J. of Supercritical Fluids 100 (2015) 105–109

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Extraction of oil and silybin compounds from milk thistle seeds using supercritical carbon dioxide

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ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 19 February 2015
Accepted 20 February 2015
Available online 27 February 2015

Keywords:
Milk thistle seeds
Silybin A
Silybin B
Supercritical extraction

ABSTRACT

Milk thistle is a plant that has been used medicinally for over 2000 years. It has been used for the treatment of many diseases such as cancer, liver, kidney, cardiac, brain. Their seeds are rich in silymarin compounds (especially silybin A and silybin B) and fatty acids. The purpose of this study was to extract silybin A and silybin B from milk thistle seeds with the supercritical CO_2. The effect of operating parameters such as temperature (40–80 °C), pressure (160–220 bar), CO_2 flow rate (3, 4 and 5 mL/min) and particle size (0.3025, 0.925 and 1.2 mm) on extracted oil, silybin A and silybin B were investigated. Fatty acid composition in milk thistle seed extract was determined at optimum conditions. The results indicated that the optimal conditions were 40 °C temperature, 200 bar pressure, 4 mL/min CO_2 flow rate and 0.3025 mm particle size. In these conditions; the amounts of oil, silybin A and silybin B were obtained 327, 2.29 and 1.92 mg/g milk thistle seeds respectively. For the fatty acids obtained by supercritical CO_2 extraction, the most abundant compounds were palmitic acid (8.15%); stearic acid (5.51%), oleic acid (24.10%); linoleic acid (54.97%) at optimum conditions.

1. Introduction

The milk thistle is an annual or biannual plant of the Asteraceae family. The milk thistle seeds are included in silymarin compounds, especially silybin A and silybin B. Silybin A and silybin B are well known as antioxidant [1,2] and have been used for the treatment of cancer, liver, kidney, cardiac, brain, cirrhosis and poisoning of alcohol, drugs or toxins [3–6]. Also the milk thistle seeds are consisting of fatty acid compounds like oleic acid, linoleic acid, etc. which are good antioxidant substances too.

Silymarin compounds [7–11] and fatty acid compounds [12–14] are generally produced by traditional extraction method from plant. Supercritical fluid method is used many industries such as medicine, chemistry, biochemistry and food. Supercritical fluid extraction method has supremacy in comparison to conventional solvent extraction methods. The most widely used supercritical fluid is carbon dioxide which is non-toxic, relatively inert, and non-flammable [15]. The extraction solvents such as petroleum ether and ethyl acetate are widely used in traditional methods but they cannot be removed completely from extract. However, carbon dioxide is gaseous at ambient conditions of temperature and pressure and completely separated from the extract in these conditions [16,17]. Several investigations were reported for extraction by supercritical CO_2 from tea [18,19], jatropha [20,21], *Stevia rebaudiana* leaves [22], grape [23], mango leaves [24] and watermelon [25]. Hadolin et al. [26] studied the extraction of milk thistle seeds by supercritical CO_2/propan to obtain vitamin E content. Szentmihályi et al. [27] studied the extraction of milk thistle seeds by supercritical CO_2/propan to obtain fatty acid, pheophytin, carotene, tocopherol content and concentrations of some metals. In addition, there are some studies that investigate the effect of supercritical CO_2 with together cosolvent such as methanol, ethanol, water on extraction process [28–31].

But there is no study in the literature regarding, the effect of parameters of temperature, pressure, particle size and CO_2 flow rate on silybin A and silybin B in oil extracted from the milk thistle seeds by supercritical CO_2 without cosolvent.

The purpose of this study was to investigate of pharmaceutical importance silybin A and silybin B were extracted from milk thistle seeds by the supercritical CO_2 without using cosolvent due to the preventing of cosolvent contamination. The purity of silybin compounds must be affected by cosolvent. The qualitative and quantitative analysis of silybin A and silybin B were performed by HPLC. The fatty acids composition in extract was analyzed by GC-FID. The effect of parameters such as temperature, pressure, particle size and CO_2 flow rate were investigated on supercritical extraction process.

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http://dx.doi.org/10.1016/j.supflu.2015.02.025
0896-8446/

2. Materials and method

2.1. Materials

Milk thistle was obtained from Muğla in Turkey. Whole seeds were separated from flowers. They were dried at room conditions and then ground up the specific particle dimension by a coffee grinder. The specific particle dimensions of samples were 0.3025, 0.925 and 12 mm. Grinding was carried out just before extraction in order to avoid oxidation.

2.2. Supercritical CO_2 extraction

Extractions were performed in laboratory scale supercritical fluid extraction system (Applied Separation Speed, USA). A 24 mL of extraction vessel was packed with 4 g of the milk thistle seeds particles. The vessel was put in a temperature-controlled oven. CO_2 was sent continuously through the system. The extract was collected in vials. The detailed description of the system was given in another our study [18]. The collection vials were changed per every 30 min. The experiments were performed among 120 min in the range of 40–80 °C, 160–220 bar, 3–5 mL CO_2/min, 0.3025–1.2 mm particle size. Extraction time was fixed at 120 min because of there were not more significant changed in the amounts of oil, silybin A and silybin B over 120 min in all experiments.

2.3. Analysis of silybin A and silybin B in the milk thistle seeds extract

Silybin A and silybin B were determined by high pressure liquid chromatography (HPLC, Dionex 680) equipped with Acclaim 120 C18 column of 150 mm × 4.6 mm and 3 μm particle size. The mobile phase consisted of solvent A (methanol:water = 20:80 (v/v)) and solvent B (methanol) and applying the following gradient: 0–40 min %80 B, 40–45 min %10 B. The detection wavelength (UVD170U detector) was 288 nm. Injection volume and mobile phase flow rate were 20 μL and 0.8 mL/min, respectively. Stock standard solution of silybin A and silybin B was prepared in methanol.

Silybin A and silybin B in milk thistle seeds extract peaks were identified by comparing their retention times with the reference standards. The analysis was carried out in triplicate. Concentrations of silybin A and silybin B in the samples were estimated with least-squares equation derived from peak area ratios of individual silybin A and silybin B.

2.4. Analysis of fatty acid in the milk thistle seeds extract

The fatty acid composition was determined by conversion of fatty acid methyl esters (FAMEs) based on the using IUPAC method 2.301 [32]. The fatty acid composition was analyzed with gas chromatography which equipped with a flame ionization detector and a HP-88 column (100 m × 0.25 mm I.D., 0.2 μm film thickness Agilent Technologies, Spain). The carrier and supporting gas were hydrogen (30 mL/min) and air (350 mL/min), respectively. The temperature of detector was set to 270 °C. The temperature program of the column was set 90 °C for 5 min and a subsequent increase to 190 °C with 10 °C/min rate. The injection was performed at 250 °C with split ratio of 1/50 and injection volume 2 μL. The individual fatty acid peaks were identified by comparison their retention times with standard fatty acids mixtures peaks (Supelco Fame Mix). The results were expressed as relative percentages of total fatty acids.

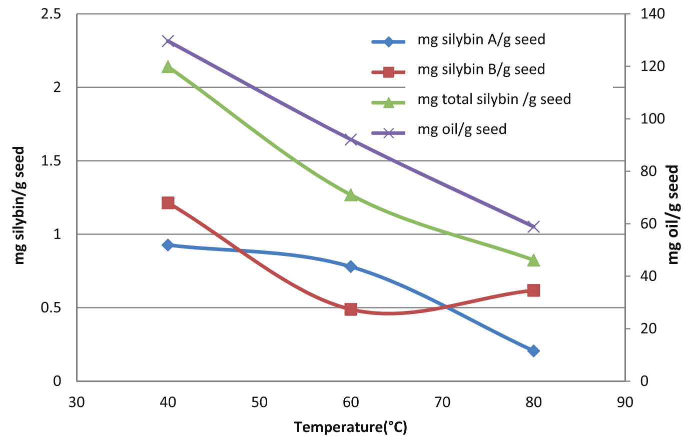

Fig. 1. The effect of temperature on amounts of silybin A, silybin B, total silybin and oil (200 bar, 4 mL/min, 0.925 mm) (×: oil, ▲: total silybin, ♦: silybin A, ■: silybin B).

3. Results and discussion

The effect of process parameters (temperature, pressure, CO2 flow rate and particle size) were investigated on supercritical CO_2 extraction of milk thistle seeds. The range of the temperature (40–80 °C), the pressure (160–220 bar), the flow rate of supercritical CO_2 (3–5 ml/min) and the particle size (0.3025–1.2 mm) were selected according to the literatures research.

Firstly, the effect of temperature was researched on the amounts of oil, silybin A and silybin B extracted from milk thistle seeds. Extractions were accomplished for 40, 60 and 80 °C temperature at 200 bar pressure, 4 mL/min CO_2 flow rate, 0.925 mm particle size. For analyzing the effect of one parameter, values of other parameters fixed at nearly middle of the range selected. The maximum amounts of oil, silybin A, silybin B and total silybin (silybin A + silybin B) were recorded as, 129.64, 0.93, 1.21 and 2.14 mg/g seeds, respectively, for temperatures of 40 °C (Fig. 1).

It can be observed that amounts of oil, silybin A and total silybin were reduced with temperature rising through 80 °C. When extraction temperature upward from 40 °C to 80 °C; the CO_2 density was decreasing ~29-fold at 200 bar pressure [33]. The decreasing of CO_2 density was shown dominant effect than vapor pressure of the active components in oil. Parameter of temperature adversely affected to the yield of oil, silybin A and total silybin. Similar results have been reported for extracted oils from different natural products. Yield of nutmeg oils from nutmeg seeds reduced with rising temperature in the range of 313–323 K at pressure of 15 Mpa [34]. The study of hemp seeds extraction with supercritical CO_2 was shown; the yield of oils reduced with increasing temperature in the range of 40–60 °C at pressure of 300 bar [35]. As a result, 40 °C was selected as an optimum temperature value in the following experiments.

Secondly, the effect of extraction pressure on the amounts of oil, silybin A and silybin B was investigated. The experiments carried out at pressure 160, 180, 200 and 220 bar at 40 °C for the temperature, 4 mL/min for the CO_2 flow rate, 0.925 mm for the particle size. The experimental results were given in Fig. 2.

It is evident from Fig. 2 that the amounts of oil and total silybin gone up significantly with increasing pressure from 160 to 180 bar at a constant temperature. The solubility of oil in supercritical CO_2 increases with rising of pressure. Above 180 bar, there were no significant rise in amounts of oil and total silybin. From 160 to 180 bar, there were meaningful increases in the amounts of oil and total silybin (23% and 25%, respectively) than from 180 to 220 bar (0.73% and 0.67%, respectively). Similar results had been also reported by Mahmudah et al. [34], Uribe et al. [36], Rebolleda et al. [37].