Multivariate optimization of microwave-assisted extraction of zinc and copper from soybean

Mostafa Khajeh

Revised: 11 March 2012 / Accepted: 11 June 2012 / Published online: 24 June 2012

© Association of Food Scientists & Technologists (India) 2012

Abstract In this study, a simple and fast microwave-assisted extraction (MAE) method combined with flame atomic absorption spectrometry (FAAS) was developed and optimized for the extraction and determination of zinc and copper in soybean. The optimization of the MAE conditions was conducted using a full factorial design and Box-Behnken matrix. Firstly, the full factorial design was carried out for preliminary evaluation of the significance of the factors, the factors chosen being: irradiation power, temperature, extraction time, and concentration of nitric acid. The results showed that all of the factors were significant. Because of this, a Box-Behnken experimental design was carried out in order to determine the optimum condition. The total 27 experiments were conducted in the study towards the construction of a quadratic model. According to analysis of variance (ANOVA) results, the proposed model can be used to navigate the design space. High regression coefficient between the variables and the response ($R^2=0.97$) indicated excellent evaluation of experimental data by polynomial regression model. The method was applied to the determination of zinc and copper in soybean samples.

Keywords Zinc · Copper · Box-Behnken design · Microwave-assisted extraction · Soybean

Introduction

The potential of soybeans as a functional food is being currently explored by the food industry. However,

soybeans and soy foods, like soymilk are widely promoted and eaten based on assumed relationships between its consumption and beneficial health influences in humans including chemoprevention of breast and prostate cancer, osteoporosis, cardiovascular disease as well as relieving menopausal symptoms (Wu et al. 2008; Rostagno et al. 2009). Evidence provided not only by epidemiological studies showing a lower incidence of these health conditions in Asian countries like Japan and China, which have high soy consumption, but also from intervention studies, is the basis of this relationship (Wu et al. 2008; Rostagno et al. 2009).

The proposition of analytical procedures for food quality control and inspection in an important method is to ensure proper micronutrient levels. Nutrition of children at school age is of public concern since are critical to their growth and development (Da-Col et al. 2009; Fraga 2005). The levels of element concentrations should be known to avoid contaminations with toxic elements and overexposure to micronutrients. Therefore, in order to develop quick and reliable methods to analyze the samples, analytical chemistry should be used (Da-Col et al. 2009; Munoz-Olivas 2004). Several methods employing, for example, mineralization with concentrated reagent [HNO_3 , HCl and HClO_4 ; (Oliveira 2003)] can be found in the previous work. These methods involve great sample mass and need both attention of the operator and long completion time (Huie 2002). However, the analytical sequence time can be reduced using a microwave oven, therefore leading to an increase in analytical sampling rate (Oliveira 2003).

The optimization of analytical systems by multivariate experimental designs is an important tool to study variables simultaneously, resulting in faster and cost effective procedures than traditional univariate optimization. Multivariate

M. Khajeh (✉)
Department of Chemistry, University of Zabol,
P. O. Box 98615-538, Zabol, Iran
e-mail: mostafa.khajeh@gmail.com

optimization appears to be more interesting and complete alternative, allowing maximum information to be obtained due to the possibility of evaluating interactions between variables (Maranhão et al. 2007). Two steps generally are involved in multivariate optimization of analytical methods. The first step is a preliminary evaluation that selects the significant variables in the method using factorial design. Afterward, a second step performs an appropriate estimation of the real functional relationship among the analytical response and significant factors. This way, the optimum values for these factors can be calculated. A Box-Behnken experimental design is a chemometric technique, which is used for the estimation of critical points. It has been widely used procedure in optimization of analytical methods (Maranhão et al. 2007; Sharma et al. 2008).

In this work, the microwave assisted extraction system was used for the extraction of zinc and copper from soybean samples and their determination by FAAS. The optimization of the experimental variables (irradiation power, time, concentration of nitric acid and temperature) will be described using a Box-Behnken matrix.

Experimental

Apparatus

The measurements were performed with a Konik Won M300 (Barcelona, Spain) flame atomic absorption spectrometer (FAAS), equipped with a conventional pneumatic nebulizer and nebulization chamber was used for the analysis. Hollow cathode lamp for determination of copper and zinc was used. Background correction was performed with deuterium lamp. The microwave extractions were carried out in an Ethos SEL (Milestone, Sorisole, Italy).

Materials

Nitric acid used was of the highest purity available from Merck (Darmstadt, Germany). Reagent grade of salts (all from Merck) were of the highest purity available. A stock solution of the copper and zinc prepared by dissolving a proper amount of the salts in doubly distilled water in a 10 mL flask. Dilute solutions were prepared by an appropriate dilution of the stock solution in doubly distilled water.

Microwave-assisted extraction procedure

An aliquot (1.0 g) of sample was transferred to the vessel of microwave assisted extraction and then 10 mL of different concentration of HNO_3 (1 to 3 mol.L⁻¹)

Table 1 Experimental design the results obtained in function of the $\mu\text{g g}^{-1}$ of zinc

No.	P (W)	T(°C)	[HNO ₃] (molL ⁻¹)	t (min)	$\mu\text{g g}^{-1}$
1	100	60	0.10	5	47.47
2	200	60	0.10	5	48.35
3	100	80	0.10	5	49.83
4	200	80	0.10	5	48.85
5	100	60	1.00	5	55.33
6	200	60	1.00	5	59.65
7	100	80	1.00	5	59.15
8	200	80	1.00	5	60.90
9	100	60	0.10	15	48.56
10	200	60	0.10	15	51.77
11	100	80	0.10	15	53.88
12	200	80	0.10	15	54.07
13	100	60	1.00	15	62.62
14	200	60	1.00	15	64.10
15	100	80	1.00	15	64.07
16	200	80	1.00	15	63.71
17	150	70	0.55	10	60.24
18	150	70	0.55	10	60.40

as extraction solvent was added. According to a preliminary experimental design, extractions were performed at various conditions of temperature ranging from 80 to 120 °C, irradiation time from 20 to 40 min, concentration of nitric acid from 1 to 3 molL⁻¹ and irradiation power from 200 to 400 W. After extraction, the irradiated sample was transferred to 10 mL volumetric flask and the volume was completed to the mark. The concentration of the analytes in this solution was analyzed by FAAS.

Fig. 1 Pareto chart of main effects obtained from 2⁴ full factorial designs. The vertical line defines the 95 % confidence interval [A = power (P), B = temperature (°C), C = concentration of nitric acid (molL⁻¹) and D = time (min)]

Results and discussion

Factorial design

The factors chosen considering the MAE system were: power (100–200 W), nitric acid concentration (0.1–1.0 molL⁻¹) time (5–15 min) and temperature (60–80 °C). A two-level full factorial design (2⁴) was carried out in duplicate to determine the effect of each factor and its interactions in the extraction system. Table 1 show the experimental design and the results derived from each run. Analysis of variance (ANOVA) was used to check the significant of the effects. The Pareto chart (Fig. 1) also shows the significance of factors. In the interpretation of this graph, it should be considered that bar lengths are proportional to the absolute value of the estimated effects. This chart demonstrates that the all of factors were significant.

Box-Behnken design

Factorial design demonstrated that the factors, namely power, temperature, nitric acid concentration and time in the studied levels required a final optimization. Then, a Box-Behnken experimental design was carried out. In this study, the three levels, four factorial Box-Behnken experimental designs was used to investigate and validate the process parameters affecting the extraction of metals: power (X_1) (200–400 W), nitric acid concentration (X_2)(1–3 molL⁻¹) time (X_3)(20–40 min) and temperature (X_4)(80–120 °C) were input variables, the factor levels were coded as −1 (low), 0 (central point) and 1 (high). The design of real experiments is given in Table 2. The MiniTab 14 software was used for statistical analysis.

The behavior of the system is explained by the following quadratic equation (Bayraktar 2001).

Table 2 Design matrix in the Box-Behnken model, observed and predicted values

Trial No.	P (W)	T(°C)	t (min)	[HNO ₃] (molL ⁻¹)	Observed, Y_a (μgg ⁻¹)		Predicted, Y_p (μgg ⁻¹)	
					Zinc	Copper	Zinc	Copper
1	200	80	30	2	89.8	8.52	92.68	8.73
2	400	80	30	2	90.81	8.81	91.51	8.81
3	200	120	30	2	89.75	10.87	89.92	11.08
4	400	120	30	2	105.22	11.15	103.21	11.15
5	300	100	20	1	58.63	9.35	59.78	9.44
6	300	100	40	1	64.86	8.24	62.00	8.15
7	300	100	20	3	78.32	4.37	82.05	4.68
8	300	100	40	3	90.19	10.26	89.91	10.39
9	200	100	20	2	76.47	8.12	76.49	7.87
10	400	100	20	2	79.89	8.31	81.32	8.24
11	200	100	40	2	79.97	10.55	80.30	10.38
12	400	100	40	2	85.84	10.16	87.58	10.16
13	300	80	30	1	68.85	8.78	70.73	8.65
14	300	120	30	1	74.46	10.61	76.87	10.49
15	300	80	30	3	98.14	7.01	97.49	6.89
16	300	120	30	3	100.41	9.85	100.29	9.73
17	200	100	30	1	60.93	8.97	59.26	9.08
18	400	100	30	1	68.95	9.81	68.06	9.95
19	200	100	30	3	88.83	8.73	87.10	8.62
20	400	100	30	3	91.36	7.98	90.41	7.90
21	300	80	20	2	91.11	6.56	86.86	6.52
22	300	120	20	2	93.29	9.74	91.22	9.70
23	300	80	40	2	92.34	9.50	91.79	9.57
24	300	120	40	2	94.74	11.01	96.37	11.08
25	300	100	30	2	90.93	9.61	90.45	9.90
26	300	100	30	2	91.76	10.02	90.45	9.90
27	300	100	30	2	88.67	10.07	90.45	9.90

^a Average of triplicate extraction

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Here Y is the process response or output (dependent variable), k is the number of the patterns, i and j are the index numbers for pattern, β_0 is the free or offset term called intercept term, $x_1, x_2, \dots, x_k$ are the coded independent variables, β_i is the first-order (linear) main effect, β_{ii} is the quadratic (squared) effect, β_{ij} is the interaction effect, and ε is the random error or allows for description or uncertainties between predicted and measured value.

Modeling

In this work, multiple regression analyses were performed using response surface methodology analysis to (1) fit mathematical models to the experimental data; (2) define the rela-

tionship between four independent variables and the response variables as shown in Table 2. The response surface analysis allowed the development of an empirical relationship where the response variable (Y) was assessed as a function of temperature (T), power (P), nitric acid concentration (N) and time (t), four first-order effects (linear term in T , P , N and t), four second-order effects (quadratic terms in T^2 , P^2 , N^2 and t^2) and six interaction effects (interactive terms in TP , TN , Tt , PN , Pt and Nt). The number of experiments required to investigate the previously noted four parameters at three levels would be 81 (3^4). However, this was reduced to 27 using a Box-Behnken experimental design. The results from this limited number of experiments provided a statistical model, which was used to identify high yield trends for the extraction process. Table 2 shows the matrix and the microgram per gram for zinc and copper. The equations below explain the relationship of the four variables, that is, irradiations power (P); time (t); concentration of nitric acid (N) and temperature (T) and amount of zinc and copper in the samples (Y).

$$Y(\text{zinc}) = 139.385 + 0.046(P) - 3.859(T) + 3.3(t) + 61.078(N) - 6.0 \times 10^{-4}(P)^2 + 0.018(T)^2 - 0.059(t)^2 - 11.117(N)^2 + 0.002(T)(P) + 0.001(P)(t) - 0.014(P)(N) + 6.0 \times 10^{-4}(T)(t) - 0.042(T)(N) + 0.141(t)(N) \quad (2)$$

$$Y(\text{copper}) = -4.52917 + 0.016(P) + 0.025(T) + 0.45158(t) - 1.92(N) - 5.0 \times 10^{-7}(P)^2 + 0.00012(T)^2 - 0.00731(t)^2 - 1.0075(N)^2 - 1.25 \times 10^{-6}(T)(P) - 0.00014(P)(t) - 0.00398(P)(N) - 0.00209(T)(t) + 0.01262(T)(N) + 0.175(t)(N) \quad (3)$$

Table 3 ANOVA analysis for zinc and copper extraction

Source	Sum of squares (SS)	Degree of freedom	Mean square (MSS)	F-value	P
For zinc					
Regression	3715.59	14	265.399	35.69	<0.0001
Linear	885.20	4	221.300	29.76	0.0001
Square	1508.23	4	377.057	50.70	0.0001
Interaction	72.06	6	12.010	1.62	0.226
Residual	89.24	12	7.437		
Lack-of-fit	84.13	10	8.413	3.29	0.256
Pure Error	5.11	2	2.557		
Adjusrt- R^2	0.95				
For copper					
Regression	58.4102	14	4.17216	89.38	<0.0001
Linear	1.8359	4	0.45899	9.83	0.001
Square	8.5868	4	2.14669	45.99	<0.0001
Interaction	13.9184	6	2.31973	49.69	<0.0001
Residual	0.5602	12	0.04668		
Lack-of-fit	0.4328	10	0.04328	0.68	0.725
Pure error	0.1274	2	0.0637		
Adjusrt- R^2	0.979				

The critical point in the surface response are founded by solving these equation systems for the condition of $\frac{\delta(Y)}{\delta(T)} = 0$, $\frac{\delta(Y)}{\delta(P)} = 0$, $\frac{\delta(Y)}{\delta(N)} = 0$ and $\frac{\delta(t)}{\delta(t)} = 0$. The way of calculating these critical points has been published in previous study (Santos et al. 2004). The calculated values for the critical point are as follows: temperature (T)= 97.0 °C, irradiation power (P)=399.0 W, concentration of nitric acid=2.6 molL⁻¹ and irradiation time (t)= 35.0 min for zinc and temperature (T)=117 °C, irradiation power (P)=396 W, concentration of nitric acid=2.5 molL⁻¹ and irradiation time (t)=40 min for copper. In order to establish a single condition that could be useful for the extraction of the samples aiming the determination of zinc and copper, working values of T, P, concentration of nitric acid and t were fixed as 107.0 °C, 398.0 W, 2.5 molL⁻¹ and 38.0 min, respectively.

The results obtained were than analyzed by ANOVA to assess the goodness of fit. Only terms found statistically significant ($p < 0.05$) were included in the reduced model. As shown in Eq. 2, the model obtained for

predicting the response variable explained the main quadratic and interaction effects of factors affecting the response variable. The analysis of variance (ANOVA) is shown in Table 3. The significance of each term was determined using the *F* ratio and *p* value as presented in Table 3. As shown in Table 3, the linear and quadratic effect had the most significant ($p < 0.05$) effects on the extraction. The interaction effect of independent variables had no significant ($p > 0.05$) influence on the MAE extraction. Also, the results showed that the regression model for the response variable was significant by the *F* test at the 5 % confidence level ($p < 0.05$). The goodness of fit of the model can be checked by the determination coefficient (R^2). The value of adjusted R^2 (0.95 for zinc and 0.979 for copper) indicated that only 5.0 % and 2.1 % of the total variations were not explained by this model for zinc and copper, respectively. Therefore, the value of determination coefficient (R^2 : 0.98 and 0.991 for zinc and copper, respectively) indicates good relation between the experimental and predicted values of the response. The lack-of-fit measures the failure of the

Fig. 2 Response surface obtained from Box-Behnken design for extraction efficiency of copper (a) and zinc (b)

model to represent data in the experimental domain at points which are not included in the regression (Yetilmezsoy et al. 2009). The non-significant value of lack-of-fit (>0.05) revealed that the quadratic model is statistically significant for the response.

The Durbin-Watson (DW) statistic (Eq. 4) indicates whether autocorrelation, or correlation between errors, is present in a model (Yetilmezsoy et al. 2009; Stickel 2007). The range of DW statistic is 0.0 to 4.0, and is employed for examine the linear association between adjacent residuals (Yetilmezsoy et al. 2009; Hewings et al. 2002). The values of DW below and above 2 can show positive and negative autocorrelation, respectively (Yetilmezsoy et al. 2009; Stickel 2007). If the DW value is around 2, this indicates a good fit of the model (Yetilmezsoy et al. 2009).

$$DW = \frac{\sum_{i=2}^n (e_i - e_{i-1})^2}{\sum_{i=1}^n e_i^2} \quad (4)$$

In this study, the DW statistic (DW=1.87 and 1.8 for zinc and copper, respectively) was determined to be very close to 2, shows the goodness of fit of the model, as similarly reported by Yetilmezsoy et al. (2009).

The three dimensional (3D) response surface plots as a function of two factors, maintaining all other factors at fixed levels are helpful in understanding both the main and their interaction effects of these two factors is shown in Fig. 2.

Table 2 indicates the observed (Y_a), predicted (Y_p) values and the percentage of the response error. As mentioned above, the high values of R^2 and R_a^2 showed that the quadratic equation can represent the system under the given experimental domain. This is also evident from the fact that the parity plot depicted in Fig. 3 indicates a satisfactory correlation between the observed and predicted values of zinc and copper extraction efficiency. As seen in Fig. 3, the points cluster around the diagonal line shows a good fit of the model, since the deviation between the experimental and predicted values was less, as similarly reported by Yetilmezsoy et al.

The significance each coefficient was determined by Student's t -test and p -values that are listed in Table 4. The p value is used as a tool to check the significance of each of the coefficients. The greater the magnitude of the t value and the smaller the p value, the most significant are the parameters in the regression mode (Yetilmezsoy et al. 2009). Based on the sum of squares obtained from the ANOVA, the percentage of contributions (PC) for each term were calculated and tabulated in Table 4.

Fig. 3 Parity plot show the correlation between the observed and predicted values for zinc (a) and copper (b)

Analytical application

The optimized MAE method was applied to the determination of zinc and copper in soybean. The results obtained are showed in Table 5.

Conclusion

The present study revealed that the extraction of zinc and copper with MAE was significantly ($p < 0.05$) influenced by the main and quadratic effects of the independent variables studied. Analysis of variance (ANOVA) showed a high overall coefficient of determination value ($R^2 > 0.97$) for the regression model. Thus, it was possible to develop the empirical equation for describing and predicting the variation of the response variables.

The application of a Box-Behnken matrix became possible, fast, economical and efficient way of an optimization

Table 4 Multiple regression results and significance of the components for the quadratic model

Factor (coded)	Parameter	SE ^a		<i>t</i> ratio		<i>p</i> -value		SS ^b		(PC%) ^c PC = $\frac{SS}{\sum SS} \times 100$	
		Zinc	Copper	Zinc	Copper	Zinc	Copper	Zinc	Copper	Zinc	Copper
Intercept	β_0										
P	β_1	0.1102	0.0087	0.421	1.501	0.6809	0.159	1.32	0.11	0.07	0.460
T	B ₂	0.6726	0.0533	-5.737	1.35	<0.0001	0.202	244.75	0.085	13.50	0.355
t	B ₃	1.1021	0.0873	2.994	5.172	0.0112	0.0002	66.67	1.25	3.68	5.227
[HNO ₃]	B ₄	10.1427	0.8036	6.022	-2.389	<0.0001	0.034	269.68	0.27	14.87	1.129
P ²	B ₁₁	0.0001	0.00001	-2.651	-0.053	0.0211	0.958	52.28	1.33×10^{-4}	2.88	0.001
T ²	B ₂₂	0.003	0.00023	5.934	0.521	<0.0001	0.612	261.83	0.013	14.44	0.054
t ²	B ₃₃	0.0118	0.00094	-4.998	-7.816	0.0003	<0.0001	185.78	2.85	10.24	11.919
[HNO ₃] ²	B ₄₄	1.1808	0.0936	-9.415	-10.769	<0.0001	<0.0001	659.14	5.41	36.35	22.624
PT	B ₁₂	0.0007	0.00005	2.651	-0.023	0.0211	0.982	52.27	2.5×10^{-5}	2.88	0.000
Pt	B ₁₃	0.014	0.00011	0.449	-1.342	0.6613	0.204	1.5	0.084	0.08	0.351
P[HNO ₃]	B ₁₄	0.0136	0.0011	-1.007	-3.68	0.3340	0.003	7.54	0.63	0.419	2.635
Tt	B ₂₃	0.006	0.00054	0.004	-3.865	0.9685	0.002	0.012	0.7	0.001	2.927
T[HNO ₃]	B ₂₄	0.0682	0.0054	-0.612	2.337	0.5517	0.038	2.79	0.26	0.15	1.087
t[HNO ₃]	B ₃₄	0.1364	0.0108	1.034	16.199	0.3215	<0.0001	7.95	12.25	0.44	51.229

^a Standard error^b Sum of squares^c Percentage contribution (%)

Table 5 Determination of zinc and copper in the soybean

Sample no.	$(\mu\text{g g}^{-1})(\text{RSD}\%)$	
	Zinc	Copper
Sample 1	88.1 $\pm$ 0.12	62.4 $\pm$ 0.14
Sample 2	90.7 $\pm$ 0.13	60.9 $\pm$ 0.09

These samples were selectively purchased from a local market

strategy of the proposed procedure. The MAE procedure developed for the determination of zinc and copper in the soybean.

References

- Bayraktar E (2001) Response surface optimization of the separation of DL-tryptophan using an emulsion liquid membrane. *Process Biochem* 37:169–175
- Da-Col JA, Domene SMA, Pereira-Filho ER (2009) Fast determination of Cd, Fe, Pb, and Zn in food using AAS. *Food Anal Methods* 2:110–115
- Fraga CG (2005) Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 26:235–244
- Hewings GJD, Changnon S, Dridi C (2002) Testing for the significance of extreme weather and climate event on the state economies. In: *The regional economics application laboratory*, REAL 00-T-6, S. Mathews, Urbana, IL, September, 2002, pp 1–16
- Huie CW (2002) A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. *Anal Bioanal Chem* 373:23–30
- Maranhão TA, Martendal E, Borges DLG, Carasek E, Welz B, Curtius AJ (2007) Cloud point extraction for the determination of lead and cadmium in urine by graphite furnace atomic absorption spectrometry with multivariate optimization using Box-Behnken design. *Spectrochim Acta B At Spectrosc* 62:1019–1027
- Munoz-Olivas R (2004) Screening analysis: an overview of methods applied to environmental, clinical and food analyses. *Trends Anal Chem* 23:203–216
- Oliveira E (2003) Sample preparation for atomic spectroscopy: evolution and future trends. *J Braz Chem Soc* 14:174–182
- Rostagno MA, Villaresa A, Guillaumona E, Garcia-Lafuentea A, Martinez JA (2009) Sample preparation for the analysis of isoflavones from soybeans and soy foods. *J Chromatogr A* 1216:2–29
- Santos WLD, Santos CMMD, Costa JLO, Andrade HMC, Ferreira SLC (2004) Multivariate optimization and validation studies in on-line pre-concentration system for lead determination in drinking water and saline waste from oil refinery. *Microchem J* 77:123–129
- Sharma P, Singh L, Dilbaghi N (2008) Optimization of process variables for decolorization of Disperse Yellow 211 by *Bacillus subtilis* using Box-Behnken design. *J Hazard Mater* 164:1024–1029
- Stickel D (2007) Alaska's non-petroleum corporate income tax, trends in collections by sector and revised corporate income tax forecast model, in: *Alaska Department of Revenue-Tax Division, FTA Revenue Estimation Conference*, September, 2007, pp 1–20
- Wu AH, Yu MC, Tseng CC, Pike MC (2008) Epidemiology of soy exposures and breast cancer risk. *Br J Cancer* 98:9–14
- Yetilmezsoy K, Demirel S, Vanderbei RJ (2009) Response surface modeling of Pb(II) removal from aqueous solution by *Pistacia vera* L.: Box-Behnken experimental design. *J Hazard Mater* 171:551–562