lower levels in SC-CO_2 extracted oil). The effects of extraction procedures on oxidative stability of crude SC-CO_2 oil were studied. Ensuring that all pieces of the extractor in contact with the oil were in stainless steel; cleaning the separator, i.e., washing with KOH, rinsing, purging with N_2 and CO_2, and heating; performing a couple of extractions before the main extraction; and achieving the extraction without interruption all positively influenced the oxidative stability of the oil. Conversely, increasing CO_2 purity above 99.5% had no effect. Oxidative stability of the SC-CO_2 oil extracted under these conditions was only slightly lower than that of the oil extracted with petroleum ether.

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KEY WORDS: Free fatty acids, millet bran, oil extraction, oxidative stability, supercritical carbon dioxide.

Millets constitute one of the world's most important groups of food plants. They are common in much of the tropical and subtropical world, where they are cultivated in poor soils in areas of low or inconsistent rainfall (1). Millet grains have been dietary staples for centuries in parts of Africa, India, and China. Today this crop is a significant contributor to the protein and energy requirements of millions of people. Millet is processed into a wide array of foods. For production of most foods, the grains are decorticated using mortars and pestles or mechanical dehullers. This treatment removes the outer layers of the grains, i.e., the bran. Millet bran is therefore a by-product of millet-based food manufacturing. It is usually fed to animals. Lipids are relatively minor constituents in cereal grains. Whole millet grain contains about 6% (dry weight basis) oil (2). Extracting this oil requires the use of a solvent, e.g., hexane.

An alternative processing method is supercritical (SC) fluid extraction. Carbon dioxide (CO_2) is employed as a supercritical fluid because it has a low critical temperature (31.1°C) and pressure (7.28 MPa), which makes it an ideal solvent for extracting thermally sensitive materials. CO_2 is also nontoxic, nonflammable, and low cost. In addition, products obtained by SC-CO_2 extraction are completely free of solvent residues. On the contrary, conventionally solvent-extracted products must be desolventized before they are suitable for consumption. SC-CO_2 defatted meal can therefore be directly used in low-calorie foods. Also, crude oils obtained by SC-CO_2 extraction are generally more easily refined than conventionally extracted oils as they contain fewer impurities. However, SC-CO_2 extraction has a serious disadvantage in that it entails the large capital costs associated with a high-pressure operation. Accordingly, although the list of potential uses of this technology is large, only a few examples of commercial-scale SC-CO_2 processes exist.

SC-CO_2 extraction of lipids has been extensively studied in the laboratory. For example, use of SC-CO_2 to extract oil from raw materials in general (3), and from oilseeds (4–7) in particular, is frequently reported in the literature. SC-CO_2 extraction can also be used for the recovery of residual oil after press extraction (3), which usually requires organic solvents. SC fluid extraction of oils from cereal seeds with low oil content has been described in various papers (8,9). Although SC-CO_2 extraction of oat (10), corn germ (11), wheat germ (12), and rice bran (13) has been extensively described in the literature, no work has yet been published on oil extraction from millet by SC-CO_2.

The present work was undertaken to investigate on a laboratory scale SC-CO_2 extraction of millet bran. The influence of a number of experimental parameters, i.e., pressure, temperature, flow and purity of the CO_2, extraction time, drying, and milling of raw material, on oil recovery and oil quality

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was determined. The quality of oil extracted by SC-CO_2 under optimal conditions was compared to that of oil obtained by Soxhlet extraction using petroleum ether.

MATERIALS AND METHODS

Materials. Pellets (10 × 5 mm) of Proso millet bran [*Panicum miliaceum* (L.)], variety Dakota White, were obtained from E. Zwicky AG (Müllheim-Wigoltingen, Switzerland) and stored at 6°C before use. They contained 7–8% moisture (Karl Fischer) and 7% oil (Soxhlet extraction with petroleum ether, bp 40–60°C). They were used either as such or after drying for 3 h at 105°C (residual moisture 1–2%). Alternatively, they were ground using a kitchen Moulinex (model 53402/2.20; Moulinex, France): 80% of the particles of the meal were between 290 and 1200 μm.

Oil extraction. Lipids were removed either by Soxhlet or SC-CO_2 extraction. In the first case, lipids were extracted from ground pellets in a Soxhlet extractor with petroleum ether, bp 40–60°C (E. Merck, Darmstadt, Germany) (14). Pellets or meals, either unprocessed or dried, were used for SC-CO_2 extraction. Lipids were extracted either using an Isco (Isco Inc., Lincoln, NE) SFX-200 extractor (extraction volume 10 mL, service pressure 60–500 bar) or a Separex (Champigneulles, France) SFE 500 extractor (extraction volume 350 mL, three-step decompression system, three 100-mL cyclonic separators, service pressure 60–300 bar, service temperature 20–70°C, total solvent recycling). During extraction with the SFE 500, oil was distributed within the three separators. Food grade (99.5%, <5,000 ppm O_2/N_2, <400 ppm H_2O) CO_2 (Ossigeno SA, Magadino, Switzerland) was used for SC-CO_2 extractions. For comparison, some extractions were performed using either 99.99% or 99.995% CO_2 (Ossigeno SA). Oil extracted with the small-scale extractor was separated from water and from the solid phase by centrifugation at 6,000 rpm using a Wifug X1 101-38 centrifuge (Wifug, Stockholm, Sweden), and stored under nitrogen at 6°C before being analyzed. The amount of oil recovered by SC-CO_2 extraction was reported as percent oil obtained by Soxhlet extraction.

Lipid classes. Lipids of the SC-CO_2- and petroleum ether-extracted oils were analyzed by high-performance thin-layer chromatography (HPTLC) coupled with scanning densitometry as previously described (15). Samples (oil dissolved in 2:1 vol/vol chloroform/methanol) were spotted using a Linomat IV (Camag AG, Muttenz, Switzerland) onto a 10 × 20 cm HPTLC silica gel plate (Merck # 1.05642) that was previously cleaned by onefold elution with chloroform/methanol 1:1 (vol/vol) and dried for 15 min at 100°C. To separate hydrocarbons, sterol esters, and waxes, the plate was first developed to 98 mm with toluene/hexane/formic acid 140:60:1 (vol/vol/vol). After drying under N_2, the plate was developed to 52 mm with hexane/diethylether/formic acid 60:40:1 (vol/vol/vol) to separate sterols, diacylglycerols, free fatty acids, and triacylglycerols. Spots were revealed using a dip-in copper sulfate phosphoric acid reagent (10% $CuSO_4$ in 8% H_3PO_4) and charring for 9 min at 180°C. They were identified by analyzing a mix of cholesteryl palmitate, tripalmitin, palmitic acid, cholesterol (Mix A from Supelco, Bellefonte, PA), squalene (Supelco), 1,2-dipalmitoyl-*sn*-glycerol (Sigma, Buchs, Switzerland), and oleic acid stearyl ester (Sigma) under the same conditions. Optical density (λ = 547 nm) of the spots was measured in reflectance mode using a CD-60 densitometer (Desaga, Heidelberg, Germany).

Phospholipids were separately analyzed by HPTLC coupled with scanning densitometry as previously reported (16). Oil samples were spotted on a boric acid-impregnated silica gel plate (Merck # 1.05642) using a Linomat IV. The plate was first eluted with hexane/ether 6:4 (vol/vol) to remove excess neutral lipids from the over-loaded sampling zone and then with chloroform/ethanol/methanol/triethylamine/water 30:25:10:38:8 (by vol).

Phospholipid spots were visualized using a molybdate dip-in reagent and scanned at λ = 595 nm, using the CD-60 densitometer in reflectance mode. They were identified by analyzing a mix of phosphatidylcholine, lysophosphatidylcholine (LPC), phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, lyso-*N*-acyl-phosphatidylethanolamine (mixed soy phospholipid reference standard; Spectral Service GmbH, Köln-Ehrenfeld, Germany), synthetic monopalmitoylphosphatidylcholine (Avanti Polar Lipids Inc., Pelham, AL) under the same conditions.

Fatty acid composition. Fatty acid methyl esters (FAME) were prepared and analyzed by gas chromatography (GC) (17). FAME were prepared by reacting the oil in *n*-hexane with 2 N methanolic potassium hydroxide and injected into a Carlo Erba HRGC chromatograph (CE Instruments, Rodano/MI, Italy) equipped with a flame-ionization detector. Separation was achieved on a wall-coated open-tubular fused-silica capillary column coated with 100% cyanopropyl polysiloxane (CP-Sil 88 50 m × 0.32 mm i.d., 0.20 μm film thickness, type FAME; Chrompack, Middelburg, The Netherlands). Carrier gas was hydrogen (purity >99.9997 vol%) at 80 kPa. The oven temperature program was as follows: 70°C, 2 min iso, 30°C/min → 135°C, 1 min iso, 3°C/min → 180°C, 15°C/min → 220°C, 5 min iso. Data acquisition and peak integration were done using a Chrom-Card version 1.19 (CE Instruments). Peaks were identified by comparison of retention times with standard GLC-85 (Nu-Chek-Prep, Elysian, MN).

Tocopherols and tocotrienols. Tocopherols and tocotrienols were measured by high-performance liquid chromatography (HPLC) using a LaChrom acquisition system from Merck-Hitachi (E. Merck; Hitachi Ltd., Tokyo, Japan) (17). The conditions for HPLC measurements were as follows: column, Lichrospher Si 60 (5 μm), 250 × 4 mm (direct phase); precolumn, Si 60 (5 μm), 4 × 4 mm (both columns were purchased from E. Merck); mobile phase, mixture of *n*-hexane and dioxane; program, *n*-hexane/dioxane 95:5 (vol/vol) for 23 min, *n*-hexane/dioxane 50:50 (vol/vol) for 5 min, and *n*-hexane/dioxane 95:5 (vol/vol) for 10 min; flow rate, 1.6 mL/min; fluorescence excitation, 295 nm; and fluorescence emission, 330 nm. Peaks were identified by compar-

ison with a standard, i.e., a solution of each, tocopherol and tocotrienol homolog (E. Merck) diluted to 0.005 mg/mL in *n*-hexane.

Metals. Metals were quantified by inductively coupled plasma mass spectrometry according to a standard method (18). A number of metals were screened in one run. Semi-quantitative results, i.e., ± 30%, were obtained.

Physicochemical characteristics. Peroxide value (PV) expressed as meq O_2/kg oil was measured according to the American Oil Chemists' Society (AOCS) official method (19). Free fatty acid (expressed as wt% oleic acid) was measured according to International Union of Pure and Applied Chemistry standard method 2.201 (20). Unsaponifiables were determined according to a standard method (21). Moisture content (expressed in wt%) was determined using a Karl Fischer apparatus Multidosimat 645 (Metrohm AG, Herisau, Switzerland) according to the AOCS official method (22) but with a ready-to-use reagent, Hydranal Composite 2, (Riedel-de-Haën, Seelze, Germany).

Oxidative stability index. Oxidative stability of the oil was determined at 110°C with the Rancimat (Metrohm AG) apparatus, according to the AOCS official method (23).

RESULTS AND DISCUSSION

Process parameters. Two phases were observed during SC-CO_2 extraction of oil from millet bran (Figs. 1 and 2). Initially, the kinetics of the oil mass transfer were high. This period, called the washing period (8,9), lasted until around 60–70% of the total oil present in the millet bran had been extracted. During this period, the fluid phase resisted mass transfer (4). This resulted in a linear relationship between the mass of the extracted oil and the quantity of solvent used. Then, when around 30% of the total oil remained in the sample, the kinetics of the oil mass transfer slowed down and followed an asymptotic curve. In this second period, called the diffusion period, the mass transfer is controlled by the resistance of the solid matrix to diffusion (4). It is necessary to extract more than 70% of the oil to obtain a well-defatted cake. This implies the use of a large amount of solvent and a long extraction time: for example, extracting the last 14% of oil increases the extraction time by about 30%, thereby correspondingly reducing the daily amount of raw material processed in an industrial plant.

It is known that the rate of extraction with SC-CO_2 can be greatly enhanced by raising the pressure of the fluid to 900 bar (24). This is due to the increase of oil solubility in CO_2 as the pressure increases (4,24). However, working with pressures higher than 300–400 bar is not practical (very high investment costs); therefore, lipid extractions are usually carried out at pressures of 300–400 bar (24). In this work, the effect of pressure was investigated between 100 and 500 bar. As can be seen in Figure 1, the higher the pressure, the higher the kinetics of the oil mass transfer during the washing period.

The influence of temperature was investigated between 40 and 60°C (Fig. 2). A decrease in the kinetics of the oil mass

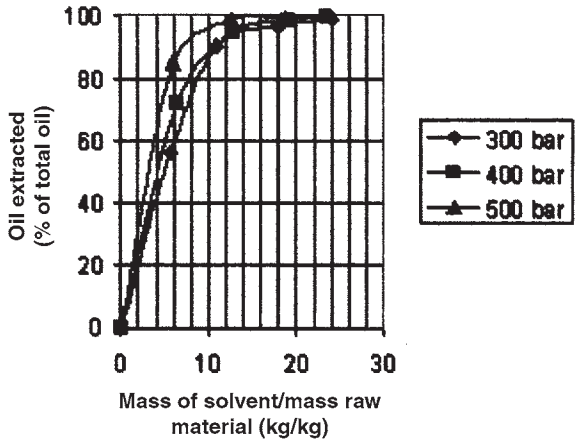

FIG. 1. Effect of pressure on oil extraction from millet bran (raw material, 4 g dried and ground pellets; extractor, Isco (Lincoln, NE); temperature 40°C; solvent flow, 1 mL/min).

transfer was observed when the temperature was raised. Contrary to the pressure, temperature had almost no effect during the washing period, although it increased the extraction rate during the diffusion period. This is in agreement with previously reported data showing that between 300 and 350 bar extraction is favored at lower temperatures (4). This is important for complete defatting of the raw material and also for the extraction of unsaponifiables like sterols (25).

The effect of specific solvent flow (solvent flow/mass raw material, $kg \cdot h^{-1} \cdot kg^{-1} = h^{-1}$) was investigated between 1 and 20 h^{-1}. Relatively slow kinetics of oil mass transfer were recorded with specific solvent flow between 15 and 20 h^{-1} (Fig. 3). The kinetics were, however, much higher when the specific

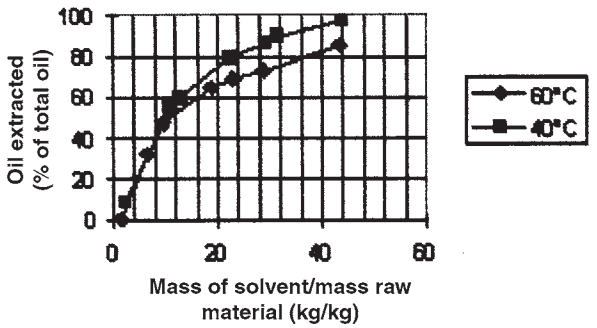

FIG. 2. Effect of temperature on the oil extraction from millet bran (raw material, 220 g undried pellets; extractor, Separex (Champigneulles, France); extraction pressure, 300 bar; pressures in the three separators, 100, 100, and 60 bar, respectively; specific solvent flow, 1 h^{-1}).

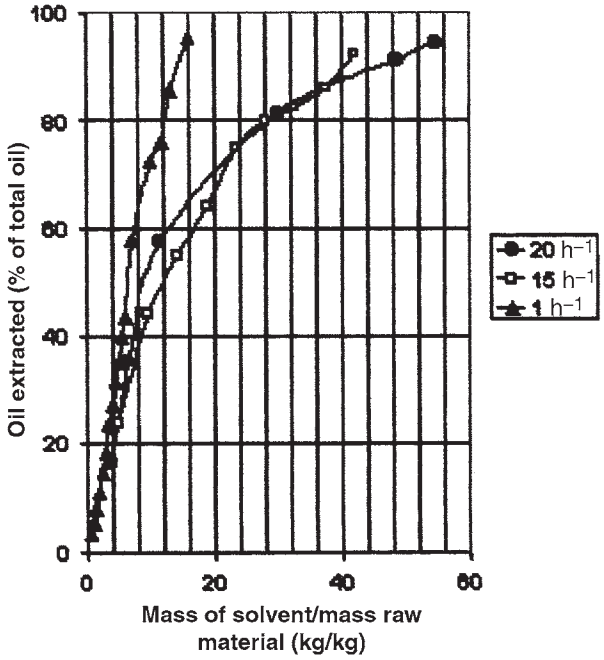

FIG. 3. Effect of specific solvent flow on the oil extraction from millet bran (raw material, 220 g of ground and dried pellets; extractor, Separex; temperature, 40°C; extraction pressure, 300 bar; pressures in the three separators, 100, 100, and 60 bar, respectively). For manufacturer see Figure 2.

solvent flow was 1 h^{-1}. A low specific solvent flow corresponds to a long solvent residence time in the extraction vessel, which favors oil mass transfer from the sample to the solvent. Accordingly, a low specific solvent flow improves the extraction performance, particularly during the diffusion period, and decreases the total solvent mass required to extract a given amount of oil (Fig. 4).

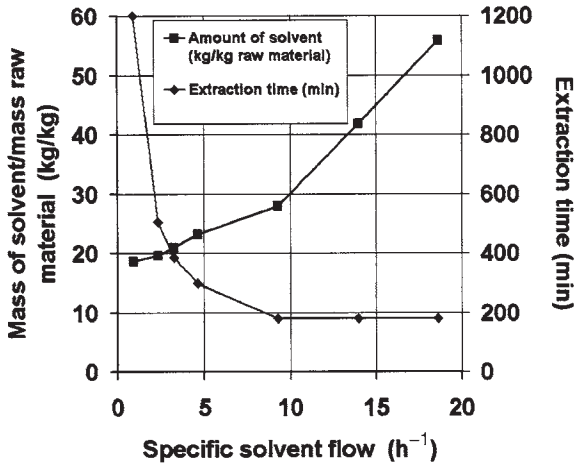

FIG. 4. Amount of solvent mass and time needed for the extraction of millet bran in function of solvent specific flow (raw material, 215–220 g dried and ground pellets; extractor, Separex; temperature, 40°C; extraction pressure, 300 bar; pressures in the three separators, 100, 100, and 60 bar, respectively). For manufacturer see Figure 2.

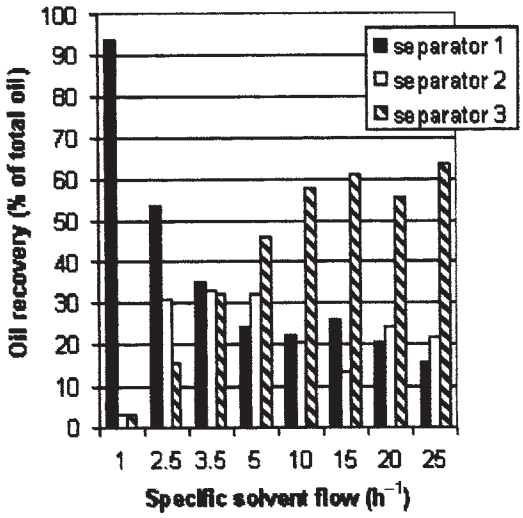

FIG. 5. Distribution of the extract between the three separators as a function of the solvent flow rate (raw material, ground and dried pellets; extractor, Separex; temperature, 40°C; extraction pressure, 300 bar; pressures in the three separators, 100, 100, and 60 bar, respectively). For manufacturer see Figure 2.

Moreover, together with pressure, the specific solvent flow also influences the distribution of the oil and water between the three separators. This distribution is determined by the pressure (solubility) and by the specific solvent flow (dragging). For example, about 90% of the oil was recovered in the first two separators when the specific solvent flow was below 2 h^{-1} (Fig. 5). Under these conditions the major part of the water was collected in the third separator, whatever the moisture content of the raw material. By comparison, only half of the oil was collected in the first two separators when the specific solvent flow was 5 h^{-1} (Fig. 5).

However, as specific solvent flow decreased, the extraction time increased (Fig. 4) and the process became uneconomic due to low throughput. Accordingly, to obtain an efficient extraction the solvent flow must be optimized (4). The present study shows that for economic operation the specific solvent flow should be between 2 and 10 h^{-1}.

In summary, optimal process parameters for extracting oil from millet bran with the Separex SFE-500 instrument (maximal working pressure: 300 bar) were pressure, 300 bar; temperature, 40°C; and specific solvent flow, 2–10 h^{-1}. Under these conditions a complete de-oiling of millet bran could be achieved within 200 and 500 min and with the use of 20–30 kg CO_2/kg raw material (Fig. 4).

Features of the raw material. Moisture and particle size are the main features of the raw material that influence SC-CO_2 oil extraction from plant material (4). However, reducing the moisture content from 8 to 1–2% did not modify the oil mass transfer during SC-CO_2 extraction of ground pellets

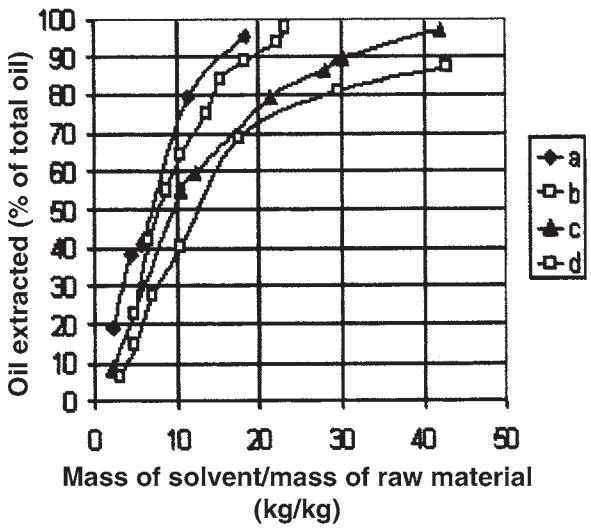

FIG. 6. Effect of milling and drying on the solvent mass needed (extractor, Separex; temperature, 40°C; extraction pressure, 300 bar; pressures in the three separators, 100 bar, 100 bar, and 60 bar, respectively; a: ground/dried; b: ground/not dried; c: pellets/not dried, d: pellets/dried).

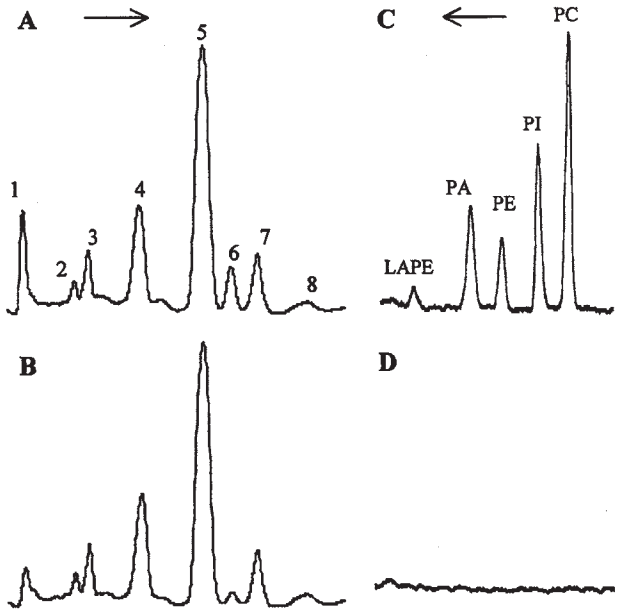

FIG. 7. High performance thin layer chromatography (HPTLC) separation of lipid classes (A and B) and phospholipids (C and D) in Soxhlet- (A and C) and SC-CO_2-extracted (B and D) crude oils. Lipid classes comprised phospholipids and unknown compounds (1), sterols (2), diacylglycerols (3), free fatty acids (4), triacylglycerols (5), waxes (6), sterol esters (7), and hydrocarbons (8). Phospholipids present in peak no. 1 included phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidic acid (PA), and lyso-N-acyl-PE (LAPE). The elution direction of the HPTLC solvent is shown by an arrow.

of millet bran (Fig. 6). This result is in agreement with previously reported data showing that moisture between 3 and 12% has little effect on oil mass transfer during SC-CO_2 extraction (26). Surprisingly, the oil mass transfer was slightly more efficient in pellets that were not dried (moisture around 8%) compared to dried pellets (1–2% moisture). It is worthwhile also to mention that drying of cereals, millet bran in particular, before oil extraction may produce a bitter taste in the cake (1).

Grinding the pellets improved the SC-CO_2 extraction performance by extending the washing period (Fig. 6). This is likely due to disruption of the oil-containing cell structure (26,27). Grinding the pellets before extraction has, however, practical drawbacks, such as loading difficulties and pressure instability during extraction.

Comparison of SC-CO_2- and petroleum ether-extracted crude oils. Composition of the crude oil obtained by SC-CO_2 extraction with the Separex SFE 500 under optimal conditions (pressure, 300 bar; temperature, 40°C; specific solvent flow, 2–10 h^{-1}; raw material, not dried ground pellets) was compared with that of crude oil obtained by Soxhlet extraction using petroleum ether, bp 40–60°C. Analysis of neutral lipids by HPTLC showed, in addition to acylglycerols, the presence of sterols, waxes, sterol esters, and hydrocarbons in both oils. Apart from a lower content of waxes in SC-CO_2-extracted oil, amounts of other neutral lipids were not markedly different in the two oils (Fig. 7A,B).

As already reported for other oils (7,10,11,28), phospholipids are essentially absent from SC-CO_2-extracted crude millet bran oil. This was confirmed in our SC-CO_2 extracts, which contained only minor amounts of compounds at the start of the chromatogram (Fig. 7B). Since their reaction with the molybdate reagent was negative (Fig. 7D), it is thought that they included monoacylglycerols and the least polar glycolipid species such as esterified sterylglycosides. Conversely, all major phospholipid classes were detected in a crude oil obtained by Soxhlet extraction (Fig. 7C), except for LPC, whose extraction requires chloroform/methanol solvents. It was reported that extracting phospholipids is feasible either by using high pressures or a co-solvent, e.g., hexane and ethanol (10,29).

In other respects, the compositions of the two oils were similar. For example, no significant differences were observed between their fatty acid profiles. Similarity in the fatty acid profile of SC-CO_2- and Soxhlet-extracted oils was already observed (10), although the fatty acid composition of SC-CO_2-extracted oil has been reported to vary slightly with temperature and pressure (30,31). The major fatty acids present in these two oils were linoleic (64%), oleic (23%), and palmitic acid (7%), which agrees with a previous study (32). Similarity between these two oils was also observed for free fatty acids (FFA) (~12% in each oil), unsaponifiable matter (5.9% for the oil obtained by Soxhlet extraction compared to 5.1% for the SC-CO_2-extracted oil), peroxides (3–6 meq O_2/kg), and tocopherols (350–400 mg/kg in each oil, of which about 70% is γ-tocopherol) (33). Tocopherol recovery during SC-CO_2 extraction was shown to decrease drastically when

the temperature increased (11,25). At low temperatures, however, amounts of tocopherols in SC-CO_2-extracted oils were similar, or even higher, than in oils extracted conventionally (11,25,28,34).

Except for phospholipids (which were almost absent), waxes, and metals (which were present at lower levels), crude SC-CO_2-extracted oil had a similar composition to that obtained by Soxhlet extraction using petroleum ether. It is worthwhile noting that the amount of FFA is as high in crude SC-CO_2 oil as in Soxhlet oil. In spite of the relatively high amount of FFA, refining of crude SC-CO_2 oil should be much easier than refining of crude Soxhlet oil due to the absence of gums in SC-CO_2 oil and to its low content of waxes and metals. Also, refining of crude SC-CO_2 oil should entail much less loss on oil than that of crude petroleum ether oil.

Oxidative stability of the SC-CO_2 crude oil. SC-CO_2 extraction has been associated with oxidative instability of the oil (7,34,35). Various reasons have been put forward to explain this lower stability: selectivity of SC-CO_2 as a solvent (7); lower content of phospholipids known to have a synergistic antioxidative effect with tocopherols (35); presence of oxygen in commercial CO_2 used for extraction (34); introduction of oxygen into the apparatus during the extraction (7); and contamination of the oil with heavy metals from the equipment (7). Food grade (99.5%) CO_2 was used in our experiments. However, using CO_2 of higher purity (99.9% and 99.995%) did not improve the oxidative stability of the extracted oil.

A slightly shorter induction period was recorded for the SC-CO_2-extracted millet bran oil as compared to the Soxhlet-extracted oil (mean value 6 h and 7 h at 100°C, respectively). This relatively highly stable SC-CO_2-extracted oil can be obtained provided some precautions are taken. First, all pieces of equipment in contact with the oil during extraction must be made of stainless steel. This results in a lower concentration of metals in the SC-CO_2-extracted oil than in the control oil (Table 1). Second, the extractor must be thoroughly cleaned before collection of samples can start. The circuit was thus washed with a solution of 0.1 N KOH until the solution at the outlet had no turbidity; then, it was rinsed with distilled water until neutrality, purged with nitrogen and CO_2, and finally heated at 40–50°C overnight. The conditioning was completed by performing two oil extractions of millet bran before the main run. Indeed, oil extracted immediately after washing, rinsing, purging, and drying was shown to be less stable than samples obtained during successive extractions. Third, extractions should be achieved without interruption. Stopping the extraction for some time (e.g., for a few hours) was shown to have a detrimental effect on oil stability, even when the extractor was kept under pressure of CO_2 during the interruption. Fourth, co-extracted water has to be separated from the oil as soon as possible. Accordingly, samples were centrifuged immediately after extraction and the water removed. These results indicate that contamination of oil by impurities present on the surface of the extractor circuit (e.g., metals and water) and in the gas phase (e.g., oxygen and vapor) are important factors influencing the oxidative stability of oils extracted by SC-CO_2.

TABLE 1
Concentration of Metals in Millet Bran Oils (mg/kg)

Metals	SC-CO_2-extracted oil[a]	Petroleum ether-extracted oil
Magnesium	0.1	200
Iron	0.2	19
Zinc	0.6	16
Manganese	0.2	7
Copper	0.2	1
Nickel	0.7	0.6
Aluminum	0.2	1
Platinum	<0.005	<0.005

[a]SC-CO_2, Supercritical carbon dioxide.

The slight difference in oxidative stability observed between the SC-CO_2 oil and the Soxhlet oil does not come from tocopherols, as similar levels of these antioxidants were measured in the two oils, or from metals (these contaminants were at a lower level in SC-CO_2 oil). Accordingly, the most likely explanation lies in the absence of phospholipids in SC-CO_2 oil, which are known to act synergistically with tocopherols (35). Provided some precautions are taken during extraction, the oxidative stability of crude SC-CO_2 oil is similar to that of the Soxhlet oil.

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