SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF BORAGE SEED OIL

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The oil from the seeds of *Borago officinalis L.* (borage) is a major commercial source of ?-linolenic acid. The fatty acid has a great potential therapeutic value in the treatment of several diseases.
In the present work the efficiency of high pressure extraction of borage seed oil containing ?-linolenic acid in high concentrations was investigated. Extraction was performed by semi-continuous flow apparatus using supercritical carbon dioxide at pressures from 200 to 300 bar and temperatures 40°C to 60°C. The flow rate of carbon dioxide was maintained constant at 3.5 g/min. Composition of oil extracted was determined by gas chromatographic analysis.
The best results of borage seed oil extraction were obtained at 300 bar and 40°C, where the quality of oil due to ?-linolenic acid content was the highest. The yield obtained by supercritical extraction was very similar to that obtained by conventional extraction using n-hexane as solvent.
Dynamic behaviour of the extraction runs was analysed by a mathematical model and diffusion coefficients of the components within the solid and in the bulk were determined. Results show that the cumulative rate data calculated by the mathematical model are in fairly good agreement with the data obtained experimentally.

INTRODUCTION

Borage (*Borago officinalis L.*) is annual or biannual plant that has sky blue flowers and green leaves with white hairs. It is native to Europe, Asia Minor and North Africa and has been used for centuries as a culinary herb of purposed medical value. The oil from borage seeds contains mainly triacylglycerols (95%) consisting of C16-C20 fatty acids. The remaining 5% presents minor components composed of phospolipids, tocopherols, flavonoids, sterols, free fatty acids and also mono- and diacylglycerols.
The oil from borage seeds is rich on ?-linolenic acid (GLA) which is one of the fatty acids found in seeds oils. GLA (cis-6,9,12-octadecatrienoic acid) is an essential fatty acid in the omega-6 family that is found in plant-based oils. The main sources of GLA are oils of evening primrose, borage and black currant plants. The studies have shown that GLA can help people with benign breast diseases, skin problems, cardiovascular diseases, high blood pressure and is useful in treating neurological problems related to diabetes [1].
Supercritical fluid extraction (SFE) is an alternative to supplement or to substitute the conventional separation systems involving natural products, which are temperature and oxidation dependant [2]. Due to pure thermal stability of GLA the extraction process using

CO_2 as solvent should be carried out at moderate conditions, with special attention to the temperature.
Several authors have studied supercritical extraction of seed oils: sunflower [3], soybean [4], rapeseed [5], evening primrose [6,7], wheat germ [8], grape seed [9]. The borage seed oil extraction uding CO_2 was already preformed in wide ranges of pressure, temperature, flow rate and seed size by Gomez et.al. [10], and Daukšas et.al. [11] where the infuence of entrainer was tested. A large number of species have been processed using supercritical CO_2 however the mass transport models reported in the literature for describing supercritical extraction of seed oil are limited (rapeseed [5], sunflower [3], fennel seed [12], soybean [13], evening primrose [14], almond oil [15]).
In this work high pressure extraction of borage seed oil containing ?-linolenic acid was investigated. Extraction experiments were performed by semicontinuous flow apparatus using supercritical carbon dioxide at pressures 200 and 300 bar and temperatures 40 and 60°C. The flow rate of carbon dioxide and particle size were maintained constant and were 3.5 g/min and 1125 μm, respectively. Composition of oil extracted was determined by GC analysis. The course of extraction curves obtained was approximated by mathematical model and diffusion coefficients were determined.

MATERIALS AND METHODS

The Borage seeds were supplied by John K.King&sons Limited (Coggeshall, Colchester, Essex, UK). All chemicals used for analysis were purchased from Merck (Darmstadt, Germany). CO_2 (purity of 99.5%) was obtained from Messer (Ruše, Slovenia).
The seeds were grounded and the particle size distribution and median particle size of ground material was determined by sieve analysis. Moisture content of raw material was determined by the Karl-Fischer method.
The extraction experiments with conventional solvents were performed in a Soxhlet-type apparatus using *n*-hexane as solvent. For extraction 50 g of borage seeds and 200 mL of *n*-hexane were used. Extraction time was 10 hours what allowed full depletion of the grain and so maximum possible extraction yields were obtained. After the extraction process has finished, the solvent was evaporated and extract was analysed.
The SFE experiments using CO_2 were performed by semicontinuous flow apparatus [16]. The apparatus was home build for a maximum pressure of 500 bar and a temperature of 100°C. Approximately 20 g of ground material was charged into the extractor (V = 60 mL). The temperature in the water bath was regulated and maintained at constant level (±0.5°C, LAUDA DR.R Wobser GmbH &Co. KG, Lauda Königshofen, Germany). The apparatus was purged first with nitrogen and later with the gas used for extraction. In the next step, liquefied gas (CO_2) was continuously pumped with a high pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebrasca, P_{max} = 450 bar) through the preheating coil and over the bed of sample in extractor. The solvent flowrate was measured with a flow- meter (ELSTER HANDEL GmbH, Mainz, Germany). The product precipitated in separator (glass trap), where the separation was performed at 1 bar and at temperature of 25°C. The product collected in the glass trap was weighted (±0.1 mg) and yield was calculated. The extract obtained was immediately analysed.
Extracted crude oil was analysed on free fatty acids (FFA) composition. The analyses were performed with GC model 5890 Hewlett-Packard (Pittsburgh, PA, USA) with FID temperature set at 300°C and column (HP-FFAP 30 m x 0.25 mm x 0.25 μm). The oven time-temperature profile was as follows 80°C (1min), 25°C per min to 180°C (1min), 5°C per min

to 220°C (10 min), 5°C per min to 230°C (30 min). The carrier gas was nitrogen with total flow through the column 61.0 mL/min. The samples were analysed in *n*-hexane solutions immediately after extraction process. The quantification of FFA was made using calibration curves.

For modelling extraction curves the unsteady state mass transfer model was used. With this model it was assumed that the diffusion inside the solid phase controls the rate of the process and can be derived from the Ficks second law of diffusion. The diffusion equation was proposed by Hong et.al. [13].

$$\frac{m(?)}{m_0} = \exp(-K?) \quad (1)$$

$$K \cong F_0 / ? \quad (2)$$

$$F_0 = D_s ?/d^2 \quad (3)$$

where *m(?)* is the amount of oil in grams that remains in the seeds at time *?* (in s), m_0 is the initial amount of oil (in g) in the seeds at time zero, F_0 is the Fourier number that results from a series solution to the original differential equation and D_s is the diffusivity in the solid phase (in m^2/s). The parameters can be estimated using linear regression of experimental data in diffusion-controlled section.

CONCLUSION

Supercritical fluid extraction. **Figures 1** and **2** show the influence of pressure on the amount of extracted borage seed oil at 40°C and at 60°C, respectively. As can be seen from this figures the yield increases with increasing pressure. At both temperatures researched the highest yield is achieved at 300 bar where 98 % of total oil can be obtained after using 35 - 40 g of CO_2/g of raw material. In the beginning of the process the constant extraction rate at 40°C is higher as at 60°C, what is consequence of higher density of CO_2 at lower temperature. Therefore, the rate of the process is lower at higher temperature.

Fatty acid composition of the extracts obtained at different pressures and temperatures is presented in **Table 1**. The results show, that GLA content increases from 11.65 to 16.89% by increasing the pressure from 200 to 300 bar at temperature 40°C and slightly increases from 13.75 to 14.37% by increasing the pressure from 200 to 300 bar at temperature 60°C.

It can be assumed that the maximum amount of oil from the seeds can be obtained already at 40°C and 300 bar. The yield obtained at this conditions is similar as in the case of solvent extraction whit *n*-hexane using Soxhlet apparatus (approximately 31 %). Further, in the case of CO_2 extraction of borage seeds at 40°C and 300 bar the total content of fatty acids in oil obtained is lower as in case of conventional extraction with *n*-hexane, however the composition of fatty acids is similar.

Conventional extraction. The quality of borage seed oil extracted with *n*-hexane using Soxhlet apparatus is similar to that obtained with SFE, however longer times and higher temperatures are required to obtain the same quality and quantity of the oil. The results are shown in **Table 1**. In extract obtained with SC CO_2 at 300 bar and 40°C the fraction of GLA in FFA (16.89 %) is somewhat higher compared to that obtained with conventional extraction with *n*-hexane (15.11%).

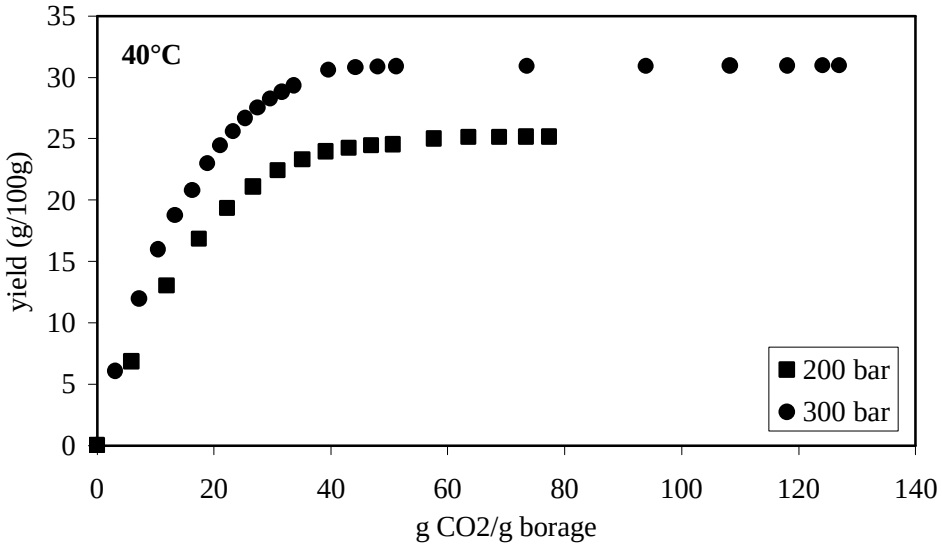

Figure 1: Extraction of borage seed oil using CO_2 at 40°C.

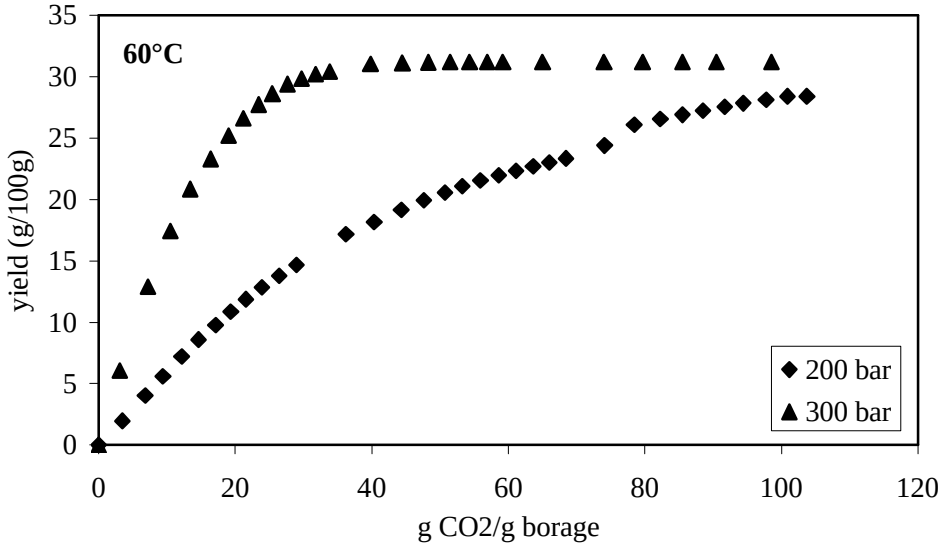

Figure 2: Extraction of borage seed oil using CO_2 at 60°C.

Table 1: Fatty acid composition of oil using *n*-hexane and SFE.

Oil	Total yield (%)	FFA (%)	FFA composition (%)				
			Palmitic	Stearic	Oleic	Linoleic	GLA
n-hexane, Soxhlet	31.02	4.98	16.61	5.07	21.24	41.96	15.11
CO_2, 200bar, 40°C	25.17	7.16	16.70	4.43	22.92	44.29	11.65
CO_2, 200bar, 60°C	28.41	6.90	14.27	4.87	19.80	47.30	13.75
CO_2, 300bar, 40°C	30.97	8.94	15.61	4.83	21.61	41.06	16.89
CO_2, 300bar, 60°C	31.19	6.29	16.09	4.79	19.18	45.56	14.37

Mathematical model. To describe the extraction curves it is assumed that the diffusion inside the solid phase controls the rate of the process and unsteady state mass transfer model (Eq. 1-3) was used. The parameter D_s for unsteady mass transfer was determined from experimental data (**Table 2**).

The agrement of the experimental and calculated extraction curves is presented on Figures 3 and 4. For extractions at 200 bar and 60°C and 300 bar and 40°C good agreement between the experimental data and model curves is obtained with AARD 3.64 % and 2.02 %, respectively.

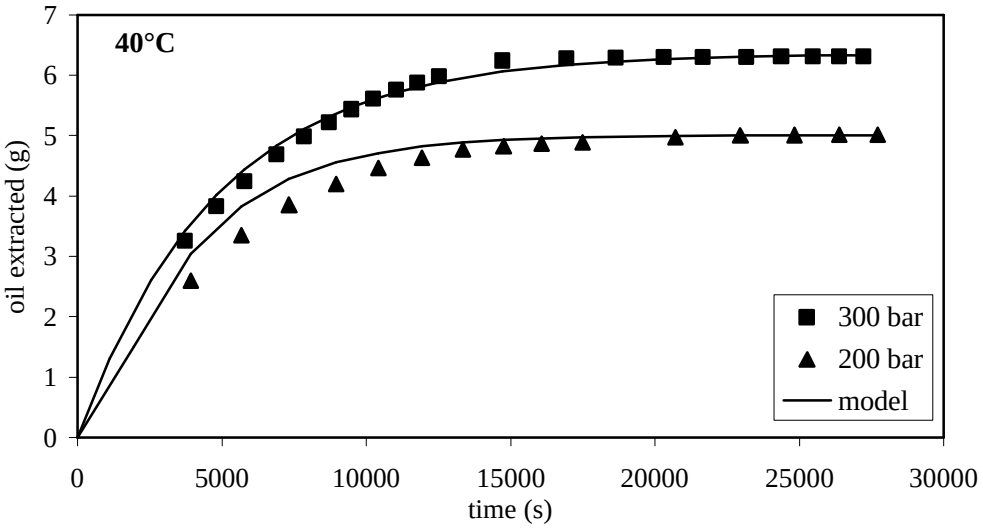

Figure 3: Extraction of borage seed oil using CO_2: comparison between experimental and calculated extraction curves at 40°C.

Table 2: Diffusion coefficients for unsteady state

P (bar)	T (°C)	q (g/min)	F_0	$D_s \times 10^{-10}$ (m^2/s)
200	40	3.332	8.079	**3.689**
200	60	3.232	2.317	**0.740**
300	40	3.267	5.648	**2.626**
300	60	3.196	6.935	**4.019**

The diffusion coefficient D_s, representing the transport of the oil components within the solid particle, vary from 2.63×10^{-10} to 4.02×10^{-10} m^2/s. Exception is diffusion coefficient at pressure 200 bar and temperature 60°C, where the extraction rate is and diffusion coefficient calculated is approximately ten times smaller then others. We assumed that lower density of CO_2 causes lower extraction rate and slow diffusion from the solid particles. The deviation of the model from the experimental data is the highest at the beginning of extraction.

Results shows that the cumulative rate data calculated by the mathematical model for diffusion inside the solid phase controlled process are in fairly good agreement with the data obtained experimentally.

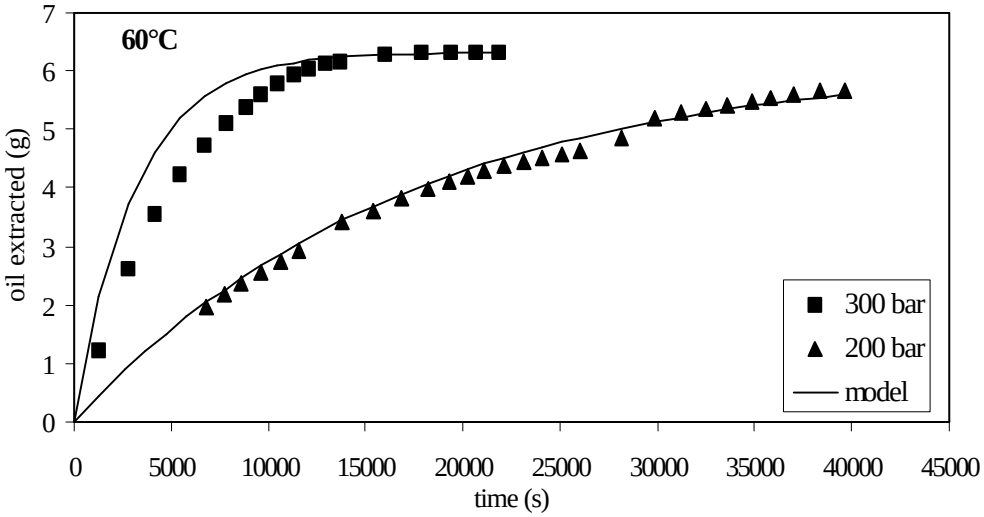

Figure 4: Extraction of borage seed oil using CO_2: comparison between experimental and calculated extraction curves at 60°C.

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