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Chemometric tools applied to the development and proximal and sensory characterization of chocolate cakes containing chia and azuki

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ABSTRACT. A 2^2 full factorial design (two factors at two levels) with duplicate was performed to investigate the influence of the factors percentage of chia and percentage of azuki added to gluten-free chocolate cake on the proximal composition, energy and sensory aspects. Partially defatted chia flour was used in the formulations. Both factors were significant and an increase in their value contributed positively to the measured responses. The percentage of chia was the factor that most contributed to the majority of the responses, except for the nitrogen free extract (nifext). The percentage of azuki did not significantly affect cake moisture and energy. Principal component 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 assays.

Keywords: *Salvia hispanica*, L., *Vigna angularis*, gluten-free food, response surface methodology, principal components analysis.

Ferramentas quimiométricas aplicadas no desenvolvimento e caracterização proximal e sensorial de bolos de chocolate contendo chia e azuki

RESUMO. Um planejamento fatorial 2^2 completo (2 fatores em 2 níveis) com duplicata foi realizado, para investigar a influência dos fatores porcentagem de chia e de azuki adicionados em 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 porcentagem de chia e de azuki foram significativos e o aumento desses valores contribuiu positivamente nas respostas. A porcentagem de chia foi o fator que mais contribuiu para a maioria das respostas, exceto para o extrato não-nitrogenado (nifext). O efeito principal da porcentagem de azuki não foi significativo para as respostas umidade e energia. A análise de componentes principais distinguiu as amostras com maior teor de chia, principalmente, pela proteína bruta e 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 apresentaram 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.

Introduction

Life nowadays continues apace and causes daily changes on the world population, mainly on eating habits. Currently, most foods do not have the minimum nutrients essential for the maintenance of human health, a fact that aroused interest in developing enriched foods and with good acceptance (GOHARA et al., 2013; PAGAMUNICI et al., 2014; SOUZA et al., 2014a).

Cakes ready for consumption have been acquiring great importance among 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, 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 (LEE et al., 2007).

Azuki (*Vigna angularis*) is a legume widely produced and consumed in Asia, used in various manufacturing products, 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; COATES, 2005).

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 for pattern recognition, the gathering of information, reduction of data dimensionality and also the organization of data in a simpler structure, easier to understand. Principal component analysis (PCA) is based on performing linear comparisons of the original variables. The principal components (PC) are mutually orthogonal, and explained variance decreases with an increase in PC number (CORREIA; FERREIRA, 2007).

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

Material and methods

Sampling

The grains of azuki used in this study were cultivated in the region of Maringá, Paraná State, and were 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 Ltd. (St. Angelo-Rio Grande do Sul State). The other ingredients were obtained in retail stores in Maringá.

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, nitrogen free extract (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	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 white, 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 a factor of 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 calculations 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. These analyses were used for all cakes formulations and raw materials (chia, azuki and rice).

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 the State University of Maringá, 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 analyses 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_o is a constant; β_1, β_2, β_{11}, β_{22} and β_{12} are the regression coefficients; and x_1 and x_2 are the levels of independent variables (GRANATO et al., 2010).

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 analyses were conducted using the software Statistica, version 7.0 (STATSOFT, 2007), 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 the 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 100 g^{-1} of sample and energy as kJ 100 g^{-1} of cake.

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 100 g^{-1} of sample); [d](kJ 100 g^{-1} of sample); [e]Control sample (100% rice flour).

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 a 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 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.

Response	Effects		
	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 were negative for most of the responses. Table 6 indicated that azuki main effects on moisture and energy were not significant ($p < 0.05$), with a contribution lower than 1%. Although these results did not influence significantly these responses, the interaction chia X azuki presented a significant contribution ($p < 0.05$) to the models (12.70% for moisture and 10.87% for energy). This coefficient was maintained in order to better fit the linear model without affecting the R-squared, as described by Souza et al. (2014b).

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 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 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 contribution of high levels of proteins and lipids by chia (Tables 2, 5 and 6), as shown by Capitani et al. (2012) for defatted chia flour.

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

This fact can be confirmed by analyzing the PC1 versus PC2 (Figure 1A and B), which showed samples 3, 4, 7 and 8, with higher concentrations of chia, separated by quadrants from the other samples. In addition, Table 4 showed a positive interaction between the factors studied and the response surfaces (Figure 2C and D) indicating the same samples as the optimal points for the lipid and protein responses.

The PC1 in Figure 1, which explains 57.22% of the data variance, was able to distinguish 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 (Figure 1A and B), the response surfaces (Figure. 2), and the effects from Table 4, higher lipid and nifext contents and energy value were evidenced with an increased percentage of defatted chia and azuki flours.

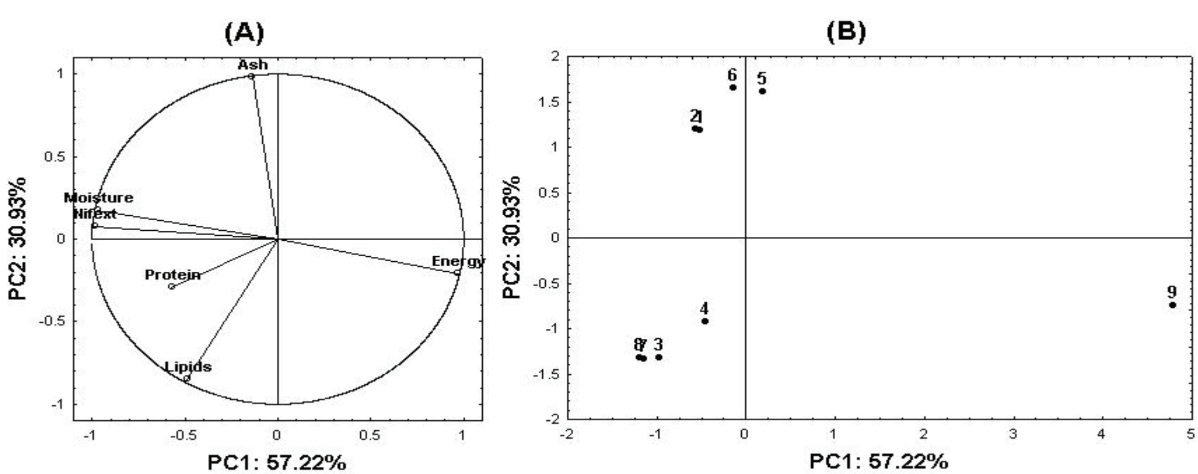

Figure 1. Principal components analysis for the responses studied in the 2^2 factorial design. PC: principal component. loadings, scores.

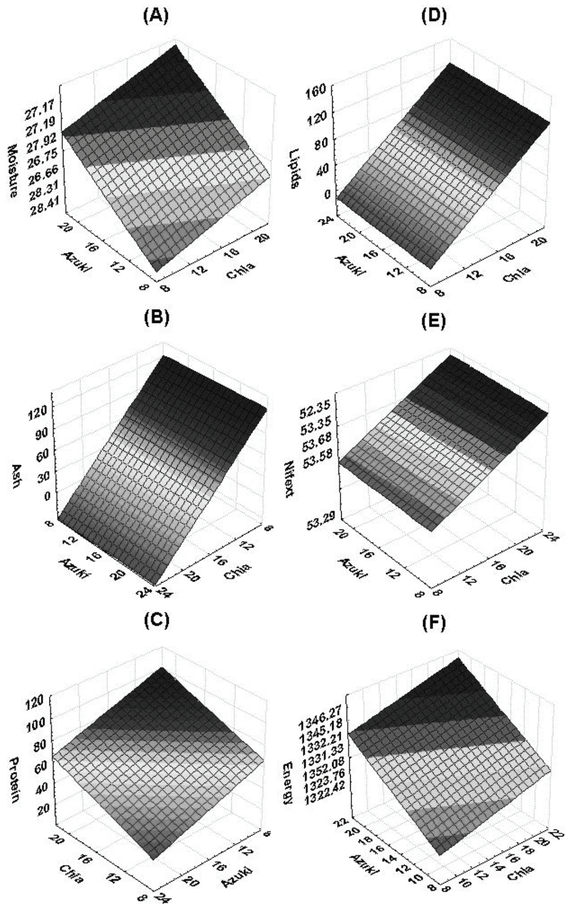

Figure 2. Response surfaces for contents of moisture (A), ash (B), protein (C), lipids (D), nifext (E) and energy (F) according to the concentration of chia and azuki flour.

Table 7 shows that by-product of the cold extraction of oil from chia (the defatted chia flour) has more content of ash, crude proteins and lipids than other flours. Ixtaina et al. (2010) described the prevalence of unsaturated fatty acids in the lipid fraction of chia, especially alpha-linolenic acid (44.4 -63.4%).

Table 8 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. Proximal composition and energy of principal ingredients in the chocolate cakes.

Parameters	Chia	Azuki	Rice
Moisture	7.99 ± 0.02	14.48 ± 0.08	11.14 ± 0.03
Ash	6.29 ± 0.05	3.59 ± 0.04	0.37 ± 0.04
Protein	28.72 ± 0.03	22.94 ± 0.22	7.18 ± 0.06
Lipids	10.80 ± 0.11	0.42 ± 0.05	0.65 ± 0.03
Nifext	46.20 ± 0.07	58.58 ± 0.24	80.66 ± 0.05
Energy	1661.64 ± 3.09	1380.82 ± 3.08	1495.51 ± 1.59

Means and standard deviations.

The responses obtained in the sensory analysis (Table 8) 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), effective tests directly access 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 8), 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 chia and azuki flours obtained very similar averages and standard deviation.

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, cake 1 had the higher contribution of color and texture attributes (Figure 3A) and had the highest averages for both (Table 8).

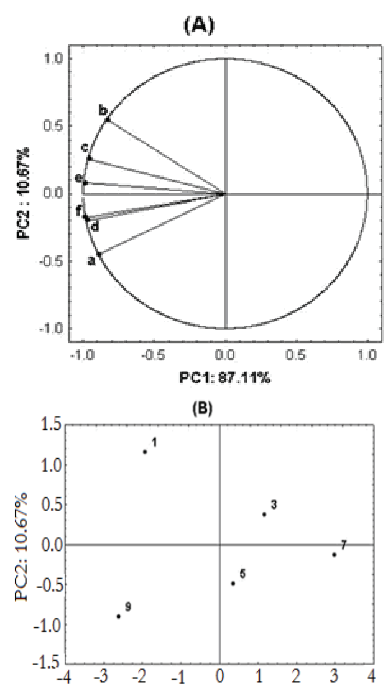

Figure 3. Principal component analysis for the responses studied in the 2^2 factorial model. PC: principal component. Figure 1A: loadings. Figure 1B: scores. a: smell; b: color; c: appearance; d: flavour; e: texture; f: overall acceptance.

Table 8. 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.

Conclusion

Chia flour was the factor that contributed the most the responses, except for nifext.

The main effect of azuki was not significant for the moisture and energy responses. The principal component analysis distinguished samples with a higher content of chia mainly due to crude protein and lipid 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 chia and azuki provided characteristics similar to control assays.

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