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Extraction of Lutein Diesters from *Tagetes Erecta* using Supercritical CO_2 and Liquid Propane

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Dedicated to the memory of the late Prof. Dr. Valentin Koloini

Abstract

The efficiency of high pressure extraction of lutein diesters from marigold (*Tagetes erecta*) flower petals has been investigated. The solvents used for extraction were supercritical carbon dioxide and liquid propane. Operating parameters were 300 bar and 40, 60 and 80 °C for CO_2 and 100, 150, 200 bar and 40 and 60 °C for propane, respectively. The influence of process parameters on the total yield of extraction and content of lutein diesters in the extracts was investigated. The results show, that solvent power of propane for lutein diesters is approximately 3.5 times higher than of CO_2. The calculation procedure based on the Fick's second law was applied to determine the diffusivities of lutein diesters during extraction from marigold flower petals for both extraction stages: a constant rate stage followed by a stage of decreasing rate. The mathematical model based on the Fick's second law well described the experimental extraction results.

Keywords: Lutein diesters, *Tagetes erecta*, extraction, supercritical CO_2, subcritical propane.

1. Introduction

In recent years, an increasing interest in xanthophylls for application in human food, mainly lutein, as well as for production of animal feed is observed.[1] Lutein is the predominant xanthophyll in most green and leafy plants and in petals of flowers. The main natural source are marigold flowers (*Tagetes erecta* L.), where lutein is acylated with different fatty acids. The marigold oleoresin is extracted from dried marigold flower petals with hexane and contains lutein, lutein esters, other carotenoids and waxes. Purified lutein is obtained from the oleoresin by saponification and crystallisation. Thus, lutein can be found in its native esterified or in its free form on the market.[1]

As reported, this traditional process results in at least 50% losses of carotenoids,[2] depending on conditions for silaging, drying and extraction. Another drawback of the extraction process is the use of hexane as solvent. Because of these problems, the interest has arisen to improve xanthophyll production process and to develop new alternative methods.

One possibility which appears promising is the use of degrading-cell wall enzymes with mixed activities (hemicellulase, cellulase, pectinase, proteinase) to enhance carotenoid extraction.[2] Investigations show that the extraction yields of xantophyls are improved by enzymatic treatment of marigold prior the solvent extraction.[2–7] It is reported by Barzana et al., that by applying enzymatic treatment of flowers prior the extraction, a carotenoid recovery of 97% was obtained, while in absence of enzymes the carotenoid recovery was only 44%.[4]

Due to legal limitations of solvent residues and solvents which can be used for food-, drug-, and cosmetic industry the use of supercritical fluid (SCF) technology has attract higher interest and is becoming, beside biotechnology and membrane technology, one of the most promising fields of research. In addition, recent publications, which investigate the costs of SCF processing,[8,9] report that processing costs can be very competitive to other processes.[9] Costs reduction can be accomplished by scale up the process, continuous mode operation, by using propane-CO_2 mixture instead of pure CO_2, by adsorbing the solute and using adsorbate as product, and by operating at low pressure drop along the solvent cycle what can be accomplished by using membranes for separation of solute from the supercritical solvent.[9]

Extraction with CO_2, which is the most often used SCF, is a particularly suitable isolation method for natural materials[10] and gives an alternative to replace the non-polar organic solvents. Extractions with supercritical (SC) CO_2 result in solvent-free products and avoid deteriorating reactions, due to low process temperatures. The CO_2 is readily available, relatively cheap and accepted as a solvent in the food industry. It is selective for lipophilic compounds of lower molecular weight such as hydrocarbons, halogenated hydrocarbons, ethers, esters, ketones and aldehydes. When polar groups such as hydroxyl-, carboxyl-, amino- are present, the solubility of compound decreases. In this case it is common to increase the polarity of SCF by addition of small amounts of polar modifiers (cosolvents) such as ethanol, acetone, and water.

In literature the investigations of using SC CO_2 without and with cosolvents for extraction of lutein and lutein diesters from *Menta spicata*[11] and marigold[12–14] are reported. It was found that by using chloroform as cosolvent, the amount of solubilized lutein diesters was increased for 2.8 times compared to pure CO_2, and 65% of the amount extracted with hexane was obtained.[13] The soybean oil used as cosolvent at low to medium concentrations was able to substantially increase the yield of lutein.[14] At optimal conditions of 355 bar, 58.7 °C and 6.9% of soybean oil as cosolvent, the yield of lutein was 1.04%.[14] Ultrasound assisted SC CO_2 extraction of lutein esters from marigold was studied by Gao et al.,[15] and the same yield of lutein esters was achieved at lower pressure or temperature than traditional SC CO_2 process.

Another lipophilic solvent accepted in the food industry is propane; however investigations using propane for the extraction of xantophylls are limited. Daood et al. studied the extraction of oleoresin from pungent spice paprika powder using SC CO_2 and subcritical propane.[16] They found, that in general, the color content of propane-extracted oleoresin was 4–5 times higher than that of SC CO_2 extracted ones and that SC CO_2 was inefficient for the extraction of diesters of xantophylls.

In the present work CO_2 and propane have been used for extraction of marigold flower petals and the recovery of lutein diesters and concentration of extracts obtained at different operating conditions was researched.

2. Experimental

2. 1. Materials

The marigold flower petals were purchased from local market. The content of lutein diesters in the material, determined by multiple step extraction with hexane, was 4.51%. The marigold flower petals were ground and sieve analysis of ground material was carried out to determine the particle size distribution. Experiments were made in a laboratory scale, in small quantities and the heating due to grinding the raw material was minimal. Moisture content of plant material was determined using Karl Fisher Titrator (Mettler Toledo DL31).

CO_2 (purity 99.97 %), propane (purity 99.95 %) and nitrogen (purity 99.999 %) were obtained from Messer-Griesheim Ruše.

2. 2. Methods

Extraction apparatus and experimental procedure. The extraction experiments with SC CO_2 and liquid propane were performed on a semicontinuous apparatus, which is presented in Fig. 1. The apparatus was home built for a maximum pressure of 300 bar and a temperature of 100 °C.

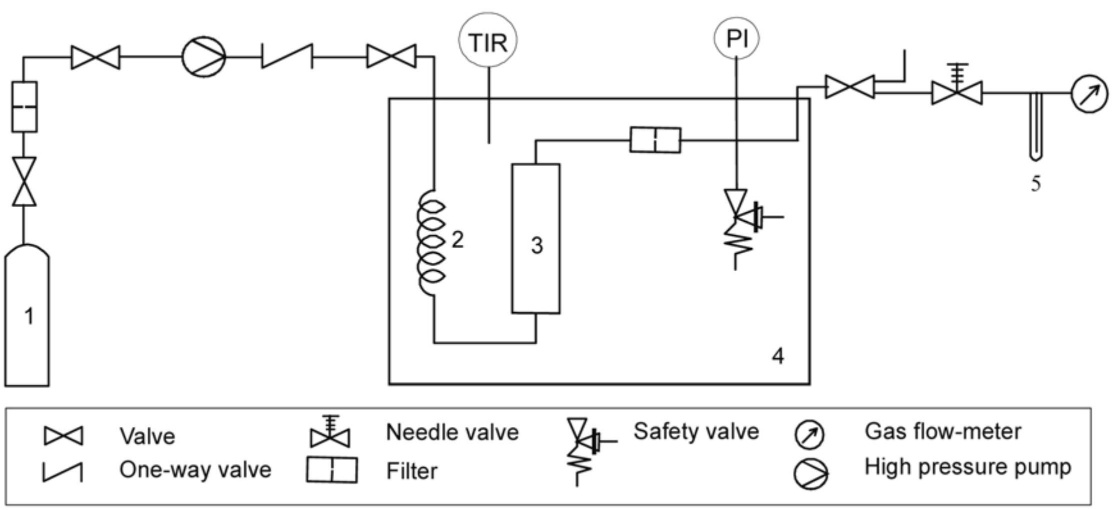

Figure 1. Apparatus for semi-continuous high pressure extraction: 1 – gas cylinder, 2 – preheating coil, 3 – extractor, 4 – water bath, 5 – trap.

Approximately 20 g of ground material 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 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 propane) 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 was weighed (±0.1 mg) and yield of extraction was calculated by the formula:

$$\text{Yield}(\%) = \frac{m_{\text{extract}}}{m_{\text{raw material}}} \cdot 100\% \qquad (1)$$

where $m_{extract}$ is mass of the extract and $m_{raw\ material}$ is mass of the raw material (marigold flower petals) extracted.

Yield of lutein diesters (LD) was calculated as:

$$\text{Yield}_{\text{LD}}\ (\%) = \text{Yield}(\%) \cdot w_{\text{LD}} \qquad (2)$$

where w_{LD} is weight fraction of lutein diesters in the extract and was determined by spectrophotometer.

Analysis of extract. Content of lutein diesters in extracts was analysed spectrophotometrically. 0.1–0.2 g of sample was weighed into a small beaker and heated to 50 °C in water bath until material was a paste. The paste was dissolved in hexane and the solution was transferred into a 50 ml volumetric flask and filled to 50 ml with hexane. 1 ml of solution was transferred into a 100 ml volumetric flask and diluted to 100 ml with hexane. The absorbance was measured with the UV/VIS spectrophotometer at 445 nm. The concentration of lutein esters in extract was calculated as follows:

$$w_{LD}(\%) = 1.8\frac{F \cdot A_{445}}{2550 \cdot m_{\text{sample}}} \qquad (3)$$

where m_{sample} is mass of the sample, *A* is the absorbance and *F* is the dilution factor (*F* = 5000).

2. 3. Mathematical Model

The diffusion model of solid-liquid extraction derived from Fick's second law and proposed by Crank was applied.[17] The presence of two parallel diffusion processes inside the solid; one faster and one slower were considered[18] and the mass transfer from the sphere particles was described by the following equation:

$$\frac{c_\infty - c}{c_\infty} = \frac{6}{\pi^2}\left[f_1 \exp\left\{-\frac{\pi^2 D_1 t}{R^2}\right\} + f_2 \exp\left\{-\frac{\pi^2 D_2 t}{R^2}\right\}\right] \qquad (4)$$

where *c* and c_∞ are the concentration of the extracted constituent in the solution at time *t* and after infinite time, respectively. *R* is the radius of sphere particles and f_1/π and $6f_2/\pi$ are the fractions of the solute, which are extracted with diffusion coefficients D_1 and D_2, respectively. In later stages of the extraction, only the second term on the right-hand side of Eq. (4) remains significant. The parameter D_2 is obtained from the slope and the parameter f_2 from the intercept of the curve where $\ln[c_\infty / (c_\infty - c)]$ is plotted as function of time *t*. In earlier stages of the extraction, the second exponential term is close to unity and D_1 and f_1 can be determined.

3. Results and Discussion

The diameter of ground marigold flower petals particles was in the range from 0.2 to 2.5 mm and the median particle size, determined by sieve analysis, was 1.04 mm. Moister content of plant material was 7.08 % (w/w).

Table 1. Extraction of lutein diesters from marigold flower petals with SC CO_2 and liquid propane.

Solvent	*P* (bar)	*T* (°C)	ρ^* (kg/m^3)	Q_V^{**} (L/min)	Yield (%)	w_{LD} (%)
CO_2	300	40	909.9	0.774	5.10	11.48
	300	60	829.7	0.774	5.46	8.64
	300	80	745.6	0.863	5.04	8.31
Propane	100	40	495.5	0.420	5.99	42.48
	150	40	506.9	0.497	9.02	41.23
	200	40	516.5	0.528	8.78	43.58
	150	60	482.1	0.175	7.85	42.69

* density of solvent obtained from NIST Chemistry WebBook[19] at constant temperature (*T*) and pressure (*P*).
** volume flowrate of solvent at atmospheric conditions

Results of semicontinuous extraction of marigold flower petals with SC CO_2 and liquid propane at different operating conditions are presented in Table 1. In Figs. 2 and 3 extraction curves of *T. erecta* obtained by plotting yield of extract versus solvent to feed ratio (S/F in kg/kg) are presented. It can be observed that, by using CO_2 as solvent at constant pressure of 300 bar (Fig. 2), maximum yield obtained does not change much with increasing temperature from 40 to 80 °C and is between 5.04% to 5.46%. In the case of propane (Fig.3) at 40 °C, by increasing the pressure from 100 to 150 bar the yield increases from 5.99% to 9.02% and stays approximately constant with further increase of pressure. At constant pressure 150 bar, the maximum yield is independent of temperature and does not change much with increasing the temperature to 60 °C.

Another important fact, which can also be seen from the extraction results is, that the amount of propane needed for the extraction of marigold flower petals is generally much lower (approx. 6 times lower) than that of CO_2.

In Table 1 the yield and concentration of lutein diesters in the obtained extracts can be observed. In case of CO_2 at constant pressure 300 bar, the content of lutein diesters in extract is relatively low at all temperatures investigated. The maximal yield of lutein diesters (Fig.4) is attained at 40 °C and 300 bar (0.59%), where the density of CO_2 is the highest. The concentration of lutein diesters in the obtained extract at these conditions is 11.48 % and the recovery is 12.27%. With the increase of temperature to 60 and 80 °C, the concentration of lutein diesters in extract decreases to 8.64 and 8.31%, respectively.

In case of propane the concentration of lutein diesters in the extract is considerably higher than in CO_2 extracts at all conditions investigated. The maximal yield in respect to lutein diesters is attained at 40 °C and 200 bar and is 3.83% what is 43.58% of total extract. The recovery of lutein diesters at these conditions is 84.92% from maximal amount obtained by repeated experiments with hexane as solvent.

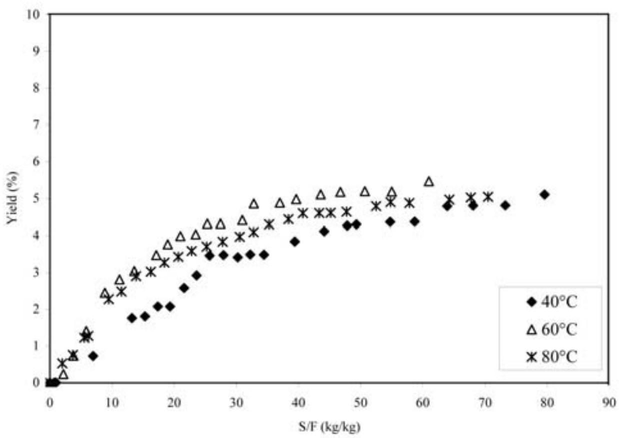

Figure 2. Extraction of marigold flower petals with CO_2: yield of extraction versus solvent to feed ratio (S/F).

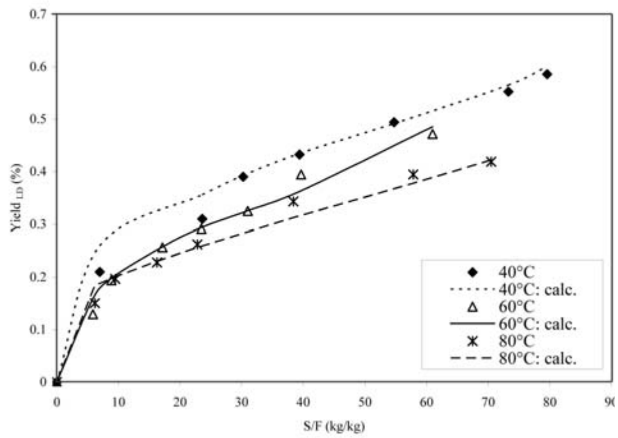

Figure 4. Extraction of marigold flower petals with CO_2 at 300 bar: yield of lutein diesters (LD) versus solvent to feed ratio (S/F).

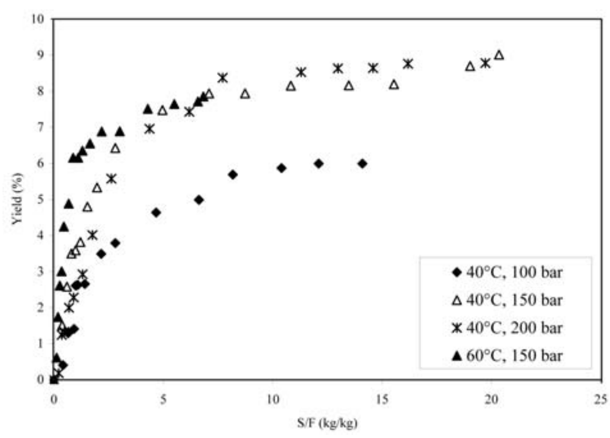

Figure 3. Extraction of marigold flower petals with propane: yield of extraction versus solvent to feed ratio (S/F).

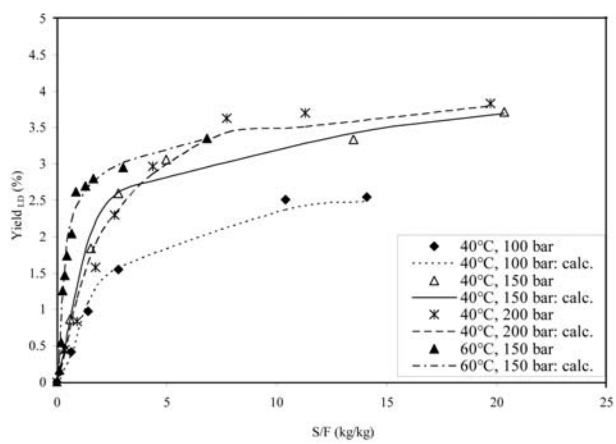

Figure 5. Extraction of marigold flower petals with propane: yield of lutein diesters (LD) versus solvent to feed ratio (S/F).

Table 2. Diffusion coefficients of lutein diesters obtained for extraction of marigold flower petals with SC CO_2 and liquid propane.

T (^{0}C)	*P* (bar)	f_1 /	f_2 /	$D_1 \times 10^{10}$ (m^2/s)	$D_2 \times 10^{12}$ (m^2/s)	AARD* (%)
Carbon dioxide						
40	300	0.102	1.543	0.142	0.118	6.38
60	300	0.071	1.574	0.186	0.118	5.32
80	300	0.058	1.574	0.454	0.118	5.85
Propane						
40	100	0.871	0.778	0.289	0.675	6.48
40	150	0.933	0.691	0.581	5.23	4.36
40	200	1.294	0.440	0.317	3.72	10.65
60	150	0.962	0.782	0.865	4.05	18.57

$$^{*}\ \mathrm{AARD}(\%) = \frac{100}{N}\cdot\sum_{i=1}^{N}\frac{\left|\mathrm{yield_{calc}} - \mathrm{yield_{exp}}\right|}{\mathrm{yield_{exp}}}$$

Extraction kinetic curves for lutein diesters were analysed for both, constant and decreasing extraction rate periods and diffusion coefficients for both stages D_1 and D_2 were calculated, respectively, and are presented in Table 2. The diffusion coefficients obtained for propane are higher as for CO_2, what is especially evident for the second stage of decreasing rate, were up to 50 times higher values were obtained in the case of propane. For both solvents D_1 increases with temperature. Furthermore, the fraction of solute extracted in first period ($6f_1/\pi$) generally increases with increasing density and is generally lower than 0.1 in the case of CO_2 (from 0.04 to 0.06), while in the case of propane it is higher than 0.5 (from 0.53 to 0.79). The comparison between experimental and calculated data for yield of lutein diesters using the previous described model can be observed from Figs. 4 and 5. The performance of the model approximation is presented by average absolute relative deviation (AARD) in Table 2. The deviation of the model from the experimental data is between 5.3–6.4% for CO_2 and 4.4–18.6 % for propane.

4. Conclusions

Study of high pressure extraction of marigold flower petals showed that liquid propane possess high solvent power for lutein diesters. By using propane as solvent at 40°C and 200 bar the recovery of lutein diesters was 85% and the extracts obtained contain high amount of lutein diesters (43.6%). The amount of propane needed for obtaining the maximal yield was approximately 8 kg/kg feed. Oppositely, the content of lutein diesters in supercritical CO_2 extracts was generally low (up to 11.5% at conditions investigated) and the recovery was only around 12.3%. The amount of CO_2 needed for obtaining this recovery was approximately 80 kg/kg feed. The results of mathematical analyses of extraction runs showed, that diffusion coefficients of lutein diesters are generally higher in propane as in CO_2. The chosen model gives good approximation between experimental and calculated data.

In the future, the optimization of SCF extraction with propane has to be done, in order to determine optimal operating parameters where maximal recovery of lutein diesters is obtained.

5. Acknowledgement

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Povzetek

Raziskali smo učinkovitost visokotlačne ekstrakcije diestrov luteina iz cvetov žametnice (*Tagetes erecta*). Topila, ki smo jih uporabili za ekstrakcijo so bila superkritični ogljikov dioksid in tekoči propan. Obratovalni parametri so bili tlak 300 bar in temperatura 40, 60 in 80 °C v primeru CO_2 ter 100, 150 in 200 bar in 40 in 60°C za propan. Raziskali smo vpliv obratovalnih parametrov na izkoristek ekstrakcije in vsebnost luteinskih diestrov v ekstraktih. Rezultati so pokazali da je propan boljše topilo za diestre luteina kot CO_2, njegova topnostna moč je približno 3,5 krat višja od CO_2. Difuzivnost luteinskih diestrov med ekstrakcijo cvetov žametnice smo izračunali z uporabo modela, ki temelji na 2. Fickovem zakonu in sicer za dve stopnji ekstrakcije: stopnjo konstantne ekstrakcijske hitrosti, ki ji sledi stopnja padajoče ekstrakcijske hitrosti. Matematični model na osnovi 2. Fickovega zakona je dobro opisal eksperimentalne ekstrakcijske podatke.