



Optimization of Digestion Method for Determination of Copper in Shrimp Paste Sample Using Flame Atomic Absorption Spectrometry

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Sample preparation using microwave assisted digestion has been a well-known procedure worldwide. Nowadays, experimental designs have been used to facilitate this process. This study uses factorial design approach to study the concentration of copper in shrimp paste sample. The aim of the study was to optimize the digestion procedures for digesting the shrimp paste samples. Flame atomic absorption spectrometry was used to study the copper concentration. DOE software-Design Expert®7.0 was used to interpret all the data obtained. The combination of 4 mL HNO₃, 2 mL of H₂O₂, 0.1 g of sample weight, 1400 W of power and duration of 5 min were found to be optimum in digesting shrimp paste sample for copper analysis.

Keywords: Microwave assisted digestion, Optimization, Factorial design, Copper, Atomic absorption spectrometry, Shrimp paste.

INTRODUCTION

Copper is one of the essential elements [1] that has been found to be important in our biological systems that acts as a cofactor in more than 30 types of enzymes [2]. It involves in iron utilization [2,3], respirations, oxidative stress protection, pigmentation [1] and necessary in hemoglobin synthesis [4]. It also exists in animals, plants and microorganisms. Its deficiency has been identified to cause ischaemic heart disease, anemia and bone disorders. However, copper also poses toxic when its concentration reaches above the permissible limits [2]. Excessive intake of copper always associated with liver cirrhosis, dermatitis and neurological disorders [5-13]. The differences between both permissible and perilous levels are basically very small [6,7]. Therefore, incessant efforts in controlling the heavy metal concentrations on the level of ng g⁻¹ or ng mL⁻¹ in soil, water, plants and food products is of paramount. To achieve this, precise, accurate [6], simple, sensitive, selective and inexpensive [8] analytical methods should be practiced.

Sample preparation has been found to very important step in any analytical procedure. Nowadays, the use of microwave digestion system has been known to improve the process of digestion. It provides shorter time, better recovery, minimal contaminations, lower consumption of reagents and minimal residue or waste [9].

The aim of the study was to optimize the digestion procedures for digesting the shrimp paste samples for copper

analysis. Flame atomic absorption spectrometry was used to study the copper concentration. There were five variables selected to be studied; volume of HNO₃, volume of H₂O₂, weight of sample, power of digestion and time of digestion.

EXPERIMENTAL

A microwave reaction system (Anton Paar Multiwave 3000) was used in this study to digest the sample. The digestate sample solution was then diluted to 50 mL with ultrapure water Mili-Q® System Milipore (PURELAB classic, ELGA). Copper was finally determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 800). Instrumental conditions for the Flame Analysis on the AAnalyst 800 are tabulated in Table-1.

All chemicals were of analytical grade and used as received. 65 % HNO₃ and 30 % H₂O₂ (Analytical grade, Fisher Scientific) were used. Standard solution of copper was prepared by diluting a stock solution of 1000 mg/L supplied by Perkin Elmer. Ultrapure water Mili-Q® System Milipore (PURELAB classic, ELGA) was used throughout the experiment. All glassware and quartz vessels were cleaned by soaking with 10 % (v/v) HNO₃, rinsed with ultrapure water and dried before used. Shrimp paste (belacan) samples purchased from local shops at Nilai, Negeri Sembilan, Malaysia. Samples were dried more than 24 h at 115 to 125 °F using an Excalibur® Food Dehydrator Parallelex (USA). The samples were homogenized using Panasonic blender (MX-335) [9].

TABLE-1
INSTRUMENTAL CONDITIONS FOR THE
FLAME ANALYSIS ON THE AANALYST 80

Element	Copper
Wavelength (nm)	324.8
Slit (nm)	0.7
Mode	AA
Flame	Air-acetylene
Burner	10 cm universal
Calibration	Non-linear through zero
Lamp	HCL
Lamp current (mA)	15
Standards (mg/L)	1.0, 2.0, 3.0, 4.0
Spike concentration (mg/L)	20
Read time (s)	3.0
Replicates	3
Air flow (L/min)	17.0
Acetylene flow (L/min)	2.0

Microwave digestion procedure: This study followed exactly the design procedure from our previous study [9] except for copper element. A half factorial design for five factors 2^{5-1} (16 runs) was developed in order to determine the influence of the factors and their interactions to the copper concentration. Low and high levels of each factor as listed in Table-2 were chosen, which the negative and positive signs showing the low and high levels, respectively. The low and high level values for all variables or factors involved in digestion procedures were as follows: Factor A (volume of HNO_3) were 2 and 4 mL, respectively. Factor B (volume of H_2O_2) were 1 and 2 mL, respectively. Factor C (weight of sample) were 0.1 and 0.5 g, respectively. Factor D (power) were 600 and 1400 W, respectively and factor E (time) were 5 and 10 min, respectively. The two-level fractional factorial design was employed in planning the experiments for studying the effect of the variables mentioned above to the recovery efficiency of copper analyzed in sample.

RESULTS AND DISCUSSION

Table-2 shows the results of copper concentration from 16 experimental runs with the lowest concentration achieved was 3.43 mg/L (experiment 1) and the highest concentration found was 24.5 mg/L (experiment 6). The average of the copper concentration was 10.41 mg/L. The lowest concentration of copper analyzed involved main factors A (HNO_3 volume), D (power) and E (time) at its high levels and the other two main factors B (H_2O_2 volume) and C (sample weight) at its low levels, respectively, while the highest copper concentration of copper analyzed involved main factors A, B and D at its high level and main factors C and E at its low levels.

It is important to identify which main factors or parameters and interactions between 2-factors had the largest effects on the copper concentrations in the samples. Therefore, estimate effects were calculated to achieve the purpose. Table-3 summarized the 15 estimate effects from 16 experiments, alongside sum of squares and percent contribution. The negative sign (-) and positive sign (+) indicates that the effects gave better performance at low levels and high levels, respectively.

Seven estimate effects, having negative signs and eight effects having positive signs indicating that 46.7 % of the total

TABLE-2
CONCENTRATIONS OF COPPER IN SAMPLES
DIGESTED FOLLOWING 16 EXPERIMENTAL
RUNS DEVELOPED EMPLOYING FIVE FACTORS

Exp.	A: HNO_3 volume	B: H_2O_2 volume	C: Sample weight	D: Power	E: Time	Copper (mg/L)
1	+	-	-	+	+	3.43
2	-	-	+	-	-	9.27
3	-	-	-	-	+	13.3
4	-	+	-	-	-	13.5
5	-	-	-	+	-	5.94
6	+	+	-	+	-	24.5
7	-	+	-	+	+	14.7
8	-	+	+	-	+	8.43
9	-	+	+	+	-	9.30
10	+	+	+	+	+	9.05
11	+	-	+	-	+	8.56
12	+	+	-	-	+	13.1
13	+	-	+	+	-	8.81
14	-	-	+	+	+	8.24
15	+	-	-	-	-	7.99
16	+	+	+	-	-	8.39

(+) high level; (-) low level

TABLE-3
SUMMARY OF THE ESTIMATE EFFECT FOR
COPPER CONCENTRATION IN SAMPLES

Model term	Effect estimate	Sum of squares	Contribution (%)
A	0.14	0.083	0.02
B	4.43	78.46	23.1
C	-3.30	43.59	12.83
D	0.18	0.128	0.04
E	-1.11	4.94	1.45
AB	2.13	18.21	5.36
AC	-0.25	0.25	0.07
AD	1.76	12.37	3.64
AE	-2.78	30.83	9.08
BC	-4.36	75.91	22.35
BD	3.35	44.99	13.24
BE	-1.49	8.90	2.62
CD	0.01	0.00	0.00
CE	0.74	2.18	0.64
DE	-2.17	18.86	5.55

estimate effects were found to be better performances at low levels and 53.3 % of the total estimate effects were found to be better performances at high levels for copper analysis.

To diagrammatically observing the effects, a normal probability of the effect was plotted in Fig. 1. In the figure, the estimate effects that were negligible were assumed to be normally distributed, with mean zero and variance σ^2 would tend to fall along a straight line on the plot, whereas significant effects would have nonzero means and would not lie along the straight line [10]. From the figure, it showed that the main factors and 2-interactions which assumed to be significant were factors B, C, E, AB, AD, AE, BC, BD, BE, CE and DE.

Analysis of variance (ANOVA): ANOVA was done to determine the magnitude of the effects. Since the normal probability plot in Fig. 1 was plotted to determine which effects and 2-interaction were assumed to be significant, the ANOVA performed would only include these significant effects to

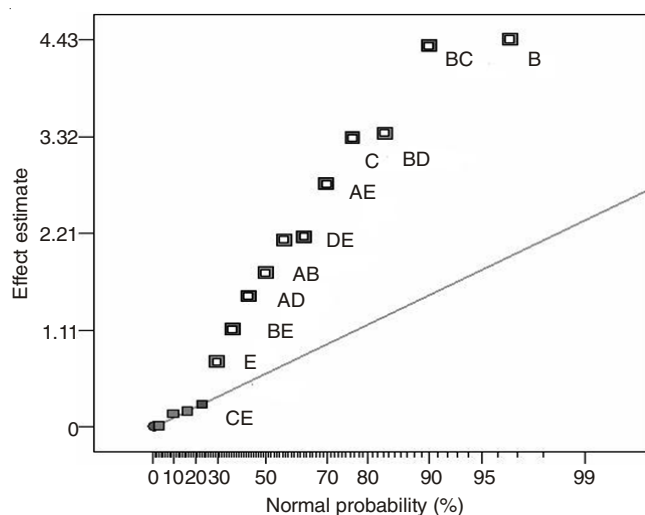


Fig. 1. Normal probability of effect to estimate the copper

ensure the assumptions made were true [10]. Table-4 summarized the ANOVA for copper concentrations. From the table, it could be seen that the model was significant with p -value of < 0.0001 .

TABLE-4
ANALYSIS OF VARIANCE FOR THE
COPPER CONCENTRATION DATA

Source	Sum of squares	df	Mean square	F value	p-value
Model	339.24	11	30.84	266.28	< 0.0001
B	78.46	1	78.46	677.40	< 0.0001
C	43.59	1	43.59	376.39	< 0.0001
E	4.94	1	4.94	42.65	0.0028
AB	18.21	1	18.21	157.24	0.0002
AD	12.37	1	12.37	106.83	0.0005
AE	30.83	1	30.83	266.19	< 0.0001
BC	75.91	1	75.91	655.40	< 0.0001
BD	44.99	1	44.99	388.46	< 0.0001
BE	8.90	1	8.90	76.80	0.0009
CE	2.18	1	2.18	18.85	0.0122
DE	18.86	1	18.86	162.82	0.0002
Residual	0.46	4	0.12		
Total	339.70	15			

Table-5 summarized the observed, predicted and residual values for copper concentrations. The observed values were the actual values obtained from the experiments and the predicted values were obtained from the regression model in eqn. 1. This equation is important to gain the predicted values so that the values can be used to determine the optimum condition for main factors which not significant in the ANOVA. The residual values were the difference between both observed and predicted values [10]. It could be shown that the lowest and highest predicted values were achieved in experiments 1 and 6 with the values of 3.14 and 24.24 mg/L, respectively. The average for predicted value was also 10.41 mg/L and similar with the average of observed values, meaning that the regression model used in obtaining the predicted copper concentrations was true.

$$\hat{y}_{\text{copper}} = 10.41 + (2.21)x_2 - (1.65)x_3 - (0.56)x_5 + (1.07)x_1x_2 + (0.88)x_1x_4 - (1.39)x_1x_5 - (2.18)x_2x_3 + (1.68)x_2x_4 - (0.75)x_2x_5 + (0.37)x_3x_5 - (1.09)x_4x_5 \quad (1)$$

TABLE-5
OBSERVED, PREDICTED AND RESIDUAL
VALUES FOR COPPER CONCENTRATION

Exp.	Observed value, y	Predicted value, \hat{y}	Residual, $e = y - \hat{y}$
1	3.43	3.14	0.29
2	9.27	9.32	-0.05
3	13.3	13.60	-0.30
4	13.5	13.78	-0.28
5	5.94	6.06	-0.12
6	24.5	24.24	0.26
7	14.7	14.80	-0.10
8	8.43	8.46	-0.03
9	9.30	9.16	0.14
10	9.05	9.00	0.05
11	8.56	8.72	-0.16
12	13.1	12.98	0.12
13	8.81	8.78	0.03
14	8.24	8.10	0.14
15	7.99	7.88	0.11
16	8.39	8.54	-0.15

where 10.41 is the average from the total 16 observations and the x_2 represents B_{effect} , x_3 represents C_{effect} , x_5 represents E_{effect} , x_1x_2 represents $AB_{\text{interaction}}$, x_1x_4 represents $AD_{\text{interaction}}$, x_1x_5 represents $AE_{\text{interaction}}$, x_2x_3 represents $BC_{\text{interaction}}$, x_2x_4 represents $BD_{\text{interaction}}$, x_2x_5 represents $BE_{\text{interaction}}$, x_3x_5 represents $CE_{\text{interaction}}$ and x_4x_5 represents $DE_{\text{interaction}}$. \hat{y}_{copper} represents the predicted copper concentration.

Although there were eight 2-interactions were found to be significant in ANOVA, it was not necessarily to study all the interactions, because the main factors B, C and E were found significant at their particular levels in which factor B was optimized at high level, factor C was optimized at low level and factor E was optimized at low level, respectively. To help in understanding of this situation, the response surface and contour plots was employed to find the optimum condition for factors A and D which found insignificant in ANOVA study. Thus, response surface and contour plots of AB, BD and CE were only selected.

In response surface of AB interaction in Fig. 2, horizontal axes represent the factors of A and B whereas vertical axis represents the predicted copper concentration. It could be observed that the highest predicted concentration could be found at the area where both factors at high levels. It confirmed that factor B was best performed at high level. Factor A also could be assumed better at high level.

In contour plot of AB interaction, horizontal axes represent the factor A and copper concentration and vertical axis represents the factor B. The predicted values increased from bottom to top of factor B which from low to high level of the factor. The increased values of the concentration are mutually with the increasing value of factor A from low to high level. It confirmed that factor A also was optimum at high level.

In response surface of BD interaction in Fig. 3, horizontal axes represent the factors of B and D whereas vertical axis represents the predicted copper concentration. The highest predicted value of copper could be found at both high levels of factors B and D.

In contour plot of BD interaction, horizontal axes represent the factor B and copper concentration and vertical axis

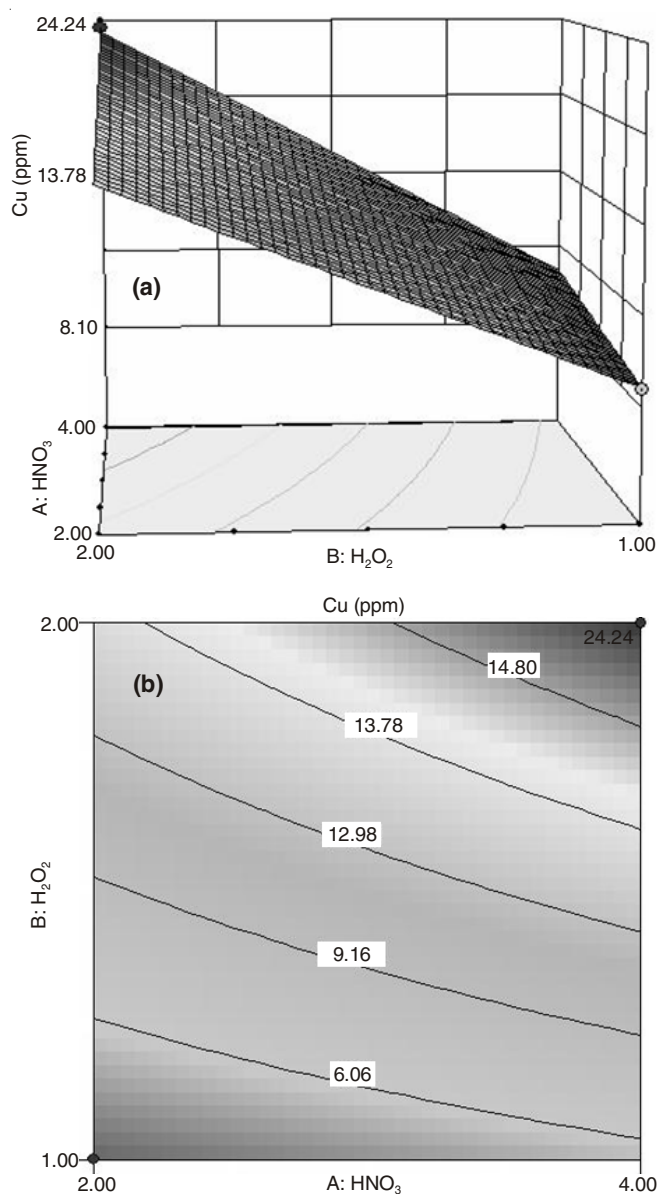


Fig. 2. (a) Response surface for AB interaction (b) Contour plot of AB interaction

represents the factor D. The highest concentration was achieved at both factors at high levels which predicted values displayed ascending order from left to right and bottom to top of factor B and D, respectively. It confirmed that factor D was optimum at high level.

In response surface of CE in Fig. 4, horizontal axes represent the factors of C and E whereas vertical axis represents the predicted copper concentration. Both factors were found significant in ANOVA study. This figure would only show how both factors was optimum in working at low levels.

In contour plot of CE interaction, horizontal axes represent the factor C and copper concentration and vertical axis represents the factor E. the predicted values was arranged in ascending order from right to left and top to bottom of factor C and E, respectively.

Hence, 4 mL HNO₃ (A: high level), 2 mL (B: high level), 0.1 g of sample weight (C: low level), 1400 W of power (D: high level) and duration of 5 min (E: low level) were

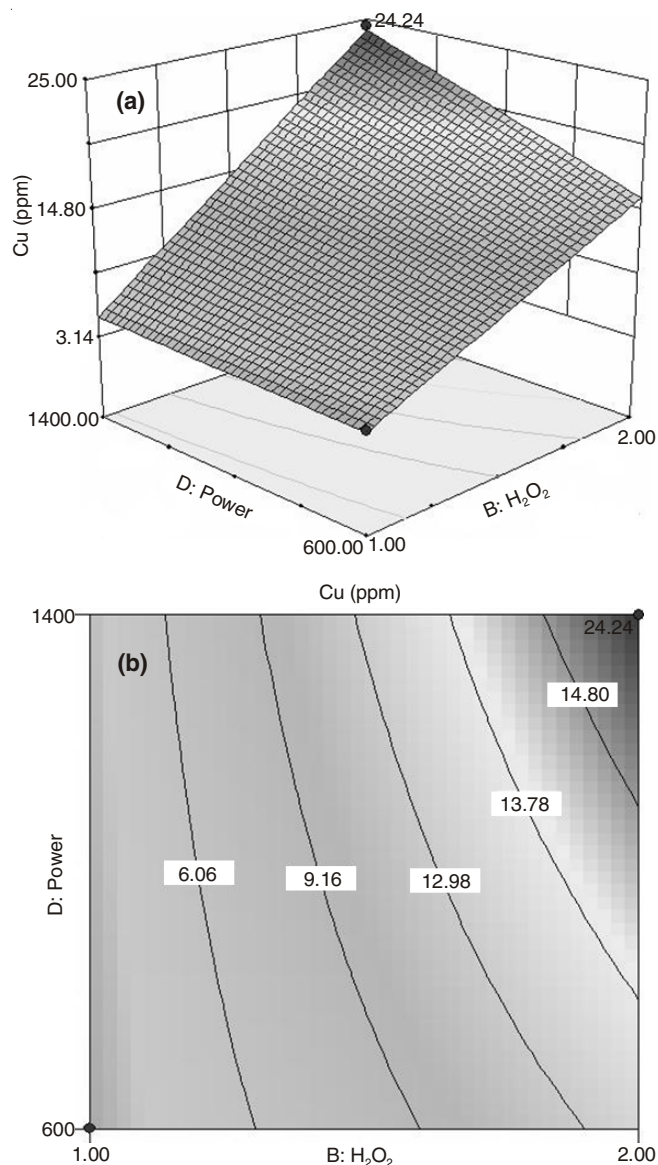


Fig. 3. (a) Response surface of BD interaction (b) contour plot of BD interaction

recommended to be the optimum parameters in digesting shrimp paste sample for copper analysis.

Recovery studies: Optimum digestion parameters from the previous discussion were used to conduct recovery studies of copper analysis. This experiment was intentionally done to ensure that the optimization parameters are valid to be used in an experiment. To meet the purpose, seven shrimp paste samples were randomly purchased and digested following the optimization parameters set for copper element concluded from the previous study.

Following stringent samples preparation procedures as described earlier in the experimental section, duplicate of individual samples were spiked with 20 mg/L of the standard copper element and undergo the digestion procedures devised accordingly based on the findings in the earlier discussion.

Table-6 shows the results of the recovery studies for copper. This study showed that the method had attained good recoveries for seven samples studies. It meant that the optimum condition was applicable to be used in any experiments.

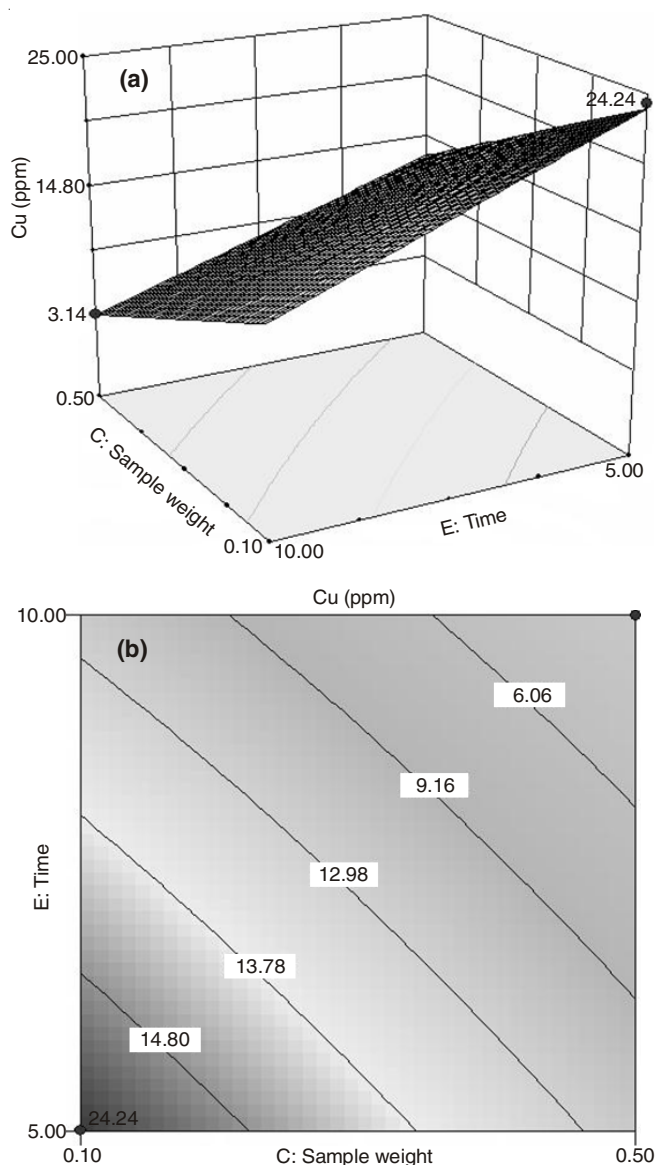


Fig. 4. (a) Response surface of CE interaction (b) Contour plot CE interaction

TABLE-6
RESULT OF RECOVERY STUDIES ON COPPER

Recovery 1 (%)	Recovery 2 (%)	Mean (%)	SD ^a	RSD (%) ^b
93.40	92.95	93.18	0.32	0.34
104.04	103.32	103.68	0.51	0.46
99.35	99.24	99.30	0.08	0.08
97.02	99.70	98.36	1.90	1.93
103.77	103.18	103.48	0.42	0.40
98.10	99.01	98.56	0.64	0.65
106.37	107.97	107.17	1.13	1.05

^aStandard deviation, ^bRelative standard deviation

The precision of the replicate analyses of recoveries were defined as relative standard deviation, which is calculated from standard deviation divided by the mean and multiplied by hundred. It could be found that relative standard deviation values for all elements were lower than or equal to 5 % showing that the precision of recovery studies were very good [9].

Conclusion

Factorial designs approaches have facilitated many researchers to study the combinations of parameters simultaneously specially in sample preparations. In this study, ANOVA was able to determine significant effects of both main factors and 2-interaction factors. The study indicates that factors B (H₂O₂ volume), C (sample weight) and E (time of digestion) were found to be significantly different between low and high levels, respectively. Regression model equation was important to predict the copper concentration in the samples. By employing the response surface and contour plots it was possible to evaluate the influence of each variables and the combination of variables in the analysis of copper. It was demonstrated that all parameters involves are important in optimizing the digestion method for determination of copper in shrimp paste sample. From this study, it is concluded that the optimum digestion parameters for determination of copper are 4 mL HNO₃, 2 mL H₂O₂, 0.1 g of sample weight, 1400 W power and 5 min digestion time. Thus any sample consist of similar sample matrices such as used here could apply this operating parameter in preparation of sample using microwave digestion method.

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REFERENCES

1. K. Balamurugan, D. Egli, H. Hua, R. Rajaram, G. Seisenbacher, O. Georgiev and W. Schaffner, *EMBO J.*, **26**, 1035 (2007).
2. S.H. Abbasi, H. Khani and R. Tabaraki, *Food Chem.*, **123**, 507 (2010).
3. U. Celik and J. Oehlenschlager, *Food Contr.*, **18**, 258 (2007).
4. P. Sivaperumal, T. Sankar and P. Viswanathannair, *Food Chem.*, **102**, 612 (2007).
5. M.M. Storelli, G. Barone, R. Garofalo and G.O. Marcotrigiano, *Food Chem.*, **100**, 1337 (2007).
6. J.W. Chwastowska, E. Skwara, J. Sterlinska and M. Dudek, *Chem. Anal. (Warsaw)*, **53**, 887 (2008).
7. V.A. Lemos, D.R. Vieira, C.G. Novaes, M.E. Rocha, M.S. Santos and R.T. Yamaki, *Mikrochim. Acta*, **153**, 193 (2006).
8. D. Citak and M. Tuzen, *Food Chem. Toxicol.*, **48**, 1399 (2010).
9. C.W.Z.C.W. Ngah and M.A. Yahya, *Food Chem.*, **134**, 2406 (2012).
10. D.C. Montgomery, *Design and Analysis of Experiments*, John Wiley & Sons, USA, edn 8 (2001).
11. H.M. Zalloum, Z. Al-Qodah, M.S. Mubarak, *J. Macromol. Sci., Part A*, **46**, 46 (2008).
12. M.A. Yahya, Z. Al-Qodah and C.W.Z. Ngah, *Renew. Sustain. Energy Rev.*, **46**, 218 (2015).
13. Z. Al-Qodah, M. Al-Busoul and M. Al-Hassan, *Powder Technol.*, **115**, 58 (2001).