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Optimization of Microwave-Assisted Extraction of Cherry Laurel (*Prunus laurocerasus* L.) Fruit Using Response Surface Methodology

Ivana T. Karabegović, Saša S. Stojičević, Dragan T. Veličković, Nada Č. Nikolić, and Miodrag L. Lazić

***Abstract*—**Optimization of a microwave-assisted extraction of cherry laurel (*Prunus laurocerasus*) fruit using methanol was studied. The influence of process parameters (microwave power, plant material-to-solvent ratio and the extraction time) on the extraction efficiency were optimized by using response surface methodology. The predicted maximum yield of extractive substances (41.85 g/100 g fresh plant material) was obtained at microwave power of 600 W and plant material to solvent ratio of 0.2 g/cm^3 after 26 minutes of extraction, while a mean value of 40.80±0.41 g/100 g fresh plant material was obtained from laboratory experiments. This proves applicability of the model in predicting optimal extraction conditions with minimal laborious and time consuming. The results indicated that all process parameters were effective on the extraction efficiency, while the most important factor was extraction time. In order to rationalize production the optimal economical condition which gave a large total extract yield with minimal energy and solvent consumption was found.

***Keywords*—**Cherry laurel, Extraction, Multiple regression modeling, Microwave.

I. Introduction

CHERY laurel (*Prunus laurocerasus* L.) is an evergreen shrub belongs to the *Rosaceae* family with natural habitat in Asia Minor, the Caucasus, Iran, Northern Ireland, Peloponnese, Bulgaria and Serbia [1]. In traditional Turkish medicine all parts of the plant species were used for centuries for the problems with digestive and respiratory organs (leaf), in the treatment of stomach ulcers, bronchitis, eczemas and hemorrhoids (fruit, seed) [2]–[4]. Cherry laurel fruit is mostly consumed in fresh or dried form, pickled or processed into jam, marmalade and liqueur [1], [5]. Fruit is a good source of nutrients, contains fructose, glucose, sorbitol [6], mannitol, ascorbic acid [7], dietary fiber, some minerals [1], [8], and the most common phenolic acids are benzoic, vanillic and caffeic [9].

In order to increase the yield and improve the quality of extracts, to enhance pollution prevention, reduce the extraction time and solvent consumption a various non-conventional extraction techniques, such as supercritical fluid extraction [10], extraction under the influence of an electric field [11], microwaves [12]-[14] and ultrasound [15], [16], have been developed. Among these, microwave-assisted extraction (MAE) is the simplest and the least expensive technique for the extraction of nutraceuticals [17]. The major benefits of MAE are the considerable reduction in time and solvent consumption with better extraction yield [18], [19].

Microwaves are already used for the extraction of bioactive substances which are of interest for the food and pharmaceutical industries, for example, polyphenols from aromatic plants [20], apple fruit [21], grape seed [22], and green tea leaves [23], antioxidants from rice bran [24], chlorogenic acid from *Lonicera japonica* flower buds Thunb. [25], solanesol from tobacco leaves [26], and isoflavones from soybeans [27].

Extraction of plant materials is affected by many factors (extraction method, solid-to-solvent ratio, time, type of solvent, temperature) whose effects (individual and combined) can be estimated by statistical methods. Response surface methodology (RSM) is a statistical-mathematical method which uses quantitative data in an experimental design to determine and solve multivariable equations in order to optimize processes with avoiding the limitations of classical methods [28]. It has been successfully used to model and optimize the extraction of total polyphenol content [29], flavonoids [30], anthocyanins [31], carbohydrates [32], polysaccharides [33] or natural dye for textiles [34] from different plant materials.

The aim of this study was to investigate the applicability of microwave-assisted extraction to the extraction of cherry laurel fruit and to optimize the effects of processing parameters of extraction on the yield of extractive substances by using the response surface methodology. The phenolic composition and antioxidant activity of the extract obtained under optimal conditions were also evaluated.

II. Experimental

A. Materials

Cherry laurel (*P. laurocerasus* L.) fruit were collected at the full ripening stage from natural habitats (South Serbia). Fresh plant material was milled immediatly before extraction. Moisture content determined by drying at 105 ^{o}C to constant weight was 77.4±3.2 %.

Ivana T. Karabegović, Saša S. Stojičević, Nada Č. Nikolić, Miodrag L. Lazić are with the University of Niš, Faculty of Technology, 124 Bulevar oslobodjenja St., Leskovac, Serbia (phone: +38116247203; fax: +38116242859; e-mail: ivana.karabegovic@tf.ni.ac.rs).

Dragan T. Veličković, was with College of Agriculture and Food Technology, 1 Ćirila i Metodija St., 18400 Prokuplje, Serbia.

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B. Microwave Assisted Extraction

To perform microwave extraction modified microwave oven with variable magnetron input voltage ("SAMSUNG", type M1712N, Malaysia) was used. A vertical condenser was linked to the Erlenmayer flasks placed in microwave oven throw the circular hole (diameter 55 mm) which was made on the upper wall of the oven. Ground plant material (20, 40 or 80 g) and methanol (400 cm^3) were put in a series of the Erlenmayer flasks (500 cm^3) to make ratio of plant material to solvent 0.05, 0.1 or 0.2 g/cm^3. The extraction was performed for 10, 20 and 30 minutes. At the end of the extraction cycle the liquid extract was separated from the solid residue by vacuum filtration. The solid residue was washed twice with fresh solvent (20 cm^3). The filtrates were collected and the solvent was evaporated in a vacuum evaporator at 40 °C.

C. Experimental Design for Response Surface Methodology

Experiments were planned according to 3^3 factorial experiment design to study the influence of microwave power, plant material to solvent ratio and extraction time on extraction yield in order to predict optimal extraction conditions. The independent parameters: microwave power (300–600 W, X_1), plant material to solvent ratio (0.2-0.05 g/cm^3, X_2), and extraction time (10–30 min, X_3) were determined by the preliminary experiments. The extraction yield (dependent parameter, Y) was determined three times, and means were used for the regression analysis.

To find the optimal extraction conditions and to describe the behavior of the system, a second-order model was used according to the following equation:

$$Y= \beta_o+ \Sigma\beta_i X_i +\Sigma\beta_{ii} X_i^2+\Sigma\beta_{ij} X_i X_j \quad (1)$$

where Y is the predicted response (yield of extractive substances in g/cm^3), β_o, β_i, β_{ii}, and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively and X_i and X_j are the actual levels of the independent variables.

The statistical software Design Expert (Trial version 7.0.0, STAT-EASE Inc.) was used to analyze the experimental data, for analysis of variance (ANOVA), regression coefficient calculations and for graphical analysis (response surfaces and contour plots) of the experimental data. In order to determine adequacy of the fitted model the experimental and predicted values were compared.

III. Results and Discussions

The yield of extractive substances of cherry laurel fruit (experimental and predicted) under various extraction conditions according to the factorial design is shown in Table I. The highest yield of extractive substances was recorded at 600 W, 0.05 g/cm^3 and 30 min (experiment 4).

A quadratic polynomial equation was generated to predict the yield of extractive substances from cherry laurel fruit by applying multiple nonlinear regression analysis on the experimental data obtained in 3^3 designed experiments.

TABLE I
Experimental Design Matrix and Results

Run	Design matrix: Coded factors: Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	Uncoded factors: Microwave power, W (X_1)	Plant material to solvent ratio, g/cm^3 (X_2)	Time, min (X_3)	Response: Yield[a], g/100 g fresh plant material: Experimental	Predicted
1	-1	0	+1	300	0.10	30	37.83	37.94
2	-1	+1	0	300	0.20	20	34.27	35.00
3	0	0	0	450	0.10	20	37.87	38.03
4	+1	-1	+1	600	0.05	30	40.80	40.95
5	0	0	0	450	0.10	20	37.87	38.03
6	+1	+1	-1	600	0.20	10	23.90	23.89
7	+1	0	0	600	0.10	20	38.50	39.17
8	0	-1	0	450	0.05	20	38.60	38.38
9	-1	-1	-1	300	0.05	10	22.57	23.68
10	0	+1	+1	450	0.20	30	37.77	37.49
11	0	0	-1	450	0.10	10	24.50	25.14
12	0	0	0	450	0.10	20	37.87	38.03
13	0	-1	+1	450	0.05	30	39.33	39.68
14	0	0	0	450	0.10	20	37.87	38.03
15	0	+1	-1	450	0.20	10	23.25	23.1
16	+1	0	+1	600	0.10	30	39.67	40.42
17	0	0	0	450	0.10	20	37.87	38.03
18	+1	-1	-1	600	0.05	10	28.13	26.97
19	-1	0	-1	300	0.10	10	23.87	23.52
20	+1	+1	0	600	0.20	20	36.65	36.77
21	-1	-1	0	300	0.05	20	37.27	36.63
22	-1	+1	+1	300	0.20	30	36.22	36.5
23	-1	0	0	300	0.10	20	36.30	36.51
24	0	+1	0	450	0.20	20	35.40	36.07
25	-1	-1	+1	300	0.05	30	38.20	38.02
26	0	0	0	450	0.10	20	37.87	38.03
27	+1	+1	+1	600	0.20	30	38.83	38.09
28	-1	+1	-1	300	0.20	10	22.57	21.94
29	0	0	+1	450	0.10	30	38.87	39.37
30	+1	-1	0	600	0.05	20	39.47	39.74
31	0	0	0	450	0.10	20	37.87	38.03
32	+1	0	-1	600	0.10	10	25.10	26.37
33	0	-1	-1	450	0.05	10	25.20	25.52

[a] The mean of three replicates

$$Y=37.70+1.22X_1-1.15X_2+7.14X_3-0.34X_1X_2-0.090X_1X_3+0.056X_2X_3-0.19X_1^2-0.48X_2^2-5.78X_3^2 \quad (2)$$

The results of the analysis of variance (ANOVA) for fitting the selected quadratic polynomial model by a mean square method are summarized in Table II.

The significance of each of the factors and interactions are checked by p-values (probability of error value), where the values less than 0.0001 indicates high significance in predicting the response values and the suitability of the deduced model.

The high F value (458.71) with very low probability ($p < 0.0001$) indicates the high significance of the chosen model. The smaller the P value for a parameter the more significant is

TABLE II
ANALYSIS OF VARIANCE (ANOVA) FOR RESPONSE SURFACE QUADRATIC MODEL

Source of variation	Sum of squares	Degrees of freedom (df)	Mean squares	F value	P value
Model	1261.13	9	140.13	458.71	< 0.0001
A-Power	26.81	1	26.81	87.76	< 0.0001
B-Plant material to solvent ratio	23.84	1	23.84	78.05	< 0.0001
C-Time	916.40	1	916.40	2999.86	< 0.0001
AB	1.36	1	1.36	4.44	0.0474
AC	0.098	1	0.098	0.32	0.5775
BC	0.037	1	0.037	0.12	0.7312
A^2	0.26	1	0.26	0.86	0.3645
B^2	1.65	1	1.65	5.39	0.0304
C^2	242.57	1	242.57	794.05	< 0.0001
Residual	6.42	21	0.31		
Lack of fit	6.42	17	0.38		
Pure error	0.000	4	0.000		
Cor total	1268.13	32			
CV = 1.62%	R^2 = 0.9949	Adj R^2 = 0.9928	Predicted R^2 = 0.9808		

the parameter, hence reflecting the relative importance of the term attached to that parameter [35].

All considered factors were statistically significant, the interaction of microwave power and ratio of plant material to solvent, as well as the second-order effect of ratio of plant material to solvent (X_2^2) and time (X_3^2). The most significant factor was extraction time. However, while microwave power and extraction time seem to have positive effect, ratio of plant material to solvent had a significant negative influence on yield which is in agreement with the fact that the extractive substances yield increased with decreasing the ratio of plant material to solvent due to a higher driving force for extractive substances transfer in the more diluted solution.

The factors and interactions which are statistically insignificant with p-values greater than 0.05 were omitted in order to get a simpler model, which is as follows:

$$Y = 37.70+1.22X1-1.15X2+7.14X3-0.34X1X2-0.48X22-5.78X32 \quad (3)$$

Predicted values were calculated by using (3). The plot of experimental versus calculated values of yield (g/100 g fresh plant material) was shown in Fig. 1. It shows that the experimental values are distributed close to the straight line with relatively high values of the coefficient of determination (R^2=0.9949), indicating that 99.49% of the variability in the response could be explained by the model. The adjusted coefficient of determination (Adj R^2 = 0.9928) is also close to 1.0 which indicates that results of the fitted model are reliable and support the significance of the model. At the same time, a high value of predicted R^2 (0.9808) is also an indication of reasonable precision of the model fitted. The coefficient of variation had low value (CV = 1.62%), showing greater reliability.

As the fitted model provides a good approximation to the experimental condition, the model was employed to find the values of the process variables for maximum yield.

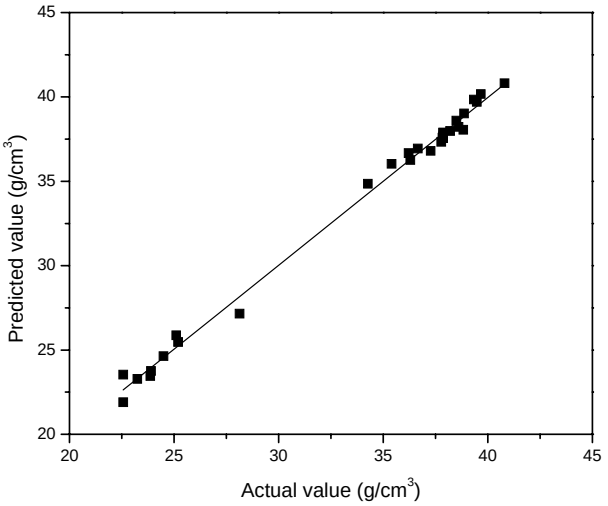

Fig. 1 Correlation of calculated versus experimental values for yield of cherry laurel fruit extractive substances

The graphical representations of the regression equation, three-dimensional (3D) response surfaces and two-dimensional (2D) contour plots, were obtained using the statistical software Design-Expert. They visualize the relationship between the response and each variable, interactions between any two tested variables and the optimum values of the independent variables under which dependent variable could arrive the maximum response. In the present study, the results of extraction yield affected by microwave power, plant material to solvent ratio and extraction time are presented in Fig. 2.

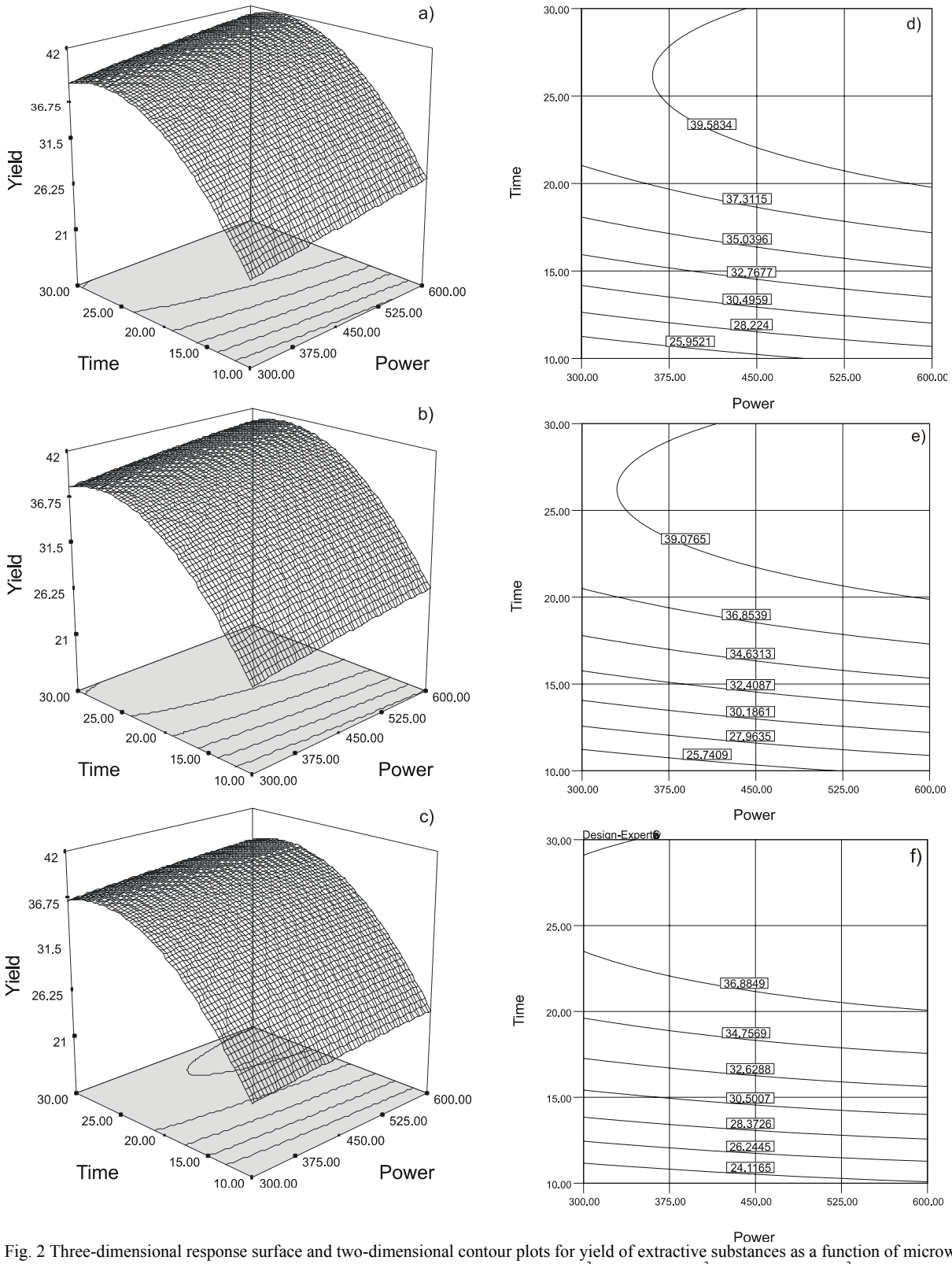

Fig. 2 Three-dimensional response surface and two-dimensional contour plots for yield of extractive substances as a function of microwave power, extraction time and plant material to solvent ratio of 0.05 g/cm^3 (a, d), 0.1 g/cm^3 (b, e) and 0.2 g/cm^3 (c, f)

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Fig. 2 presents the response surfaces and contour plots for extractive substances yield as a function of the microwave power and extraction time while plant material to solvent ratio was kept constant at three levels (0.05, 0.1 and 0.2 g/cm^3). Analyses of the 3D response surfaces and their respective 2D contour plots allowed us to conveniently investigate the interactions between any two variables, and locate the optimum ranges of the variables efficiently such that the response was maximized.

From those figures, it can be concluded that the yield of extractive substances, independently of microwave power and plant material to solvent ratio, increased rapidly with increase of extraction time from 10 to 26 minutes, then slightly decreased from 26 to 30 minutes.

It is noticed from the response surface plots that regardless of the extraction time the yield of extractive substances increased with increasing both microwave power from 300 to 600 W and plant material to solvent ratio from 0.05 g/cm^3 to 0.2 g/cm^3. Maximal yield of extractive substances (41.85 g/100 g fresh plant material) was achieved at the microwave power of 600 W and plant material to solvent ratio of 0.2 g/cm3 after 26 minutes of extraction. Under those conditions, the predicted yield was 41.85 g/100 g fresh plant material, while a mean value of 40.80±0.41 g/100 g fresh plant material (n = 5) was obtained from laboratory experiments.

In order to rationalize production and cost minimization each variable level was set manually on desired values by the use of the statistical software. Precisely, microwave power and time were set on minimal, and plant material to solvent ratio was set on its maximal value. The new criteria were used to find the extraction conditions that would give minimal energy and solvent consumption while maintaining a large total extract yield (optimal economical condition). For that case the model predicts that the highest yield of extractive substances (33.52 g/100 g fresh plant material) can be obtained in 18.2 min under the 300 W and plant material to solvent ratio of 0.2 g/cm^3. This means that two times less power input, four times less solvent consumption and about 40% short time gives yield which was lower for about 20%. By estimation of energy input per unit of final product it was calculated that for the case of optimal condition and economical conditions of microwave extraction of extractive substances specific energy input was 0.031 kWh and 0.0034 kWh, respectively. It means that under chosen economical conditions obtaining extractive substances is more than 9 times cheaper.

IV. Conclusion

The response surface methodology was successfully employed to optimize the microwave-assisted extraction of extractive substances from cherry laurel fruit. A second order model was obtained to predict maximum yield of extractive substances as a function of microwave power, plant material to solvent ratio and extraction time. Results confirmed the plosive effects of microwave power and extraction time and negative effect of plant material to solvent ratio, while the extraction time was the most important factor affecting extraction efficiency. Based on the proposed model, the most efficient conditions for microwave assisted extraction of cherry laurel fruit were found to be at 600 W, with plant material to solvent ratio of 0.02 g/cm^3 and 26 minutes. The model was found to describe adequately the experimental range studied. By an economic evaluation of process parameters, taking into account the cost of the energy input, economical conditions of microwave extraction of extractive substances was found as follows: 300 W, 0.5 g/cm^3 and 18.2 minutes.

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