INTRODUCTION

During Croatian history hemp was grown and had large share in the industrial production. In past few decades the variety of hemp Cannabis sativa L. was unfairly neglected because of the similarity with the kind of hemp (Cannabis indica L.) that is illegal and is used as a narcotic.

The objective of this work was to evaluate the concentration of tocopherols and fatty acids in different fractions of hemp seed oil obtained by supercritical CO₂ at different extraction process conditions. The composition of hemp seed oil obtained with supercritical CO₂ was compared with the hemp seed oil extracted by nhexane.

PLANT MATERIAL PREPARATION

Hemp seeds (Cannabis sativa L.) were obtained from family farm Organic Vita (Vranešac, Croatia) in 2013. Moisture content of the hemp seeds (8.09 ± 0.09%) was determined according to AOAC Official Method 925.40 (2000). Samples were cleaned from impurities and grounded using laboratory mill (IKA Basic A11, Germany) and sieved using a vertical vibratory sieve shaker (Labotechnik GmbH, Lennern, Germany) for 20 min.

ORGANIC SOLVENT EXTRACTION

The initial oil content in hemp seeds was measured by automatic extraction systems Soxtern by Gerdhart with nhexane. 5 g of ground hemp seeds was extracted with 130 ml solvent, until totally depolymerized according to HRN ISO 6892 (2000). The whole process took 2 h and 45 min, at 180°C. The measurement was done in duplicate. The average of the initial oil content for two replicates was 33.34 ± 0.23%.

DETERMINATION OF TOCOPHEROLS

Preparation of samples for GC-MS analysis has been preceded by saponification of 0.5 g of sample in 50 ml of potassium hydroxide, and then by extraction unprofitable components using diethyl ether as extraction solvent. For analysis of tocopherol Agilent 7890A GC equipped with Agilent 5975 MSD has been used. For this analysis GC-MS was fitted with HP-5MS (Agilent J&W 30 m. (100 μm, 0.25 μm). The temperatures of injection port was 280°C, splitless injection. The temperature of transfer line was 280°C. The initial oven temperature was set at 200°C for 3 min, and then programmed as 8°C/min to 280°C. The carrier gas was He. MS conditions were: scan (95 to 550 amu), threshold 100 MS, quad 150 MS, source 250°C. Injected sample volume was 1 μl. The identification of components was carried out based on computer matches with NIST 2005 MS Library. Standard compounds were dissolved in nhexane to prepare six different extraction concentrations of each component. m/z for each calibration curve was 0.999. All analyses were performed in triplicate.

DETERMINATION OF FATTY ACIDS COMPOSITION

Prepared fatty acid methyl esters (EN ISO 5509:2000 standard) were analyzed by gas chromatography according to EN ISO 5509-1993. It was used 5890B gas chromatograph (Agilent Technologies, Lake Forest, USA) with a capillary column, HP-1 100 m long with a diameter of 0.25 mm and the thickness of the stationary phase 0.10 μm (Agilent Technologies, Lake Forest, USA), a split-splittless injector (temperature 250°C) and a flame-ionization detector (temperature 220°C). A sample (1 μl) was injected with a split ratio of 1:50. Start column temperature was 120°C with holding time for 1 minute. The oven temperature was increased with a rate of 10°C/min to 175°C/min, holding for 10 minutes, then at a rate of 5°C/min was heated to 210°C, holding for 5 minutes. Carrier gas was helium (99.999%) at constant flow rate of 2 ml/min. The hydrogen flow was 40 ml/min, air flow was 350 ml/min, and the makeup gas flow (nitrogen) was 30 ml/min. Fatty acid methyl esters were identified by comparison with retention times of 37 fatty acid methyl ester standard compounds analyzed at the same conditions. For analysis, each sample, 1 μl of the sample, and for every analysis, calibration reference material (CRM), was prepared and analyzed at the same conditions. The result is expressed as percentage (mg/kg) individual fatty acids to totally fatty acids determined. The detection limit method by GC/MS was 0.01%. Values determined in the validation process for parameter truthfulness were compared with the criteria of the Guidelines for the implementation of analytical methods and interpretation of results (AN ON 2/2005), that to prove the truth of the proportion of the weight by > 10 mg/kg may vary from ±20% to ±10% as compared to the certified value.

STATISTICAL ANALYSIS

Regression analysis was used to predict the amount of extracted substance (fatty acids and tocopherols) during the extraction process. Overall fitting of the model was tested with the analysis of variance (ANOVA) with IBM SPSS for Windows software.

Paired-samples T-test was used to examine if different temperatures and pressures in the process itself affect on the amount of extracted tocopherols and fatty acids. Because individual protocols vary in number of performed measurements, what consequently mean difference in the results pairs, some variations in the calculated arithmetic means and standard deviations occurred.

RESULTS

The effect of extraction time on amount of extracted tocopherols at temperature 40°C and pressure 300 bar

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The effect of temperature (a) and pressure (b) on the extraction yield of hemp seed oil

Table 1. The effect of different pressures on the extraction of fatty acids

Table 2. The effect of different temperatures on the extraction of fatty acids

Table 3. The effect of different temperatures on the extraction of tocopherols

Table 4. The effect of different pressures on the extraction of fatty acids

Table 5. The effect of extraction time on amount of extracted fatty acids at temperature 60°C and pressure 300 bar

Table 6. The effect of extraction time on amount of extracted fatty acids at temperature 60°C and pressure 400 bar

Table 7. The effect of extraction time on amount of extracted fatty acids at temperature 60°C and pressure 400 bar

Table 8. The effect of extraction time on amount of extracted tocopherols at temperature 40°C and pressure 300 bar

Table 9. The effect of extraction time on amount of extracted tocopherols at temperature 40°C and pressure 400 bar

Table 10. The effect of extraction time on amount of extracted tocopherols at temperature 60°C and pressure 300 bar

Table 11. Fatty acid and tocopherol content in hemp seed oil

CONCLUSIONS

Supercritical extraction and fractionation of natural compounds is one of the early and most studied applications in the field of supercritical fluids. Fractionation of the oil is very important for producing products with physical or nutritional properties of interest to the food industry. Supercritical CO₂ was carried out at homemade supercritical fluid extraction system where almost completely oil was extracted from hemp seeds and the obtained oil had higher tocopherol content compared to cold pressing. Low temperatures of extraction, reduced energy consumption, high product quality and absence of solvent in extracts are numerous advantages that supercritical fluid extraction technique can provide as opposed to traditional methods of extraction.