Fractionation of Free Fatty Acids and β-sitosterol from Rapeseed Oil by Supercritical Carbon Dioxide

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The fractionation of minor components from vegetable oils was performed using two step extraction procedures. The vegetable oil used in our study was crude rapeseed oil with the content of FFA and β-sitosterol 31.0 wt.% and 0.28 wt.%, respectively The optimal extraction conditions were determined according to extraction yield and content of FFA and β-sitosterol. The extraction yields obtained from the first and second step extraction decrease with increasing temperature and increase with increasing pressure at constant temperature. Generally, the oil with high content of both, FFA and β-sitosterol, was obtained in extract after two step extraction at following conditions: first step at pressure 80 bar and temperature 40 °C, and second step extraction at pressure 150 bar and temperature 40°C, where 84.59 wt.% of free fatty acids and 0.99 wt.% of β-sitosterol was obtained.

INTRODUCTION

Free fatty acids (FFA) and β-sitosterol are minor components in vegetable oil. Usually, in edible oils phytosterols and fatty acids are present in free or esterified forms and are important in pharmaceutical, nutrition and cosmetic industry [1]. Several procedures for isolation of those compounds can be found in the literature, where different supercritical procedures were used [2]. One of the procedures is continuous countercurrent supercritical extraction developed by Dunford et.al. [3], where with a two-step continuous supercritical carbon dioxide fractionation process the enrichment of phytosterol esters in vegetable oils was reached. In the literature, the enrichment of minor components in crude palm oil can be found, where authors used multi-step separation for concentrating carotenoids and tocochromanols in oil [4]. Therefore, the enrichment of minor components in vegetable oils increased the interest of our research in the field of supercritical fluid fractionation.
The aim of our work was to determine the extraction conditions for separation and/or enrichment of FFA and β-sitosterol in rapeseed oil. Extraction experiments were performed by lab-scale semi continuous flow apparatus using supercritical carbon dioxide as solvent in two steps at temperatures 40, 60 and 80 °C. The first step of extraction was performed at pressures 80, 100 and 140 bar, and the second step at pressures 150, 250 and 300 bar, respectively. The temperature was maintained constant during two step extraction. The separation was carried out until constant mass of extract was reached. The crude oil, extracts and residues obtained by extraction were analysed on FFA and β-sitosterol content.

MATERIALS AND METHODS

Materials: Following free fatty acids were included in research: Palmitic acid (Cat.No.76122, purity 97%), Palmitoleic acid (Cat.No. 76169, purity 98.5%), Stearic acid (Cat.No. 85680, purity 98.5%), Oleic acid (Cat.No. 75090, purity 99%), Linoleic acid (Cat.No. 62230, purity

99%) and Linolenic acid (Cat.No. 62160, purity 98.5%) and were purchased from Fluka. β-sitosterol (Cat. No. 854151, purity 60%) was also purchased from Fluka. CO_2 (2.5), He (6.0), Air (5.0) and N_2 (5.0) were purchased from Messer, Slovenia. All organic solvents used for analysis were purchased from Merck (Darmstadt, Germany).

Methods:

Gas chromatography. The analyses of FFA were performed with GC model 6890 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 120°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 helium with total flow through the column 64.0 mL/min. The samples were analyzed in *n*-hexane solutions.[5]

HPLC-ELSD. The analysis of β-sitosterol was performed with HPLC 1200 Agilent coupled with ELSD detector and chromatographic column Agilent SB C18 (150 x 4.6 mm with 3.5 μm particles). The chromatographic elution was isocratic with methanol – water (95:5 v/v) at flow rate 1 mL/min [6]. The temperature was maintained at 40 °C and the injection volume was 10 μL. The UV detection was set on 208 nm and then, the chromatographic effluent was subjected to detection by ELSD (Agilent, USA). The nebulisation was performed at 65 °C and constant N_2 flow at pressure 3.5 bar. The detector was set at a gain 10 and filter 4s.

Saponification. 1. FFA were prepared using saponification process of the oil suggested by Ju et.al. [7]. A NaOH solution was prepared by dissolving 48 g NaOH and 0.5 g Na_2EDTA in 160 mL water. To this solution, 160 mL ethanol was added. To 100 g of oil 200 mL of NaOH solution was added and then heated at 60°C with magnetic stirring at 550 rpm for 1 h. After 1 h 40 mL water and 400 mL hexane was added and the solution was stirred for 1 h at room temperature. The upper layer containing unsaponifiable matter was removed. To the lower layer 160 mL water was added and 12N hydrochloric acid was added until the pH equalled 1. The resulting lower layer was removed by a separating funnel and discarded. The FFA-containing upper layer was dried with anhydrous Na_2SO_4, and solvent was evaporated in a vacuum rotary evaporator. FFA obtained from saponification of oil was immediately analysed.
2. β-sitosterol was saponified [8] before qualitative and quantitative analysis on HPLC-ELSD. 5 g of vegetable oil was saponified by refluxing with 50 mL of ethanolic solution of KOH 2 M for 1 h. After cooling at room temperature, 100 mL of water was added. The phases were separated using separation funnel; the aqueous phase was washed three times with diethyl ether. The diethyl ether fractions were collected and dried with anhydrous sodium sulphate, filtered and evaporated in a vacuum rotary evaporator. The residue was dissolved in 1 mL of chloroform.

Supercritical fluid extraction (SFE). The SFE experiments using CO_2 were performed on a semi continuous flow apparatus (Fig. 1). Approximately 50 g of crude oil was charged into the extractor (V = 60 mL). The temperature of the water bath was regulated and maintained at constant level (± 0.5 °C, LAUDA Königshofen, Germany). The liquefied gas (CO_2) was continuously pumped with a high pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebraska) through the preheating coil and over the bed of sample in extractor. The solvent flow rate was measured with a flow meter. The product precipitated in separator (glass trap), where the separation was performed at 1 bar and at room temperature.

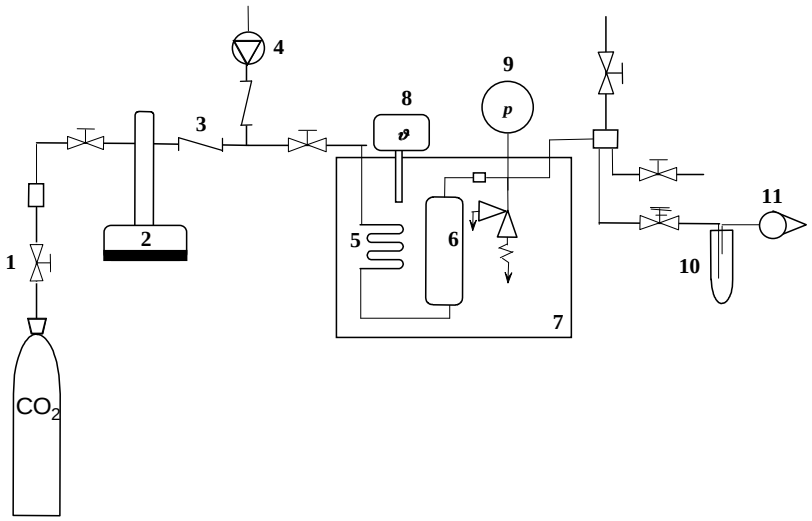

Figure 1: Semi continuous flow apparatus; 1- CO_2 tank, 2-high pressure pump, 3-valve, 4-cosolvent pump, 5-coil, 6-high pressure autoclave, 7-heating bath, 8-temperature control, 9-pressure control, 10-separator (glass trap), 11- gas flow meter.

RESULTS

The two step extractions were carried out by a semicontinuous flow apparatus (Fig. 1). The operating conditions are presented in Table 1.

Table 1: Operating conditions of two step extraction procedure.

Temperature (°C)	Pressure at 1.step (bar)	Pressure at 2.step (bar)
40	80	150
40	100	250
40	140	300
60	80	150
60	100	250
60	140	300
80	80	150
80	100	250
80	140	300

The first step separation was carried out at lower pressure than the second extraction step. Both extractions were performed at constant temperature. The residue obtained from first step extraction was treated in a second step at different pressure. The procedure is presented in Fig.2.

The extraction yields obtained from the first step extraction decrease with increasing temperature. In first step separation the highest extraction yield was obtained at temperature 40 °C and 100 bar (29.70 wt.%). In second step separation, where the residual from first step separation was extracted, the same trend can be observed, with increasing temperature extraction yields decrease. The exception was separation at 250 bar, where the extraction yield at 60 °C was lower than at 80 °C. That can be explained with the comparison of density of CO_2 and the density of the oil. At 250 bar and 60 °C the density of CO_2 is comparable to the density of oil, what can cause phase separation problems. This phenomenon should be further investigated. At constant temperature of 60 °C, the increase of pressure in first step

extraction, the extraction yields increased. The same trends can be observed in second step extraction.

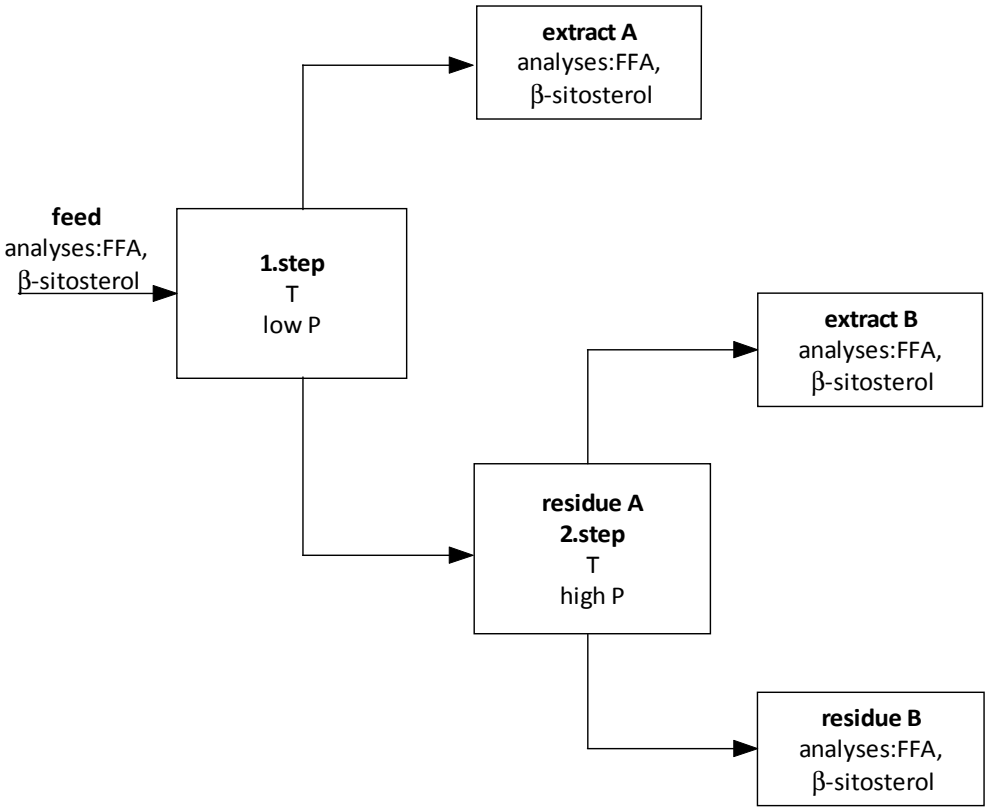

Figure 2: Schematic review of two-step fractionation of crude rapeseed oil.

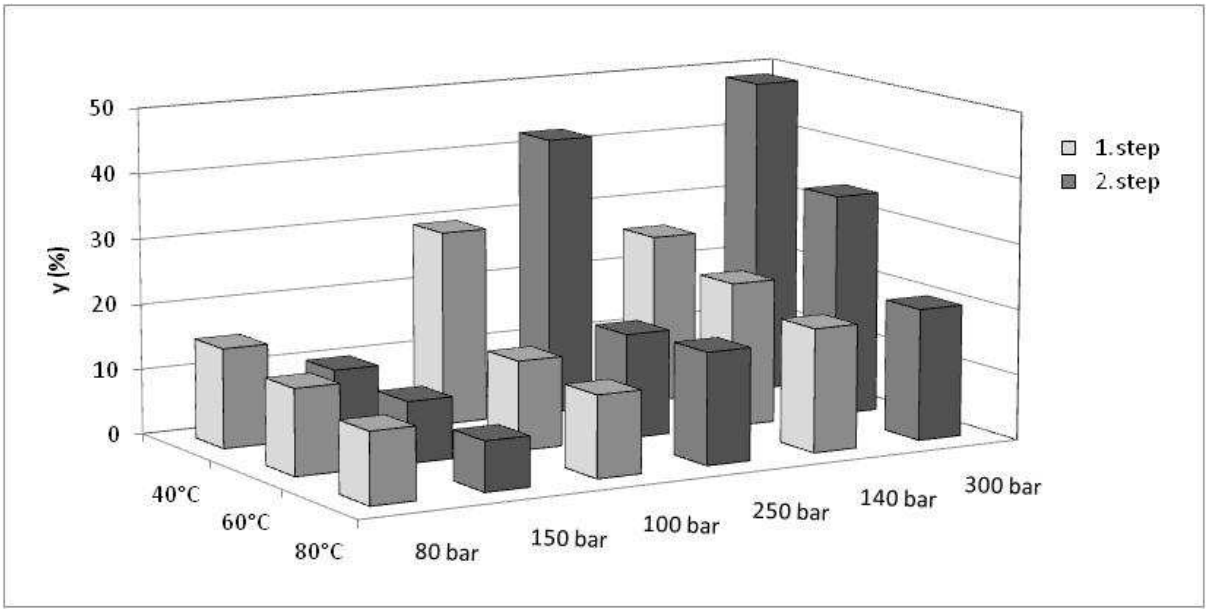

Figure 3: Extraction yields dependent on temperature and pressure.

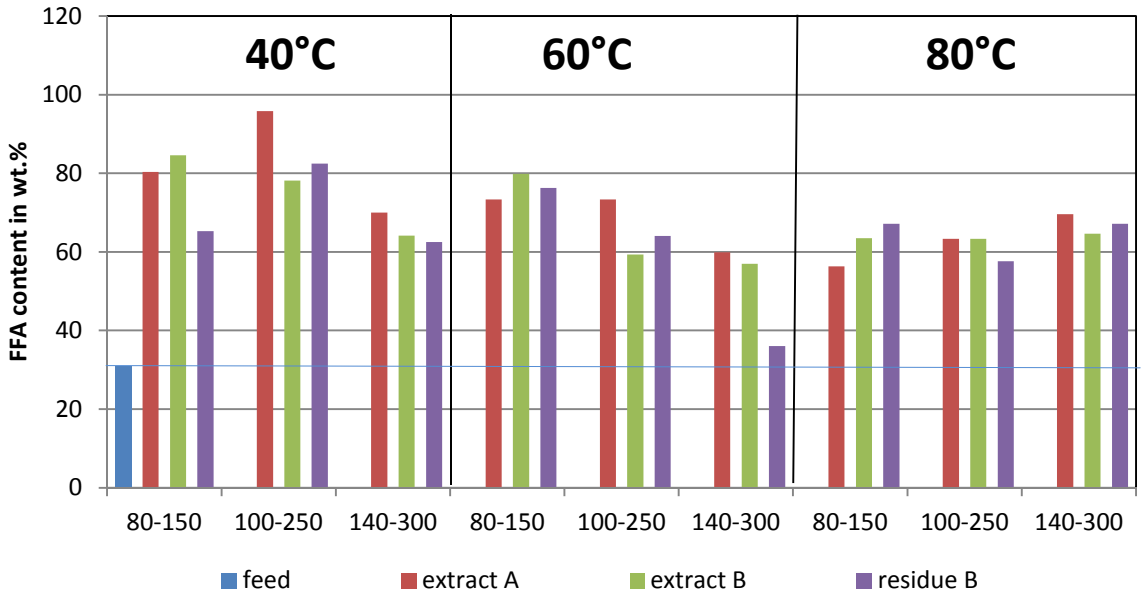

Figure 4: Content of FFA (in wt.%) at different stage of two step fractionation.

Even though the extraction yields are high, the content of investigated compounds shows different results. The content of FFA and β-sitosterol of crude rapeseed oil used in experiments was 31 wt.% and 0.28 wt.%, respectively. The best result of extraction in case of FFA (Fig. 4) was obtained in experiment performed at following conditions: first step of extraction at 100 bar and 40 °C, where the content of FFA in extract was 95.82 wt.% and the content of β-sitosterol was 0.51 wt.%. The highest content of β-sitosterol (Fig. 5) was obtained in residue after second step at 150 bar and 40 °C, and it was 1.16 wt.% and the content of free fatty acids was 65.3 wt.%.
Generally, the oil with high content of both, FFA and β-sitosterol, was obtained in extract after two step extraction at following conditions: first step at pressure 80 bar and temperature 40°C, and second step at pressure 150 bar and temperature 40°C, where extract with 84.59 wt.% of free fatty acids and 0,99 wt.% of β-sitosterol was obtained.

CONCLUSION

This study presents the preliminary results on fractionation of minor components from vegetable oils by supercritical fluid extraction with CO_2. Higher content of those compounds can contribute to higher quality of the oil. The aim was to determine the optimal extraction conditions for concentration of FFA and β-sitosterol in crude rapeseed oil. By using two step extraction procedures at different pressures and temperatures the satisfying results were obtained. The best results in case of β-sitosterol were obtained with extraction at temperature 40 °C and pressure 150 bar, where the content in residual oil was 1.16 wt.%. In case of FFA, the best result was obtained at temperature 40 °C and pressure 100 bar, where FFA were concentrated in extract after first step extraction. It can be concluded that FFA are more soluble in CO_2 at moderate conditions as sterols and with further research not only concentration but also separation of those compound could be achieved.

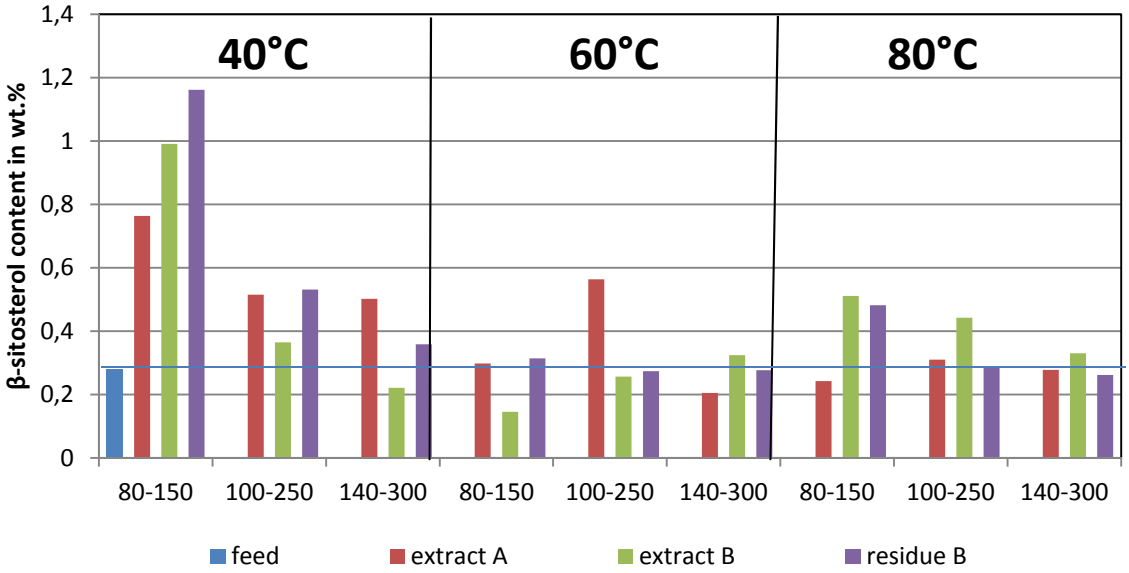

Figure 5: Content of β-sitosterol (in wt.%) at different stage of two step fractionation.

REFERENCES :

[1]. FERNANDES, P., CABRAL, J.M.S., Bioresource Technology, Vol. 98, **2007**, p. 2335.
[2].SNYDER, J.M., KING, J.W., TAYLOR, S.L., NEESE, A.L., Journal of American Oil Chemists Society, Vol. 76, **1999**, p. 717.
[3].DUNFORD, N.T., TEEL, J.A., KING, J.W., Food Research International, Vol. 36, **2003**, p. 175.
[4].CHUANG, M., BRUNNER, G., Journal of Supercritical Fluids, Vol. 37, **2006**, p. 151.
[5].KOTNIK, P., ŠKERGET, M., KNEZ, Ž., European Journal of Lipid Science and Technology, 108, **2006**, p. 569.
[6].NAIR, V.D.P., KANFER, I., HOOGMARTENS, J., Journal of Pharmaceutical and Biomedical Analysis, Vol. 41, **2006**, p. 731.
[7]. JU, Y., CHEN, T., Journal of American Oil Chemists Society, Vol. 79, **2002**, p. 29.
[8]. CANABATE-DIAZ, B., SEGURA CARRETERO, A., FERNANDEZ-GUTIERREZ, A., BELMONTE VEGA, A., GARRIDO FRENICH, A., MARTINEZ VIDAL, J.L., DURAN MARTOS, J., Food Chemistry, Vol. 102, **2007**, p. 593.