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EXTRACTION OF WHEAT GERM OIL USING SUPERCRITICAL CARBON DIOXIDE (SC-CO_2) AND ITS DETAILED COMPARATIVE ANALYSIS WITH CONVENTIONAL HEXANE EXTRACTED OIL

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ABSTRACT

Supercritical Carbon dioxide (SC-CO_2) is gradually gaining its importance as green solvent. Throughout the world efforts are being made by the researchers to find alternative solvents to hexane – the most commonly used solvent for extraction of vegetable oils. SC-CO_2 extraction has already been used for extraction of oils having higher nutritive values like flaxseed oil, fish oil etc. Extraction of oil from wheat germ was also studied as it contains higher amount of tocols (vitamin E) and Alpha Linolenic Acid (ALA). However, there was no thorough analysis of quality parameters of SC-CO_2 extracted oil in comparison to hexane extracted oil. In the present study, SC-CO_2 extraction was carried out to extract oil from wheat germ at various ranges of operating parameters such as pressure of 300 to 500 bar, temperature of 40 to 60 C and CO_2 flow rate of 20 to 30 g min^{-1}. At 500 bar, 40 C and 30 g min^{-1}, maximum oil yield of 9.9% was obtained. Simultaneously, wheat germ was extracted with conventional hexane that resulted 9.87% of oil yield. The detailed physico-chemical characteristics of the oils obtained both ways of extraction were evaluated and compared. Tocols (vitamin E) contents of both extracted oil sample were evaluated using high performance liquid chromatography. The fatty acid composition of oils was analysed using gas chromatography. Molecular species of TAG were estimated using reverse phase high performance liquid chromatography. The data obtained has immense importance as phosphorous content of the SC-CO_2 extracted oil was found to be significantly low. It will, definitely, help in designing commercial refining process.

KEYWORDS: Phosphorus content, Tocols content, Gas chromatography, High performance liquid chromatography.

INTRODUCTION

Supercritical carbon dioxide (SC-CO_2) extraction is steadily gaining attention as an alternative technique for extraction of natural products having higher nutritional and medicinal values. This process is environment-friendly and no traces of solvent are found in the extracted oil/product. With the advancement of engineering practices, the capital investment is also gradually coming down. The SC-CO_2 extraction plants are now equipped with sate of the art safety devices. In last few years huge numbers of commercial plants based on this technique were commissioned. This particular technique was explored for extraction of specific components from various natural products (Cavalcanti *et al*., 2012; Santos *et al*., 2012; Poontawee *et al*., 2015; Saldana *et al*., 2000; James *et al*. 2016; Asheh *et al*. 2012; Vardanega *et al*. 2014; Nobre *et al*., 2006; Chatterjee *et al*., 2013; Peusch *et al*., 1997; Duarte *et al*., 2004). SC-CO_2 extraction process was also tried for extraction of oils and fats (Friedrich *et al*., 1982; Bozan *et al*., 2002; Roy *et al*., 2006; Sarmento *et al*., 2006; Han *et al*., 2009; Rodriguez *et al*., 2012). This process may be commercially attractive for extraction of oils having higher nutritive value. Conventionally, the extraction of oils and fats is carried out either by mechanical expelling or by conventional solvent extraction. The major demerits of the conventional extraction techniques are more time of operation, poor quality of oil and presence of organic solvents. Carbon is non-toxic and inexpensive gas which can be used as super critical fluid above critical conditions. The supercritical state of carbon dioxide is formed above the pressure of 73.96 bar and temperature of 31 C. At these conditions, CO_2 have high density, high penetration power, high mass transfer and solubilising properties. The CO_2 is readily available and its low temperature and pressure for supercritical conditions compared to the other supercritical fluids makes this solvent commercially suitable for industrial operations. SC-$CO_{2,}$ hence, is one of the most feasible alternatives for extraction as it will nullifies all the disadvantages of hexane. Higher initial investment is the only disadvantage of the process. Wheat germ is the reproductive part of the wheat grain and this is also considered to be the most nutritive part. It contains vitamins, nutrients and minerals. This is added to to enhance the quality of food products (Pomeranz *et al*., 1970; Hooti *et al*., 2002; Rizzello *et al*., 2010; Gimenez *et al*., 2014). Wheat germ contains around 10% of oil (Swern, 1979). This oil contains high amount of tocols (vitamin E) that aids neutralization of free radicals (Begum *et al*, 2002). It contains up to 2500 ppm of vitamin E (Shuler, 1990). This oil shows moisturizing and sun protection effects (Kumar *et al*,. 2015; Zalatnai, 2001).

It is also reported that this oil can be used as an inhibitor to colon carcinogenesis (Kapoor *et al*., 2010). The higher amount of α- linolenic acid in wheat germ oil makes this a healthier source of dietary fat. Some researchers found that it has anti-oxidant properties (Tracy *et al*., 1944; Saleh *et al*., 2010; Karabacak *et al*., 2011; Mahmoud *et al*., 2015; Jyotsna *et al*., 2016). It has been shown that wheat germ oil supplementation of diet for patients with hypercholesterolemia reduced both oxidative stress and platelet formation (Alessandri *et al*., 2006).

Some authors investigated the super critical extraction of wheat germ oil. However, they have not done without proper physico-chemical characterization of SC-CO_2 extracted wheat germ oil compared to hexane extracted oil. The process would be understood in more details if this knowledge gap is filled. The thorough analysis of oil will definitely help in designing the subsequent refining techniques. In the present investigation, systematic SC-CO_2 extraction data of pressure, temperature and CO_2 flow rate for optimum oil recovery are reported. The physico-chemical properties of both hexane and SC-CO_2 extracted oils were thoroughly investigated and compared to find out the possible added advantages.

MATERIALS & METHODS

Materials

Freshly produced wheat germ was procured from a local wheat processing industry situated at Andhra Pradesh, India. All the chemicals and solvents used in this study were procured from M/s. Sd fine Chem. (Mumbai, India) and were of laboratory reagent grade.

Methods

Solvent (hexane) Extraction

Initially, the oil was extracted from wheat germ in a soxhlet apparatus with hexane for 8 hours at 60 C and the extraction procedure was continued up to 8 hrs to extract the maximum amount oil. The oil content was determined as a percentage of the extracted oil to the sample weight (w/w). The extracted oil was stored at 4 C in a glass bottle under nitrogen blanket for further analysis.

SC-CO_2 Extraction

Wheat germ was extracted in a PLC controlled SC-CO_2 extractor (SFE 500 ml) supplied by Waters Corporation, Milford, USA. The SFE unit is assembled with chiller, CO_2 pump, co-solvent pump, heat exchanger, extraction vessel, ABPR (automatic back pressure regulator), fraction collector etc. The system is designed to withstand pressure up to 600 bar and temperature up to 80 C. Initially, 150 g of seed sample was taken in a 0.45 µm cotton filter bag and inserted in the extraction chamber and then the chiller temperature was brought down to 5 C. The CO_2 filled cylinder was connected to unit. The CO_2 gas was pre-liquefied by passing through shell side heat exchanger and then pumped to extraction vessel where the temperature and pressure were maintained at above critical conditions. The oil extraction was carried out at different operating conditions of pressure, temperature and CO_2 flow rate. Each experiment was run for obtaining the maximum possible oil yield. The extraction time was set to 3 hours. Oil was then collected in a sample bottle from collection vessel after each run and weighed to get the oil yield. The sample bottle was tightly sealed and kept in a refrigerator for further analysis.

Physico-chemical Characteristics

Physico-chemical characteristics of the extracted oils were measured and analysed by the standard prescribed procedure which are Acid value (AOCS, 1994), Iodine value (AOCS, 1994), Refractive Index (AOCS, 1994), Saponification value (AOCS, 1994), Moisture (AOCS, 1994), Unsaponifiable matter (AOCS, 1994), Phosphorous (Pacquot and Hautfenne, 1987), Peroxide value (AOCS, 1994). Density was measured according to ASTM D 4052 method (ASTM, 1984). Colour was measured as per AOCS method using Lovibond Tintometer (Lovibond model PFX 995) (AOCS, 1994). Kinematic viscosity was measured as per ASTM standard method D-445 (Cannon Instrument Co., State College, PA) (ASTM, 1970).

High Performance Liquid Chromatography (HPLC) Analysis of Tocols

Tocopherol and tocotrienol of oil samples were analysed by high performance liquid chromatography (HPLC) using prescribed analytical method was used (AOCS, 1994). An Agilent 1100 series HPLC unit equipped with fluorescence detector. The normal phase silica column (LiChroshper Si-60; 250 mm × 4.0 mm × 5 µm) (Merck Millipore, UK) was used to separate the tocopherol and tocotrienol. The excitation and emission wave length of the detector was set at 292 and 330 nm respectively. Hexane and isopropyl alcohol (99.5:0.5, vol/vol) was used at a flow rate of 1.0 ml/min as mobile phase. The total tocopherol and tocotrienol content was expressed as total tocols (vitamin E) content in ppm.

Gas Chromatography Analysis for Fatty Acid Composition

Fatty acid methyl esters (FAME) of oil samples were prepared using the solution of methanolic sulphuric acid (2% v/v) (Christie, 1982). The analysis was performed on Agilent 6890 gas chromatograph assembled with flame ionization detector (FID) and a capillary column (DB-225; 30 m × 0.25 mm × 0.25 µm). The temperatures of oven were programmed for 2 min at 160 C, raised to 230^{o}C at 5 C/min and finally hold at 230 C for 15 min. The injector and detector temperatures were set as 230 and 270 C respectively, with a split ratio of 10:1. Nitrogen (N_2) was used as carrier gas and that maintained at 1 mL/min. The flow rates of air and hydrogen were 300 mL/min and 30 mL/min respectively. The identification of fatty acid composition was recorded by comparing the retention times of standard FAMEs, C_4-C_{24} (Supelco, USA). Injection was performed in triplicate for each sample and average values are reported.

High Performance Liquid Chromatography (HPLC) for Determination of TAG Molecular Species

The reversed phase high performance liquid chromatography (RP-HPLC) analysis was performed on Waters semi prep HPLC. That was integrated with an evaporative light scattering detector (Waters 2424 (ELSD) with a quaterny pump). The oil samples (about 10 µl of 1 mg/ml concentration) were injected in to RP column (C18-RP). Mobile phase of acetone (100%) at a flow rate of 1 ml/min was used. The operating conditions for ELSD were: drift tube temperature 50ºC, flow of nitrogen 50 psi with gain 100. The molecular species of oils were identified by their equivalent carbon numbers (ECN) and the elution order was predicted according to a method designed earlier (Reena *et al*., 2009).

Statistical Analysis

The reported values of physic-chemical characteristics are the means of the three replicates, presented as means ± standard deviations (SD) and those were also analyzed by a paired Student's t-test to evaluate the statistical significance.

RESULTS & DISCUSSION

In the present experimental study, oils from wheat germ were extracted with both hexane and SC-CO_2. The oil samples obtained from SC-CO_2 extraction under selected operating conditions and conventional hexane extraction were analysed for physico-chemical characteristics, fatty acid composition and TAG molecular species. Wheat germ was extracted initially using conventional hexane in 5 L capacity soxhlet apparatus. 64.9 g of wheat germ oil was obtained from 650g of wheat germ at 60°C operating temperature and an extraction time of 8 hours. The oil content was found to be 9.87%. The extracted oil sample was thoroughly analysed for physico-chemical characteristics which are tabulated in the Table 1.

TABLE 1: Physico-chemical characteristics of conventional hexane and SC-CO_2 extracted wheat germ oil

Characteristics	Hexane Extracted Oil	SC-CO_2 Extracted Oil*
Oil content (wt %)	9.87 (±) 0.10	9.9 (±) 0.07[a]
Moisture content (wt %)	6.3 (±) 0.03	3.5 (±) 0.05[d]
Acid value (mg KOH g^{-1})	13.9 (±) 0.02	10.2 (±) 0.06[d]
Iodine value	134.68 (±) 0.07	134.21 (±) 0.28[a]
Saponification value	194.6 (±) 0.61	195 (±) 0.43[b]
Peroxide value (ppm)	2.37 (±) 0.10	2.17 (±) 0.08[d]
Unsaponifiable matter(wt%)	5.5 (±) 0.04	4.5 (±) 0.05[d]
Phosphorous content (ppm)	931.7 (±) 0.26	35.7 (±) 0.09[d]
Colour in 1" cell (Y+5R)	56.0 (6.2R, 25.0Y) (±) 0.17	49.0 (5.2R, 23.0Y) (±)0.1[d]
Density at 40°C (gm cm^{-3})	0.90725 (±) 0	0.90402 (±) 0
RI at 25°C	1.4738 (±) 0	1.4731 (±) 0

**at 500 bar, 40°C and 30 g min^{-1}. Values are means ± SD for three samples. [a]Significantly different from hexane extracted wheat germ oil: $P > 0.05$. [b]Significantly different from hexane extracted wheat germ oil: $P < 0.05$. [c]Significantly different from hexane extracted wheat germ oil: $P > 0.001$. [d]Significantly different from hexane extracted wheat germ oil: $P < 0.001$.*

Some samples of wheat germ was then extracted using SC-CO_2 unit at various processing conditions such as pressures (300 to 500 bar), temperatures (40 to 60 C) and CO_2 flow rates (10 to 30 g min^{-1}) to obtain maximum possible oil yield. The extraction run time was fixed to 3 hours for all experiments based on some initial experimental trials. Initially, the SC-CO_2 extraction was carried out at three different temperatures (40, 50 and 60 C) and three different pressures (300, 400 and 500 bar) with constant CO_2 flow rate of 20 g min^{-1}. The oil yields were found to be 5.0, 8.8 and 9.6% at 300, 400 and 500 bar respectively at 40 C. On increasing the temperature to 50 C, a decreasing trend there in oil yields was observed as 4.96, 8 and 8.6% respectively at 300, 400 and 500 bar. For further increase in extraction temperature to 60 C, the yields decreased to 4.8, 7.2 and 8.2% respectively at 300, 400 and 500 bar. It is, therefore, clearly observed that with the increase of temperature the oil yield is reduced. This may be due to reduction of CO_2 density. On the other hand, yield was found to be increased with increase in operating pressure. At increased pressure, the compressed liquid was having high density. Moreover, diffusivity and penetration power of solvent were high, that lead to higher yield. In an effort to study the effect of the flow rate of CO_2 on yield, experiments were carried out at increased flow rate of 30 g min^{-1}. At 40 C with a flow rate of 30 g min^{-1}, the oil yields were found to be 5.59, 9.33 and 9.9% at 300, 400 and 500 bar respectively. On increasing the temperature to 50 C and maintaining the same flow rate of CO_2, the extracted oil yields decreased to 5.5, 8.2 and 9.8% at 300, 400 and 500 bar respectively. However, considerable decrease in extracted oil yield of 5, 8 and 8.66% at 300, 400 and 500 bar respectively were observed on further increase in extraction temperature to 60 C at the same flow rate of 30 g min^{-1}. The effects of temperature, pressure and CO_2 flow rate on oil yield are shown in Figure 1 and 2. Thus the maximum possible extraction of oil (9.9%) from wheat germ was achieved at extraction pressure of 500 bar, temperature of 40 C and at a flow rate of 30 g min^{-1}. At these operating conditions, oil was extracted and physico-chemical properties of extracted oils were thoroughly analysed using standard prescribed methods. Table 1 shows the physico-chemical properties of wheat germ oil extracted with SC-CO_2 as well as conventional hexane extraction. From this data, it is observed that peroxide value, iodine value, saponification value, unsaponifiable matter, colour (in 1″ cell), density, refractive index and viscosity were found to be similar for both hexane and SC-CO_2 extracted oils. Moisture content was found to be higher in case of hexane extracted oil compared to that of SC-CO_2 extracted oil. SC-CO_2 extraction produced comparatively less coloured oil. A major and interesting observation was the non-extractability of phospholipids in the oil extracted by SC-CO_2 when compared to conventional hexane extracted oil. During SC-CO_2 extraction, the phospholipids are not getting extracted into the oil like hexane. This is because of the nature of the CO_2 which does not dissolve the phospholipids contained in the wheat germ. The values were 931.7 and 35.7 ppm for hexane and SC-CO_2 extracted oils respectively. Due to this reason, the colour of SC-CO_2 extracted oil was light yellow and was better in appearance when compared to conventional hexane extracted oil. Lesser amount of phosphorous indicates that degumming which an important unit operation in vegetable oil is refining can be avoided when the oil is extracted using SC-CO_2.Tocols content of SC-CO_2 extracted oil was found to be less when compared to hexane extracted oil which is shown in Table 2. The values were 1887 ppm for hexane extracted oil and 1580.5 ppm for SC-CO_2 extracted oil at selected conditions. However, due to the presence of higher amount of phosphorous (931.7 ppm). The oil obtained by extraction with hexane has to be subjected to degumming. The hexane extracted oil was degummed using 3% water as per standard procedure (Sharqi *et al*. 2014) and the degummed oil was found to have 1523.3 ppm of tocols content along with phosphorous content of 110 ppm.

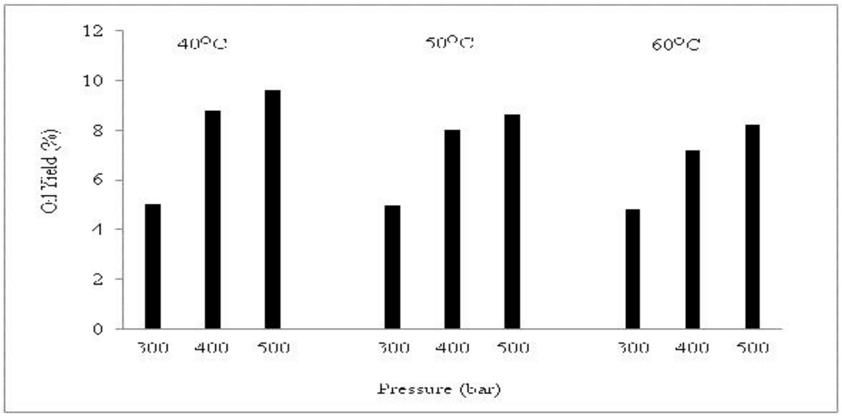

FIGURE 1. Effect of temperature and pressure on oil yield at CO_2 flow rate of 20 g min^{-1}

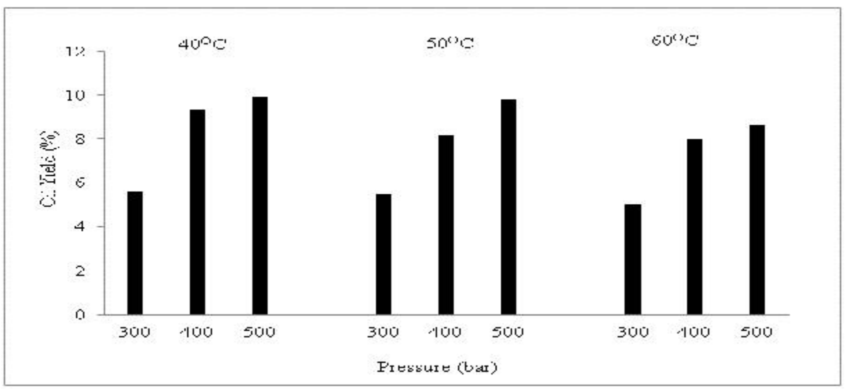

FIGURE 2. Effect of temperature and pressure on oil yield at CO_2 flow rate of 30 g min^{-1}

TABLE 2: Tocols content (ppm) of wheat germ oil samples.

Sample	α- Tocopherol	β- Tocopherol	γ- Tocotrienol	Total Tocols
CHEO	1369 (±) 0.17	469 (±) 0.18	49 (±) 0.34	1887 (±) 0.08
WDHEO	1050.2 (±) 0.3	446.1 (±) 0.36	27 (±) 0.2	1523.3 (±) 0.26
SCEO*	789.5 (±) 0.15	720 (±) 0.08	71 (±) 0.14	1580.5 (±) 0.43

**at 500 bar, 40°C and 30 g min-1, Results represent mean ± SD of three replicates, CHEO= Conventional Hexane Extracted Oil, WDHEO= Water Degummed Hexane Extracted Oil, SCEO= SC-CO_2 Extracted Oil*

TABLE 3: Fatty acid composition of conventional hexane and SC-CO_2 extracted wheat germ oil.

Fatty Acid	Hexane Extracted Oil	SC-CO_2 Extracted Oil*
C14:0	0.10	0.11
C16:0	17.57	18.73
C16:1	0.16	0.18
C18:0	0.60	0.55
C18:1	13.94	13.41
C18:2	57.92	57.04
C18:3	8.30	8.76
C20:0	0.09	0.08
C20:1	1.18	1.01
C20.2	0.11	0.13

** 500 bar, 40°C and 30 gmin-1*

This process also incurred around 1.2% of loss of oil. SC-CO_2 extracted oil (with 'p' content of 35.7 ppm) need not be subjected to degumming operation. This will ensure the tocols content to be intact. The subsequent refining protocol would be same for both types of oils. Hence, SC-CO_2 extraction of oil will give better yield with superior nutritive quality. The fatty acid composition of both the extracted oils is almost similar and is shown in Table 3. These wheat germ oil was found to contain primarly linoleic acid, palmitic acid, oleic acid and linolenic acid.

The triacylglycerols (TAG) molecular species were determined by using reversed phase HPLC for both types of oils and the results obtained are given in Table 4. The molecular species are shown as effective carbon number (ECN) of triacylglycerol. The results clearly showed that the molecular species of the wheat germ oil obtained in 8 types which were in the range of triglyceride having ECN C36-C50. The species of triglyceride of the seed oils having C44 is the main component, followed by C42 and C46. The molecular species were found to have much similar characteristics for both oils. However, C48 species were present in higher quantities in SC-CO_2 extracted oil.

TABLE 4: TAG molecular species of conventional hexane and SC-CO_2 extracted wheat germ oils

ECN	Expected Molecular Species	Hexane Extracted Oil (%)	SC-CO_2 Extracted Oil* (%)
C36	LnLnLn	2.8	3.5
C38	LnLnL	5.0	4.5
C40	LLLn	6.1	6.1
C42	LLL/LnLP	24.1	24.2
C44	POLn/PLLn/LnPP/PLL/OLL	36.6	37..2
C46	POL/LPP/LOO/PLP	13.0	13.0
C48	POP/PPP/POO/OOO/PPO	3.6	9.0
C50	SOO	0.4	0.5
-	Unidentified	8.4	2.0

** 500 bar, 40^oC and 30 gmin^{-1}*

CONCLUSION

Physical and chemical characteristics of crude vegetable oils play crucial role in designing further refining protocol. Irrespective of the method of extraction, the quality of crude oils is to be thoroughly evaluated before refining. This is more important for oils having superior nutritional properties like wheat germ oil. Based on the quality of oil, the most benign methods of refining can be chosen for keeping the nutritive value intact. Though SC-CO_2 was employed for extraction of wheat germ oil, no thorough comparative evaluation was done between hexane extracted wheat germ oil and SC-CO_2 extracted wheat germ oil. In this present investigation, a detailed comparison of the physico-chemical properties of wheat germ oils extracted both by SC-CO_2 and hexane was presented. In the current investigation, the maximum oil yield of 9.9% was obtained from SC-CO_2 extraction under the pressure of 500 bar, temperature of 40 C and CO_2 flow rate of 30 g min^{-1} which was almost similar compared to conventional hexane extracted oil yield of 9.87%. One very important observation was that the phosphorous content of the conventional hexane extracted oil was significantly higher compared to the SC-CO_2 extracted oil. Therefore, degumming for removal of phosphorous from hexane extracted oil will be an important step in refining. This will definitely result in oil loss and also reduction of tocols content of the oil significantly. Whereas, by using the SC-CO_2, it is possible to eliminate degumming step. This will definitely result in better quality wheat germ oil with higher yield. The other characteristics of SC-CO_2 extracted oil were also found to be little better compared to the hexane extracted oil. Thus, the detailed evaluation of physico-chemical properties will definitely help in designing the further processing of the wheat germ oil to obtain oil having higher nutritive values.

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