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Solvent extraction study of antioxidants from Balm (*Melissa officinalis* L.) leaves

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Abstract

In the paper, the extraction of antioxidants from Balm (*Melissa officinalis* L.) leaves with ethanol is presented. Effects of particle size, amount of solvent and temperature on the extraction rates and concentrations of antioxidants in the extracts were studied and kinetics was determined. Individual antioxidants (carnosic, ursolic and oleanolic acids) were identified by high performance liquid chromatography. Results showed that the intraparticle diffusion was the rate-governing step of the extraction process. The extractions all proceeded in three stages: an initial washing stage, a fast stage and a slower stage. Experimental extraction curves were analysed with a mathematical model derived from Fick's second law, and diffusion coefficients of the antioxidants within the particles under different operating conditions in ethanol were determined.

Keywords: *Melissa officinalis* L.; Antioxidants; Semi-batch extraction; Phase equilibrium

1. Introduction

Antioxidants are organic substances. By adding them to fats, the autoxidation process is retarded and so the shelf life of food products is extended. The antioxidants can be of synthetic or natural origin. The use of synthetic antioxidants is restricted in several countries, because of their possible undesirable effects on human health (Branen, 1975; Chen, Shi, & Ho, 1992; Kahl & Kappus, 1993). As a result, there is a great interest in obtaining and utilising the antioxidants from natural sources. In herbs from the Labiatae family (rosemary, sage, thyme, balm) large quantities of antioxidants (Economou, Oreopoulou, & Thomopoulos, 1991) can be detected. Many rosemary extracts, for use in food systems, are today available in the market (Bauman, Hadolin, Rižner Hraš, & Knez, 1999).

The Latin name ''Melissa'' (balm) has its roots in the Greek word ''meliteia'', from ''meli, melitos'' (honey) and refers to the great attraction of the plant for bees. The term ''officinalis'' (officinal) was first added at the time of Linnaeus. It was first mentioned in a pharmacopoeia in France in 1733. The meaning of the French word ''officine'' for ''apothecary, laboratory'' was first documented in 1812. The name ''balm'' is a short form of ''balsam'', the most important of the sweet-smelling oils (Koch-Heitzmann & Shultze, 1988).

Balm (*Melissa officinalis* L.) is an aromatic (lemony) perennial herb, up to about 1 m high, growing in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. Parts mostly used are dried leaves, often having flowering tops (Leung & Foster, 1996).

Balm is very useful for nervous agitation, and for promoting sleep, and ameliorates functional gastrointestinal complaints (Kümel, Stoll, & Brendel, 1991). In folk medicine, balm is recommended as a plant juice, cream or tea infusion for nervous complaints, lower abdominal disorders, gastric complaints, hysteria and melancholia, chronic bronchial catarrh, migraine, nervous debility, toothache, earache, headache and high blood pressure and, externally, for rheumatism, nerve pains and stiff necks (compress) (Cohen, Kucera, & Herrmann, 1964).

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Nomenclature

Symbols

A	surface area (m^2)
c	concentration of the extracted constituent in the solution (g dm^{-3})
C	concentration of component within a flat plate (g dm^{-3})
D_{fast}	diffusion coefficient in short time period (cm^2 s^{-1})
D_{slow}	diffusion coefficient in long time period (cm^2 s^{-1})
k_{obs}	overall rate constant (s^{-1})
K	partition coefficient of the extracted constituent between the solvent and Balm leaf, (-)
k_1, k_{-1}	first order rate constant for transfer of the constituent across the interface from leaf to the solution and in the opposite direction (m s^{-1})
L	leaf half thickness (mm)
m	mass (g)
M	mass transferred from the flat plate (g)
R	ratio volume of solvent per kg of raw material (dm^3 kg^{-1})
T	temperature (°C)
t	time (s)
V	volume of solvent (dm^3)
w_i	concentration of component i isolated per kg of raw material (%)

Greek

δ	thickness of Nernst layer (mm)

Balm contains volatile oil, glycosides of the alcoholic or phenolic components of the volatile oil (eugenol glucoside) (Sarer & Kokdil, 1991), caffeic acid derivatives (rosmarinic acid), flavonoids (cynaroside, cosmosin, rhamnocitrin, isoquercitrin), phenolic acid (carnosic acid), and triterpene acids (ursolic and oleanolic acid). The last two compounds are well known antioxidants (Schultze, König, Hilker, & Richter, 1995).

In the present work, the effects of process parameters (particle size, amount of solvent and temperature) on the extraction rate and concentrations of antioxidants in extracts from Balm leaves were studied. Individual antioxidants [carnosic acid (CA), ursolic acid (UA) and oleanolic acid (OA)] were identified by HPLC. Experimental curves were analysed with a mathematical model (Crank, 1975) derived from Fick's second law and the diffusion coefficients of the antioxidants under different operating conditions in conventional solvent (ethanol) were determined.

2. Materials and methods

2.1. Materials

Melissa officinalis L. (crop 2000) was supplied by Droga (Portorož, Slovenia). All chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Apparatus and experimental procedure

The extraction experiments with conventional solvents were performed with a batch extractor. The batch extraction system used in the study was composed of a 500 ml round-bottomed flask with a three-necked top connected to the condenser, a magnetic stirrer and a boiler.

The Balm leaves were ground and sieve analysis of the ground material was carried out to determine the particle size distribution. Experiments were performed on a laboratory scale, in small quantities and the heating due to grinding of the raw material was minimal.

The batch extractor was first filled with a solvent and raw material. The content was heated to the desired temperature and mixed. The vapours condensed in a condenser and, after that, they were returned to the flask. The temperature in the flask was controlled with a thermometer. At specific time intervals, 2 ml of the solution were taken from the batch extractor, filtered and analysed by HPLC. Ethanol was evaporated and the mass of the extract was determined. The yield of extraction was calculated by the formula:

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

where m_{extract} is the mass of the extract and $m_{\text{raw material}}$ is the mass of the raw material (Balm leaves) extracted. The amount of individual antioxidant isolated per kg of raw material w_i was calculated with the equation:

$$w_i(\%) = \frac{m_{i,\ \text{extract}}}{m_{\text{raw material}}} \cdot 100 \quad (2)$$

where i represents CA, UA or OA, respectively, and $m_{i,\ \text{extract}}$ is a mass of the individual component in the extract.

Each extraction run was repeated and the relative standard deviation between two experiments ranged from 2.1 to 8.4%.

To determine the content of antioxidants in the raw material, 100 mg of Balm leaves were exhaustively pulverised, mixed into 100 ml aliquots of ethanol:methanol:2-propanol [90:5:5 (v/v)] and extracted in an ultrasonic bath for 1 h. Afterwards, the sample was filtered and analysed by HPLC.

2.2.2. HPLC analysis of balm extracts

For quantitative determination of the contents of individual antioxidants in the extracts and in the raw material, high performance liquid chromatography (HPLC) (Rižner Hraš, Hadolin, Knez, & Bauman, 2000) was used. The HPLC system consisted of a consta-Metric 3000 (Milton Roy) pump, a spectro Monitor 3100 (Milton Roy) UV-Vis detector, an HP 3396 integrator (Hewlett Packard) and a Rheodyne injector (Cotati, CA). A LiChrosorb RP-18 (250×4 mm, 7 μm) column (Merck, Germany) was used. The mobile phase was a mixture of acetonitrile and water [65:35 (v/v)] and contained 0.5% phosphoric acid and 1 mM EDTA. The flow rate was 2 ml/min and the detection wavelength was 230 nm.

The HPLC method was validated and, on a 95% confidence basis, showed no statistical differences. Each sample was analysed three times and relative standard deviation between measurements was 2.1%.

2.3. Mathematical model

Extraction runs were analysed with a mathematical model (Crank, 1975) derived from Fick's second law. The model is based on the following assumptions:

1. solid particles are considered as flat plates with a thickness of 2L,
2. active ingredient is initially homogeneously contained in the solid,
3. the content of active ingredient in the solid varies with time and distance,
4. the extraction of active ingredient occurs in a two step process:
   - a constant rate stage
   - a decreasing rate stage
5. at the interface, thermodynamic equilibrium is established,
6. porous solid is considered as a pseudo-homogeneous medium.

Application of the steady-state model (Spiro & Jago, 1982) leads to the first-order rate equation:

$$\ln\left(\frac{c_\infty}{c_\infty - c}\right) = k_{\text{obs}} \cdot t \tag{3}$$

where c is the concentration of the extracted constituent in the solution at time t and c_∞ is its concentration at equilibrium ($t = \infty$). The theory also shows that the overall rate constant k_{obs} is given by

$$\frac{1}{k_{\text{obs}}}\left(\frac{A}{V} + \frac{K}{L}\right) = \frac{1}{k_{-1}} + \frac{KL}{2D} + \frac{\delta}{D_{\text{soln}}} + \frac{K\delta^2}{2D_{\text{soln}}L} \tag{4}$$

where A is total surface area and V is volume of solvent. K is the partition coefficient of the extracted constituent between the solvent and Balm leaf. k_1 and k_{-1} are the first order rate constants for the transfer of the constituent across the interface from leaf to the solution and in the opposite direction, respectively. The last term on the right-hand side of Eq. (4) can usually be neglected, as it will almost always be much smaller than the δ/D_{soln} term. The other three terms correspond to the three rate governing steps of the process: surface-controlled infusion, diffusion of the soluble constituent through the leaf with a diffusion coefficient D, and diffusion of the constituent through the Nernst layer of thickness δ with a diffusion coefficient D_{soln}. If the second step alone is rate determining, then

$$k_{\text{obs}} = \frac{2D}{KL}\left(\frac{A}{V} + \frac{K}{L}\right) \tag{5}$$

A similar equation can be derived by the application of Fick's second law (Crank, 1975):

$$D_j\left[\frac{\partial^2 C_j}{\partial x^2}\right] = \frac{\partial C_j}{\partial t} \tag{6}$$

When capital C denotes the concentration within a flat plate, the initial and boundary conditions are:

- $t = 0 \quad -L \leqslant x \leqslant L \quad C = C_0$
- $\forall t \rangle 0 \quad x = \pm L \quad C = C_i = 0$ (constant content at interface)
- $\forall t \rangle 0 \quad x = 0 \quad (\partial C/\partial x) = 0$ (symmetry of the system).

The general solution of Eq. (6) is given by Crank (1975), as follows:

$$\frac{C - C_0}{C_i - C_0} = 1 - \left[\frac{4}{\pi}\sum_{n=0}^{n=\infty}\frac{(-1)^n}{2n+1}\cos\frac{(2n+1)\pi \cdot x}{2L} \exp\left(-\frac{(2n+1)^2\pi^2}{4L^2}\right)Dt\right] \tag{7}$$

where C is the concentration at any given time at a distance x from the centre within the plate ($x = \pm L$). By integrating the concentration over the thickness, the mass transferred from the plate at any time (M) can be calculated. The mass transferred at time t relative to the total amount transferred after infinite time (M_∞) is expressed as:

$$\frac{M}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{n=\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2}{4L^2}Dt\right) \tag{8}$$

After a short period, all terms except the first become negligible, so that, by considering that the mass transferred

from the plate at time t equals the concentration in the solution (c), the following equation is obtained (Spiro, 1988):

$$\ln\left(\frac{c_\infty}{c_\infty - c}\right) = 0.210 + \frac{9.87Dt}{4L^2} \quad (9)$$

When $\ln(c_\infty/[c_\infty - c])$ is plotted against time, the points fall on to two intersecting straight lines, the first with a relatively steep slope and the second with a relatively shallow one. The points of intersection of the $\ln(c_\infty/[c_\infty - c])$ vs. t plots for the fast and slow stages are termed transition points (Kandiah & Spiro, 1990; Spiro & Kandiah, 1989; Spiro, Kandiah, & Price, 1990).

Osburn and Katz (1944), dealing with the extraction from lamina soybean flakes, found it necessary to consider the presence of two parallel diffusion processes inside the solid; one faster and one slower. Their method of treatment can equally well be applied to the extraction from flat particles with the following form:

$$\frac{c_\infty - c}{c_\infty} = \frac{8}{\pi^2}\left[f_1 \exp\left(-\frac{\pi^2 D_1 t}{4L^2}\right) + f_2 \exp\left(-\frac{\pi^2 D_2 t}{4L^2}\right)\right] \quad (10)$$

where f_1 and f_2 are the fractions of the solute, which are extracted with diffusion coefficients D_1, and D_2, respectively.

In the later stages of the extraction only the second term on the right-hand side of Eq. (10) remains significant. A plot of the ln function against time will thus provide D_2 from the slope and f_2 from the intercept if c_∞ has been determined separately. In the earlier stages of the extraction, on the other hand, the exponential second term is close to unity and D_1 and f_1 can be determined.

3. Results and discussion

3.1. General

For the extraction experiments, three different particle sizes (0.20–0.25, 0.25–0.315 and 0.315–0.400 mm) of Balm leaves were taken. HPLC analyses of the raw material showed that Balm leaves contained 0.045% (w/w) CA, 0.450% (w/w) UA and 0.350% (w/w) OA.

3.2. Extraction kinetics study

In order to study the extraction kinetics of balm leaves with ethanol under various process conditions, the yield and the amount of antioxidative components extracted per kg of raw material are plotted as a function of time. In all cases, the yield increases rapidly at the beginning of the process and slows down afterwards. The rapid variation could be explained by recovery of the solute from the superficial sites of the plant. The second slower step corresponds to the extraction of the solute stored in the internal sites.

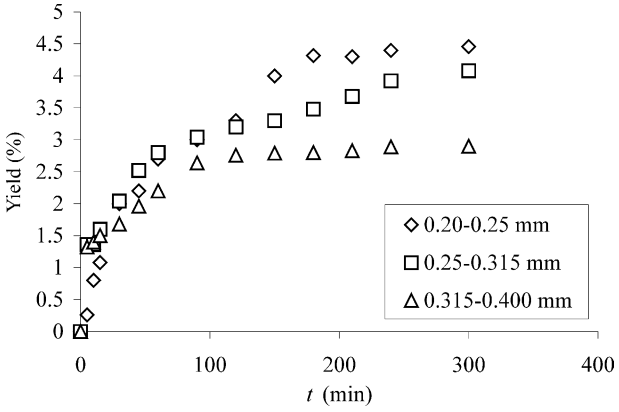

Fig. 1. Kinetics of extraction of Balm leaves with ethanol at 20 °C and $R = 4$ dm^3 kg^{-1}: influence of particle diameter.

3.3. Particle size effect

The influence of particle size on the yield of extraction was studied at 20 °C and the volume of ethanol per kg of raw material was equal to 4 ($R = 4$). The results are presented in Fig. 1. It can be observed that the initial extraction rate (at the beginning of the experiment) does not depend on particle size, however, the total yield of extraction increases with the decrease of particle size. These results indicate that, in the latter stage of the extraction process, the diffusion of the solvent into the particle and the solvent–solute diffusion out of the particle are rate-governing steps of the process.

The effects of particle size on the amounts of individual antioxidants (CA, UA and OA) extracted per kg of raw material at 20 °C and $R = 4$ are presented in Fig. 2a–c. It can be observed that, in the case of small particles with sizes 0.20–0.25 mm, the extraction rates and total yields in respect to a particular component are high for CA while, for UA and OA, the extraction rate is low. For UA and OA, the extraction rates and yields are higher when particles of larger size are extracted. Results showed that, at 20 °C and $R = 4$ maximum, 91% of the CA initially contained in the Balm leaves was isolated from particles with sizes 0.20–0.25 mm (Fig. 2a). Further, in the case of particle sizes 0.315–0.400 mm, a maximum of 77% of OA and only 27% of UA was isolated.

3.4. Effect of ratio volume of ethanol per kg of raw material (R)

Generally, during the conventional extraction processes, the percentage of active component in the liquid phase will increase until equilibrium is reached. In order to determine the optimal amount of solvent per kg of raw material, the effects of R on the extraction rate,

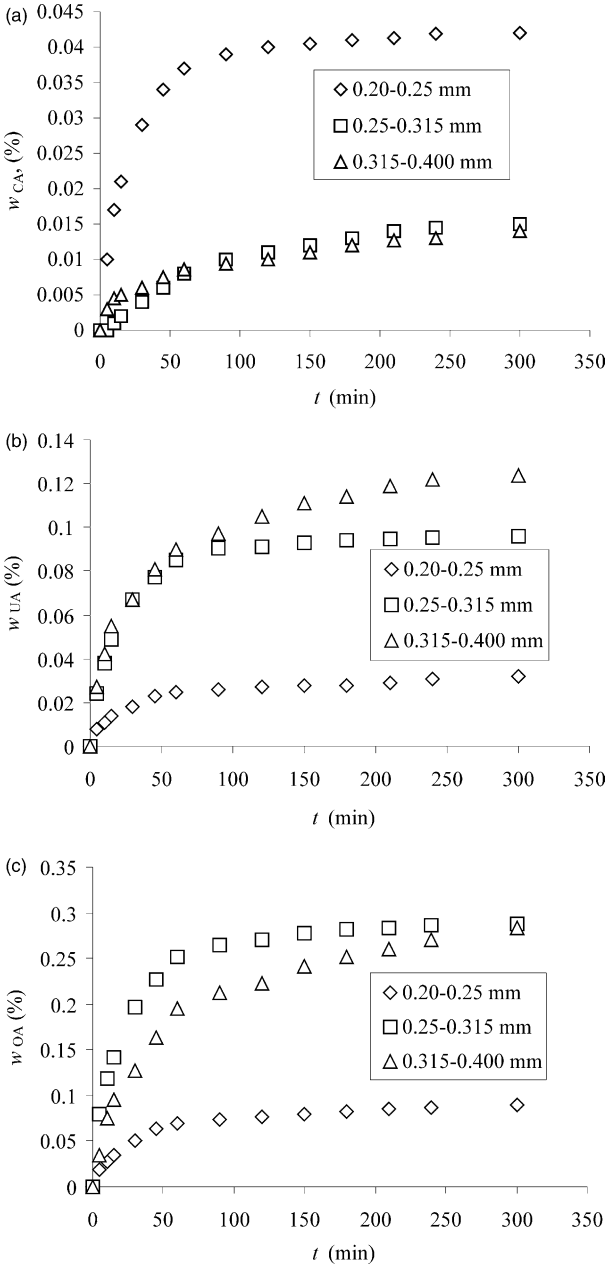

Fig. 2. Kinetics of extraction of antioxidants from Balm leaves with ethanol at 20 °C and $R = 4$ dm^3 kg^{-1}: influence of particle diameter: (a) CA, (b) UA and (c) OA.

yield and amount of extracted antioxidative components were examined. The results show that the initial extraction rate is independent on R. However, the total yield increases with the increase of R (Fig. 3). The effect of R on the amount of active component extracted per kg of raw material is shown in Fig. 4.

The results show that the final composition of the extracts depends on the amount of the solvent used per kg of raw material. The total amount of antioxidative components extracted decreases with the increase of the amount of the solvent, from 6 to 10 l per kg of raw material.

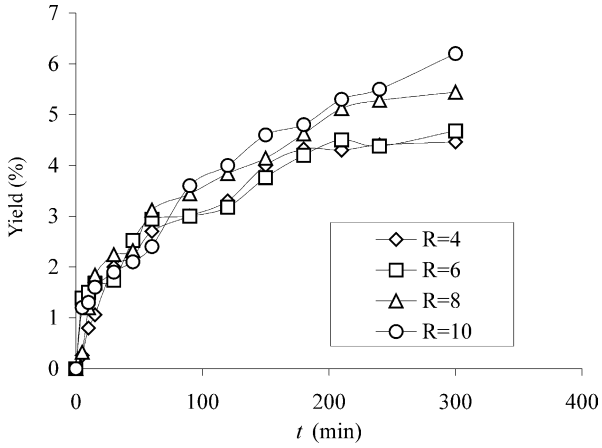

Fig. 3. Kinetics of extraction of Balm leaves with ethanol at 20 °C and particle size 0.20–0.25 mm: influence of ratio volume of ethanol:mass of raw material (R).

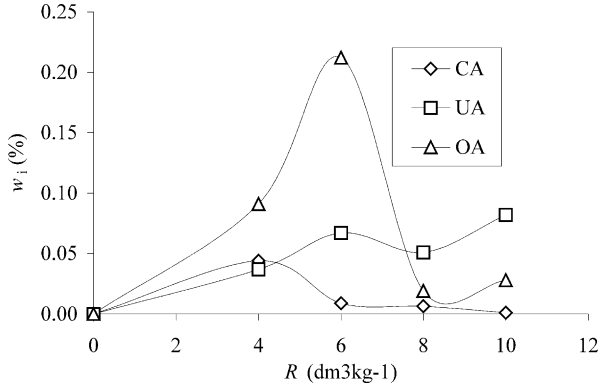

Fig. 4. Extraction of Balm leaves with ethanol at 20 °C and particle size 0.20–0.25 mm: influence of R on total amount of antioxidants extracted per kg of raw material.

3.5. Temperature effect

Generally, temperature has a positive effect on extraction yields and rates when it is not too high, as some of balm active components degrade with temperature.

The yield of extracted CA increases with a temperature increase from 0 to 20 °C, where the best results are obtained. With a further increase of the temperature, the concentration of CA in the extract decreases (Fig. 5).

The yield of the extracted UA is highest at 0 °C and decreases with increase of temperature from 0 to 20 °C, where the lowest % of UA was isolated. With increase of temperature to 40 °C, a small increase in the yield can be observed and it remains constant with a further rise of temperature to 80 °C.

The yield of the extracted OA decreases significantly with increase of temperature from 0 °C to 40 °C and remains constant with any further increasing of the temperature to 80 °C (Fig. 5).

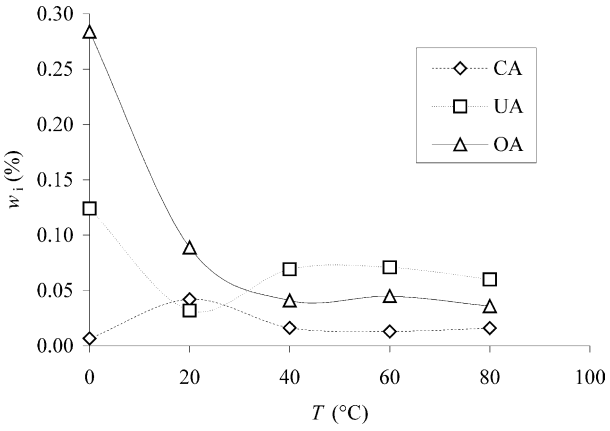

Fig. 5. Extraction of Balm leaves with ethanol at $R=4$ and particle size 0.20–0.25 mm: influence of temperature on total amount of antioxidants extracted per kg of raw material.

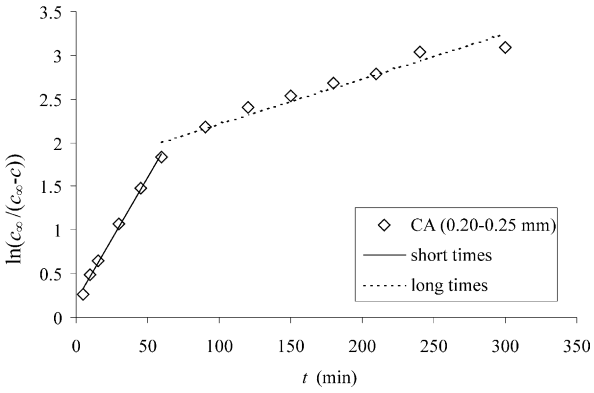

Fig. 6. First-order plot for the fast and slow stage of the CA extraction from 0.20 to 0.25 mm Balm particles extracted with ethanol at 20 °C and $R=4$ dm^3 kg^{-1}.

3.6. Mathematical model

The kinetic plots showed the presence of two extraction stages: a constant rate stage followed by a stage of decreasing rate. It can be assumed that the transfer of active components from balm can be described as followed:

- A solid-liquid transfer, corresponding to the extraction of the oil situated in exogenous glands by simple washing. During the first stage, the extraction rate is the highest.
- A molecular diffusion of the oil through the porous media. During the second stage, the extraction rate depends on time.

Plots of the ln function [Eq. (9)] against time display three features: an intercept at $t=0$, which indicates a very rapid initial extraction, a fast extraction stage represented by a straight line with relatively high slope and, finally, a slow extraction stage, represented by a straight line with a much lower slope. Fig. 6 shows the corresponding plot of the function $\ln(c_\infty/[c_\infty - c])$ against time for the fast first stage of the extraction and for the later slower second stage. The rate constants for individual runs obtained from these linear plots can be described by Eq. (9). The diffusion coefficients for both periods were calculated with Eq. (10) and are summarised in Tables 1–3.

It can be seen from Table 1 that D_{fast} for CA, UA and OA increases with the increase of the particle size from

Table 1
Diffusion coefficients of antioxidants obtained for conventional ethanol extraction of Balm leaves at 20 °C and $R=4$ dm^3 kg^{-1}: influence of particle size

	CA		UA		OA	
	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})
0.20–0.25 mm	0.42	0.039	0.48	0.032	0.69	0.084
0.25–0.315 mm	1.24	0.077	0.53	0.066	0.63	0.094
0.315–0.400 mm	1.44	0.032	0.58	0.042	1.10	0.064

Table 2
Diffusion coefficients of antioxidants obtained for conventional ethanol extraction of Balm leaves with particle diameter 0.20–0.25 mm and at 20 °C: influence of ratio volume of ethanol per kg of raw material (R)

	CA		UA		OA	
	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})	$D_{fast}\times10^7$ (cm^2 s^{-1})	$D_{slow}\times10^7$ (cm^2 s^{-1})
$R=4$ dm^3 kg^{-1}	0.42	0.039	0.48	0.032	0.69	0.084
$R=6$ dm^3 kg^{-1}	0.73	0.049	0.49	0.074	1.90	0.119
$R=8$ dm^3 kg^{-1}	1.52	0.046		0.060	2.59	0.069
$R=10$ dm^3 kg^{-1}	3.07	0.061	4.29	0.106	0.277	0.045

Table 3
Diffusion coefficients of antioxidants obtained for conventional ethanol extraction of Balm leaves with particle diameter 0.20–0.25 mm and $R=4$ dm^3kg^{-1}: influence of temperature

	CA		UA		OA	
	$D_{fast}\times10^7$ ($cm^2\ s^{-1}$)	$D_{slow}\times10^7$ ($cm^2\ s^{-1}$)	$D_{fast}\times10^7$ ($cm^2\ s^{-1}$)	$D_{slow}\times10^7$ ($cm^2\ s^{-1}$)	$D_{fast}\times10^7$ ($cm^2\ s^{-1}$)	$D_{slow}\times10^7$ ($cm^2\ s^{-1}$)
0 °C	0.37	0.013	0.59	0.041	1.72	0.068
20 °C	0.42	0.039	0.48	0.032	0.69	0.084
40 °C	0.52	0.024	0.62	0.029	0.40	0.056
60 °C	0.50	0.039	0.45	0.027	0.58	0.067
80 °C	0.29	0.028	0.63	0.029	1.26	0.044

0.20–0.25 to 0.315–0.400 mm by the factors 3.4 for CA, 1.2 for UA and 1.6 for OA. Similarly, D_{slow} increases with the increase of the particle size from 0.20–0.25 to 0.25–0.315 mm by a factor 1.1–2.1 for all components and decreases again with a further increase of particle size.

From Table 2 it can be concluded that CA and UA diffuse best in the solvent with $R=10$. By increasing the amount of the solvent per kg of raw material, i.e. with the increase of R from 4 to 10, the D_{fast} and D_{slow} increase by factors 8.9 and 3.3, respectively, for UA and by factors 7.3 and 1.6 for CA. In the case of OA, D_{fast} increases with the increase of R from 4 to 8 by the factor 3.8. Similarly, D_{slow} increases with the increase of R up to 6 by the factor 1.4 and afterwards they both decrease.

The diffusion coefficients of CA, UA and OA, calculated from the results of ethanol extraction of Balm at different temperatures, are summarised in Table 3. It can be observed that D_{fast} of CA increases with the increase of temperature from 0 to 40 °C by the factor 1.4 and afterwards it decreases. D_{fast} of UA varies with temperature from 0.45×10^{-7} to 0.63×10^{-7} $cm^2\ s^{-1}$. For OA, the highest diffusion coefficient, D_{fast}, is obtained at 0 °C. With a further increase of temperature, the diffusion coefficients slowly decrease. D_{slow} of CA is lowest at 0 °C and increases with increase of temperature to 20 °C. Oppositely, D_{slow} decreases with increase of temperature from 0 to 40 °C by the factor 1.4 and remains constant thereafter. D_{slow} of OA varies with temperature from 0.044×10^{-7} to 0.084×10^{-7} $cm^2\ s^{-1}$.

4. Conclusion

The aim of the present work was to study the effects of the operating conditions on the conventional ethanol extraction of antioxidants from Balm leaves. A positive effect was shown by decreasing the particle size diameter and temperature and increasing the amount of the solvent. As a result, the best operating conditions found were at particle size diameter between 0.20 and 0.25 mm, $R=4$ and temperature 20 °C for the extraction of CA, UA and OA. The study also showed that ethanol was a very suitable solvent for the extraction of CA from Balm leaves while 91% of CA and only 28% of UA and 77% of OA were isolated.

The kinetic study showed the presence of three extraction stages: an initial washing stage, a fast stage and a slower stage. The analyses of the extraction curves in dependence of particle size, R and temperature showed that, in this extraction process, the intraparticle diffusion resistance was dominant.

The diffusion coefficients were calculated for the fast and the slow stage: D_{fast} corresponded to the diffusion in short time periods and D_{slow} corresponded to the diffusion occurring in long time periods. D_{fast}, at operating conditions investigated, was in the order of magnitude from 0.37×10^{-7} to 3.07×10^{-7} $cm^2\ s^{-1}$ for CA, from 0.45×10^{-7} to 4.29×10^{-7} $cm^2\ s^{-1}$ for UA and from 0.4×10^{-7} to 2.59×10^{-7} $cm^2\ s^{-1}$ for OA. Similarly, D_{slow} was in the order of magnitude from 0.013×10^{-7} to 0.077×10^{-7} $cm^2\ s^{-1}$ for CA, 0.027×10^{-7} to 0.106×10^{-7} $cm^2\ s^{-1}$ for UA and 0.044×10^{-7} to 0.119×10^{-7} $cm^2\ s^{-1}$ for OA.

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References

Bauman, D., Hadolin, M., Rižner Hraš, A., & Knez, Ž (1999). Supercritical fluid extraction of rosemary and sage antioxidants. *Acta Alimentaria*, *28*, 15–28.

Branen, A. L. (1975). Toxicology and biochemistry of butylated hydroxy-anisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society*, *52*, 59–63.

Chen, Q., Shi, H., & Ho, C. T. (1992). Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. *Journal of the American Oil Chemists' Society*, *69*, 999–1002.

Cohen, R. A., Kucera, L. S., & Herrmann, E. (1964). Antiviral activity of Melissa officinalis (Limon Balm) extract. *Proceedings of the Society for Experimental Biology and Medicine*, *117*, 431–434.

Crank, J. (1975). *The mathematics of diffusion*. Oxford: Oxford University Press.

Economou, K. D., Oreopoulou, V., & Thomopoulos, C. D. (1991). Antioxidants activity of some plant extracts in the family Labiatae. *Journal of the American Oil Chemists' Society*, *68*, 109–113.

Kahl, R., & Kappus, H. (1993). Antioxidanten BHA and BHT im Vergleich mit dem natürlichen Antioxidant Vitamin E. *Zeitschrift für Lebensmittel- Untersuchung und Forschung*, *196*, 329–338.

Kandiah, M., & Spiro, M. (1990). Extraction of ginger rhizome: kinetic studies with supercritical carbon dioxide. *International Journal of Food Science and Technology*, *25*, 328–338.

Koch-Heitzmann, I., & Schultze, W. (1988). 2000 Jahre Melissa officinalis. Von der Bienenpflanze zum Virustatikum. *Phytotherapie*, *9*, 77.

Kümel, G., Stoll, L., & Brendel, M. (1991). Therapie mit rezeptfreien Topika. *Deutsche Apotheker Zeitung*, *131*, 1609.

Leung, Y., & Foster, S. (1996). *Encyclopedia of common natural ingredients used in food, drugs and cosmetics*. New York: John Wiley & Sons.

Osburn, J. O., & Katz, D. L. (1944). Structure as a variable in the application of diffusion theory to extraction. *Transactions of the American Institute of Chemical Engineers*, *40*, 511–531.

Rižner Hraš, A., Hadolin, M., Knez, Ž., & Bauman, D. (2000). Comparison of antioxidative and synergistic effects of rosemary extracts with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chemistry*, *71*, 229–233.

Sarer, E., & Kokdil, G. (1991). Constituents of the essential oil from Melissa officinalis. *Planta Medica*, *57*, 89.

Schultze, W., König, W. A., Hilker, A., & Richter, R. (1995). Melissenöle. *Deutsche Apotheker Zeitung*, *135*, 557–577.

Spiro, M. (1988). The rate of caffeine infusion from Kenyan arabica coffee beans. *12th Colloque Scientifique International du Café*, 260–264.

Spiro, M., & Jago, D. S. (1982). Kinetics and Equilibria of Tea Infusion. *Journal of the Chemical Society, Faraday Transactions I*, *78*, 295–305.

Spiro, M., & Kandiah, M. (1989). Extraction of ginger rhizome: kinetic studies with acetone. *International Journal of Food Science and Technology*, *24*, 589–600.

Spiro, M., Kandiah, M., & Price, W. (1989). Extraction of ginger rhizome: kinetic studies with dichloromethane, ethanol, 2-propanol and an acetone water mixture. *International Journal of Food Science and Technology*, *25*, 157–167.