http://dx.doi.org/10.5935/0103-5053.20130101

J. Braz. Chem. Soc., Vol. 24, No. 5, 771-776, 2013.
Printed in Brazil - ©2013 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Chemometric Methods Applied to the Mineral Content Increase in Chocolate Cakes Containing Chia and Azuki

Aline K. Gohara,[a] Aloisio H. P. Souza,[a] Ângela C. Rodrigues,[c] Gisely L. Stroher,[d] Sandra T. M. Gomes,[b] Nilson E. Souza,[e] Jesuí V. Visentainer[b] and Makoto Matsushita*,[b]

[a]Center of Agricultural Sciences and [b]Department of Chemisty, State University of Maringá, Av. Colombo, 5790, 87020-900 Maringá-PR, Brazil

[c]Federal Technological University of Paraná, Av. Brasil, 4232, 85884-000 Medianeira-PR, Brazil

[d]Federal Technological University of Paraná, Department of Chemistry, Rua Marcílio Dias, 635, 85884-000 Apucarana-PR, Brazil

[e]Federal Technological University of Paraná, Av. Pioneiros, 3131, 86036-370 Londrina-PR, Brazil

Um planejamento fatorial completo 2^2 (dois fatores em dois níveis) com duplicata foi realizado para investigar a influência das porcentagens de chia e azuki (os dois fatores) adicionados ao bolo de chocolate isento de glúten no teor mineral (Ca, Cu, Fe, K, Mg, Mn e Zn). No estudo foi utilizada farinha parcialmente desengordurada de chia e farinha integral de azuki. Os fatores porcentagem de chia e azuki foram significativos, e os aumentos destes valores contribuíram positivamente para as respostas. O efeito de interação não foi significativo para as respostas de Ca, Fe, Mg e Zn. A análise de componentes principais (PCA) distinguiu as amostras com teor mais elevado de chia através do PC1, e PC2 separou as formulações de teores mais elevados de azuki das de menores teores. Através de análise de variância (ANOVA) e de superfícies de respostas, foi possível concluir que o aumento no teor mineral foi maior com a adição de 20% de ambas as farinhas.

A 2^2 full factorial design (two factors at two levels) with duplicate was performed to investigate the influence of the percentages of chia and azuki (the two factors) added to gluten-free chocolate cake on the mineral contents (Ca, Cu, Fe, K, Mg , Mn and Zn). In the study, partially defatted chia flour and wholemeal azuki flour were used. The percentage factors of chia and azuki were significant and the increases in these values contributed positively to the responses. The interaction effect was not significant for the Ca, Fe, Mg and Zn responses. The principal component analysis (PCA) distinguished samples with higher content of chia by means of PC1, and PC2 separated the formulations with the highest levels of azuki from the ones with the lowest levels. Using analysis of variance (ANOVA) and response surface analyses, it was possible to conclude that the increase in mineral content was higher with the addition of 20% of both flours.

Keywords: *Salvia hispanica* L., *Vigna angularis*, response surface methodology, principal component analysis

Introduction

Current changes in the world population reflect drastic transformations in many aspects of life, especially in food consumption. Presently, most foods do not have the minimum nutrients essential for the maintenance of human health, a fact that has aroused interest, mainly by the food industries, in developing enriched foods with functional appeal.

Cakes ready for consumption have been acquiring great prominence among bakery products[1] since they are heavily marketed and hold second position as the most consumed product in this category, ranking only behind bread. There are gluten-free versions that can be consumed by celiac patients; however, these are still poor in many nutrients because they are composed primarily of rice flour.[2]

Azuki (*Vigna angularis*) is a legume widely produced and consumed in Asia, used in the manufacture

*e-mail: mmakoto@uem.br

of various products, especially in typical sweets.[3-6] Chia (*Salvia hispanica L.*) is an angiosperm plant from the mint family (*Lamiaceae*) characterized as a grain from tropical and subtropical climates and widely consumed in pre-Columbian America by the Aztecs, in the region that includes Mexico and Guatemala.[7,8] Both grains, azuki and chia, are considered rich sources of many nutrients essential for maintenance of good health.[9-12]

One of the most relevant aspects regarding nutrients is their mineral composition. Several minerals are essential for the maintenance of biological systems because they participate in metabolic reactions as cofactors. In celiac patients, mineral intake must be higher because they present a lower absorption of nutrients due to the inflammatory process in the small intestine caused by the disease. Consumption of foods rich in minerals may reduce the risk of coronary heart disease, anemia, osteoporosis and prostate cancer by boosting the immune system.[13]

The aim of this study was to apply chemometric method to investigate the influence of the percentage factors of chia and azuki added to gluten-free chocolate cakes for determinations of the Ca, Cu, Fe, K, Mg, Mn and Zn minerals.

Experimental

Sampling

The azuki grain used in this study was cultivated in the region of Maringá city (Paraná state, Brazil) and purchased at the local market. Approximately 6 kg of grain were ground in a hammer mill to obtain homogeneous flour that was sieved using a 20 mesh sieve. The chia flour used in this study was partially defatted since it was a byproduct of the oil extraction process by cold pressing. The latter ingredient was supplied by the Giroil Agroindustria Ltda. (Santo Angelo, Rio Grande do Sul state, Brazil) company. The other ingredients were obtained at retail stores in Maringá.

Experimental design

A 2^2 full factorial design (two factors at two levels) with duplicate was performed to investigate the influence of two factors on the mineral composition of chocolate cakes. The two factors were the concentrations of azuki and chia flours, as shown in Table 1. The total flour in each formulation was kept constant by adding rice flour. The responses analyzed were the Ca, Cu, Fe, K, Mg, Mn and Zn concentrations.

Table 1. The factors investigated and the levels used for the development of a 2^2 full factorial design with duplicates

Factor	Unit	Symbol	Type	Level	
				–1	+1
Chia flour	%	C	numeric	10	20
Azuki flour	%	A	numeric	10	20

Development of the cakes

All ingredients were previously weighed separately. The rice, azuki and chia flours, at the respective percentage for each formulation, were mixed to obtain a homogeneous fraction (28.80% of the whole formulation); egg white (8.70%) was then added to the mix to form a solid phase. The egg yolk (5.80%), butter (5.80%) and sugar (16.90%) were then homogenized to form a cream to which the mixture of flour, chocolate powder (8.00%), cocoa powder (3.80%), egg whites, water (19.08%), milk powder (2.12%) and baking powder (1.00%) were slowly added to form a homogeneous mass. The cake mass was transferred to a rectangular baking dish and baked in a conventional oven for 30 min at 200 °C, with subsequent cooling to room temperature (25 °C).

Mineral quantification

For the mineral composition analysis, the samples were digested by the dry method according to the Association of Official Agricultural Chemists (AOAC).[14] All samples were calcined in a muffle furnace at 550 ºC for 6 h and recovered with nitric acid solution (5% v/v). Ca, Cu, Fe, K, Mg, Mn and Zn were quantified in an atomic absorption spectrophotometer AA240FS (Varian, USA) as mg of mineral *per* 100 g of product using technical parameters of calibration according to Table 2.

Table 2. Technical parameters of the calibration for the atomic absorption spectrophotometer

Element	Wavelength / nm	Spectral bandwidth / nm	Method
Ca	239.9	0.2	flame - acetylene/nitrous oxide
Cu	324.8	0.5	flame - acetylene
Fe	248.3	0.2	flame - acetylene
K	404.4	0.5	flame - acetylene
Mg	202.6	1.0	flame - acetylene
Mn	279.5	0.2	flame - acetylene
Zn	213.9	1.0	flame - acetylene

Statistical analysis

All analyses were carried out in triplicate. Initially, values for the main effects, interactions and ANOVA were obtained. Thereafter, all variables had their normality and homogeneity of variance assessed by residual plots. Then, analysis of variance (ANOVA between groups) was performed for all the answers. To evaluate the effect of independent variables on the responses, response surface methodology (RSM) was applied. The basic model equation used to fit the data was:

$$E(y) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (1)$$

where E(y) is the expected response, β_0 is a constant, β_1, β_2, β_{11}, β_{22} and β_{12} are regression coefficients, and x_1, x_2 are the levels of independent variables.[15]

The multivariate analysis was performed by applying principal component analysis (PCA) in order to facilitate the selection of the optimal region. Means of the analyses in triplicate of two cakes for each formulation were used to compose the responses. Means were auto-scaled, so all variables presented the same weight, and two-dimensional graphs of PCA were obtained. All statistical analyses were conducted using Statistica software version 7.0 (StatSoft, USA) with a 5% ($p < 0.05$) significance level for rejection of the null hypothesis.

Results and Discussion

The concentrations of the metals contained in chia and azuki flours are presented in Table 3.

Table 3. Concentrations of the metals in the chia and azuki flours

Element	Azuki flour / (mg *per* 100 g of sample)	Chia flour / (mg *per* 100 g of sample)
Ca	95.60 ± 1.86	889.21 ± 37.63
Cu	0.47 ± 0.01	1.93 ± 0.03
Fe	6.05 ± 0.27	4.85 ± 0.20
K	1282.26 ± 20.18	9399.08 ± 37.34
Mg	85.75 ± 1.82	255.74 ± 9.07
Mn	0.49 ± 0.02	1.83 ± 0.15
Zn	2.05 ± 0.05	6.94 ± 0.12

The equations for each model along with their coefficients of regression (R^2) are listed in Table 4. The data belonging to independent variables and the responses were analyzed to acquire linear regression equations (Table 4). The values for each main effect, the interactions between these effects and also the percentage contribution of each effect to the model were analyzed using ANOVA.

Table 4. Mathematical equations for all the responses applying the response surface model

Parameter	Equation	R^2
Ca	$Ca = 205.43 + 13.56 x_1 + 3.59 x_2 - 0.50 x_1 x_2$	0.985
Cu	$Cu = 0.39 + 0.03 x_1 + 0.03 x_2 - 0.02 x_1 x_2$	0.966
Fe	$Fe = 2.22 + 0.14 x_1 + 0.08 x_2 - 0.03 x_1 x_2$	0.977
K	$K = 333.04 + 18.03 x_1 + 21.67 x_2 - 12.67 x_1 x_2$	0.999
Mg	$Mg = 36.44 + 3.58 x_1 + 1.48 x_2 - 7.00 \times 10^{-3} x_1 x_2$	0.996
Mn	$Mn = 0.88 + 0.04 x_1 + 5.69 \times 10^{-3} x_2 + 5.69 \times 10^{-3} x_1 x_2$	0.995
Zn	$Zn = 1.35 + 0.10 x_1 + 0.02 x_2 - 6.08 \times 10^{-3} x_1 x_2$	0.989

x_1: chia; x_2: azuki; R^2: coefficient of regression.

Table 5 shows the conditions of the 2^2 factorial model (in duplicate), applied to the experiments, and the values obtained for all the studied responses: Ca, Cu, Fe, K, Mg, Mn and Zn as mg *per* 100 g of sample.

The graphs of the residuals for each response indicated that the data exhibited very satisfactory normality and

Table 5. Parameters of the 2^2 full factorial design (in duplicate) and the responses obtained in the experiments

Assay	Independent variable		Response / (mg *per* 100 g of sample)						
	Numeric level								
	Chia / %	Azuki / %	Ca	Cu	Fe	K	Mg	Mn	Zn
1	10	10	187.03	0.32	1.99	281.60	31.28	0.83	1.24
2	10	10	188.55	0.32	1.94	279.34	31.46	0.84	1.22
3	20	10	217.88	0.41	2.28	343.34	38.89	0.92	1.44
4	20	10	213.92	0.41	2.33	341.19	38.21	0.91	1.43
5	10	20	198.58	0.41	2.19	349.04	34.30	0.83	1.26
6	10	20	193.35	0.42	2.18	350.05	34.38	0.84	1.30
7	20	20	222.86	0.44	2.38	358.78	41.12	0.94	1.46
8	20	20	221.29	0.41	2.45	360.97	41.86	0.93	1.46

Table 6. ANOVA results, *F*-test and *p*-value for the responses studied in the 2^2 factorial modeling

Source	*F*-test						
	Ca	Cu	Fe	K	Mg	Mn	Zn
Regression	87.84	38.37	57.53	1320.14	306.02	239.90	117.56
Chia	245.94	42.95	125.59	1340.94	784.30	695.92	340.45
Azuki	17.23	45.57	42.36	1936.75	133.77	11.89	10.86
Chia × Azuki	0.34	26.60	4.65	682.72	3.00×10^{-3}	11.89	1.38
Source	*p*-value						
	Ca	Cu	Fe	K	Mg	Mn	Zn
Regression	0.0004	0.0021	0.0010	< 0.0001	< 0.0001	< 0.0001	0.0002
Chia	< 0.0001	0.0028	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Azuki	0.0142	0.0025	0.0029	< 0.0001	0.0003	0.0261	0.0301
Chia × Azuki	0.5928	0.0067	0.0974	< 0.0001	0.9590	0.0261	0.3053

homogeneity of variance, showing that all models were significant and indicated no significant lack of fit. The R^2 value and the *F*-value for each model, obtained by ANOVA and shown in Tables 4 and 6, respectively, also indicate the positive significance of the models.

Table 7 shows the values of the main effects and their interactions for all the responses. Tables 8 and 6 present the results obtained by ANOVA analysis for each of the responses studied in duplicate in the 2^2 full factorial design.

Analyzing Table 7, it is possible to observe that the interaction chia × azuki was negative for most of the responses. Table 8 indicates that the contributions for Fe, Mg, Mn and Zn were less than 3%. Although this result did not significantly affect these responses, this factor allows a better 'straightening' of the linear model, whereas its absence could compromise the R-squared value.

Table 7. Main and interaction effects calculated for the 2^2 factorial design

Response	Effect		
	Chia	Azuki	Chia × Azuki
Ca	27.11	7.18	–1.00
Cu	0.05	5.61×10^{-3}	–0.04
Fe	0.28	0.16	–0.05
K	36.06	43.34	–25.73
Mg	7.16	2.96	–0.01
Mn	0.09	0.01	0.01
Zn	0.19	0.03	–0.01

The ANOVA results (Table 8) indicate that the main factors were significant for all the responses, and that the interaction between the main factors was not significant for the Ca, Fe, Mg and Zn responses.

Table 8. ANOVA results, sum of square and mean square for the responses studied in the 2^2 factorial modeling

Source	DF	Sum of square						
		Ca	Cu	Fe	K	Mg	Mn	Zn
Regression	3	1574.94	0.01	0.22	7682.47	120.07	0.02	0.08
Chia	1	1469.92	5.29×10^{-3}	0.16	2601.18	102.58	0.02	0.07
Azuki	1	103.00	5.61×10^{-3}	0.05	3756.94	17.50	2.59×10^{-4}	2.33×10^{-3}
Chia × Azuki	1	2.01	3.27×10^{-3}	5.90×10^{-3}	1324.35	3.92×10^{-4}	2.59×10^{-4}	2.96×10^{-4}
Pure error	4	23.91	4.93×10^{-4}	5.08×10^{-3}	7.76	0.52	8.72×10^{-5}	8.59×10^{-4}
Total	7	1598.85	0.02	0.22	7690.23	120.60	0.02	0.08
Source		Mean square						
		Ca	Cu	Fe	K	Mg	Mn	Zn
Regression		524.98	4.72×10^{-3}	0.07	2560.82	40.02	5.23×10^{-3}	0.03
Chia		1469.92	5.28×10^{-3}	0.16	2601.18	102.58	0.02	0.07
Azuki		103.00	5.61×10^{-3}	0.05	3756.94	17.50	2.59×10^{-4}	2.33×10^{-3}
Chia × Azuki		2.01	3.27×10^{-3}	5.90×10^{-3}	1324.35	3.92×10^{-4}	2.59×10^{-4}	2.96×10^{-4}
Pure error		5.98	1.23×10^{-4}	1.27×10^{-3}	1.94	0.13	2.18×10^{-5}	2.15×10^{-4}

DF: degree of freedom.

The percentage of chia was the factor that contributed the most to the majority of the mineral responses, except for potassium as shown in Table 7. In Figure 1, principal component 1 (PC1), which explains 78.43% of the data variance, was able to distinguish the samples with the highest level of chia from the others. This was possible due to the loadings (Figure 1a), which showed all the minerals contributing to the scores (Figure 1b) of samples 3, 4, 7 and 8.

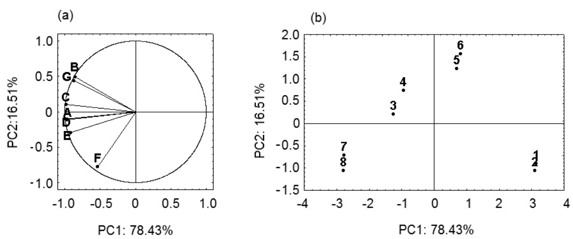

Figure 1. Principal component analysis for the responses studied in the 2^2 factorial design: (a) loadings and (b) scores (Ca (A), Cu (B), Fe (C), K (D), Mg (E), Mn (F) and Zn (G)).

By analyzing PC1 *vs.* PC2 (Figures 1a and 1b), there is a new separation through the quadrants. The samples with the highest concentration of chia and the lowest concentration of azuki (samples 3 and 4, respectively) are explained by by copper, iron and potassium. According to the ANOVA table (Table 8), the percentage contributions of the chia variable for Cu, Fe and K were 52.9, 73.73 and 33.86%, respectively. The azuki variable had percentage contributions of 56.10% (Cu), 22.73% (Fe) and 48.90% (K). The interaction effect had the least influence, and the table of ANOVA indicated that there was a positive contribution by this effect, increasing the values of the responses (less than 33%).

Response surfaces were constructed for the independent variables and levels, as shown in Figure 2. Analyzing the response surfaces (Figure 2), table of effects (Table 7) and principal component, due to the arrangement of samples 7 and 8 in PC1 and PC2 (Figures 1a and 1b), it could be determined that the increase in the chia and azuki flour concentrations evidenced a greater incorporation of the Cu, Fe, K and Mg minerals. According to the quadrant analysis in Figure 1a (loadings), the contents of Ca, Mg, Mn and Zn contributed to the separation of the cakes containing the highest concentration of chia and azuki (Figure 1b). According to Capitani *et al.*,[16] partially defatted chia flour can be considered an excellent source of minerals, but information about these compounds in azuki beans were not found in the literature. According to researchers and the organizers of TACO (Tabela Brasileira de Composição de Alimentos),[17] to achieve food and nutrition security, it is essential that the population knows the composition of the consumed food. TACO is one of the best references for food composition and analysis in Brazil. As chia and azuki become more and more ubiquitous in Brazilian diets, they could be incorporated in this table. Through the 2^2 factorial design, it was determined that higher concentrations of chia and azuki introduced higher mineral contents in gluten-free chocolate cakes. According to the results obtained in this study, it is evident that these grains are good alternatives to substitute common flours in food products, including gluten-free foods for celiac patients.

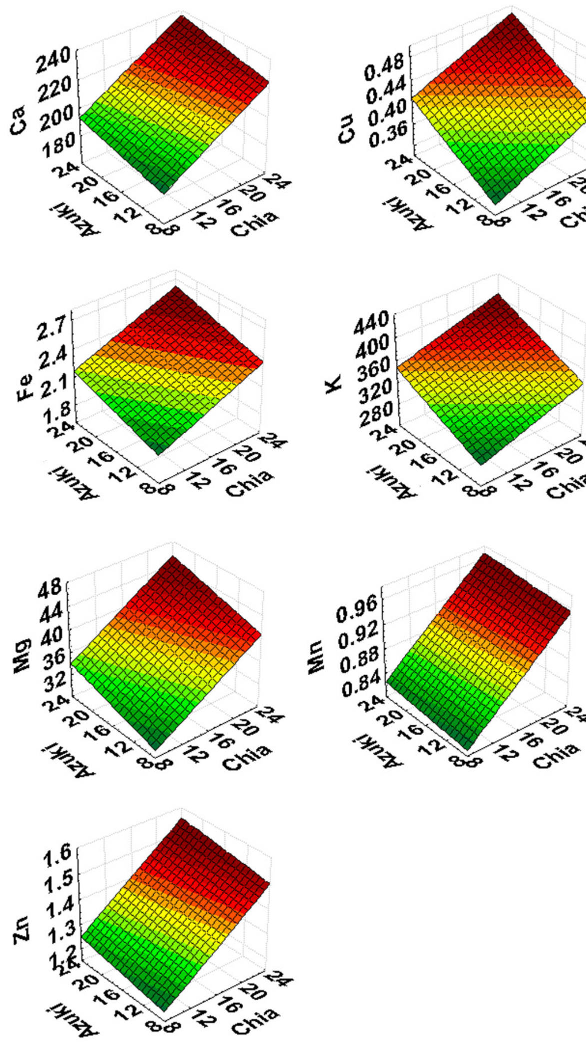

Figure 2. Response surfaces for the contents of Ca, Cu, Fe, K, Mg, Mn and Zn in mg *per* 100 g of sample according to the percentages of chia and azuki.

Conclusions

The factorial design conducted to incorporate minerals in chocolate cake showed that the factors with the highest percentage of chia and azuki flour were significant, and the increase of these factors contributed positively to the Cu, Fe, K and Mg responses. However, the maximum and minimum levels of azuki did not influence the increase in the Ca, Mn and Zn minerals. The interaction effect was not significant only for the Ca, Fe, Mg and Zn responses. PCA distinguished samples with a higher content of chia by means of PC1, and PC2 separated those formulations with the highest level of azuki from that with the lowest level. ANOVA and response surface analyses showed that the highest mineral contents were found in the formulations with the highest concentrations of both flours. These grains are good alternative for common flours in food products, including gluten-free foods to increase their mineral contents. However, studies must still be performed to evaluate the bioavailability of microelements in these foods.

Acknowledgments

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação Araucária for the financial support and the Complexo Central de Apoio à Pesquisa (COMCAP, State University of Maringá) for the availability of resources and technology for the development of this research.

References

1. Osawa, C. C.; Fontes, L. C. B.; Miranda, E. H. W.; Chang, Y. K.; Steel, C. J.; *Cienc. Tecnol. Aliment.* **2009**, *29*, 92.
2. Lee, A. R.; Ng, D. L.; Zivin, J.; Green, P. H.; *J. Hum. Nutr. Diet.* **2007**, *20*, 423.
3. Kubota, K.; *J. Fac. Appl. Biol. Sci.* **1979**, *18*, 161.
4. Sperbeck, J.; *New Farm*. **1981**, *3*, 48.
5. Hoshikawa, K. In *Edible Crops*, vol. 1, 2nd ed.; Chiyachin, K.; Ogata, K., eds.; Yokendou: Tokyo, Japan, 1985.
6. Shi, Y. In *National Cooperating Symposium of Reproduction and Selection for Edible Legume Germplasm Resources and Identification of Agronomic Characteristics*, vol. 1, 1st ed.; Long, L.; Lin, L.; Xushen, H.; Shi, Y., eds.; Science Publishing House: Beijing, China, 1988.
7. Ayerza, R.; *J. Am. Oil Chem. Soc.* **1995**, *72*, 1079.
8. Cahill, P. J.; *Genet. Res. Crop. Evol.* **2004**, *51*, 773.
9. Hsieh, H. M.; Pomeranz, Y.; Swanson, B. G.; *Cereal Chem.* **1992**, *69*, 244.
10. Tjahjadi, C.; Lin, S.; Breene, W. N.; *J. Food Sci.* **1988**, *53*, 1438.
11. Tosco, G.; *Atual. Ornit.* **2004**, *119*, 7.
12. Weber, C. W.; Gentry, H. S.; Kohlhepp, E. A.; McCrohan, P. R.; *Ecol. Food Nutr*. **1991**, *26*, 119.
13. Hathcock, J. N.; *Vitamin and Mineral Safety*, vol. 1, ed. 2; Council for Responsible Nutrition: Washington, USA, 2004.
14. Association of Official Agricultural Chemists (AOAC); *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th ed.; AOAC: Arlington, USA, 1995.
15. Granato, D.; Bigaski, J.; Castro, I. A.; Masson, M. L.; *Food Chem*. **2010**, *121*, 899.
16. Capitani, M. I.; Spotorno, V.; Nolasco, S. M.; Tomás, M. C.; *LWT--Food Sci. Technol*. **2012**, *45*, 94.
17. http://www.unicamp.br/nepa/taco accessed in January 2013.

Submitted: November 28, 2012
Published online: April 26, 2013