

Optimisation of Antioxidant Extraction from Lemon Balm (*Melissa officinalis*)

MIROSLAV ONDREJOVIČ¹, FILIP KRAIC², HANA BENKOVIČOVÁ² and STANISLAV ŠILHÁR¹

¹Biocentre Modra, Food Research Institute, Modra, Slovak Republic;

²Department of Biotechnology, Faculty of Natural Sciences, University of ss. Cyril and Methodius, Trnava, Slovak Republic

Abstract

ONDREJOVIČ M., KRAIC F., BENKOVIČOVÁ H., ŠILHÁR S. (2012): **Optimisation of antioxidant extraction from lemon balm** (*Melissa officinalis*). Czech J. Food Sci., **30**: 385–393.

The effects of the propan-2-ol proportion in the extraction solvent (PPES), solid to liquid ratio (SLR), and extraction temperature on the extraction yield of antioxidants measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and β -carotene-linoleic acid bleaching inhibition activity (BCLM) were evaluated. Secondly, total polyphenol and flavonoid contents were determined to find possible relations of these parameters with antioxidant activity. The optimal conditions for the extraction were determined using response surface methodology (RSM). The optimal conditions for the extraction of antioxidants measured by radical scavenging activity (DPPH) were PPES 50.2% (v/v), 33.8°C, and SLR 1:147 (w/v). The optimal conditions for the extraction of antioxidants measured by BCLM were PPES 1.15% (v/v), 61.8°C, and SLR 1:153 (w/v). The optimal conditions for the extraction of total polyphenols and total flavonoids were 23.3% (v/v) (PPES), 67.5°C, 1:148 (w/v) (SLR); 1.15% (v/v) (PPES), 80°C, 1:179 (w/v) (SLR); respectively. The experimental values agreed with the predicted ones within a 95% confidence interval.

Keywords: DPPH; BCLM; polyphenols; flavonoids; response surface methodology

Lemon balm (*Melissa officinalis* L.), also known as common balm or sweet balm, is a perennial lemon-scented herb in the mint family native to the Mediterranean and to Southern Europe (SMALL 2006). *M. officinalis* is an aromatic (lemony) perennial herb, up to about 1 m high, growing in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. The parts mostly used are dried leaves often with flowering tops (LEUNG & FOSTER 2003).

M. officinalis has been used in traditional medicine dating back as far as ancient Greek and Roman times for treating disorders of the nervous system and melancholy (GRIEVE 1998). Today, this plant is very useful for treating nervous agitation and for promoting sleep, and it ameliorates functional

gastrointestinal complaints (KÜMEL *et al.* 1991). It is recommended in the form of 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 *et al.* 1964). Recently, *M. officinalis* has also shown anxiolytic, mood, and memory-enhancing effects (BALLARD *et al.* 2002; KENNEDY *et al.* 2002, 2003, 2004, 2006; AKHONDZADEH *et al.* 2003) as well as sedative (SOULIMANI *et al.* 1991), antimicrobial, and antitumor actions (DRAGLAND *et al.* 2003; MIMICA-DUKIC *et al.* 2004; de SOUSA *et al.* 2004). The important biological activity is its

remarkable strong antioxidant activity (HIRASA & TAKEMASA 1998) due to which this plant has more from the described curative effects.

Crude extracts of these plants rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (JAVANMARDI *et al.* 2003). In addition, the potential of the antioxidant constituents of the plant materials for the maintenance of health and protection against coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (LÖLIGER 1991; FIALOVÁ *et al.* 2008; LAHUCKY *et al.* 2010).

Recently, there has been a growing interest in the search for natural antioxidants for three principal reasons: (i) numerous clinical and epidemiological studies have demonstrated that the consumption of fruits and vegetables is associated with reduced risks of developing chronic diseases such as cancer, cardiovascular disorders, and diabetes, (ii) safety consideration regarding the potential harmful effects of the chronic consumption of synthetic antioxidants in foods and beverages, and (iii) the public's perception that natural and dietary antioxidant are safer than synthetic analogues. The result has been an increased interest in spices and aromatic and medicinal plants as sources of natural antioxidants (DASTMALCHI *et al.* 2008).

M. officinalis contains volatile oil (e.g. eugenol glucoside) (SARER & KOKDIL 1991), glycosides of alcoholic triterpene acids (e.g. ursolic and oleanolic acid), and phenolic compounds such as phenolic acids (e.g. carnosic acid, rosmarinic acid), and flavonoids (e.g. cynaroside, cosmosin, rhamnocitrin, isoquercitrin). The last two compound groups are well known antioxidants (SHAHIDI *et al.* 1992; PIETTA 2003).

The aim of this study was to evaluate the effects of the solvent composition, solid to liquid ratio, and extraction temperature on the extraction yield of antioxidants. Antioxidant activity can be explained as the potential of various compounds for: (i) preventing radical formation by preventing lipid peroxidation (BCLM method), (ii) scavenging the formed radicals (DPPH method) and (iii) the regeneration of oxidised compounds by reducing power. For these purposes, different antioxidants can be used. In the presented work, we studied only the radical scavenging and preventing of lipid

peroxidation by antioxidants from lemon balm (*Melissa officinalis*). Secondary, total polyphenol and flavonoid contents were determined to find possible relations between these parameters and antioxidant activity.

MATERIAL AND METHODS

Chemicals. Propan-2-ol, methanol, trichloroacetic acid (TCA), ethanol, chloroform (all Mikrochem, Pezinok, Slovak Republic), Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), gallic acid quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rutin, β -carotene, linolenic acid, Tween 20 (all Sigma-Aldrich, Steinheim, Germany), aluminum chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate (all Lachema, Brno, Czech Republic).

Plant material. Adult leaves of *M. officinalis* (Slovak University of Agriculture in Nitra, IVVP, Kolíňany, Slovakia) were harvested with the twigs and dried at 40°C. Thereafter, the leaves were separated from the twigs and cut in small squares < 0.5 mm. This extraction material was stored in a dark powder flask at laboratory temperature and in normal atmosphere for up to 3 weeks.

Experimental design for the response surface methodology (RSM). The RSM used a three-factor and central composite design in three blocks consisting of 17 experimental runs with three replications at the centre point. In all experiments, the extraction was done in experimental microtubes and terminated by a rapid decantation of the extracts. The design variables were the solvent composition – the propan-2-ol proportion in the extraction solvent (PPES) (propan-2-ol: water, 1–95% (v/v); X_1), the temperature (20–80°C; X_2), and the solid-liquid ratio (SLR) (16–184 g of ml of the extraction solvent to 1 g of dry extraction material; X_3) (Table 1). The experimental design of the described independent variables in their original and coded forms is shown in Table 2. The variables particle size (< 0.5 mm) and extraction time (2 h) were kept at constant values. The dependent variables were polyphenol and flavonoid contents and antioxidant activity determined as DPPH radical-scavenging activity and β -carotene-linoleic acid bleaching inhibition.

The experimental data were fitted to the following second-order polynomial model (Eq. (1)) and regression coefficients were obtained.

Table 1. Independent variables and their coded and actual values used for optimisation

Independent variable	Coded levels						
	–1.682	–1	0	1	1.682	step	mean
Solvent composition [% (v/v)]	1.2	20	48	76	94.9	28	48
Temperature (°C)	19.9	32	50	68	80.1	18	50
Solid to liquid ratio (ml/g)	16.3	50	100	150	183.7	50	100

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k b_{ij} X_i X_j \quad (1)$$

where:

$X_1, X_2, \dots, X_k$ – independent variables affecting the response Y

$b_0, b_i (i = 1, 2, \dots, k), b_{ii} (i = 1, 2, \dots, k), b_{ij} (i = 1, 2, \dots, k-1; j = 2, 3, \dots, k)$

– regression coefficients for intercept, linear, quadratic, and interaction terms

k – number of variables

The optimum conditions of antioxidant extraction were looked for using Statgraphic, 5.1 (Stat-Point Technologies, Inc., Warrenton, USA). The verification of the validity and adequacy of the predictive extraction model was carried out in these optimum conditions of solid-liquid ratio, solvent composition, and extraction temperature.

Three experimental replicates were performed under optimised conditions and the experimental and predicted values were compared.

Analyses of the response variables

Determination of total polyphenols. The total polyphenols content was determined by the Folin-Ciocalteu method (SINGLETON & ROSSI 1965). 0.5 ml of diluted sample was mixed with 0.5 ml of Folin-Ciocalteu reagent and 5 ml of 20% (w/v) water solution of Na_2CO_3 . After 30 min of incubation at laboratory temperature in the dark, the absorbance of the reaction mixture was measured at 700 nm using the spectrophotometer (Genesys 10 UV; Thermo Fisher Scientific, Waltham, USA).

Table 2. Central composite design setting in the original and coded (numbers in round brackets) form of the independent variables (X_1 , X_2 and X_3)

Standard order	Factor 1 (X_1) solvent composition [% (v/v)]	Factor 2 (X_2) temperature (°C)	Factor 3 (X_3) solid to liquid ratio (ml/g)
1	20.0 (–1)	32.0 (–1)	50.0 (–1)
2	76.0 (1)	68.0 (1)	50.0 (–1)
3	76.0 (1)	32.0 (–1)	150.0 (1)
4	20.0 (–1)	68.0 (1)	150.0 (1)
5	48.0 (0)	50.0 (0)	100.0 (0)
6	20.0 (–1)	68.0 (1)	50.0 (–1)
7	48.0 (0)	50.0 (0)	100.0 (0)
8	20.0 (–1)	32.0 (–1)	150.0 (1)
9	76.0 (1)	32.0 (–1)	50.0 (–1)
10	76.0 (1)	68.0 (1)	150.0 (1)
11	48.0 (0)	80.1 (1.682)	100.0 (0)
12	1.2 (–1.682)	50.0 (0)	100.0 (0)
13	48.0 (0)	19.9 (–1.682)	100.0 (0)
14	48.0 (0)	50.0 (0)	100.0 (0)
15	48.0 (0)	50.0 (0)	16.3 (–1.682)
16	48.0 (0)	50.0 (0)	183.7 (1.682)
17	94.9 (1.682)	50.0 (0)	100.0 (0)

The polyphenol content was expressed as mg of gallic acid equivalents per 1 g of the dry extraction material.

Determination of total flavonoids. The total flavonoids content was determined by the colorimetric method according to QUETTIER-DELEU *et al.* (2000). 0.1 ml of diluted sample was mixed with 0.02 ml of 5% (w/v) aluminium chloride methanolic solution. After 30 min of incubation at laboratory temperature, the absorbance of the reaction mixture was measured at 405 nm using the spectrophotometer (Genesys 10 UV; Thermo Fisher Scientific, Waltham, USA). The flavonoid content was expressed as mg of quercetin equivalents per 1 g of dry extraction material.

Determination of antioxidant activity. In the literature, the radical scavenging activity and lipid peroxidation prevention are often described as Inhibition Capacity (IC) which is only the percentage portion of the absorbance of the reaction solution (DPPH/ β -carotene) with and without antioxidant incubated during the given time (Eqs 3 and 6). But by using these equations, the sample dilution cannot be implemented into the calculation. Therefore, for the calculation of antioxidant activities by these methods, we used equations based on measuring the mass of the inhibited DPPH or protected β -carotene (Eqs 2 and 5). These equations allow evaluating the influence of the sample dilution and extraction yield of antioxidants from the extraction material by the Dilution Factor (DF) (Eqs 4 and 7).

DPPH radical-scavenging activity. The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical DPPH was estimated by the method according to YEN and CHEN (1995). 100 μ l of the diluted sample was mixed with 4 ml of DPPH methanol solution (0.25 g/l). After 10 min of reaction at room temperature in the dark, the absorbance was measured at 515 nm using the spectrophotometer (Genesys 10 UV; Thermo Fisher Scientific, Waltham, USA). The antioxidant content was expressed as mg of inhibited DPPH per 1 g of dry extraction material. The scavenging activity was calculated using the equations (Eqs 2–4):

$$m(\text{DPPH}) = m(\text{DPPH})_{\text{start}} \times IC \times DF \quad (2)$$

$$IC = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \quad (3)$$

$$DF = 10 \times D \times V_{\text{extract}} / m_{\text{ext. mat.}} \quad (4)$$

where:

- $m(\text{DPPH})_{\text{start}}$ – mass of DPPH in initial DPPH reagent
- IC – coefficient of inhibition capacity of sample
- DF – dilution factor
- Abs_{sample} – absorbance of reaction mixture with sample
- Abs_{control} – absorbance of reaction mixture with methanol instead of sample
- D – rate of sample dilution
- V_{extract} – volume of crude extract
- $m_{\text{ext. mat.}}$ – mass of extraction material in crude extraction

β -carotene-linoleic acid bleaching inhibition (BCLM).

The β -carotene-linoleic acid bleaching inhibition was estimated by the method according to WANASUNDARA *et al.* (1994). 2 mg of β -carotene were dissolved in 20 ml of chloroform. 4 ml of this solution was pipetted into a round-bottom flask. After the removal of chloroform using a rotary evaporator, 40 mg of linoleic acid, 400 mg of Tween 20, and 100 ml of distilled water were added. Aliquots (1.15 ml) of the prepared emulsion were transferred into tubes with 0.1 ml of the diluted sample. The absorbance of the reaction mixture was measured at 470 nm using the spectrophotometer (Genesys 10 UV, Thermo Fisher Scientific, Waltham, USA) at the start of the reaction and after incubation at 50°C for 90 minutes. The antioxidant content was expressed as mg of protected β -carotene from the oxidation product of unsaturated fatty acid peroxidation per g of extraction plant material. The β -carotene-linoleic acid bleaching inhibition was calculated using the equations (Eq. 5–7):

$$m(\beta\text{-carotene}) = m(\beta\text{-carotene})_{\text{start}} \times IC \times DF \quad (5)$$

$$IC = \frac{([\ln(a/b)/90]_{\text{control}} - [\ln(a/b)/90]_{\text{sample}})}{[\ln(a/b)/90]_{\text{control}}} \quad (6)$$

$$DF = 10 \times D \times V_{\text{extract}} / m_{\text{ext. mat.}} \quad (7)$$

where:

- $m(\beta\text{-carotene})_{\text{start}}$ – mass of β -carotene in initial β -carotene-linoleic acid reagent
- IC – coefficient of inhibition capacity of sample
- DF – dilution factor
- a – absorbance of reaction mixture with sample or control (with methanol instead of sample) at the start of reaction
- b – absorbance of reaction mixture with sample or control (with methanol instead of sample) after 90 min

D – rate of sample dilution
 V_{extract} – volume of crude extract
 $m_{\text{ext.mat.}}$ – mass of extraction material in crude extraction

RESULTS AND DISCUSSION

Selection of experimental ranges

The extraction efficiency of natural compounds from the plant material is affected by multiple parameters such as temperature, time, solid to liquid ratio, solvent polarity and others, whose effects may be either independent or interactive. The influence of the described parameters on the extraction yield of antioxidants from *M. officinalis* was partly reported in the literature in some articles, but optimal conditions for the extraction of these compounds have not yet been reported.

As stated in the literature, the antioxidative effects of lemon balm preparations are mainly due to certain compounds, such as flavonoids, phenolic acids, and terpenes (carnosic acid, ursolic acid, oleanolic acid). They have various physical-chemical properties and therefore optimal parameters of their extraction from plant material are different. The crude extraction of these compounds was realised by extraction solvents such as methanol, ethanol, acetone, propanol, and their water solutions (HERODEŽ *et al.* 2003; JAVANMARDI *et al.* 2003; DASTMALCHI *et al.* 2008; PEREIRA *et al.* 2009). The selection of optimal extraction solvent was made from the range of water solutions of propan-2-ol because water solutions of this organic solvent can simulate the polarity of all extraction solvents described. Moreover, propan-2-ol is environmental friendly, cheap and with a potential using by another purification steps.

The extraction temperature was varied from 20°C to the boiling point of the extraction solvents used

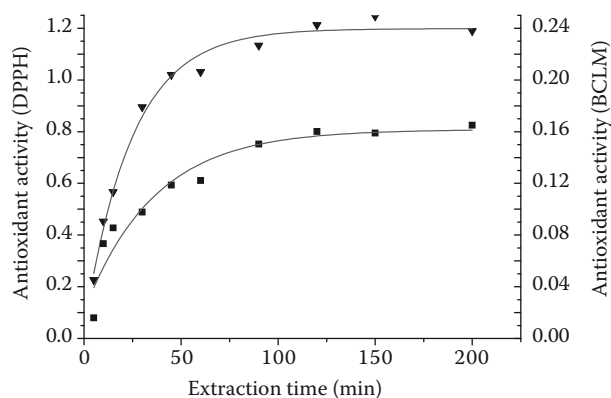


Figure 1. Kinetics of antioxidant extraction from *M. officinalis* with 50% (v/v) water solution of propan-2-ol at 50°C during 200 min by solid-liquid ratio 1:50 (w/v)

■ – β-carotene-linoleic acid bleaching inhibition in mg of protected β-carotene from the oxidation product of unsaturated fatty acid peroxidation per g of dry extraction material;
 ▼ – DPPH radical-scavenging activity in mg of inhibited DPPH per 1 g of dry extraction material

and the extraction time was in the range from 1 h to 7 days. The extraction time is dependent on the extraction temperature due to their effects on the diffusion of compounds from the extraction material. Therefore, the kinetics of antioxidant extraction was performed for the determination of the extraction time as a fixed parameter in the optimisation. In the kinetic experiments, the plant material was extracted with 50% (v/v) water solution of propan-2-ol at 50°C and solid-liquid ratio 1:50 (w/v) during 200 minutes. According to Figure 1, a rather asymptotic region was reached after 90 min of extraction for the two methods of antioxidants determination. Nonlinear regression was applied to the data using the exponential model of the first order. The coefficients of determination R^2 and determined models are given in Table 3.

Table 3. Exponential models for the kinetics of extraction of antioxidants from *Melissa officinalis* measured by two methods (BCLM – β-carotene-linoleic acid bleaching inhibition and DPPH radical-scavenging activity)

Antioxidant method y	$y = a + b \times \exp(-x/c)$			R^2
	a	b	c	
DPPH	1.198 ± 0.022	-1.163 ± 0.054	24.348 ± 2.541	0.9898
BCLM	0.161 ± 0.010	-0.141 ± 0.014	36.025 ± 10.243	0.9335

x – extraction time from kinetics of extraction of antioxidants; y – response of extracted antioxidants; a , b , c – coefficients of exponential models for the extraction kinetics

Optimisation of extraction by RSM

The antioxidant extraction from *M. officinalis* was optimised through the RSM approach. In RSM, natural variables are transformed into coded variables that have been defined as dimensionless with a mean zero and the same spread or standard deviation (MYERS & MONTGOMERY 2009). This method was used in many studies focused upon the optimisation of natural compounds extraction (LIYANA-PATHIRANA & SHAHIDI 2005; MIKULA-JOVÁ *et al.* 2007; SILVA *et al.* 2007).

In our study, the independent variables were the solvent composition, solid to liquid ratio, temperature and a fixed extraction time (2 h) and a fixed particle size (< 0.5 mm) were chosen. The resulting extraction yields of antioxidants (BCLM, DPPH) and total flavonoids (TFL) and polyphenols (TP) contents as dependent variables for all runs are reported in Table 4.

They were first submitted to simple linear regression, which gave coefficients of determination

Table 4. Experimental data for the response antioxidants (DPPH, BCLM), total flavonoids (TFL) and total polyphenols (TP) in extracts prepared by extraction conditions shown in Table 2

Standard order	Dependent variables			
	DPPH (g/g)	BCLM	TP	TFL
		(mg/g)		
1	4.551	0.504	50.89	6.43
2	3.803	0.331	47.89	7.44
3	6.311	0.427	37.06	1.37
4	5.837	0.695	79.86	14.43
5	5.989	0.576	69.79	8.32
6	4.235	0.537	70.9	13.6
7	6.012	0.581	70.23	8.41
8	6.259	0.655	58.81	6.47
9	4.516	0.305	29.55	1.62
10	5.487	0.461	56.42	7.98
11	4.744	0.533	68.77	14.72
12	5.572	0.686	67.49	10.32
13	5.739	0.474	35.39	2.85
14	5.813	0.543	65.82	8.12
15	1.423	0.187	43.25	6.91
16	6.622	0.628	72.73	8.42
17	5.281	0.311	29.84	0.76

Table 5. The coefficients of determination (R^2) for linear relations of dependent variables (BCLM, DPPH, TFL and TP)

	DPPH	BCLM	TP	TFL
DPPH	1	0.50	0.10	0.02
BCLM	0.50	1	0.63	0.32
TP	0.10	0.63	1	0.77
TFL	0.02	0.32	0.77	1

(R^2) for the linear relations of dependent variables (Table 5). This means that the extraction using the selected conditions could be selective for all dependent variables tested with regard to TFL and TP.

Multiple linear regression using the second-order polynomial model (Eq. 1) was performed with the results given Table 4. The response variability was explained by the model, the coefficient of multiple determination (R^2) being 0.85, 0.91, 0.96, and 0.99 for DPPH, BCLM, TP, and TFL, respectively.

Analyses of the regression coefficients and the response surface. The regression coefficients of the model for antioxidant isolation obtained by the multiple linear regression are reported in Table 3. The variables in their coded forms (Table 2) permitted direct interpretability of the effects (linear, quadratic and interaction) of the independent variables, and the surface plot (Figure 2) facilitated the visualisation of the statistically significant factors (marked by bold letters in Table 6) derived from the statistical analysis.

Determination and experimental validation of the optimal conditions

In order to verify the predictive capacity of the model, optimal conditions were determined using the simplex method and the maximum desirability for all dependent variables, and were used for an extraction test (Table 7). The measured values lay within a 95% mean confidence interval of the predicted values of the dependent variables. These results confirm the predictability of the model for the extraction of antioxidants from *M. officinalis* leaves in the experimental conditions used. This model of antioxidant extraction can be used for the development of industrial extraction processes for the production of natural preparation with a high antioxidant activity

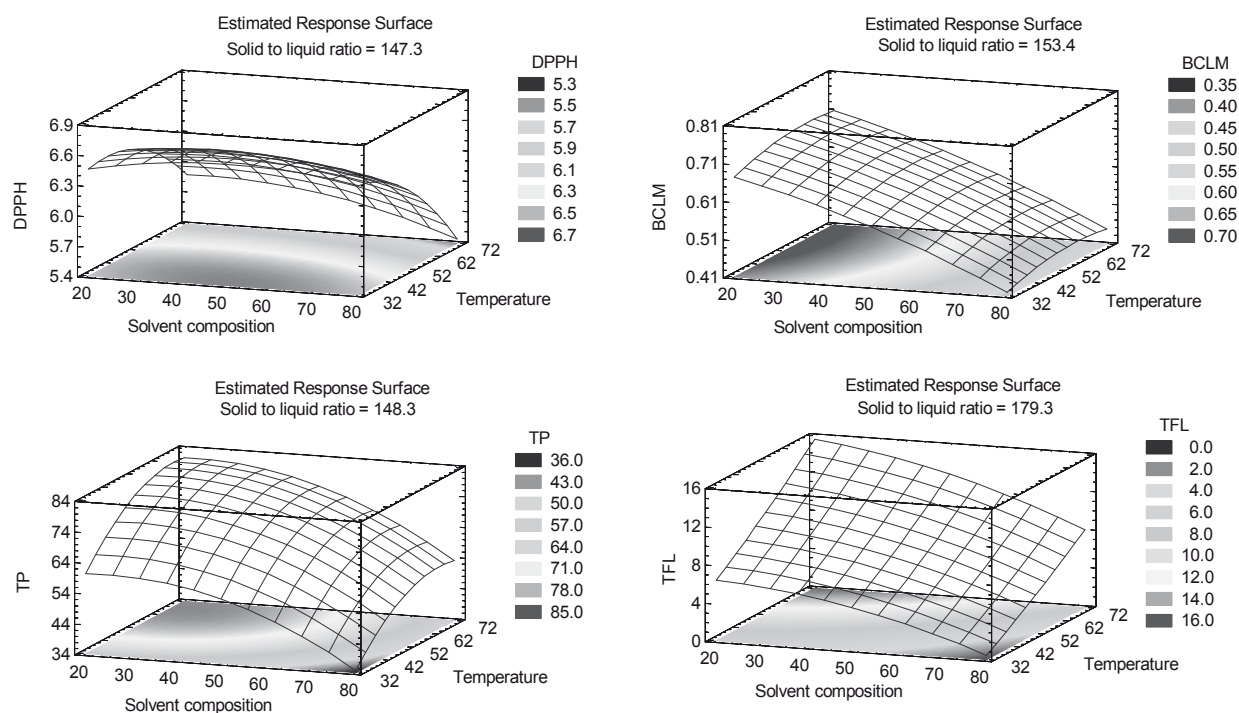


Figure 2. Response surface and contour plots for the effects of solvent composition and temperature at a constant optimal value of solid to liquid ratio for each dependent variable

from *M. officinalis* which can be useful as a food additive or supplement.

CONCLUSIONS

The response surface methodology was successfully employed to optimise the antioxidant extraction from *M. officinalis* leaves. The second-order

polynomial model gave a satisfactory description of the experimental data. The optimised conditions for maximum extraction of antioxidant compounds measured by various methods differed. The optimal conditions for the extraction of antioxidants measured by radical scavenging activity (DPPH) were PPES 50.2% (v/v), 33.8°C and SLR 1:147 (w/v). The optimal conditions for the extraction of antioxidants measured by BCLM were PPES 1.15% (v/v),

Table 6. Regression coefficients of second-order model for dependent variables (DPPH, BCLM, TP, TFL)

Model parameters		Coefficients			
		DPPH	BCLM	TP	TFL
Constant		-0.117768	0.110206	-27.224	0.29027
Linear	X_1 : solvent composition	0.0190646	-0.0010098	0.46162	0.05312
	X_2 : temperature	0.0519929	0.0059999	2.23416	0.13774
	X_3 : solid to liquid ratio	0.0727902	0.0061169	0.375	0.01178
Quadratic	X_1X_1	-0.0001453	-2.34E-05	-0.0085	-0.0012
	X_2X_2	-0.0005556	-5.11E-05	-0.0167	0.00066
	X_3X_3	-0.0002462	-2.033E-05	-0.0013	-7E-05
Interaction	X_1X_2	-0.0001982	-3.22E-06	-0.0008	-0.0007
	X_1X_3	1.509E-05	-5.09E-06	-8E-05	-5E-05
	X_2X_3	-3.014E-05	2.083E-06	0.00029	0.00022

Regression coefficients with a statistically significant at $P < 0.05$ are printed in bold

Table 7. Comparison of predicted and experimental values for the response variable

Optimised conditions	DPPH	BCLM	TP	TFL
Solvent composition (%)	50.2	1.15	23.3	1.15
Temperature (°C)	33.8	61.8	67.5	80.0
Solid-liquid ratio (ml/g)	147.3	153.4	148.3	179.3
Predicted value (mg/g)	6600	0.763	81.31	18.42
Experimental value (mg/g)	6 820 ± 211	0.734 ± 0.033	79.5 ± 2.3	17.7 ± 0.2

61.8°C and SLR 1:153 (w/v). The optimal conditions for the extraction of total polyphenols and total flavonoids were 23.3% (v/v) (PPES), 67.5°C, 1:148 (w/v) (SLR); 1.15% (v/v) (PPES), 80°C, 1:179 (w/v) (SLR); respectively. The experimental values agreed with the predicted ones within a 95% confidence interval. This study can be useful for the development of industrial extraction processes, including further studies concerning the optimal number of sequential steps to enhance the efficiency of a large-scale extraction system.

References

- AKHONDZADEH S., NOROOZIAN M., MOHAMMADI M., OHADINIA S., JAMSHIDI A.H., KHANI M. (2003): *Melissa officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. *Journal of Neurology and Neurosurgery and Psychiatry*, **74**: 863–866.
- BALLARD C.G., O'BRIEN J.T., REICHELT K., PERRY E.K. (2002): Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of double-blind, placebo-controlled trial with *Melissa*. *Journal of Clinical Psychiatry*, **63**: 553–558.
- COHEN R.A., KUCERA L.S., HERRMANN E. (1964): Antiviral activity of *Melissa officinalis* (lemon balm) extract. *Proceedings of the Society for Experimental Biology and Medicine*, **117**: 431–434.
- DASTMALCHI K., DORMAN H.J.D., OINONEN P.P., DARWIS Y., LAAKSO I., HILTUNEN R. (2008): Chemical composition and *in vitro* antioxidative activity of lemon balm (*Melissa officinalis* L.) extract. *LWT-Food Science and Technology*, **41**: 391–400.
- DRAGLAND S., SENOO H., WAKE K., HOLTE K., BLOMHOFF R. (2003): Several culinary and medicinal herbs are important sources of dietary antioxidants. *Journal of Nutrition*, **133**: 1286–1290.
- FIALOVÁ S., TEKELOVÁ D., MRLIANOVÁ M., GRANČAI D. (2008): The determination of phenolics compounds and antioxidant activity of mints and balms cultivated in Slovakia. *Acta Facultatis Pharmaceuticae Universitatis Comenianae*, **55**: 96–102.
- GRIEVE M. (1998): *A Modern Herbal*. Tiber Books International PLC, Twickenham.
- HERODEŽ Š.S., HADOLIN M., ŠKERGET M., KNEZ Ž. (2003): Solvent extraction study of antioxidants from balm (*Melissa officinalis* L.) leaves. *Food Chemistry*, **80**: 275–282.
- HIRASA K., TAKEMASA M. (1998): *Spice Science and Technology*. Marcel Dekker, New York.
- JAVANMARDI J., STUSHNOFF C., LOCKE E., VIVANCO J.M. (2003): Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry*, **83**: 547–550.
- KENNEDY D.O., SCHOLEY A.B., TILDESLEY N.T., PERRY E.K., WESNES K.A. (2002): Modulation of mood and cognitive performance following acute administration of *Melissa officinalis* (lemon balm). *Pharmacology Biochemistry and Behavior*, **72**: 953–964.
- KENNEDY D.O., WAKE G., SAVELEV S., TILDESLEY N.T.J., PERRY E.K., WESNES K.A., SCHOLEY A.B. (2003): Modulation of mood and cognitive performance following acute administration of single doses of *Melissa officinalis* (lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology*, **28**: 1871–1881.
- KENNEDY D.O., LITTLE W., SCHOLEY A.B. (2004): Attenuation of laboratory induced stress in humans after acute administration of *Melissa officinalis* (lemon balm). *Psychosomatic Medicine*, **66**: 607–613.
- KENNEDY D.O., LITTLE W., HASKELL C.F., SCHOLEY A.B. (2006): Anxiolytic effects of a combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. *Phytotherapy Research*, **20**: 96–102.
- KÜMEL G., STOLL L., BRENDL M. (1991): Therapie mit rezeptfreien Topika. *Deutsche Apotheker Zeitung*, **131**: 1609.
- LAHUCKY R., NUERNBERG K., KOVAC L., BUCKO O., NUERNBERG G. (2010): Assessment of the antioxidant potential of selected plant extracts – *in vitro* and *in vivo* experiments on pork. *Meat Science*, **85**: 779–784.
- LEUNG A.Y., FOSTER S. (2003): *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics*. John Wiley and Sons, New York.

- LIYANA-PATHIRANA C.M., SHAHIDI F. (2005): Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, **93**: 47–56.
- LÖLIGER J. (1991): *Free Radicals and Food Additives*. Taylor and Francis, London.
- MIMICA-DUKIC N., BOZIN B., SOKOVIC M., SIMIN N. (2004): Antimicrobial and antioxidant activities of *Melissa officinalis* L. (*Lamiaceae*) essential oil. *Journal of Agricultural and Food Chemistry*, **52**: 2485–2489.
- MIKULAJOVÁ A., TAKÁCSOVÁ M., ALEXY P., BRINDZOVÁ L. (2007): Optimalizácia extrakcie fenolových zložiek z pohánky na základe výsledkov plánovaného experimentu. *Chemické listy*, **101**: 563–568.
- MYERS R.H., MONTGOMERY D.C. (2009): *Response Surface Methodology: Process and Product Optimization using Designed Experiments*. John Wiley and Sons, New York.
- PEREIRA R.P., FACHINETTO R., DE SOUZA PRESTES A., PUNTEL R.L., DA SILVA G.N.S., HEINZMANN B.M., BOSCHETTI T.K., ATHAYDE M.L., BÜRGER M.E., MOREL A.F., MORSCH V.M., ROCHA J.B.T. (2009): Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*. *Neurochemical Research*, **34**: 973–983.
- PIETTA P.G. (2003): Flavonoids in medicinal plants. In: RICE-EVANS C.A., PACKER L. (eds): *Flavonoids in Health and Disease*. Dekker, New York: 61–110.
- QUETTIER-DELEU C., GRESSIER B., VASSEUR J., DINE T., BRUNET C., LUYCKX M., CAZIN M., CAZIN J.C., BAILLEUL F., TROTIN F. (2000): Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, **72**: 35–42.
- SARER E., KOKDIL G. (1991): Constituents of the essential oil from *Melissa officinalis*. *Planta Medica*, **57**: 89.
- SHAHIDI F., JANITHA P.K., WANASUNDARA P.D. (1992): Phenolic antioxidants. *Critical Reviews of Food Science and Nutrition*, **32**: 67–103.
- SILVA E.M., ROGEZ H., LARONDELLE Y. (2007): Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology*, **55**: 381–387.
- SINGLETON V.L., ROSSI J.A. (1965): Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**: 44–158.
- SMALL E. (2006): *Culinary Herbs*. NRC Research Press, Ottawa.
- SOULIMANI R., FLEURENTIN J., MORTIER F., MISSLIN R., DERRIEU G., PELT J.M. (1991): Neurotropic action of the hydroalcoholic extract of *Melissa officinalis* in the mouse. *Planta Medica*, **57**: 105–109.
- SOUSA A.C. DE, ALVIANO D.S., BLANK A.F., ALVES P.B., ALVIANO C.S., GATTASS C.R. (2004): *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. *Journal of Pharmacy and Pharmacology*, **56**: 677–681.
- YEN G.C., CHEN H.Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agriculture and Food Chemistry*, **43**: 27–32.
- WANASUNDARA U., AMAROWICZ R., SHAHIDI F. (1994): Isolation and identification of an antioxidative component in canola meal. *Journal of Agricultural Food Chemistry*, **42**: 1285–1290.

Received for publication November 10, 2010
Accepted after corrections September 15, 2011

Corresponding author:

RNDr. MIROSLAV ONDREJOVIČ, Ph.D., Food Research Institute, Biocentrum Modrá, Kostolná 5,
900 01 Modra, Slovak Republic
tel. + 421 336 472 973, e-mail: ondrejovic@vup.sk
