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Supercritical CO_2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes

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ABSTRACT

Flax oil is commonly used in food due to high percentage of omega-3-fatty acid and omega-6-fatty acid. In the present work the flax seed was extracted using green solvent viz. supercritical CO_2 and compared with soxhlet and mechanical screw press methods. The chemical compositions of the oils were determined by CHNS analyser, GC-FID, GC/MS and ^{1}H NMR. The supercritical CO_2 process selectively extracted the fatty oils with high percentage of omega-3-fatty acid and omega-6-fatty acids. The chemical composition of screw press oil is close to that of supercritical CO_2 extracted oil, whereas the yield is nearly 27% less in comparison to the supercritical CO_2 method.

1. Introduction

Flaxseed or linseed (*Linum usitatissimum* L.), which is a member of the *Linaceae* family is an important oilseed crop in the world. The plant is not a new crop and native to West Asia and the Mediterranean region. It is mainly grown in Canada, Argentina, America, China and India (Wang et al., 2007). Flax is the Canada's third major oilseed crop after canola and soybean. Flax is an economically important oilseed crop, especially for Canada, which produced about 40% of the world's flaxseed and is the world's largest exporter, representing about 75% of the global flax trade (Oomah and Mazza, 1999). The world demand for flaxseed is currently dominated by the industrial use of flaxseed oil. However, flaxseed is making great strides in the world's food supply, and demand for human food and livestock markets is expected to increase owing to the unique properties of this ancient crop (Oomah, 2001).

Flaxseed is rich in fat, protein and dietary fibre. The compositions of flaxseed averaged 30–40% fat, 20–25% protein, 20–28% total dietary fibre, 4–8% moisture and 3–4% ash, and the oil contains vitamins A, B, D and E, minerals, and amino acids (Bhatty, 1997). Traditionally, flaxseed has been grown for its oil, which is used in the manufacture of paints, varnishes and linoleum, because of its drying and hardening properties when exposed to the air and sunlight. Flaxseed oil is used as a purgative for sheep and horses. There is a market for flaxseed meal as both animal feeding, human nutrition and also as poultry feed since it increases levels of omega-3-fatty acid in eggs (Rebolé et al., 2002). It is the most prominent oilseed studied to date as a functional food, as it is a leading source of the omega-3-fatty acid, which is known as α-linolenic acid (ALA) (52% of total fatty acids) (Oomah and Mazza, 1998).

Commercial production of vegetable oils is based on mechanical pressing and extraction. The mechanical expression of oil from oilseeds is one of the method mostly used in the removal of oil from oil-bearing materials. This method which offers the possibility of using the cake residue has relatively low initial and operational costs and produces uncontaminated oil (Fasina and Ajibola, 1989). However, mechanical oil-expression equipment and processes presently available are not considered adequate for this purpose, as their oil extraction efficiency is quite low (<70% oil extraction) (Bargale et al., 1999; Willems et al., 2008). The yield obtained by mechanical pressing as usually lower than those extracted by solvents viz. hexane and pentane. It is only in the last century that solvent extraction has been used in this field. The advantage of solvent extraction is the high yield that can be obtained economically with this method (>99 wt.%), but this is at the expense of a reduced oil quality. This quality reduction is caused by the extensive solvent recovery processes that are necessary and the fact that the solvent co-extracts undesired components from the seeds. Especially for high value added oils this quality reduction is unacceptable, limiting the production process to mechanical expression (Venter et al., 2007; Willems et al., 2008).

The supercritical-fluid extraction technique has been studied extensively as an alternative to conventional methods of oil extrac-

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tion (King and List, 1993). Supercritical fluids have gas-like diffusivities but liquid-like densities. These properties vary as a function of pressure and temperature. Supercritical carbon dioxide (SC-CO_2) has been the most frequently used supercritical fluid for oil extraction, since it is nontoxic, nonflammable, inexpensive, and easily separated from the extract (Naik et al., 1989; Rout et al., 2008). Furthermore, the low critical temperature of CO_2 (31 °C) allows extraction of thermolabile compounds without degradation (Rizvi, 1994). A number of other plant oils including sunflower, rapeseed (Stahl et al., 1980), corn (Friedrich and Pryde, 1984), wheat germ (Taniguchi et al., 1985), palm (Kalra et al., 1987), canola (Fattori et al., 1988), cottonseed (Bhattacharjee et al., 2007), safflower seed (Han et al., 2009) were also successfully extracted with supercritical CO_2. Reverchon and De Marco (2006) reviewed, the numerous works carried on the application of supercritical CO_2 in food processing, pharmaceuticals and nutraceutical. This process has an advantage to isolate unsaturated fatty acids (omega-3 and omega-6) at lower temperature from flaxseed.

Presently, we extracted the flax seed by supercritical CO_2 extraction process and compared with soxhlet extraction, mechanical screw pressing and also determined their chemical compositions.

2. Materials and methods

Flaxseed used in this study was a commercial seed, procured from agricultural farm, Saskatoon, Canada. The sample was cleaned manually to remove all foreign materials such as dust, stones, dirt, immature seed. The clean and graded seeds were kept in an air tight plastic vessel and stored at 5 °C for study. Before starting a test the seeds were allowed to warm up under ambient room conditions (22–25 °C, 30–40% RH) to the equilibrium moisture. Then the samples were taken for experimental purpose. A screw press (Mini 40 screw press, Simon Rosedowns Ltd., Hull, England) was used to press the flaxseed in one pass. Carbon dioxide (99.9%) was obtained from Praxair (Edmonton, Canada). The standard fatty acid methyl esters, hexane (>99%) and analytical reagents were purchased from Sigma chemicals. The seeds were ground by using a laboratory grinder (Thomas Scientific, USA), which were used for CO_2 and solvent extraction. Moisture content was determined according to AOCS Method 2-54 (AOCS, 1993).

2.1. Extraction of fatty oils

The extraction was carried out by soxhlet method, screw press method and supercritical CO_2 (SC-CO_2) method. The flaxseeds used in each of the method were of the same batch and the yields reported are average of three experiments.

2.1.1. Soxhlet extraction

The flaxseed (50 g) was taken in a soxhlet extraction apparatus and extracted with hexane for 10 h. Then, solution was filtered and then followed by solvent removal in a rotary evaporator under *vacuo* at 40 °C. The yield of the oil was 19.4 ± 0.4 g. The oil samples were kept at 5 °C in a refrigerator for further analysis.

2.1.2. Screw pressing

Flaxseed oil recovered by pressing was obtained from a laboratory mechanical screw press as shown in Fig. 1. The capacity of the screw press was 5 kg seed/h. Samples (1000 g) with moisture content of 8.2% (d.b.) were fed from the hopper to the screw press on demand by gravity and the oil is collected at oil outlet point. The yield of the oil was 255 ± 1.6 g. The oil samples were kept at 5 °C in a refrigerator for further analysis.

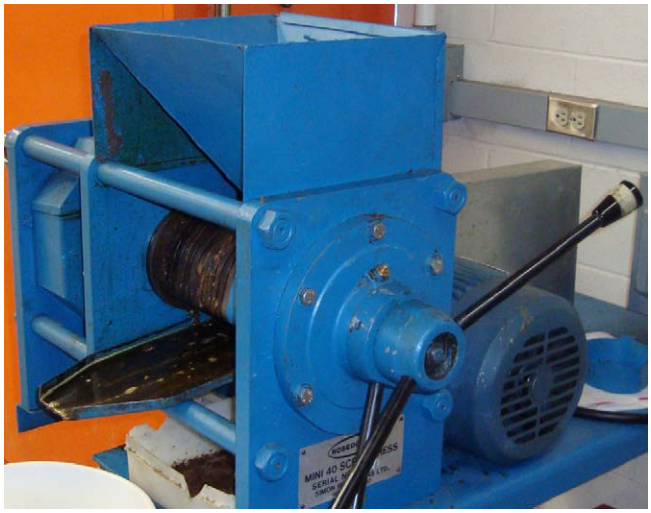

Fig. 1. Laboratory mechanical screw press oil expeller.

2.1.3. Supercritical CO_2 extraction

The CO_2 extraction was performed with a supercritical-fluid extraction system (Thar Technology, USA). The schematic diagram is presented in Fig. 2. Carbon dioxide was compressed to the desired pressure by using a diaphragm compressor. The extraction vessel was heated with a heating jacket, and temperature was controlled by a thermostat (±1 °C). Pressure was controlled by a back-pressure regulator. Ground seeds (100 g) were loaded into a 400 ml vessel covered by glass wool and extracted with CO_2 at a flow rate of 40 g/min. The temperature was kept 50 °C and the extractions were performed at a pressure of 30 MPa. The extracts were collected in another vessel attached to the depressurization valve, which were held in a circulating refrigerated bath at 0 °C. The yield of the oil was 35.3 ± 0.4 g with extraction time of 3 h. The collected fractions were stored in a refrigerator (5 °C) for further analysis.

2.2. Analysis of fatty oils

The moisture content of the oil was determined by Karl Fischer coulometer. The acid value and peroxide value of the oil were determined by ISI (1986) and AOAC (1984) methods and chemical characterisations were carried out by CHNS, GC-FID, GC/MS and ^{1}H NMR.

2.2.1. CHNS analysis

The common organic elements viz. C, H, N and S were analysed in Vario Elementar CHNS analyzer. The sample (5.0 mg) was taken in a tin capsule assortment for percentage composition of C, H, N and S and the percentage O was determined by means of difference.

2.2.2. GC and GC/MS analysis

The compositions of the oils were determined by GC-FID and GC/MS analysis. First the oils were converted to corresponding fatty acid methyl ester (FAME) by following the standard procedure (Hammond, 1993). Oil (0.5 g) was treated with 5 mL of H_2SO_4:toluene:methanol (1:10:20) reagent and reflux the mixture for 1 h. After cooling the total mixture was diluted with 5 mL of water and followed by 7 mL of hexane. The top hexane soluble layer was isolated by means of a separating funnel and dried over Na_2SO_4. The dried samples were transferred to sample vials and kept in the refrigerator for GC-FID and GC/MS analysis.

GC analysis of FAMEs were carried out on a Varian CP-3800 Gas Chromatograph equipped with a flame ionization detector (FID)

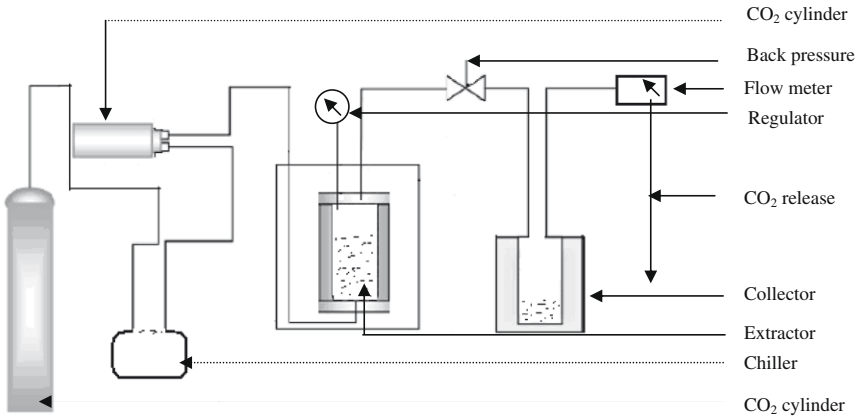

Fig. 2. Supercritical CO_2 apparatus.

and a 30 m × 0.25 mm WCOT column coated with 0.25 μm film thickness of polyethylene glycol supplied by Supelco (Supelcowax). Helium was used as the carrier gas at a flow rate of 1.0 mL/min at a column pressure of 22 kPa. Of each sample (0.2 μl) was injected into the injection port of the GC using a split ratio of 50:1. Compound separation was achieved following a linear temperature program of 160 °C (1 min), 160–240 °C (4 °C/min), 240 °C (24 min), so the total run time was 45 min. Percentage composition was calculated using peak normalization method assuming equal detector response. Each sample was injected twice in GC; thus a total of six GC analyses were performed for the process. The peaks were identified by co-eluation of standard methyl ester samples procured from Sigma–Aldrich in the same GC conditions. The GC/MS analysis was carried out on a Varian Saturn 2200 GC/MS fitted with the same column and temperature programmed as above. MS parameters: ionization voltage (EI) 70 eV, peak width 2 s, mass range 40–500 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra available on NIST-1 and NIST-II libraries.

2.2.3. ^1H NMR analysis

^1H NMR was recorded on DPX-500 Brucker UltraShield™ at 500 MHz at 25 °C. Each sample of oil (0.2 g) was dissolved in $CDCl_3$ for spectral analysis.

3. Results and discussion

In literature few works on fatty oil extraction using supercritical CO_2 were reported (Stahl et al., 1980; Fattori et al., 1988; Han et al., 2009). Stahl et al. (1980) observed that the solubility of seed oil in supercritical CO_2 was more pronounced above 25 MPa pressure and 40–50 °C. Fattori et al. (1988) reported that the optimum condition for extraction of canola seed oil in supercritical CO_2 at 50–55 °C with 30–35 MPa pressure. Recently, Han et al. (2009) reported the extraction of safflower seed oil in supercritical CO_2 at 28 MPa pressure. In considering all the above reported results, we decided to carry out the extraction of the flax seed in supercritical CO_2 at 50 °C with 30 MPa pressure.

The moisture content, acid value, peroxide value and organic element (C, H, N) are presented in Table 1. In comparing the three processes the H/C molar ratio is highest and O/C molar ratio is lowest in solvent extracted oil. The empirical formulae suggested that the solvent extracted oil contain more saturated fatty acids (11.9%) in comparison to the other two processes viz. supercritical CO_2 and screw press expression, whereas supercritical CO_2 extracted oil contains more unsaturated fatty acids.

The yields of the fatty oils and their chemical compositions are presented in Table 2. The supercritical CO_2 method gave 27.7% more yield in comparison to the screw press process. The lowest yield (25.5%) was obtained by screw pressing was most likely due to the low efficiency of the mechanical press. For instance, the yield of oil obtained by this method from flaxseed was about 34.3% less in comparison to the soxhlet method. In soxhlet extraction method, hexane was used as solvent, and it extracted mostly the neutral lipids present in the seeds. Therefore, soxhlet method of extraction was considered as the complete extraction of neutral lipids and the process was carried out for 10 h. The higher yield of lipids may be due to the extraction of neutral lipids along with few percentages of polar lipids. It was reported that in the process of oil extraction generally hexane extract contain a few percentage of glycol lipids and phospholipids in comparison to the mechanical expression (Sahoo et al., 2003). The hexane extracted oil has higher acid value (1.1) and peroxide value (3.6) in comparison to screw press and supercritical CO_2 extract oil, therefore the hexane extracted oil is inferior in quality. The yield of oil in soxhlet extraction process was in agreement with the value reported by Bhatty (1997). Whereas, the polar lipids were less soluble in supercritical CO_2 (Taniguchi et al., 1985), therefore supercritical CO_2 at the above mentioned conditions was selectively extracted the desired fatty acids such as neutral lipids.

The chemical compositions of the fatty acids in the extracts are also presented in the Table 2. There are 11 fatty acid identified by

Table 1
Moisture content and ultimate analyses [a] of oils [b].

Oils extraction method	Moisture (%)	Acid value (mg KOH/g)	Peroxide value (meq/kg)	C (%)	H (%)	N (%)	O[A] (%)	H/C molar ratio	O/C molar ratio	Empirical formulae[B]
Solvent extraction	0.01	1.1 ± 0.2	3.6 ± 0.3	78.5 ± 0.7	12.2 ± 0.3	0.02 ± 0.01	8.8 ± 0.2	1.9	0.08	$CH_{1.9}O_{0.08}$
Screw press	0.02	0.7 ± 0.1	3.1 ± 0.2	78.6 ± 0.3	11.9 ± 0.2	0.02 ± 0.01	9.4 ± 0.3	1.8	0.09	$CH_{1.8}O_{0.09}$
Supercritical CO_2 extract	0.01	0.8 ± 0.1	3.2 ± 0.2	78.8 ± 0.4	11.1 ± 0.3	0.02 ± 0.01	10.0 ± 0.1	1.7	0.1	$CH_{1.7}O_{0.1}$

[A] % of O calculated from the difference of C, H and N.
[B] N not taken into consideration.
[a] S is not detected in any of the oil samples.
[b] Data presented are average of three analyses.

Table 2
Composition of fatty acids in different methods.

Fatty acid	Soxhlet (%)	Screw expeller (%)	Supercritical CO_2 (%)	Identification
Yield	38.8 ± 0.4	25.5 ± 1.6	35.3 ± 0.4	–
Myristic acid (14:0)	0.1	t	t	Co-eluation, MS
Palmitic acid (16:0)	7.6 ± 0.8	7.1 ± 0.4	6.2 ± 0.4	Co-eluation, MS
Palmitoleic acid (16:1)	0.1	0.2 ± 0.1	t	Co-eluation, MS
Heptadecanoic acid (17:0)	t	t	t	MS
Stearic acid (18:0)	4.1 ± 0.8	3.6 ± 0.3	3.6 ± 0.3	Co-eluation, MS
Oleic acid (18:1)	16.1 ± 0.8	14.7 ± 0.5	17.5 ± 0.6	Co-eluation, MS
Omega-6-fatty acid (18:2)	14.4 ± 0.7	15.6 ± 0.4	16.2 ± 0.5	Co-eluation, MS
Omega-3-fatty acid (18:3)	50.0 ± 1.2	53.8 ± 0.8	55.0 ± 0.8	Co-eluation, MS
Eicosanoic acid (20:0)	t	0.1	t	Co-eluation, MS
Behenic acid (22:0)	0.1	t	t	MS
Erucic acid (24:0)	t	t	t	Co-eluation, MS
SFA	11.9	10.8	9.8	
MUFA	16.2	14.9	17.5	
PUFA	64.4	69.4	71.2	
Total	92.5	95.1	96.8	

Percentage of fatty acid presented is the average of six analyses.
SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, poly unsaturated fatty acid.

Table 3
^{1}H NMR of fatty oils (percentage of total hydrogen) [a].

Chemical shift ($) (ppm)	Type of hydrogen	Soxhlet	Screw expeller	Supercritical CO_2
5.2–5.7	Olefinic protons	15.1	17.3	17.5
4.1–4.5	Allylic methine protons	3.3	4.0	4.2
3.6–3.8	Methoxy protons	<0.1	<0.1	<0.1
2.0–3.0	Allylic methylene protons	22.0	23.3	24.8
1.0–1.8	CH_2 protons	49.2	45.8	44.1
0.5–1.0	CH_3 protons	10.3	9.3	9.2

[a] Reported values are average of three analyses.

comparison of mass spectra of the compound with mass spectra available in spectral libraries. The composition of most of the major compounds is reconfirmed by co-eluation of standard fatty acid methyl esters by GC-FID. The percentage of unsaturated fatty acid is higher than in supercritical CO_2 extract in comparison to the conventional methods (screw press and solvent extraction). Similarly, the percentage of PUFA is higher than in screw expression process in comparison to soxhlet extraction. PUFA consists of two important fatty acids viz. omega-6-fatty acid and omega-3-fatty acid, which are chief components and recognised for synthesis of vitamins in biological process.

^{1}H NMR studies revealed that the percentage of olefinic, allylic methine and allylic methylene protons are higher in supercritical CO_2 extracted fatty oils as presented in Table 3. So it is clear that percentage of unsaturation along with conjugated unsaturated fatty acids are higher than in supercritical CO_2 extraction process in comparison to the soxhlet and screw press extraction processes. The omega-6-fatty acid and omega-3-fatty acid belong to conjugated unsaturated fatty acids. The peak at (δ) 3.6–3.8 ppm was less than 0.1 (trace), which suggested that the absence of methoxy/fatty acid methyl ester in flax oil was. The more percentage of $-CH_3$ and $-CH_2$ protons were observed in soxhlet extraction process, so it indicated that improved recovery of saturated fatty acids in this process. Matikainen et al. (2003) reported the analytical method for determination of oxidation of methyl linoleate and methyl linolenate by ^{1}H NMR analysis.

4. Conclusions

The supercritical CO_2 process is superior in comparison to the soxhlet and screw press process in terms of yield of the important components viz. omega-6-fatty acid and omega-3-fatty acid. The process selectively extracted the neutral fatty acids in the above mentioned supercritical conditions. The chemical composition of fatty oil obtained by screw expression is comparable with supercritical CO_2 process but yield of the fatty oil is 27.8% less in screw expression process. On the other hand, it was found that the hexane extraction process gave improved yield. But the quality of the oil was inferior in terms of higher acid value (1.1%) and peroxide value (3.6%) along with less percentage of omega fatty acids (54.4%). In FAME analysis, the sum of total identified components were 92.5%, 95% and 96.8% in hexane, screw press and supercritical CO_2 extract, respectively. It indicated that some amounts of waxy components were co-extracted with hexane. The major problem is represented by hexane removal after the extraction, therefore finally the oil contained, trace amount of solvent residue. Alternatively, supercritical CO_2 is an environmentally friendly process for selectively extracting the omega-3-fatty acid and omega-6-fatty acid. The extracted oil is superior in quality without solvent residue, so the oil is most preferably used in food products.

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