Concentration of Rosmarinic Acid with Supercritical Fluids

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Rosmarinic acid is a common constituent of the *Labitae*. It has been found in many medicinal species of this family, such as rosemary, sage, thyme, balm, marjoram and oregano and have been shown to posess antioxidant, antiviral, antibacterial and anti-inflammatory properties.

In present work, the concentration of rosmarinic acid by supercritical fluids is presented. Carbon dioxide (CO_2) and dimethyl ether (DME) were used as solvents. The crude extract was prepared from rosemary with conventional extraction process. The supercritical CO_2 extraction of crude extracts was performed at pressures from 100–300 bar and temperatures of 30–60 °C. Extraction with dimethyl ether (DME) was performed at pressures from 100–200 bar and temperatures 30–60 °C.

Effects of temperature, amount of solvent, and addition of an entrainer on the concentration of rosmarinic acid in the extracts and extraction residues were studied. Content of rosmarinic acid in the samples was determined by high performance liquid chromatography.

Obtained extracts had a higher amount of rosmarinic acid, better appearance and fluid properties, no smell on essential oil, no solvent residues and were more active.

Keywords: Rosmarinic acid, natural antioxidants, high-pressure extraction, supercritical fluids

Introduction

Antioxidants are compounds used in food, pharmaceutical and cosmetic industries to delay the oxidation processes, which occurs mainly in fats and oils. The use of natural extracts of herbs commonly and traditionally used for cooking as antioxidants has held strong industrial attention inasmuch as they might substitute synthetic chemicals and still be classified as natural.

Plant phenolics are a significant group of secondary metabolites, which have diverse medicinal applications. Among various phenolics, rosmarinic acid is an important caffeoyl ester with proven medicinal properties and well-charactized physiological functions. Rosmarinic acid is found in substantial quantities in the family *Labiatae* with medicinal uses in several cultures.[5] In plants rosmarinic acid is thought to be a part of the defense system against bacterial infections and predators. The pharmacological properties of rosmarinic acid are to be antioxidant, antibacterial, antiviral, and anti-inflammatory.[4] These properties make rosmarinic acid interesting for food, cosmetic and pharmaceutical industry. Structure of rosmarinic acid is presented in Figure 1.

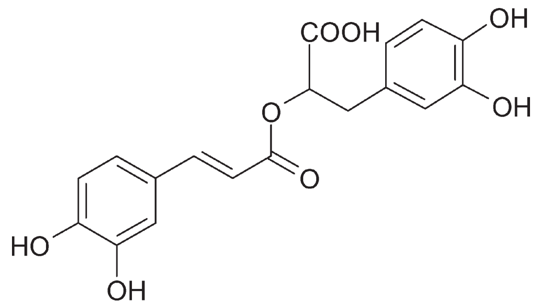

Fig. 1 – *Structural formula of rosmarinic acid (RA)*
Slika 1 – *Strukturna formula ružmarinske kiseline (RA)*

Supercritical fluids are gases with properties between that of a "normal" gas and a liquid. Variation of pressure changes properties of supercritical fluids continuously from more gas-like behaviour to more liquid-like behaviour. This behaviour may lead to new technologies in processing natural materials. Supercritical fluids can replace organic solvents in many processes such as extraction from solids (bleaching), counter current multistage separations, chromatographic separations, and others, provided the solvent properties of supercritical fluids are adequate.[2,6] High-pressure extraction is very suitable technique for the extraction and fractionation of natural thermo labile substances. The product does not contain residual organic solvents as they do in conventional extraction processes.[1,3]

Sub- and supercritical fluids have been used as a solvent for wide variety of extractive applications. Recently super-

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critical fluids have also been applied as a solvent for non--extractive applications in high-pressure micronisation, in chromatography and as a chemical and biochemical reaction media. The advantages of using sub- and supercritical fluids to perform and achieve separations are well documented in several reviews.[1]

This paper describes concentration of rosmarinic acid in crude rosemary extracts, performed with conventional extraction process. Carbon dioxide and dimethyl ether were used as supercritical fluids.

Extractions with carbon dioxide were performed at pressures 100–300 bar and temperatures 30 and 60 °C. Furthermore, the addition of an entrainer has been studied. Petrol ether, ethyl acetate and their mixture were used as entrainers. Rosmarinic acid was concentrated in the extraction residue.

Extractions of crude rosemary extracts with dimethyl ether were performed at pressure 100–200 bar and temperatures 30 and 60 °C. Rosmarinic acid was concentrated in the extract.

Materials and methods

Materials

The crude rosemary extracts originated from Pinus TKI d.d. (Rače, Slovenia). All chemicals used for analysis were purchased from Merck (Darmstadt, Germany). CO_2 (purity 2.5 (99.5 %)) and dimethyl ether (purity 99.99 %) were obtained from Linde plin (Celje, Slovenia). Nitrogen was obtained from Messer-Griesheim Ruše, Slovenia, and was 99.999 % pure.

Rosmarinic acid standard (purity 97 %, HPLC) was purchased from Sigma-Aldrich (Germany).

Methods

Apparatus and experimental procedure

The extraction experiments with dense gasses (CO_2 and dimethyl ether) were performed on a semicontinuous apparatus, which is presented in Figure 2. The apparatus was home built for a maximum pressure of 500 bar and a temperature of 100 °C. The system allows to produce natural extracts with flammable gases.

Approximately 10 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 or dimethyl ether) was continuously pumped with a high pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebrasca, p_{max} = 350 bar) through the preheating coil and over the bed of sample in the extractor. The solvent flowrate was measured with a flow-meter (ELSTER HANDEL GmbH, Mainz, Germany). The product precipitated in the separator (glass trap), where the separation was performed at 1 bar and at a temperature of 25 °C. The product collected in the glas trap or in the extractor was weighed (± 0.1 mg) and yield was calculated.

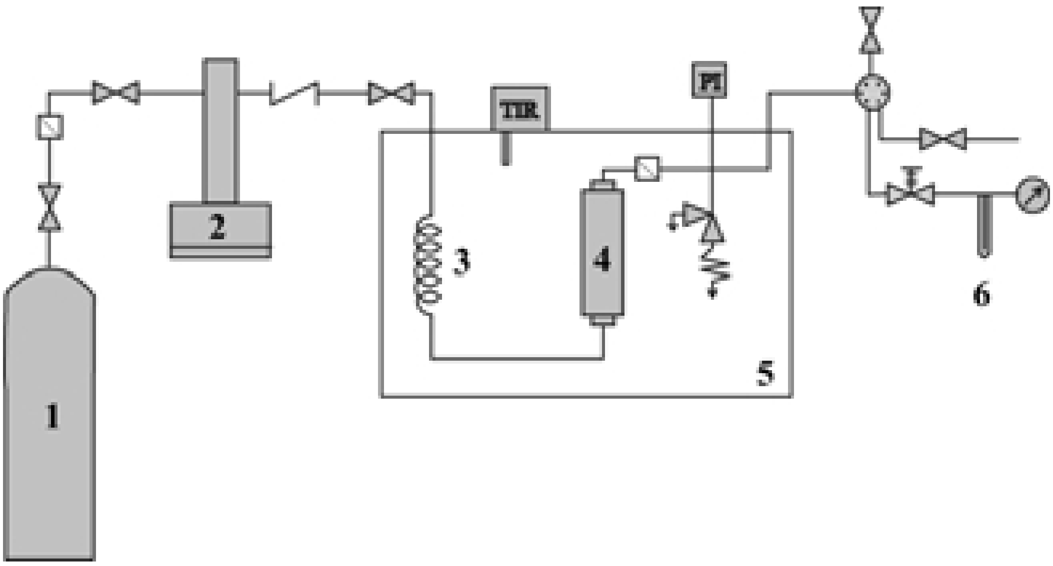
Yield of extraction of rosmarinic acid was calculated by the formula:

$$V = \frac{m_{ex}}{m_{rm}} \cdot 100 \qquad (1)$$

where m_{ex} is a mass of the rosmarinic acid in the extract or extraction residue and m_{rm} is a mass of the raw material extracted.

Analysis

HPLC – High-performance liquid chromatograph was used for the quantitative determination of rosmarinic acid con-



F i g . 2 – *Apparatus for high pressure extraction. 1 – liquid CO_2 cylinder, 2 – Isco syringe pump, 3 – preheat coil, 4 – extractor, 5 – water bath, 6 – trap*

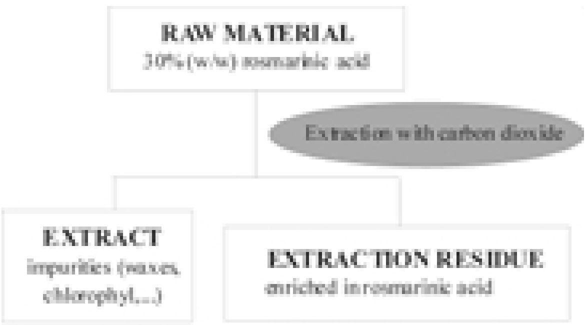
S l i k a 2 – *Aparatura za visokotlačnu ekstrakciju. 1 – bomba s tekućim CO_2, 2 – Isco ubrizgavajuča pumpa, 3 – cijev za predgrijavanje, 4 – ekstraktor, 5 – vodena kupelj, 6 – lovac ekstrakta* tent in the extracts extraction residues and raw material. The HPLC system consisted of a pump (VARIAN 9012 HPLC pump, Walnutcrek, California) and a scan detector (Spectra-Physics). A Kromasil 100 C18 column (BIA d.o.o., Ljubljana, Slovenia) with 5 μm particle diameter was used. The mobile volume phase consisted of acetonitrile: 0.03 % TFA in water $\Psi_{ACCN:TFA}$ = 25:75 (v/v). The flow rate was 1.5 ml min^{-1} and the detection was performed at 280 nm. The quantification was made with an external standard.

The HPLC method was validated and on 95 % confidence range results have no statistical differences, which mean that the accuracy is suitable. Each sample was analyzed three times and relative standard deviation between measurements was 1.6 %.

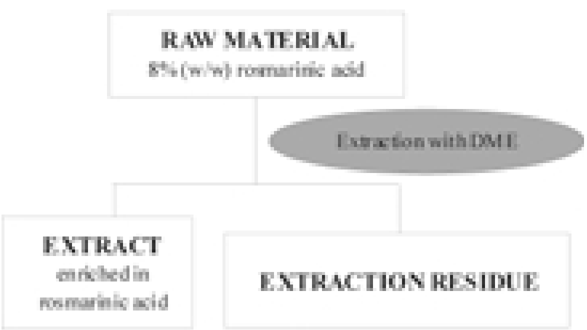
Moisture content of raw material, extracts and extraction residue was made with Karl-Fisher method.

Results and conclusion

Concentration of crude rosmarinic acid extract with rosmarinic acid content of 30 % was performed. Flow rate of carbon dioxide was 4 ml min^{-1} and flow rate of an entrainer, if used, was 0.4 ml min^{-1}. Solubility of rosmarinic acid in carbon dioxide is very low and decrease with increase of pressure. Rosmarinic acid is concentrated in extraction residue. Schema of the extraction process with carbon dioxide as a solvent is presented on Figure 3a.



a)



b)

Fig. 3 – *Schema of the concentration of rosmarinic acid (RA): a) with carbon dioxide extraction and b) with extraction with dimethyl ether (DME)*

Slika 3 – *Shema koncentracije ružmarinske kiseline (RA): a) ekstrakcijom s ugljikovim dioksidom i b) ekstrakcijom s dimetil eterom*

Effect of extraction pressure on increase of rosmarinic acid content in extraction residue at extraction temperature of 30 °C and carbon dioxide consumption of 100 g per g raw material, is presented on Figure 4. Increase of pressure causes increase of rosmarinic acid in extraction residue. Extract contains waxes, essential oil, chlorophyll and other impurities.

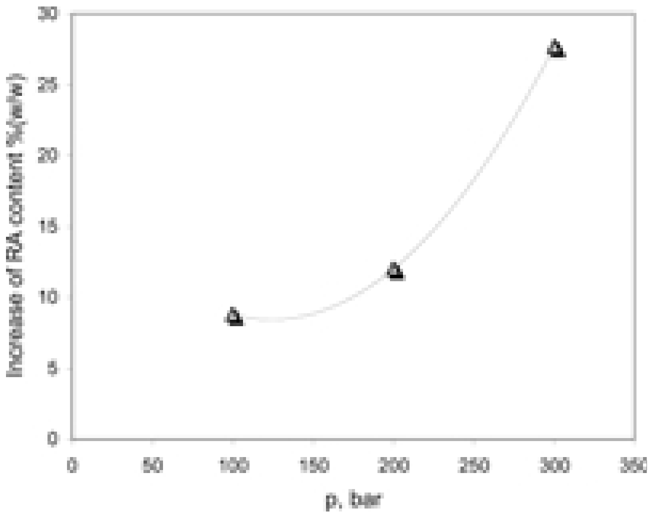


Fig. 4 – *Effect of extraction pressure on increase of rosmarinic acid (RA) mass fraction in extraction residue, T = 30 °C, consumption = 100 g CO_2 in g raw material*

Slika 4 – *Utjecaj tlaka na povećanje masenog udjela ružmarinske kiseline (RA) u ekstrakcijskom ostatku, T = 30 °C, potrošnja = 100 g CO_2 po g ulaznog materiala*

Addition of an entrainer does not lead to increase of rosmarinic acid in extraction residue, as can be seen from Figure 5. Petrol ether, ethyl acetate and mixture of petrol et-

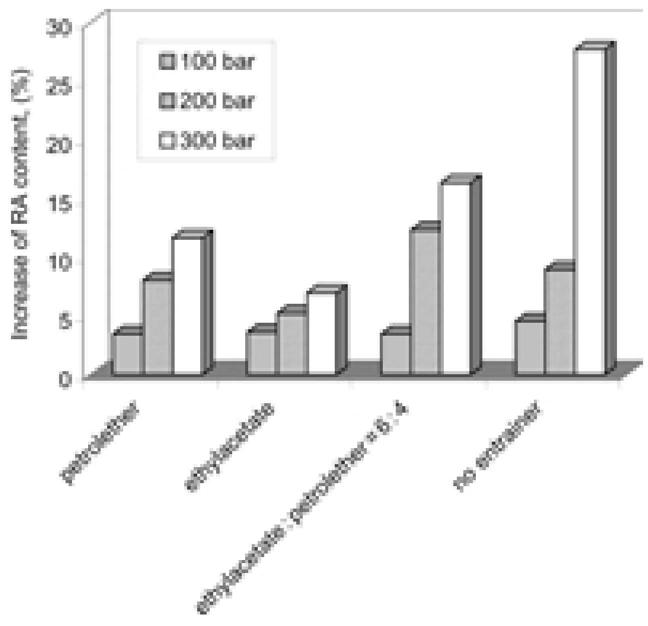


Fig. 5 – *Effect of an entrainer on increase of rosmarinic acid (RA) mass fraction in extraction residue, T = 30 °C, consumption = 100 g CO_2 in g raw material*

Slika 5 – *Utjecaj su-otapala na povećanje masenog udjela ružmarinske kiseline (RA) u ekstrakcijskom ostatku, T = 30 °C, potrošnja = 100 g CO_2 po g ulaznog materiala* her and ethyl acetate in ratio 4 : 6, were used as entrainers.

Figure 6 presents effect of carbon dioxide consumption on increase of rosmarinic acid content in extraction residue. Experiments were performed at temperature 30 °C and pressure 300 bar. As can be seen from the figure, increase of rosmarinic acid content in extraction residue depends on carbon dioxide consumption. Increase of rosmarinic acid concentration in extraction residue is quick with increase of carbon dioxide consumption up to 100 g per g raw material. With further increase of carbon dioxide consumption concentration of rosmarinic acid in extraction residue increases slowly.

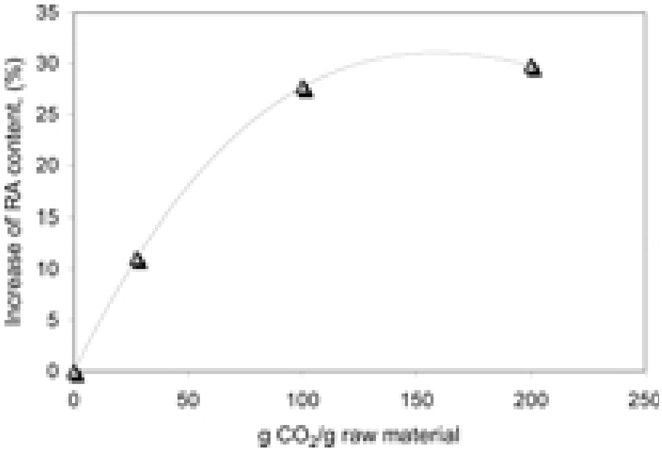


Fig. 6 – *Effect of CO_2 consumption on increase of rosmarinic acid (RA) mass fraction in extraction residue, T = 30 °C, p = 300 bar*

Slika 6 – *Utjecaj potrošnje ugljikovog dioksida na povećanje masenog udjela ružmarinske kiseline (RA) u ekstrakcijskom ostatku, T = 30 °C, p = 300 bar*

Extraction temperature affects on concentration of rosmarinic acid in extraction residue. With increase of temperature from 30 to 60 °C, concentration of rosmarinic acid decreases at extraction pressures 100 and 300 bar. Opposite behaviour was observed at pressure 200 bar, where concentration of rosmarinic acid increases with increase of temperature from 30 to 60 °C. Results are presented on Figure 7.

During the extraction of crude rosmarinic acid extract with dimethyl ether, rosmarinic acid concentrated in the extract. Crude rosemary extract with 8 % of rosmarinic acid was extracted. Flow rate of dimethyl ether was 2 ml min^{-1}. Schema of the extraction process is presented on Figure 3b.

Figure 8 presents effect of pressure and temperature on rosmarinic acid content in extract compared to raw material. Experiments were performed at dimethyl ether consumption 80 g per g raw material. Extraction pressure has effect on solubility of rosmarinic acid. The worse results were obtained at pressure of 150 bar. With further increase of extraction pressure to 200 bar rosmarinic acid concentration in the extracts increases. The same behaviour was observed with decrease of pressure to 100 bar. Extraction temperature also influences the concentration of rosmarinic acid in the extract. Rosmarinic acid content in the ex-

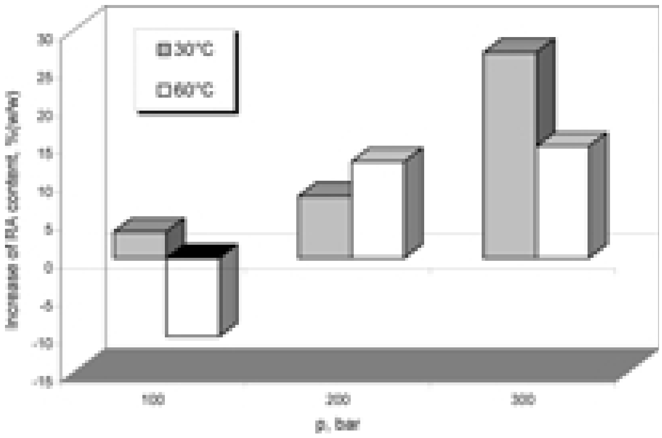


Fig. 7 – *Effect of temperature on increase of rosmarinic acid (RA) mass fraction in extraction residue, consumption = 100 g CO_2 in g raw material*

Slika 7 – *Utjecaj temperature na povećanje koncentracije ružmarinske kiseline (RA) u ekstrakcijskom ostatku, potrošnja = 100 g CO_2 po g ulaznog materiala*

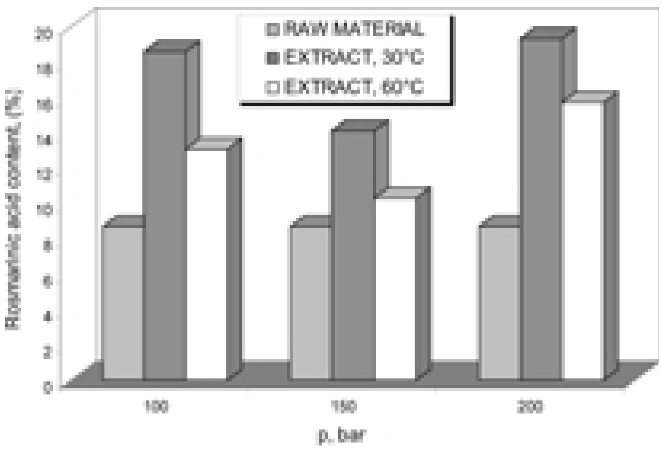


Fig. 8 – *Effect of pressure and temperature on rosmarinic acid (RA) content in the extract compared to crude extract, DME consumption = 80 g per g raw material*

Slika 8 – *Utjecaj tlaka i temperature na maseni udjel ružmarinske kiseline (RA) u ekstraktu usporedno s ulaznim materijalom, potrošnja DME = 80 g po g ulaznog materiala*

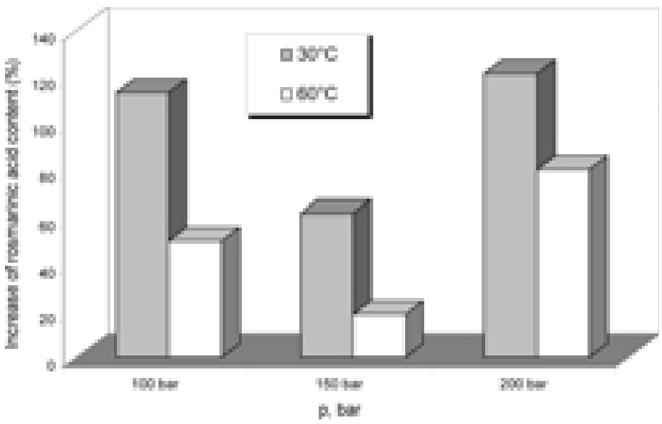


Fig. 9 – *Effect of pressure and temperature on rosmarinic acid (RA) increase in the extract, DME consumption = 80 g per g raw material*

Slika 9 – *Utjecaj tlaka i temperature na povećanje masenog udjela ružmarinske kiseline (RA) u ekstraktu, potrošnja = 80 g DME po g ulaznog materiala* tracts decreases with increase of temperature from 30 to 60 °C. Effect of pressure and temperature on rosmarinic acid increase in the extracts compared to crude rosemary extract is presented in Figure 9.

Figure 10 presents effect of pressure and temperature on yield of rosmarinic acid isolation. Experiments were performed at dimethyl ether consumption 80 g per g raw material. Yield of rosmarinic acid isolation decreases with increasing pressure from 100 to 200 bar at 30 °C. Opposite behaviour was observed at temperature of 60 °C, where yield of isolation increases with increasing pressure.

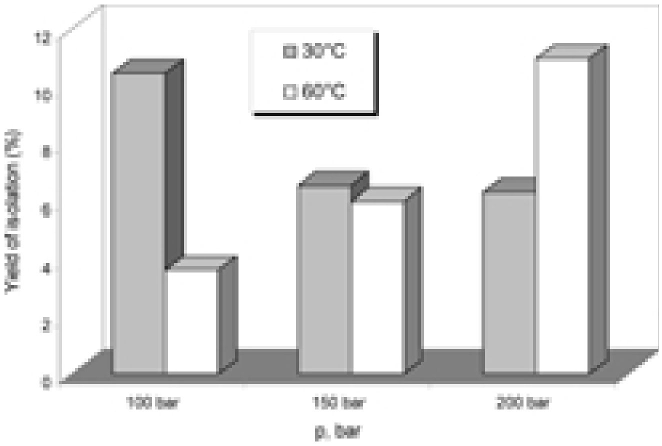


Fig. 10 – *Effect of pressure and temperature on yield of rosmarinic acid (RA) isolation, consumption = 80 g DME per g raw material*

Slika 10 – *Utjecaj tlaka i temperature na iskorištenje izolacije ružmarinske kiseline (RA), potrošnja = 80 g DME po g ulaznog materiala*

Yield of isolation also depends on dimethyl ether consumption and increase with increasing dimethyl ether consumption. Experiments were performed at temperature of 30 °C and pressure of 200 bar. Figure 11 presents the results.

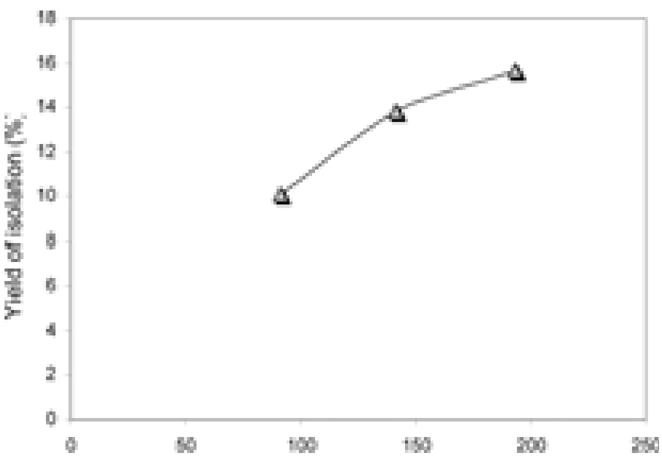


Fig. 11 – *Effect of DME consumption on yield of rosmarinic acid (RA) isolation, T = 30 °C, p = 200 bar*

Slika 11 – *Utjecaj za ekstrakciju upotrijebljenog DME na iskorištenje izolacije ružmarinske kiseline (RA), T = 30 °C, p = 200 bar*

Advantages of supercritical fluid extraction of crude rosmarinic acid extracts are:

– better appearance of the products,

– better fluidity properties of the products,

– higher concentration of rosmarinic acid in the products, and

– products do not smell on essential oil, which smell is unwanted feature for products used in cosmetic food and pharmaceutical industry.

High-pressure extraction is very appropriate technique for isolation of natural thermo labile substances. The product does not contain residual organic solvents as in conventional extraction processes, which makes these products suitable for use in food, cosmetic and pharmaceutical industry.

List of symbols
Popis simbola

RA – rosmarinic acid
– ružmarinska kiselina

DME – dimethyl ether
– dimetileter

HPLC – high performance liquid chromatography
– tekućinska kromatografija

T – temperature, °C
– temperatura, °C

p – pressure, bar
– tlak, bar

Y – yield, %
– iskorištenje, %

m – mass, g
– masa, g

w – mass fraction, %
– maseni udjel, %

References
Literatura

1. *D. Bauman, M. Hadolin, A. Rižner Hraš, Ž. Knez*, Acta Alimentaria **28** (1999) 15.
2. *Ž. Knez*, Kem. Ind. **36**:6 (1987) 243.
3. *Ž. Knez, A. Rižner*, Some novel applications of supercritical fluids in food processing. 7th International Congress on Engineering and Food. Engineering food. Part 2. Sheffield, Sheffield Academic Press, I/5-I/8, 1997.
4. *E. Kochan, H. Wysokinska, A. Chmiel, B. Grabias*, Z. Naturforsch. **54c** (1999) 11.
5. *K. Shetty*, Journal of Herbs, Spices & Medicinal Plants **8** (2001) 161.
6. *M. Škerget, Ž. Knez, Z. Novak, D. Bauman*, Acta Alimentaria **27** (1998) 149.

SAŽETAK

Koncentriranje ružmarinske kiseline sa superkritičnim fluidima

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Ružmarinska kiselina jedna je od najpoznatijih tvari u porodici *Labiatae*. Pronađena je u vrsti medicinski važnog bilja ove porodice, kao što su ružmarin, kadulja, timijan, melisa, majaron i origano. Potvrđeno je da ružmarinska kiselina ima antioksidativna, antivirusna, antibakterijska i protuupalna svojstva.

U ovom radu prikazano je koncentriranje ružmarinske kiseline u superkritičnim fluidima. Ugljikov dioksid (CO_2) i dimetil-eter (DME) upotrijebljeni su kao otapala. Sirovi ekstrakt pripremljen je pomoću konvencionalne ekstrakcije. Superkritična ekstrakcija s ugljikovim dioksidom bila je izvedena pri tlaku od 100–300 bara i pri temperaturi od 30–60 °C. Ekstrakcija s dimetileterom (DME) bila je izvedena pri tlaku od 100–200 bara i pri temperaturi od 30–60 °C.

Proučavan je utjecaj temperature, tlaka, količine otapala i upotrebe su-otapala na koncentraciju ružmarinske kiseline u ekstraktu i ekstrakcijskom ostatku. Koncentracija ružmarinske kiseline u uzorcima bila je određivana sa HPLC (tekućinska kromatografija) metodom.

Dobiveni ekstrakti imaju veću koncentraciju ružmarinske kiseline, bolji izgled, bolja tečljiva svojstva, nemaju mirisa po esencijalnom ulju, ne sadrže ostatke otapala i imaju bolju aktivnost.

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