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Programa de Pós-Graduação em Ciência de Alimentos

DESENVOLVIMENTO E CARACTERIZAÇÃO DE BOLO DE CHOCOLATE ISENTO DE GLÚTEN, CONTENDO AZUKI (*VIGNA ANGULARIS*) E CHIA (*SALVIA HISPANICA*, L.)

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Dissertação apresentada ao Programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá para obtenção do grau de Mestre em Ciência de Alimentos.

Orientador: Prof. Dr. Makoto Matsushita

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Orientador

Prof. Dr. Makoto Matsushita

BIOGRAFIA

Aline Kirie Gohara é natural de Bastos, no estado de São Paulo, Brasil. Iniciou seus estudos em 1994, na Escola Municipal de Ensino Infantil de Bastos, passando por mais 7 escolas, devido a mudanças de cidade, até concluir o Ensino Médio.

Em meados de 2005, passou no Vestibular da Universidade Estadual de Maringá, ingressando no curso de Engenharia de Alimentos no ano seguinte. Durante a graduação, participou de projetos de Ensino, Extensão e Iniciação Científica.

No ano de 2010 formou-se Engenheira de Alimentos e já em 2011 iniciou seus estudos no Mestrado em Ciência de Alimentos na mesma Universidade, adquirindo experiência em análises físico-químicas, microbiologias e sensoriais em alimentos, além do desenvolvimento de alimentos com propriedades funcionais para grupos específicos.

DEDICO

À minha amada mãe, amiga para todas as horas,
Ao meu pai (*in memorian*),
Ao meu tio Ricardo, um verdadeiro pai e amigo,
À minha querida avó Helena,
Aos meus irmãos e companheiros Alex e Anderson,
que compartilho os meus momentos de alegria e de dificuldade.
Obrigada pelo carinho, confiança, paciência, compreensão e apoio nesta fase.
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APRESENTAÇÃO

Esta dissertação de mestrado está apresentado na forma de 2 artigos científicos.

1 Aline K. Gohara, Aloisio H. P. Souza, Ângela C. Rodrigues, Gisely L. Stroher, Sandra T. M. Gomes, Nilson E. Souza, Jesuí V. Visentainer, Makoto Matsushita**. Chemometric Methods Applied on the Increasing of Mineral Content in Chocolate Cakes Containing Chia and Azuki.** Journal of the Brazilian Chemical Society (Qualis B1).

2 Aline Kirie Gohara, Aloisio Henrique Pereira de Souza, Ana Beatriz Zanqui, Nilson Evelázio de Souza, Jesuí Vergílio Visentainer, Makoto Matsushita. **Chemometric tools applied on development and proximal and sensory characterization of chocolate cakes containing chia and azuki.** Acta Scientiarum Technology (Qualis B2).

Crom Alimentos
Grupo de Pesquisa

GENERAL ABSTRACT

INTRODUCTION. The current changes in the daily of world population reflect in drastic transformations of many aspects, especially in food consumption. Nowadays, most of foods do not have the minimum nutrients essential to the maintenance of human health, fact that aroused interest in enriched food developing and with functional appeal, mainly by food industries. The cakes ready for consumption have been acquiring great importance among the bakery products, since they are largely marketed, and hold the second position of the most consumed product ranking in this category, behind only of bread. There are gluten-free versions which can be consumed by celiac patients, however, these are still poor in many nutrients because they are composed primarily of rice flour. Azuki (Vigna angularis) is a legume widely produced and consumed in Asia,

used in the various products manufacturing, especially in typical sweets. Chia (Salvia hispanica L.) is an angiosperm plant from the mint family (Lamiaceae) characterized as a grain from tropical and subtropical climate, widely consumed in pre-Columbian America by the Aztecs, in the region that includes Mexico and Guatemala. Both grains, azuki and chia, are considered rich source of many essential nutrients to good health maintenance. One of the most relevant aspects regard to nutrients is the mineral composition. Several minerals are essential for the maintenance of biological systems. Besides, sensorial aspects are very important factors for the development of new products. Factorial design enables evaluating the contribution of a specific ingredient on several characteristics of the final product and multivariate analysis enables extracting additional information when compared to the univariate analysis. This latter chemometric tool allows the pattern recognition, the gathering of information, reduction of data dimensionality and also the organization of data in a simpler structure, easier to understand. The principal component analysis (PCA) is based on perform linear comparisons of the original variables. The principal components (PC) are mutually orthogonal and explained variance decreases with increase in PC number

OBJECTIVES. The aim of this study was to apply the Chemometrics to investigate the influence of the factors: percentages of chia and azuki added on gluten-free chocolate cake for the determination of proximal composition, energy, sensory aspects and the of minerals Ca, Cu, Fe, K, Mg, Mn and Zn.

MATERIAL AND METHODS.

A 22 full factorial design (two factors at two levels) with duplicates was performed to investigate the influence of two factors on the chocolate cake proximal and mineral composition. A control assay was also prepared for comparison using Principal Components Analysis. The factors were concentrations of chia and azuki flour. All ingredients were previously weighed separately. The rice flour, azuki and chia, at the respective percentage for each formulation, were mixed to obtain a homogeneous fraction (28.80% of the whole formulation) and egg white (8.70%) was mixed to form a solid phase. The egg yolk (5.80%), butter (5.80%) and sugar (16.90%) were homogenized to form a cream on 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 added slowly to form a homogeneous mass. The cake mass was transferred to a rectangular baking dish and baked in a conventional oven for 30 minutes at 200 ° C, with subsequent cooling to room temperature (25℃). The moisture, ash, crude protein, total lipids, Nifext contents and the energy value were assessed. For mineral analysis, the samples were digested by the dry method and Ca, Cu, Fe, K, Mg, Mn and Zn were quantified in atomic absorption spectrophotometer. A group of 60 non-trained volunteer panelists and potential consumers of the products developed participated in the sensory analysis, which consisted in acceptance testing with the following attributes: smell, color, appearance, flavor, texture and overall acceptance of the cakes using a 9-point hedonic scale. The multivariate analysis was performed by applying Principal Component Analysis (PCA).

RESULTS AND DISCUSSION.

A 2^2 full factorial design (two factors at two levels) with duplicates was performed to investigate the influence of the factors: the percentage of chia and azuki added on gluten-free chocolate cake on the determination of moisture, ash, crude protein, total lipids, Nifext contents and the energy value, sensory attributes and the contents of minerals Ca, Cu, Fe, K, Mg , Mn and Zn. The formulations were prepared with partially defatted chia flour and azuki wholemeal. Both factors were significant and the increasing of their value contributed positively in the responses of proximal composition, energy and sensory aspects. The percentage of chia was the factor that most contributed to the majority of the responses, except for the nifext. Azuki principal effect was not significant for the responses moisture and energy. The principal components analysis distinguished samples with the highest content of chia mainly for the responses lipids and crude protein, and these formulations had the optimal point in response surfaces. The models for the sensory attributes were not significant and multivariate analysis showed that formulations with the lowest percentage of chia and azuki had characteristics similar to control assay. For the mineral analysis, The factors percentage of chia and azuki were significant and an increase of these values, contributed positively to the responses. The interaction effect was not significant to the responses Ca, Fe, Mg and Zn. The principal components analysis distinguished samples with higher content of chia through PC1, and PC2 separated the formulations with the highest level of azuki from the one with the lowest level. ANOVA and response surfaces analyses showed that the greatest contents of minerals were found in the formulations with the highest concentration of both flours: chia and azuki.

CONCLUSIONS.

The factorial design performed for chocolate cake enable to verify that the models for the proximal composition and energy were significant. The chia flour was the factor that most contributed for most of the responses, except for the nifext. The azuki main effect was not significant for responses moisture and energy. The principal components analysis distinguished samples with higher content of chia mainly due to crude protein and lipids contents, and these samples were the optimal points in the response surfaces of these models. Regarding sensory attributes, the models were not significant and multivariate analysis showed that the lowest percentage of the chia and azuki provided characteristics similar to control assay. 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 increasing of these factors contributes positively to all responses. The interaction effect was not significant only for responses Ca, Fe, Mg and Zn. The principal components analysis distinguished samples with higher content of chia through PC1, and PC2 separated the formulations with the highest level of azuki from the one with the lowest level. ANOVA and response surfaces analyses showed that the greatest contents of minerals were found in the formulations with the highest concentration of both flours: chia and azuki. These grains are good alternatives for common flours substitution in food products, including gluten-free ones.

Key words: *Salvia hispanica*, L., *Vigna angularis*, response surface methodology, principal components analysis.

RESUMO GERAL

INTRODUÇÃO. As atuais mudanças no cotidiano da população mundial refletiram nas transformações drásticas de muitos aspectos, especialmente no consumo de alimentos. Hoje em dia, a maioria dos alimentos não tem os nutrientes mínimos essenciais para a manutenção da saúde humana, fato que despertou o interesse no desenvolvimento de alimentos enriquecidos e com apelo funcional, principalmente pelas indústrias de alimentos. Os bolos prontos para o consumo foram adquirindo grande importância entre os produtos de panificação, uma vez que são amplamente comercializados, e ocupa a segunda posição do ranking de produtos mais consumidos nesta categoria, atrás apenas de pão. Há versões sem glúten que podem ser consumidos por pacientes celíacos, no entanto, estes ainda são pobres em nutrientes, porque eles são compostos principalmente de farinha de arroz. Azuki (*Vigna angularis*) é uma leguminosa amplamente produzida e consumida na Ásia, usado na fabricação de vários produtos, especialmente em doces típicos. Chia (*Salvia hispanica* L.) é uma planta angiosperma da família da menta (Lamiaceae), caracterizada como um grão de clima tropical e subtropical, largamente consumida no América pré-colombiana pelos astecas, na região que inclui México e Guatemala. Ambos os grãos, azuki e chia, são considerados fontes de muitos nutrientes essenciais para a manutenção da boa saúde. Um dos aspectos mais importantes diz respeito aos nutrientes é a composição mineral. Diversos minerais são essenciais para a manutenção dos sistemas biológicos. Além disso, aspectos sensoriais são fatores muito importantes para o desenvolvimento de novos produtos. Um delineamento fatorial permite avaliar a contribuição de um ingrediente específico em várias características do produto final e a análise multivariada permite extrair informações adicionais quando comparados com a análise univariada. Esta ultima ferramenta quimiométrica permite o reconhecimento de padrões, a coleta de informações, a redução da dimensionalidade dos dados e também a organização dos dados em uma estrutura mais simples, mais fácil de entender. A análise de componente principal (PCA) baseia-se em comparações lineares das variáveis originais. Os componentes principais (PC) são mutuamente ortogonais e a variância explicada diminui com o aumento do número de PC.

OBJETIVOS. O objetivo deste estudo foi aplicar a Quimiometria para investigar a influência dos fatores: percentagens de chia e azuki adicionada em bolo de chocolate sem glúten para a determinação da composição proximal, energia, aspectos sensoriais e minerais Ca, Cu, Fe , K, Mg, Mn e Zn.

MATERIAL E MÉTODOS. Um planejamento fatorial completo 2^2 (dois fatores em dois níveis), com duplicatas foi realizado para investigar a influência de dois fatores sobre a composição proximal e mineral de bolo de chocolate. Um ensaio controle também foi preparado para comparação utilizando Análise de Componentes Principais. Os fatores foram concentrações de chia e farinha de azuki. Todos os ingredientes foram previamente pesados separadamente. A farinha de arroz, e azuki chia, a respectiva percentagem para cada formulação, foram misturados para se obter uma fração homogênea (28,80% da formulação total) e a clara de ovo (8,70%) foi batida para formar uma fase sólida. A gema de ovo (5,80%), manteiga (5,80%) e açúcar (16,90%) foram homogeneizadas de modo a formar um creme no qual foram adicionados a

mistura de farinha, chocolate em pó (8,00%), cacau em pó (3,80%), clara de ovo, água (19,08%), leite em pó (2,12%) e o fermento em pó (1,00%) até formar uma massa homogénea. A massa de bolo foi transferida para uma assadeira retangular e assado num forno convencional durante 30 minutos a 200 ° C, com subsequente resfriamento até à temperatura ambiente (25 ° C). A umidade, cinzas, proteínas, lipídios totais, conteúdo Nifext e do valor energético foram avaliados. Para a análise mineral, as amostras foram digeridas pelo método seco e Ca, Cu, Fe, K, Mg, Mn e Zn foram quantificados em espectrofotómetro de absorção atómica. Um grupo de 60 provadores não treinados voluntários e potenciais consumidores dos produtos desenvolvidos participaram da análise sensorial, que consistiu em testes de aceitação com os seguintes atributos: aroma, cor, sabor, textura e aspecto global dos bolos usando escala hedônica de 9 pontos. A análise multivariada foi realizada através da aplicação de Análise de Componentes Principais (PCA).

RESULTADOS E DISCUSSÃO. Um planejamento fatorial completo 2^2 (dois fatores em dois níveis), com duplicatas foi realizada para investigar a influência de fatores: a percentagem de chia e azuki adicionada sem glúten bolo de chocolate sobre a determinação de umidade, cinzas, proteína bruta, lipídios totais , Nifext conteúdo e do valor de energia, atributos sensoriais e os teores de minerais Ca, Cu, Fe, K, Mg, Mn e Zn. As formulações foram preparadas com farinha parcialmente desengordurada de chia e integral de azuki. Ambos os fatores foram significativos e o aumento do seu valor contribuiu positivamente nas respostas de composição centesimal, energia e aspectos sensoriais. A percentagem de chia foi o factor que mais contribui para a maior parte das respostas, exceto para o nifext. O efeito principal Azuki não foi significativo para as respostas de umidade e energia. A análise de componentes principais distinguiu amostras com o maior teor de chia principalmente para as respostas lipídios e proteína bruta, e essas formulações constituíram o ponto ideal nas superfícies de resposta. Os modelos para os atributos sensoriais não foram significativos e a análise multivariada mostrou que as formulações com o menor percentual de chia e azuki tinham características semelhantes ao ensaio controle. Para a análise mineral, os fatores chia e azuki foram significativos e um aumento destes valores contribuiu positivamente para as respostas. O efeito de interação não foi significativa para a resposta Ca, Fe, Mg e Zn. A análise de componentes principais distinguiu amostras com maior teor de chia através de PC1, e PC2 separou as formulações com o nível mais alto de azuki do nível mais baixo. ANOVA e análises de superfícies de resposta mostraram que os maiores teores de minerais foram encontrados nas formulações com a maior concentração de ambas as farinhas: chia e azuki.

CONCLUSÕES. O planejamento fatorial realizado para o bolo de chocolate permitiu verificar se os modelos para a composição proximal e energia foram significativas. A farinha de chia foi o fator que mais contribuiu para a maior parte das respostas, exceto para o nifext. O efeito principal azuki não foi significativo para as respostas de umidade e energia. A análise de componentes principais distinguiu as amostras com maior teor de chia principalmente devido ao conteúdo de proteína bruta e lipídios, e estas amostras foram os pontos ótimos nas superfícies de resposta destes modelos. Quanto aos atributos sensoriais, os modelos não foram significativos e a análise multivariada

mostrou que o menor percentual da chia e azuki apresentaram características semelhantes ao ensaio controle. O planejamento fatorial realizado para incorporar minerais em bolo de chocolate demonstrou que os maiores percentuais de chia e farinha de azuki foram significativos, e o aumento destes fatores contribuiu positivamente a todas as respostas. O efeito de interação não foi significativo apenas para respostas Ca, Fe, Mg e Zn. A análise de componentes principais distinguiu amostras com maior teor de chia através de PC1, e PC2 separou as formulações com o nível mais alto de azuki do nível mais baixo. A análise de variância e das superfícies de resposta mostrou que os maiores teores de minerais foram encontrados nas formulações com a maior concentração de ambas as farinhas: chia e azuki. Estes grãos são boas alternativas para substituição de farinhas comum em produtos alimentares, incluindo aqueles sem glúten.

Palavras-chave: *Salvia hispanica*, L., *Vigna angularis*, metodologia de superfície de resposta, análise de componentes principais.

FERRAMENTAS QUIMIOMÉTRICAS APLICADAS NO DESENVOLVIMENTO E CARACTERIZAÇÃO PROXIMAL E SENSORIAL DE BOLOS DE CHOCOLATE CONTENDO CHIA E AZUKI

CHEMOMETRIC TOOLS APPLIED ON DEVELOPMENT AND PROXIMAL AND SENSORY CHARACTERIZATION OF CHOCOLATE CAKES CONTAINING CHIA AND AZUKI

Quimiometria aplicada em formulações de bolo

Chemometrics applied on cake formulations

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RESUMO

Um planejamento fatorial 2^2 completo (dois fatores em dois níveis) com duplicata foi realizado, para investigar a influência dos fatores: % de chia e azuki adicionados no bolo de chocolate isento de glúten, para a determinação da composição proximal, energia e atributos sensoriais. No estudo foi utilizada farinha desengordurada de chia e de azuki. Os fatores % de chia e azuki foram significativos, e o aumento dos valores nestes, contribuiu positivamente nas respostas. A chia foi o fator que mais contribuiu para a maioria das respostas, exceto para o nifext. O efeito principal azuki não foi significativo para as respostas umidade e energia. A análise de componentes principais distinguiram as amostras com maior teor de chia devido principalmente a proteína bruta e aos lipídios totais, sendo os pontos ótimos nas superfícies de resposta destes modelos. Para os atributos sensoriais os modelos foram não significativos e a análise

multivariada demonstrou que os menores percentuais de chia e azuki apresentam características semelhantes ao ensaio controle.
Palavras-chave: *Salvia hispanica*, L., *Vigna angularis*, alimentos isentos de glúten, metodologia de superfície de resposta, análise de componentes principais.

ABSTRACT

A 2^2 full factorial design (two factors at two levels) with duplicates was performed to investigate the influence of the factors percentage of chia and the azuki added on gluten-free chocolate cake on the proximal composition, energy and sensory aspects. A partially defatted chia flour was used in the formulations. Both factors were significant and the increasing of their value contributed positively in the responses. The percentage of chia was the factor that most contributed to the majority of the responses, except for the nifext. Azuki principal effect was not significant for the responses moisture and energy. The principal components analysis distinguished samples with the highest content of chia mainly for the responses lipids and crude protein, and these formulations had the optimal point in response surfaces. The models for the sensory attributes were not significant and multivariate analysis showed that formulations with the lowest percentage of chia and azuki had characteristics similar to control assay.
Key words: *Salvia hispanica*, L., *Vigna angularis*, response surface methodology, principal components analysis.

INTRODUCTION

The apace life of nowadays causes changes on the daily of world population, mainly on eating habits. Currently, most of foods do not have the minimum nutrients essential for the maintenance of human health, fact that aroused interest in enriched food developing and with good acceptance. The cakes ready for consumption have been acquiring great importance among the bakery products (OSAWA et al., 2009), since they are largely marketed, and hold the second position of the most consumed product ranking in this category, behind only of bread. There are gluten-free versions which can be consumed by celiac patients, however, these are still poor in many nutrients because they are composed primarily of rice flour (LEE et al., 2007).

Azuki (*Vigna angularis*) is a legume widely produced and consumed in Asia, used in the various products manufacturing, especially in typical sweets (SHI, 1988). Chia (*Salvia hispanica* L.) is an angiosperm plant from the mint family (*Lamiaceae*) characterized as a grain from tropical and subtropical climates, widely consumed in pre-Columbian America by the Aztecs, in the region that includes Mexico and Guatemala (AYERZA, 2005). Both grains, azuki and chia, are considered rich source of many essential nutrients to good health maintenance (TOSCO, 2004).

Factorial design enables evaluating the contribution of a specific ingredient on several characteristics of the final product and multivariate analysis enables extracting additional information when compared to the univariate analysis. This latter chemometric tool allows the pattern recognition, the gathering of information, reduction of data dimensionality and also the organization of data in a simpler structure, easier to understand. The principal component analysis (PCA) is based on perform linear comparisons of the original variables. The principal components (PC) are mutually orthogonal and explained variance decreases with increase in PC number (CORREIA; FERREIRA, 2007).

The aim of this study was to apply the Chemometrics to investigate the influence of the factors percentage of chia and azuki added on gluten-free chocolate cake for the determination of proximal composition, energy and sensory aspects.

MATERIALS AND METHODS

Sampling

The grains of azuki used in this study were cultivated in the region of Maringá, Paraná and purchased in the local market. Approximately 6-kg of grains were ground in a hammer mill to obtain a homogeneous flour which was sieved in a 14 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 company Giroil Agroindustria Ltda. (St. Angelo-RS). The other ingredients were obtained in retail stores in Maringá-PR.

Experimental design

A 2^2 full factorial design (two factors at two levels) with duplicates was performed to investigate the influence of two factors on the chocolate cake proximal composition. A control assay was also prepared for comparison using Principal Components Analysis. The factors were concentrations of chia and azuki flour, as shown in Table 1. The responses used were the content moisture, ash, protein, total lipids, nifext and energy.

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

Factors	Unit	Symbol	Type	Nevels	
				-1	+1
Chia flour	%	C	Numeric	10	20
Azuki flour	%	A	Numeric	10	20

Development of cakes

All ingredients were previously weighed separately. The rice flour, azuki and chia, at the respective percentage for each formulation, were mixed to obtain a homogeneous fraction (28.80% of the whole formulation) and egg white (8.70%) was mixed to form a solid phase. The egg yolk (5.80%), butter (5.80%) and sugar (16.90%) were homogenized to form a cream on 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 added slowly to form a homogeneous mass. The cake mass was transferred to a rectangular baking dish and baked in a conventional oven for 30 minutes at 200 ° C, with subsequent cooling to room temperature (25°C).

Proximal composition and energy

The moisture, ash and crude protein contents were determined according to Cunniff (1998) using factor 6.25 to convert the percentage of nitrogen into crude protein content. The total lipids were determined according to Bligh and Dyer (1959). The Nifext fractions (carbohydrates) were calculated by difference.

The energy value was obtained by the indirect method using conversion factors for each component of the product according to calculation proposed by Holands et al. (1994). The results were achieved in cal g^{-1} of food and converted to Joule, using the factor 4.1868 J to 1 cal and expressed in kJ kg^{-1} of product.

Sensory analysis

A group of 60 non-trained volunteer panelists and potential consumers of the products developed participated in the sensory analysis, which consisted in acceptance testing with the following attributes: smell, color, appearance, flavor, texture and overall acceptance of the cakes using a 9-point hedonic scale (1 = extremely dislike to 9 = extremely like). The samples were presented in random complete blocks for comparison. The index of acceptability (IA) of the products was calculated as (overall acceptance grade x 100%) / 9 (LAWLESS; HEYMANN, 2010).

Ethical aspects

The sensory testing in this study was approved by the Standing Committee on Ethics in Research Involving Human Beings of Maringá State University, CAAE File No. 02781312.0.0000.0104. All panelists signed a free and informed consent form prior to their participation in the sensory analysis.

Statistical analysis

All the analysis were carried out in triplicate. Initially, the values of the main effects, interaction and analysis of variance (ANOVA) were obtained. Thereafter, all variables had their normality and homogeneity of variance assessed by the residual plots. Then, analysis of variance (two-way ANOVA between groups) was performed for all the responses. 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 the regression coefficients and x_1, x_2 are the levels of independent variables (GRANATO et al., 2010).

The multivariate analysis for proximal composition, energy and sensory attributes were performed by applying Principal Component Analysis (PCA) on the results of the samples from the factorial design and the control cake. The means of analyses in triplicate of two cakes of each formulation were used to compose the responses. Means were auto scaled in order to provide the same weight for all the variables and two-dimensional graphs of PCA were obtained. All statistical analyzes were conducted using the software Statistica, version 7.0 (STATSOFT, USA), with 5% ($p < 0.05$) significance level for rejection of the null hypothesis.

RESULTS AND DISCUSSION

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

Table 2. Mathematical equations for all the responses by applying the response surface model.

Parameters	Equation	R^2
Moisture	Moisture = 27.54 – 0.60 * C + 8.75.10^{-3} * A + 0.23 * C * A	0.997
Ash	Ash = 1.95 + 0.12 *C + 0.05 * A - 0.03 *C * A	0.992
Protein	Protein = 9.71 + 0.60 * C + 0.26 * A + 0.07 * C * A	0.997
Lipids	Lipids = 7.58 + 0.13 * C + 0.07 * A - 0.02 * C * A	0.988
Nifext	Nifext = 53.22 - 0.25 * C - 0.39 * A - 0.24 * C * A	0.992
Energy	Energy = 1338.17 + 10.73 * C + 0.58 * A – 3.76 * C * A	0.998

C, chia = x_1; A, azuki = x_2.

Table 3 shows the conditions of the factorial model 2^2 design, applied to the experiments, in duplicate, and the values obtained for all the responses studied: moisture, ash, protein, lipids and nifext as g 100g^{-1} of sample and energy as kJ 100g^{-1} of cake.

The graphs of the residuals for each response indicated that the data exhibited normality and homogeneity of variance in a very satisfactory way, showing that all models were significant, and did not present significant lack of fit. The coefficients of regression (R^2) and the F value for each model, obtained by ANOVA and shown in Tables 2 and 6, respectively, also indicate the positive significance of the models.

Table 3. 2^2 full factorial design (in duplicate) and the responses obtained in the assays for proximal composition and energy.

Assay	Independent variables		Responses					
	Numeric levels							
	C[a] %	A[b]%	Moisture[c]	Ash[c]	Protein[c]	Lipids[c]	Nifext[c]	Energy[d]
1	10	10	28.41	1.74	8.92	7.35	53.58	1322.42
2	10	10	28.31	1.76	8.93	7.35	53.65	1323.76
3	20	10	26.66	2.08	9.94	7.64	53.68	1352.08
4	20	10	26.75	2.04	10.01	7.68	53.52	1352.08
5	10	20	27.92	1.91	9.36	7.52	53.29	1331.33
6	10	20	27.92	1.92	9.24	7.57	53.35	1332.21
7	20	20	27.19	2.10	10.65	7.75	52.31	1345.18
8	20	20	27.17	2.08	10.63	7.77	52.35	1346.27
9[e]	0	0	30.47	1.24	8.55	5.95	53.79	1267.04

[a]C: chia; [b]A: Azuki; [c](g $100g^{-1}$ of sample); [d](kJ $100g^{-1}$ of sample); [e]Control sample (100% rice flour).

Table 4 shows the values of the main and interactions effects for all the responses, and Table 5 presents the results obtained by ANOVA for the 2^2 full planning in duplicate for each of the studied responses.

Table 4. Main and interactions effects, calculated for the 2^2 factorial design.

	Effects		
Response	A=Chia	B=Azuki	A X B
Moisture	-1.20	0.02	0.46
Ash	0.24	0.10	-0.07
Protein	1.19	0.52	0.15
Lipids	0.26	0.15	-0.05
Nifext	-0.50	-0.78	-0.49
Energy	21.47	1.16	-7.52

Table 4 shows that the interactions chia X azuki was negative for most of the responses. Table 6 indicated that azuki main effect for moisture and energy were not significant ($p < 0.05$), with a contribution lower than 1%. Although these results did not influenced significantly these responses, the interaction chia X azuki presented a significant contribution ($p < 0.05$) for the models (12.70% for moisture and 10.87% for energy).

Tabela 5. ANOVA results of sum of square and mean square for the responses studied in the 2^2 factorial model.

Source	Degree of freedom	Sum of square					
		Moisture	Ash	Protein	Lipids	Nifext	Energy
Regression	3	3.29	0.15	3.44	0.19	2.20	1037.57
A=Chia	1	2.87	0.12	2.86	0.14	0.51	921.81
B=Azuki	1	$6.13*10^{-4}$	0.02	0.54	0.04	1.22	2.69
A X B	1	0.42	$9.11*10^{-3}$	0.04	$4.51*10^{-3}$	0.48	113.06
Pure error	4	$9.25*10^{-4}$	$1.25*10^{-3}$	$9.90*10^{-3}$	$2.25*10^{-3}$	0.02	1.87
Total	7	3.30	0.15	3.45	0.19	2.22	1039.44
Source		**Mean square**					
		Moisture	Ash	Protein	Lipids	Nifext	Energy
Regression		1.10	0.05	1.15	0.06	0.73	354.86
A=Chia		2.87	0.12	2.86	0.14	0.51	921.81
B=Azuki		$6.13*10^{-4}$	0.02	0.54	0.04	1.22	2.69
A X B		0.42	$0.11*10^{-3}$	0.04	$4.51*10^{-3}$	0.48	113.06
Pure error		$2.31*10^{-3}$	$3.13*10^{-4}$	$2.48*10^{-3}$	$5.63*10^{-3}$	$4.46*10^{-3}$	0.47

The ANOVA results, shown in Tables 5 and 6, indicate that the interaction of the main factors was significant for all responses, and that the main factors were not significant for the responses moisture and energy.

Table 6. ANOVA results of F test and P-value for the responses studied in the 2^2 factorial design

Source	F test					
	Moisture	Ash	Protein	Lipids	Nifext	Energy
Regression	473.84	155.45	463.15	110.13	164.70	738.61
A=Chia	1240.22	376.36	1153.96	245.00	113.17	1968.62
B=Azuki	0.26	60.84	218.51	77.36	274.42	5.75
A X B	181.02	29.16	16.99	8.02	106.51	241.46
Source	P-value					
	Moisture	Ash	Protein	Lipids	Nifext	Energy
Regression	<0.0001	0.0001	<0.0001	0.0003	0.0001	<0.0001
A=Chia	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
B=Azuki	0.6339	0.0015	0.0001	0.0009	<0.0001	0.0744
A X B	0.0002	0.0057	0.0146	0.0472	0.0005	0.0001

The percentage of chia was the factor that most contributed to the majority of the responses, except for nifext, as shown in Table 4. This is due to the great contribution of high levels of chia for proteins and lipids (Tables 2, 5 and 6), as shown by Capitani et al. (2012) for defatted chia flour. This fact can be confirmed by analyzing the PC1 versus PC2 (Figures 1A and 1B) which showed samples 3, 4, 7 and 8, with higher concentration of chia, separated by quadrants from the other samples. Besides, Table 4 showed a positive interaction between the factors studied and the responses surfaces (Figures 2C and 2D) indicated the same samples as the optimal points for the responses lipids and proteins.

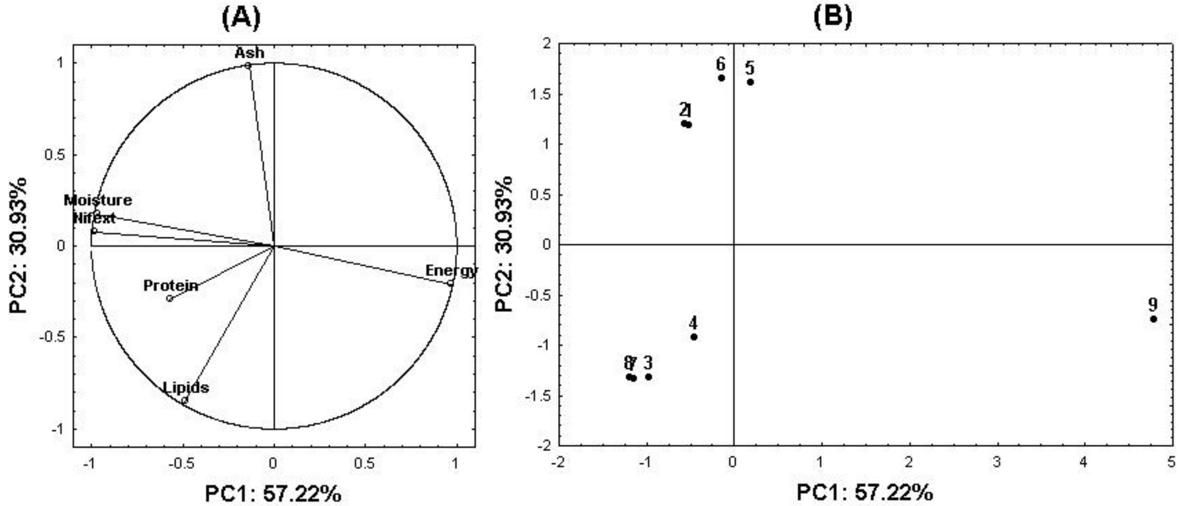

Figure 1. Principal components analysis for the responses studied in the 2^2 factorial design. PC: principal component. Fig. 1A: loadings. Fig. 1B: scores.

The PC1 in Figure 1, which explains 57.22% of the data variance, was able to distinguish the sample 9 (control), containing only rice flour, and sample 5, with the highest concentration of azuki flour. This was due to loadings graphic (Figure 1A), which presented the lowest levels of energy contributing to the scores (Figure 1B) of these samples.

Response surfaces were developed for levels and independent variables, as shown in Figure 2. By analyzing principal components, due to the disposal of samples 7 and 8 on PC1 and PC2 (Figures 1A and 1B), the response surfaces (Figure 2), and the effects from Table 4, higher lipid and nifext contents and energy value were evidenced with increasing on percentage of defatted chia and azuki flours. According to Giroil Agroindustria Ltda., the co-product of the cold extraction of oil from chia (the defatted chia flour) has a content of 10% of total lipids. Ixtaina (2010) described the prevalence of unsaturated fatty acids in the lipid fraction of chia, especially alpha-linolenic acid (44.4-63.4 %).

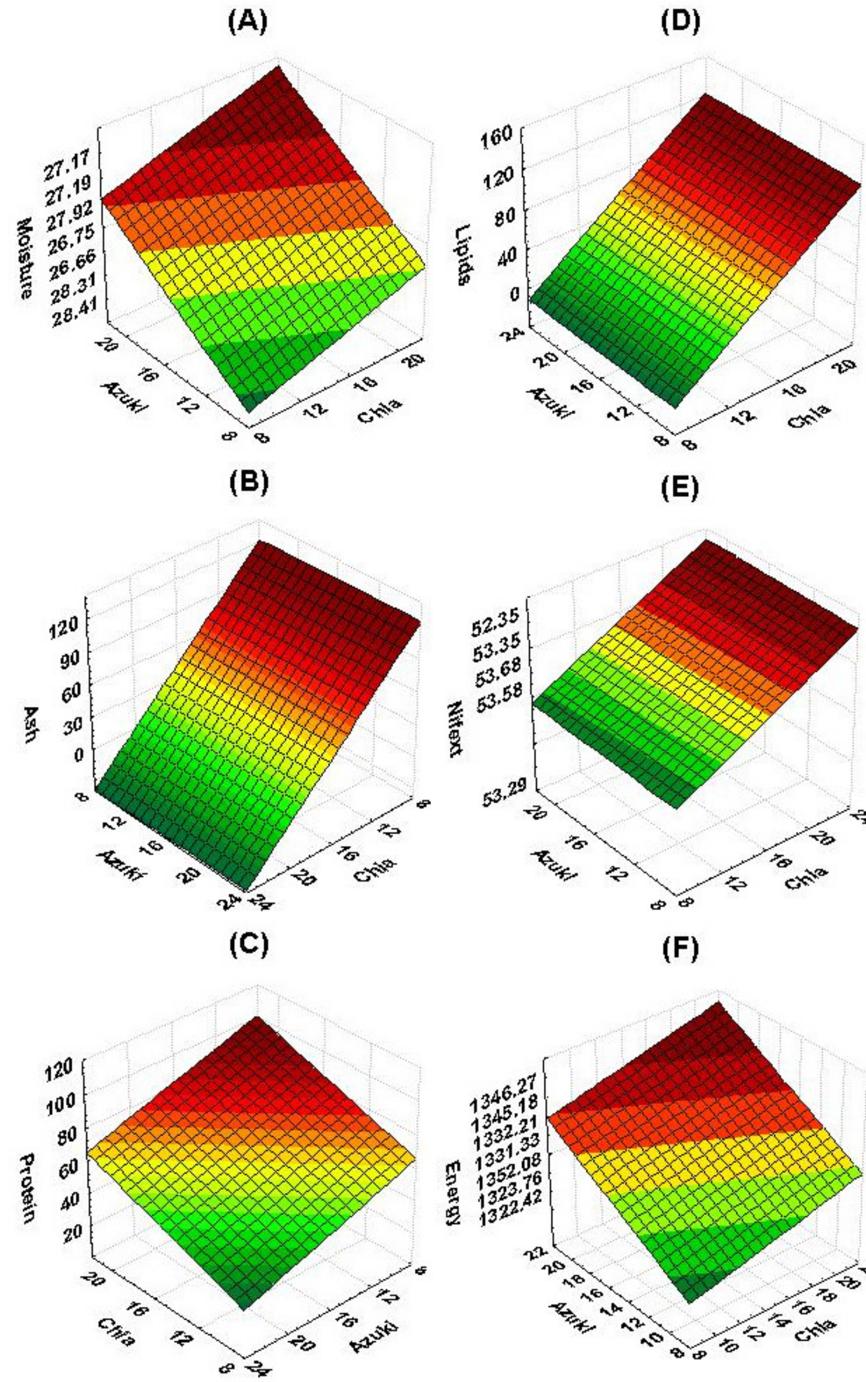

Figure 2. Response surfaces for contents of moisture (A), ash (B), protein (C), lipids (D), nifext (E) and energy (F) according to the concentration of chia e azuki flour.

Table 7 shows the responses for the sensory attributes evaluated for each chocolate cake formulation from the factorial design and for the control sample containing only rice flour.

Table 7. 2^2 full factorial design (in duplicate) and the responses for the sensory attributes obtained in the assays.

Assay	Independent variables		Responses					
	Numeric levels							
	C[a] %	A[b] %	Smell	Color	Appearance	Flavour	Texture	Overall acceptance
1	10	10	7.07 ±1.70	7.63 ±1.34	7.75 ±1.20	6.75 ±1.68	7.02 ±1.76	7.15 ±1.66
3	20	10	6.88 ±1.73	7.53 ±1.20	7.08 ±1.32	6.33 ±1.99	6.10 ±1.76	6.68 ±1.56
5	10	20	7.13 ±1.75	7.48 ±1.27	7.25 ±1.37	6.43 ±2.05	6.40 ±2.04	6.83 ±1.71
7	20	20	6.72 ±1.82	7.42 ±1.33	6.83 ±1.66	5.80 ±2.00	5.93 ±1.80	6.45 ±1.76
9[c]	0	0	7.45 ±1.40	7.55 ±1.27	7.55 ±1.21	7.27 ±1.66	7.00 ±1.87	7.48 ±1.24

[a]C: chia; [b]A: Azuki; [c]Control sample (100% rice flour); n = 60 panelists.

The responses obtained in the sensory analysis (Table 7) were analyzed, but no significant difference ($p < 0.05$) was found, consequently, the models were not significant ($p < 0.05$) and did not gather the data obtained. According to Dutcosky (2011), affective tests access directly the opinion of consumers, either the established or the potential ones, with respect to the sensory characteristics under study. The responses given by the panelists in sensory analysis presented a wide variation (Table 7), providing a significant lack of fit ($p < 0.05$) for all attributes investigated.

Rodrigues and Iemma (2009) studied the influence of the substitution of different additives in bread and proposed to conduct a parallel test to assess the properties of the products, with or without additives. When the control assay was performed, the sensory characteristics of cakes with the incorporation of the chia and azuki flours obtained average and standard deviation very similar.

Figure 3 shows the sensory attributes - loadings (Figure 3A) and samples - scores (Figure 3B). PC1 explained 88.52% of the variance, with the distinction of formulations 1 and 9 (Figure 3B), as well as all attributes contributed positively to the weight of the samples. In PC2, the cake 1 had higher contribution of color and texture attributes (Figure 3A) and had the highest averages for both (Table 7).

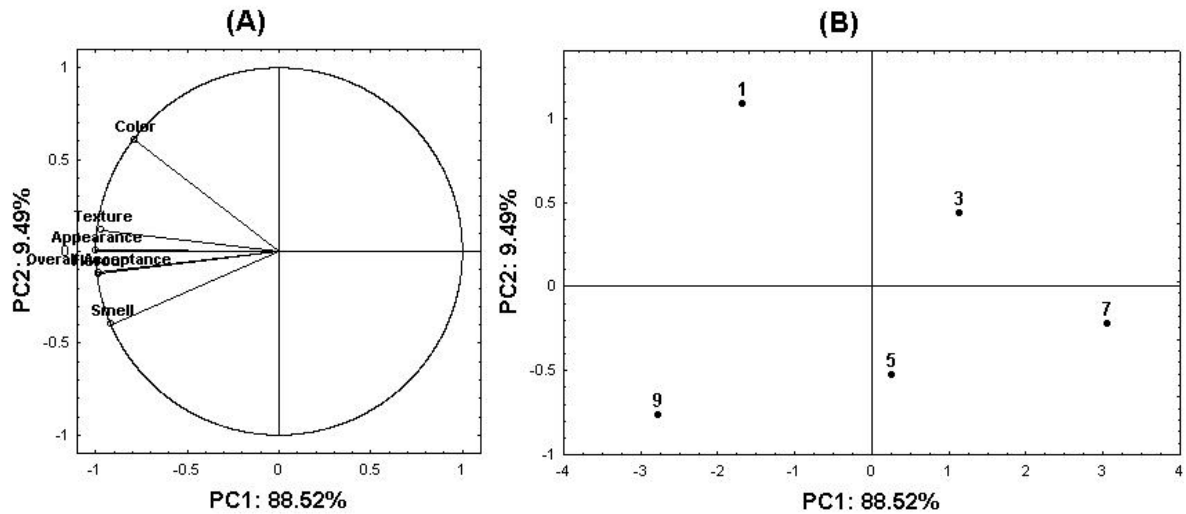

Figure 3. Principal components analysis for the responses studied in the 2^2 factorial model. PC: principal component. Fig. 1A: loadings. Fig. 1B: scores.

CONCLUSIONS

The factorial design performed for chocolate cake enable to verify that the models for the proximal composition and energy were significant. The chia flour was the factor that most contributed for most of the responses, except for the nifext. The azuki main effect was not significant for responses moisture and energy. The principal components analysis distinguished samples with higher content of chia mainly due to crude protein and lipids contents, and these samples were the optimal points in the response surfaces of these models. Regarding sensory attributes, the models were not significant and multivariate analysis showed that the lowest percentage of the chia and azuki provided characteristics similar to control assay.

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Chemometric Methods Applied on the Increasing of Mineral Content in Chocolate Cakes Containing Chia and Azuki

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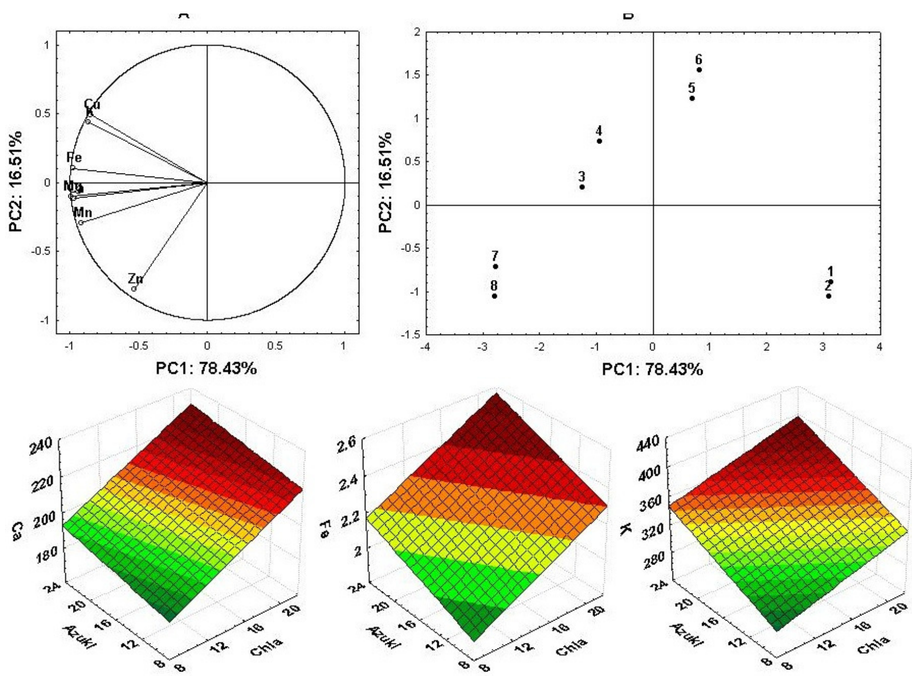

Chemometric Methods Applied on the Mineral Content Increasing in Chocolate Cakes Containing Chia and Azuki

Resumo

Um planejamento fatorial 2^2 completo (dois fatores em dois níveis) com duplicata foi realizado para investigar a influência dos fatores: % de chia e azuki adicionados no bolo de chocolate isento de glúten, para a determinação de Ca, Cu, Fe, K, Mg, Mn e Zn. No estudo foi utilizada farinha parcialmente desengordurada de chia e farinha integral de azuki. Os fatores % de chia e azuki foram significativos, e o aumento dos valores nestes, contribuiu positivamente nas respostas. O efeito de interação não foi significativo para as respostas Ca, Fe, Mg e Zn. A análise de componentes principais distinguiram as amostras com maior teor de chia através do PC1 e no PC2 os níveis superior e inferior de azuki. Através da ANOVA e das superfícies de respostas o aumento nos teores de minerais foi maior com a adição de 20% de ambas as farinhas chia e azuki.

Palavras-chave: *Salvia hispanica*, L., *Vigna angularis*, metodologia de superfície de resposta, análise de componentes principais.

Abstract

A 2^2 full factorial design (two factors at two levels) with duplicates was performed to investigate the influence of the factors: the percentage of chia and azuki added on gluten-free chocolate cake on the determination contents of minerals Ca, Cu, Fe, K, Mg , Mn and Zn. The formulations were prepared with partially defatted chia flour and azuki wholemeal. The factors percentage of chia and azuki were significant and an increase of these values, contributed positively to the responses. The interaction effect was not significant to the responses Ca, Fe, Mg and Zn. The principal components analysis distinguished samples with higher content of chia through PC1, and PC2 separated the formulations with the highest level of azuki from the one with the lowest level. ANOVA and response surfaces analyses showed that the greatest contents of minerals were found in the formulations with the highest concentration of both flours: chia and azuki.

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

Introduction

The current changes in the daily of world population reflect in drastic transformations of many aspects, especially in food consumption. Nowadays, most of foods do not have the minimum nutrients essential to the maintenance of human health, fact that aroused interest in enriched food developing and with functional appeal, mainly by food industries.

The cakes ready for consumption have been acquiring great importance among the bakery products,[1] since they are largely marketed, and hold the second position of the most consumed product ranking in this category, behind only of bread. There are gluten-free versions which 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 various products manufacturing, 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 climate, 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 source of many essential nutrients to good health maintenance.[9-12]

One of the most relevant aspects regard to nutrients is the mineral composition. Several minerals are essential for the maintenance of biological systems because they participate as cofactors in the metabolic reactions. For celiac patients, minerals 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. The 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 the Chemometrics to investigate the influence of the factors: percentages of chia and azuki added on gluten-free chocolate cake for the determination of the minerals Ca, Cu, Fe, K, Mg, Mn and Zn.

Experimental

Sampling

The grains of azuki used in this study were cultivated in the region of Maringá-PR and purchased at the local market. Approximately 6 kg of grains were ground in a hammer mill to obtain a homogeneous flour. 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 company Giroil Agroindustria Ltda. (St. Angelo-RS). The other ingredients were obtained in retail stores in Maringá-PR.

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 chocolate cake mineral composition. The factors were: concentrations of azuki and chia flour, as shown in Table 1. The total of 100% of flour for each formulation was achieved with rice flour addition. The responses analyzed were Ca, Cu, Fe, K, Mg, Mn and Zn.

Table 1. Factors investigated and their levels used for the development of 2^2 full factorial design with duplicate

Factors	Unit	Symbol	Type	Levels	
				–1	+1
Chia flour	%	C	Numeric	10	20
Azuki flour	%	A	Numeric	10	20

Development of cakes

All ingredients were previously weighed separately. The rice flour, azuki and chia, at the respective percentage for each formulation, were mixed to obtain a homogeneous fraction (28.80% of the whole formulation) and egg white (8.70 %) was mixed to form a solid phase. The egg yolk (5.80 %), butter (5.80 %) and sugar (16.90 %) were homogenized to form a cream on 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 added slowly to form a homogeneous mass. The cake mass was transferred to a rectangular baking dish and baked in a conventional oven for 30 minutes at 200 °C, with subsequent cooling to room temperature (25 °C).

Mineral quantification

In the mineral composition analysis, the samples were digested by the dry method[14] and Ca, Cu, Fe, K, Mg, Mn and Zn were quantified in atomic absorption spectrophotometer AA240FS (Varian, USA) as mg of mineral per 100 g of product using standard solutions and analytical curves.

Statistical analysis

All the analysis were carried out in triplicate. Initially, the values of the main effects, interaction and analysis of variance (ANOVA) were obtained. Thereafter, all variables had their normality and homogeneity of variance assessed by the 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, the 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} e β_{12} are the 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). The means of analyses in triplicate of two cakes of each formulation were used to compose the responses. Means were auto scaled in order to all the variables present the same weight and two-dimensional graphs of PCA were obtained. All statistical analyzes were conducted using the software Statistica, version 7.0, (STATSOFT, USA), was used with 5% ($p < 0.05$) significance level for rejection of the null hypothesis.

Results and discussion

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

Table 2. Mathematical equations for all the responses by applying the response surface model

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

Ch, chia = x_1; Az, azuki = x_2.

Table 3 shows the conditions of the 2^2 factorial model, in duplicate, applied in the experiments, and the values obtained for all the responses studied: Ca, Cu, Fe, K, Mg, Mn and Zn as mg 100 g^{-1} of sample.

The graphs of the residuals for each response indicated that the data exhibited normality and homogeneity of variance very satisfactory, showing that all models were significant, showing no significant lack of fit. The coefficients of regression (R^2) and the F value for each model, obtained by ANOVA and shown in Table 2 and 6, respectively, also indicate the positive significance of the models.

Table 3. Parameters of the 2^2 full factorial design (in duplicate) and the responses obtained in the experiments

Assay	Independent variables		Responses (mg 100 g^{-1} of sample)						
	Numeric levels								
	Ch %	Az%	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 4 shows the values of the main effects and interaction effects for all the responses. Tables 5 and 6 present the results obtained by ANOVA analysis for each one of the responses studied of 2^2 full factorial design in duplicate.

Table 4. Main effects and interaction effects calculated for the 2^2 factorial design

	Effects		
Response	A=Chia	B=Azuki	A X B
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

Analyzing Table 4, it's possible to see that the interaction chia X azuki was negative for most of the responses. Table 5 indicated that the contribution for Fe, Mg, Mn and Zn was less than 3%. Although this result had not significantly affected these responses, this factor allows a better 'straightening' of the linear model, and its absence could compromise the R-squared value.

ANOVA results, shown in Table 5, indicate that the main factors were significant for all the responses, and that the interaction between the main factors was not significant for the responses Ca, Fe, Mg and Zn.

The percentage of chia was the factor that most contributed to the majority of the responses of minerals, except for potassium as shown in Table 4. In Figure 1, principal component 1 (PC1), which explains 78.43% of 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) 0of the samples 3, 4, 7 and 8.

By analyzing the PC1 versus 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) are explained by the minerals copper, iron and potassium. According to the ANOVA table (Table 5), the contribution percentages of the variable chia for Cu, Fe and K were, respectively, 52.9, 73.73 and 33.86%. The azuki had a contribution percentage of 56.10% (Cu), 22.73% (Fe) and 48.90% (K). The interaction effect had the least influence, and Table of ANOVA indicated that there was a positive contribution of this effect to increase the values of responses (less than 33%).

Tabela 5. ANOVA results: sum of square and mean square for the responses studied in the 2^2 factorial modeling

Source	DF[a]	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
A=Chia	1	1469.92	$5.29*10^{-3}$	0.16	2601.18	102.58	0.02	0.07
B=Azuki	1	103.00	$5.61*10^{-3}$	0.05	3756.94	17.50	$2.59*10^{-4}$	$2.33*10^{-3}$
A X B	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
A=Chia		1469.92	$5.28*10^{-3}$	0.16	2601.18	102.58	0.02	0.07
B=Azuki		103.00	$5.61*10^{-3}$	0.05	3756.94	17.50	$2.59*10^{-4}$	$2.33*10^{-3}$
A X B		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	$2.18*10^{-5}$	$2.15*10^{-4}$

[a]DF=Degree of Fredo

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
A=Chia	245.94	42.95	125.59	1340.94	784.30	695.92	340.45
B=Azuki	17.23	45.57	42.36	1936.75	133.77	11.89	10.86
A X B	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
A=Chia	<0.0001	0.0028	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
B=Azuki	0.0142	0.0025	0.0029	<0.0001	0.0003	0.0261	0.0301
A X B	0.5928	0.0067	0.0974	<0.0001	0.9590	0.0261	0.3053

Response surfaces were constructed for the independent variables and levels, as shown in Figure 2. Analyzing the responses surfaces (Figure 2), table of effects (Table 4) and the principal components, due to the arrangement of samples 7 and 8 in PC1 and PC2 (Figures 1A and 1B), it was possible to realize that the increasing on chia and azuki flour concentration evidenced a higher incorporation of minerals. Through the quadrant analysis of Figure 1A loadings, the contents of calcium, magnesium, manganese and zinc 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 the this compounds in azuki beans were not found in the literature. Through the 2^2 factorial design showed that higher concentrations of chia and azuki provided higher contents of minerals in gluten-free chocolate cakes. According to the results obtained in this study, It's evident that these grains are good alternatives for common flours substitution in food products, including the gluten-free ones for celiac patients.

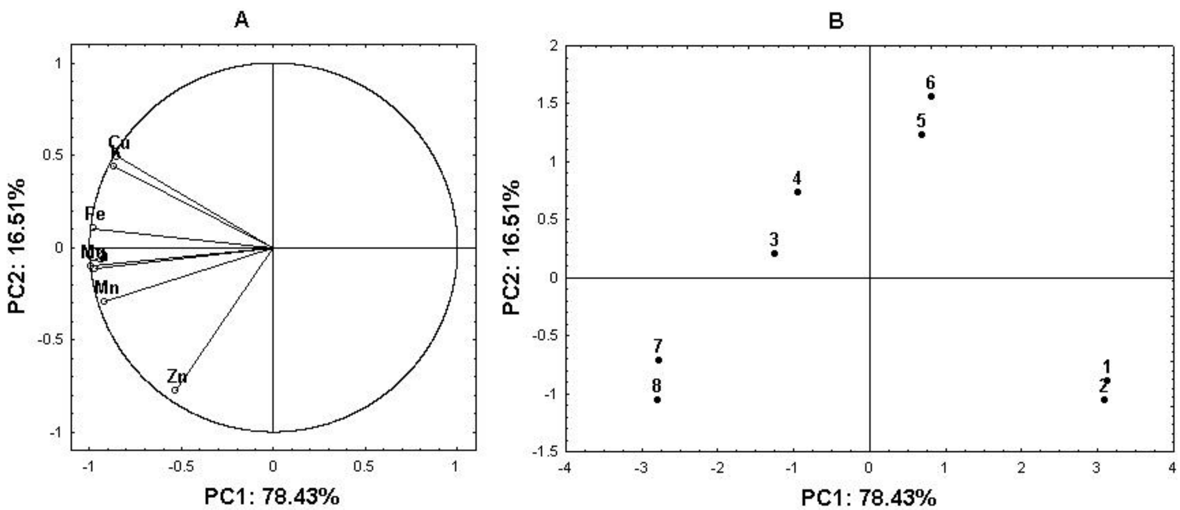

Figure 1. Principal Components Analysis for the responses studied in the 2^2 factorial design. PC= principal component. Fig. 1A: loadings. Fig. 1B: scores.

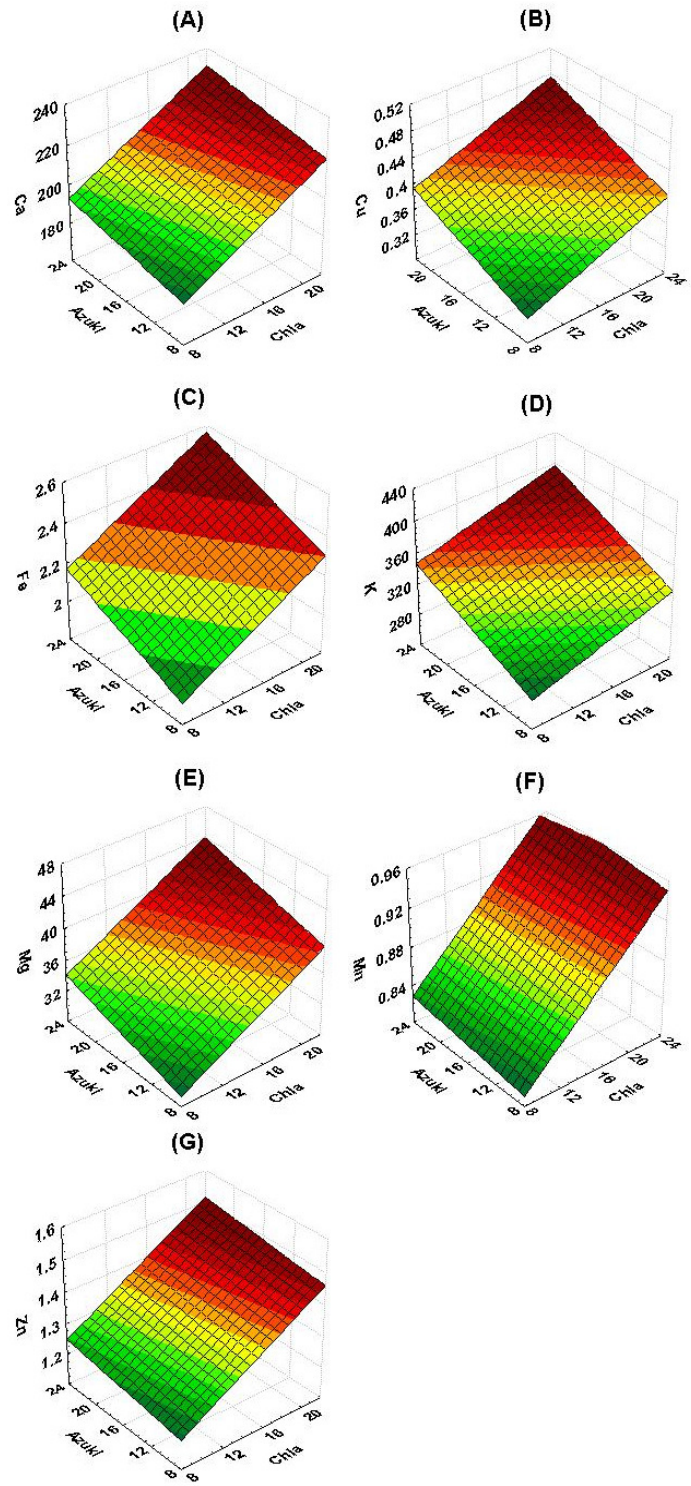

Figure 2. Response surfaces for the contents of Ca (A), Cu (B), Fe (C), K (D), Mg (E), Mn (F) e Zn (G) according to the percentage of chia and azuki.

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 increasing of these factors contributes positively to all responses. The interaction effect was not significant only for responses Ca, Fe, Mg and Zn. The principal components analysis distinguished samples with higher content of chia through PC1, and PC2 separated the formulations with the highest level of azuki from the one with the lowest level. ANOVA and response surfaces analyses showed that the greatest contents of minerals were found in the formulations with the highest concentration of both flours: chia and azuki. These grains are good alternatives for common flours substitution in food products, including gluten-free ones.

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