

## Application of Multivariate Analysis to Assess the Incorporation of Omega-3 Fatty Acid in Gluten-Free Cakes

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A complete 2<sup>2</sup> experimental planning (two factors at two levels) with central point in quintuplicate was used to investigate the influence of the factors: chia and azuki flours on fatty acids composition of gluten-free chocolate cake. Both factors were significant, but chia flour presented greater influence on the results. Increased values of both factors contributed to improve contents of polyunsaturated fatty acids (380.96 g kg<sup>-1</sup> of product), mainly n-3 series (70.25 g kg<sup>-1</sup> of product), and provided more adequate nutritional indices. The principal component analysis and desirability function indicated the sample with higher level of both factors as the optimal region. This sample showed an increase in contents of alpha-linolenic acid (188.03%) and polyunsaturated fatty acids (18.16%) when compared to control formulation. The addition of chia flour can improve nutritional characteristics of food stuffs such as bakery products, especially their lipid composition.

**Keywords:** *Salvia hispanica* L., alpha-linolenic, response surface methodology, principal components analysis, desirability function

### Introduction

Mankind has suffered lots of changes in all fields of life, such as education and job, and it has a deep reflection in eating habits. People search for products that do not need much time to prepare before eating. However, most of these foods are made of poor ingredients and they cannot offer essential nutrients for human body. In this context, food industries started developing enriched products using potential ingredients with nutritional properties.<sup>1-3</sup>

The bakery products are the most consumed by population due to their practicality and good sensory acceptance. After bread, the cakes ready for consumption are considered the preferred products in this category.<sup>4</sup>

Nowadays, there are gluten free versions of these foods and they can be consumed by celiac patients, however, almost all of these products are totally made of rice flour, which is composed basically only of carbohydrates.<sup>5</sup>

On the other hand, there are several kinds of potential ingredients which may be used to replace rice flour in gluten-free products, such as azuki and chia. Azuki (*Vigna angularis*) is a legume widely produced in Asia and generally used to make some kinds of sweets appreciated by Asiatic people.<sup>6</sup> 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.<sup>7</sup> Both grains, azuki and chia, are considered rich source of many essential nutrients. According to

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Zanqui *et al.*,<sup>8</sup> chia presents high levels of total lipids, and the lipid fraction of this grain is considered a great source of polyunsaturated fatty acids, especially alpha-linolenic acid (LNA, 18:3n-3), and other lipophilic compounds such as tocopherols and phytosterols. According to Ratnayake and Galli,<sup>9</sup> LNA is considered essential because it cannot be metabolized by the human body and must be consumed through the diet.

Factorial design is an important chemometric tool which enables evaluating the contribution of each specific ingredient to several characteristics of the final product, using a smaller number of experiments, simultaneous analysis of many variables and their effects, reliability of the results, performance of the research in stages, an interactive process of inclusion of tests to the model, main variables selection, presentation of the process through mathematical models, and conclusions from qualitative results.<sup>10</sup> Multivariate analysis enables extracting additional information when compared to the univariate analysis. The principal components analysis (PCA) allows the pattern recognition, the gathering of information, reduction of data dimensionality and also the organization of data into a simpler structure, easier to understand. This analysis is based on performing linear comparisons of the original variables. The principal components (PC) are orthogonal among each other and the explained variance decreases with increased PC number.<sup>11</sup>

This study aimed at applying chemometric methods to investigate the influence of the factors: percentages of chia and azuki flour, added in gluten-free chocolate cake for the determination of fatty acid composition and nutritional aspects.

## 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 homogeneous flour that was sieved using a 20-mesh sieve. Chia flour (*Salvia hispanica*, L.) used in this research was partially defatted, it is a

byproduct of the cold-pressing process for lipid extraction. This ingredient was provided by the company Giroil Agroindústria Ltda. (Santo Angelo, Rio Grande do Sul, Brazil). The other ingredients were obtained in retail stores in Maringá.

### Experimental design

A complete 2<sup>2</sup> experimental planning (two factors at two levels) with central point in quintuplicate was used to investigate the influence of both factors on the fatty acids composition of chocolate cake. 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 in this study were: sums of fatty acids from n-3 series, polyunsaturated fatty acids (PUFA), ratio of polyunsaturated by saturated fatty acids (PUFA / SFA), atherogenicity (IA) and thrombogenicity indices (IT) of fatty acids. A control cake (100% rice flour) was produced to be compared to experimental optimum point. The cakes were processed according to the recommendations of Gohara *et al.*<sup>12</sup>

### Formulation processing

All the ingredients were previously weighed individually. The rice flour, azuki and chia, at the respective percentage for each formulation, were mixed to obtain a homogeneous fraction (288.00 g kg<sup>-1</sup> of the whole formulation); and egg white (87.00 g kg<sup>-1</sup>) was mixed to form a solid phase. The egg yolk (58.00 g kg<sup>-1</sup>), butter (58.00 g kg<sup>-1</sup>) and sugar (169.00 g kg<sup>-1</sup>) were homogenized in a bowl to form a cream. The mixture of flours, chocolate powder (80.00 g kg<sup>-1</sup>), cocoa powder (38.00 g kg<sup>-1</sup>), egg white, water (190.80 g kg<sup>-1</sup>), milk powder (21.20 g kg<sup>-1</sup>) and baking powder (10.00 g kg<sup>-1</sup>) were added slowly to the cream 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). The sequence of ingredients mixture and temperature conditions described above followed the process performed in previous study.<sup>13</sup>

**Table 1.** Factors and levels investigated in the experimental design for the development of a 2<sup>2</sup> full factorial design

Factor	Unit	Symbol	Type	Level		
				-1	0	1
Chia flour	g Kg <sup>-1</sup>	C	numeric	100	150	200
Azuki flour	g Kg <sup>-1</sup>	A	numeric	100	150	200

### Total lipid extraction

The total lipids were extracted according to Bligh and Dyer,<sup>14</sup> using a mixture of the solvents: methanol, chloroform and water (final proportion 2:2:1.8, v/v/v).

### Fatty acid composition

The first step to determine the fatty acid composition was the conversion of the lipids into fatty acid methyl esters (FAME), according to Hartman and Lago.<sup>15</sup> The FAME were separated using a CP-3380 gas chromatograph (Varian, Walnut Creek, USA) fitted with a flame ionization detector and a CP 7420-select FAME fused-silica capillary column (100 m × 0.25 mm × 0.25 μm, cyanopropyl). The carrier gas was hydrogen at 1.4 mL min<sup>-1</sup>, the make-up gases were nitrogen at 30 mL min<sup>-1</sup> and synthetic air at 300 mL min<sup>-1</sup>, and the flame gas was hydrogen at 30 mL min<sup>-1</sup>. The sample was injected in a split ratio of 1:100. The injector and detector temperatures were 235 °C. The column temperature was maintained at 165 °C for 4 min, increased to 185 °C at 4 °C min<sup>-1</sup> and maintained for 5 min, and then increased from 185 to 225 °C at 10 °C min<sup>-1</sup> and maintained for 10 min. The retention times were compared with those of standard methyl esters (Sigma, St. Louis, USA). The fatty acids were quantified using tricosanoic acid methyl ester (Sigma) as an internal standard.<sup>16</sup> The peak areas were determined with Star 5.0 software (Sigma, Santa Clara, USA). According to Joseph and Ackman<sup>16</sup> (equation 1), correction factors of FAME for flame ionization detectors in individual fatty acids (FA) were used and their concentrations expressed in mg FA per kg<sup>-1</sup> of food:

$$M_x = \frac{A_x \times M_p \times F_{CT}}{A_p \times M_A \times F_{CEA}} \quad (1)$$

where  $M_x$ : X fatty acid mass in mg g<sup>-1</sup> of sample;  $M_p$ : internal standard mass in milligrams;  $M_A$ : sample mass in g;  $A_x$ : X fatty acid area;  $A_p$ : internal standard area;  $F_{CT}$ : theoretical correction factor;  $F_{CEA}$ : methyl ester correction factor to fatty acid.

The limits of detection (LOD) and quantification (LOQ) were estimated by triplicate analysis of diluted methyl arachidate standard solution (1.0 mg mL<sup>-1</sup>), considering the signal-noise ratio relative to the background signal as 3 and 10, respectively.<sup>17</sup>

### Nutritional quality indices of lipid fraction

The atherogenicity index (IA) and thrombogenicity index (IT) were determined as IA = [(12:0 + (4 × 14:0) +

16:0)] / (MUFA + n-6 + n-3); and IT = (14:0 + 16:0 + 18:0) / [(0.5 × MUFA) + (0.5 × n-6) + (3 × n-3) + (n-3 / n-6)], according to Ulbricht and Southgate.<sup>18</sup>

### Statistical analysis

All the analyses were done in triplicate. Fatty acid composition was demonstrated by the general average of the experiments repetitions (A: test 1; B: test 2; C: test 3; D: test 4 and E: tests 5, 6, 7, 8 and 9). Initially, the values of effects, interaction, and analysis of variance (ANOVA) were obtained. Afterwards, the whole variables had their normal and homogeneity of variance assessed through the combings. Then, the ANOVA among the groups was done for all the responses. Considering the independent variables effect on the responses, the response surface methodology was applied. The basic mathematic model used to adjust the data was (equation 2):

$$\hat{Y}_i = b_0 + b_1 x_1 + b_2 x_2 \quad (2)$$

where  $\hat{Y}_i$  is the expected response;  $b_0$  is the constant term;  $b_1$  and  $b_2$  are the terms of the regression model and  $x_1$  and  $x_2$  are the levels of the independent variables.<sup>19</sup>

The model's equations were arranged to a global response using a desirability function. The results obtained for the sums of fatty acids from n-3 series, polyunsaturated fatty acids, ratio of polyunsaturated by saturated fatty acids, atherogenicity and thrombogenicity indices were used to estimate the global response. This procedure involved a transformation of each response ( $\hat{Y}_i$ ) estimated for an individual value of desirability ( $d_i$ ), in which  $0 \leq d_i \leq 1$ , according to Derringer and Suich.<sup>20</sup>

If the objective or target  $T$  to the response  $\hat{Y}_i$  is a maximum value, then the equation 3 should be used:

$$d_i = \begin{cases} 0 & \hat{Y}_i < L \\ \left( \frac{\hat{Y}_i - L}{T - L} \right)^r & L \leq \hat{Y}_i \leq T \\ 1 & \hat{Y}_i > T \end{cases} \quad (3)$$

If the objective or target to the response  $\hat{Y}_i$  is a minimum value then the equation 4 should be used:

$$d_i = \begin{cases} 1 & \hat{Y}_i < T \\ \left( \frac{U - \hat{Y}_i}{U - T} \right)^r & T \leq \hat{Y}_i \leq U \\ 0 & \hat{Y}_i > U \end{cases} \quad (4)$$

where  $L$  and  $U$  are minimum and maximum limits, respectively.

The convenience function is linear when the weight 'r' is equal to 1. If  $r > 1$  there is more emphasis on targeting the closest value. Using  $0 < r < 1$  makes this less important.

Individual values of desirability ( $d_i$ ) were arranged through a geometric average to form a global desirability value (D), which will attend to satisfy all response simultaneously. This single value of D [0,1] gives a global assessment of convenience and the arranged response levels, and D will increase at the same time that the properties balance becomes more favorable.

PCA consisted of the sums of fatty acids from n-3 series, PUFA, PUFA / SFA, IA and IT of fatty acids - loadings. For this analysis, the average values of the five different formulations (A: test 1; B: test 2; C: test 3; D: test 4; E: tests 5, 6, 7, 8 and 9) were used - scores. Averages were autoscaled, so that the whole variables showed the same weight. In this way, PCA bidimensional graphics were obtained.

The formulation considered the best one by desirability assay was compared to the control cake. The Student's *t*-test was applied to analyze differences between the two different samples. All the statistical analyses were done using the software Statistica, version 8.0,<sup>21</sup> adopting the 5% significance level for rejection of the null hypothesis ( $p < 0.05$ ).

## Results and Discussion

The total lipids contents for samples A, B, C, D, E and control were 73.50, 76.64, 75.57, 77.65, 76.43 and 59.50 g kg<sup>-1</sup>, respectively, according to previous study.<sup>12</sup> This research showed that enriched gluten free cake presented good sensory acceptance, as well as great

contents of nutrients, such as protein and lipids, mainly the formulation with higher contents of chia flour.<sup>12</sup> Another previous study showed the great levels of essential minerals in all the formulations of cake, the minerals were Ca, Cu, Fe, K, Mg, Mn and Zn. The highest mineral contents were found in the formulations with the highest concentration of both flour (chia and azuki).<sup>13</sup> In this present study, similar results were found for essential fatty acids, as shown below.

Under the selected operation system, LOD and LOQ were estimated at 0.15 and 0.50 mg g<sup>-1</sup> of total lipids, respectively.

Gas chromatography allowed identifying and quantifying eighteen fatty acids and the major ones were: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6) and alpha-linolenic acid (LNA, 18:3n-3). All the formulations, mainly formulation with higher contents of chia, showed the prevalence of polyunsaturated fatty acids, which are involved with the smaller risk of cardiovascular diseases.<sup>9</sup>

LNA presented the highest content value in all the formulations. This fatty acid plays important roles in metabolic processes. Through desaturases and elongases enzymes, LNA can be converted into other eicosanoid fatty acids from n-3 series (omega-3), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:5n-3), which are very important for human health due to their anti-inflammatory properties.<sup>22,23</sup>

These results were similar to those found for food products enriched with omega-3 using flaxseed grain, such as panettone,<sup>24</sup> granola,<sup>25</sup> food bars<sup>26</sup> and cookies.<sup>27</sup>

Table 2 presents the conditions of the complete 2<sup>2</sup> factorial model with central point in quintuplicate, applied to the tests, as well as the values obtained for the sums, ratio, and nutritional indices of fatty acids.

**Table 2.** Complete 2<sup>2</sup> design, the obtained responses to the sums, ratios and fatty acids indices

Test	Independent variable <sup>a</sup>			Sums and ratios of fatty acids / (mg FA g <sup>-1</sup> TL)			
	x <sub>1</sub>	x <sub>2</sub>	n-3 <sup>b</sup>	PUFA <sup>b</sup>	PUFA / SFA <sup>b</sup>	IA	IT
1	-1	-1	45.83	343.55	1.06	0.47	0.66
2	-1	1	48.50	354.88	1.17	0.45	0.61
3	1	-1	67.31	366.41	1.24	0.43	0.54
4	1	1	70.25	380.96	1.35	0.39	0.49
5	0	0	59.04	367.78	1.26	0.41	0.55
6	0	0	58.30	353.71	1.13	0.44	0.62
7	0	0	57.16	349.23	1.10	0.45	0.63
8	0	0	57.18	350.18	1.11	0.45	0.63
9	0	0	59.55	363.68	1.22	0.41	0.57

<sup>a</sup>Expressed according to Table 1; <sup>b</sup>mg fatty acid *per* g<sup>-1</sup> of total lipid. x<sub>1</sub>: chia flour; x<sub>2</sub>: azuki flour; n-3: total omega-3 fatty acids; PUFA: total of polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity.

**Table 3.** Regression coefficients, confidence intervals and coefficients of determination of the responses applied to the response surface methodology

Parameter	n-3	PUFA	PUFA / SFA	IA	IT
Mean	58.13 (57.393, 58.858) <sup>a</sup>	358.93 (351.208, 366.653) <sup>a</sup>	1.18 (1.129, 1.232) <sup>a</sup>	0.43 (0.419, 0.447) <sup>a</sup>	0.59 (0.563, 0.617) <sup>a</sup>
x <sub>1</sub>	10.81 (9.709, 11.905) <sup>a</sup>	12.23 (0.650, 23.819) <sup>a</sup>	0.09 (0.014, 0.167) <sup>a</sup>	-0.03 (-0.046, -0.003) <sup>a</sup>	-0.06 (-0.112, -0.008) <sup>a</sup>
x <sub>2</sub>	1.40 (0.303, 2.500) <sup>a</sup>	6.47 (-5.116, 18,053) <sup>a</sup>	0.06 (-0.021, 0.133) <sup>a</sup>	-0.02 (-0.036, 0.006)	-0.06 (-0.064, 0.017) <sup>a</sup>
R <sup>2</sup>	0.990	0.701	0.657	0.654	0.707

<sup>a</sup>Confidence interval for 95% level. n-3: total fatty acids from omega-3 series; PUFA: total polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity; x<sub>1</sub>: chia flour; x<sub>2</sub>: azuki flour; R<sup>2</sup>: coefficient of determination.

The regression coefficients for each one of the models, their confidence intervals and coefficients of determination (R<sup>2</sup>) of the responses applied to the response surface methodology are presented in the Table 3. Values close to 1 indicate a good correlation between experimental and predicted data.<sup>28</sup> Pagamunici *et al.*<sup>29</sup> found coefficients of determination of 0.85 for instrumental data *vs.* sensory analysis as a function of time, and the models were well fitted. In this study, the values were greater than 0.60, which means that the linear model explained more than 60% of the data variability. The residual plots for each response showed normality, and homogeneity of variance was explained satisfactorily.

The limits of confidence intervals for the first-order term (x<sub>2</sub>) for the sum of polyunsaturated fatty acids, PUFA / SFA ratio, IA and IT showed values with opposite signs (Table 3). All the values are possibly within a confidence interval, therefore it is possible that this value is zero. This fact demonstrates that there was a linear correlation between the variables, so there is no statistical evidence to keep this term in the model. Nevertheless, its permanence was preferred to preserve the mathematical hierarchy.<sup>10</sup>

The effect values can be obtained by regression coefficients values (effects are twice the coefficients). Data from Table 3 show that the main effects of the two

factors (chia and azuki) were positive for fatty acids from n-3 series, PUFA and the PUFA / SFA ratio, and they were negative for IA and IT. These data enable us to estimate that greater concentration of chia and azuki may increase contents of n-3 series fatty acids and PUFA. This increase was also found for hamburgers in studies performed by Souza *et al.*<sup>30</sup> All the experimental models did not present interaction effects for the responses analyzed and were removed.

Tables 4 and 5 show the results obtained by ANOVA for each factor studied in the model response. The factor chia most influenced the increased levels of fatty acids from n-3 series, PUFA and PUFA / SFA, with contributions of 99.43, 54.78 and 47.83%, respectively (Table 4).

The values of F-test (Table 5) demonstrate the significance of coefficients of regression and lack of fit. According to data from Table 4, the lack of fit of the model was not significant for the models.

The effects may be better observed in the response surface models (Figure 1). Surfaces of n-3 series fatty acids, PUFA and PUFA / SFA ratio clearly show the positive effect caused by increasing the factors, mainly the factor chia. On the other hand, surfaces of IA and IT show a negative effect. These nutritional indices are associated with the presence of lauric (12:0), myristic (14:0), palmitic (16:0)

**Table 4.** Results of ANOVA, sum of squares of the responses obtained in full 2<sup>2</sup> factorial design with central point

Source	Sum of squares					
	DF	n-3	PUFA	PUFA / SFA	IA	IT
x <sub>1</sub>	1	467.169	598.727	0.033	0.002	0.014
x <sub>2</sub>	1	7.853	167.364	0.013	0.001	0.002
Lack of fit	2	0.181	48.346	0.004	0.000	0.001
Pure error	4	4.655	278.524	0.020	0.002	0.006
Total SS	8	469.859	1092.961	0.069	0.005	0.023

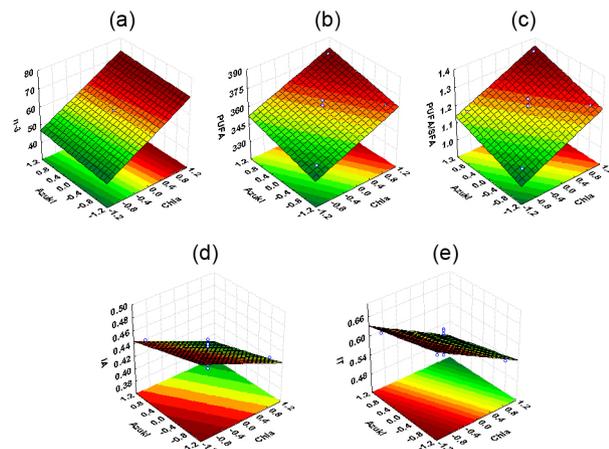
DF: degree of freedom; n-3: total fatty acids from omega-3 series; PUFA: total polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity; x<sub>1</sub>: chia flour; x<sub>2</sub>: azuki flour; SS: sum of squares.

**Table 5.** Results of ANOVA and F-test of the responses obtained in full 2<sup>2</sup> factorial design with central point

Source	F-test					
	DF	n-3	PUFA	PUFA / SFA	IA	IT
x <sub>1</sub>	1	579.584	10.990	8.304	7.436	12.003
x <sub>2</sub>	1	9.742	3.072	3.176	2.694	1.948
Lack of fit	2	0.078	0.347	0.370	0.070	0.462
Pure error	4	–	–	–	–	–

DF: degree of freedom; n-3: total fatty acids from omega-3 series; PUFA: total polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity; x<sub>1</sub>: chia flour; x<sub>2</sub>: azuki flour.

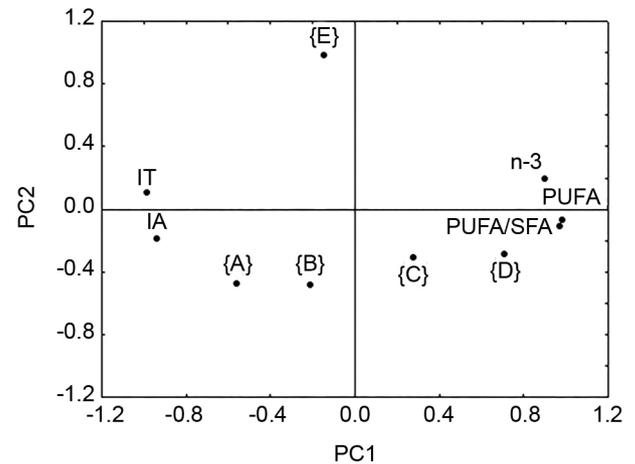
and stearic (18:0) fatty acids which are more related to the increased incidence of coronary diseases when compared to monounsaturated fatty acids, especially oleic (18:1n-9) and the series omega 3 and 6. Souza *et al.*<sup>31</sup> and Silva *et al.*<sup>32</sup> found low values of IA and IT for sacha inchi nut and perilla oil, respectively, emphasizing the direct correlation between the lowest ratio and an attenuated risk of coronary disease.



**Figure 1.** Response surfaces to sum, proportion and indices of fatty acids. n-3: sum of fatty acid from omega-3 series; PUFA: polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: atherogenicity index; IT: thrombogenicity index.

Figure 2 presents PCA. The principal components 1 and 2 (PC1 and PC2) were selected due to their statistical significance ( $p < 0.05$ ). PC1 explained 55.51% of data variance, and loadings indicated a positive contribution of the contents of n-3 series fatty acids (0.9012), total PUFA (0.9865) and PUFA / SFA ratio (0.9724), which characterized samples C and D (formulations with higher contents of chia). A negative contribution of IA (−0.9377) and IT (−0.9866) was observed on PC1, which characterized formulations A and B (formulations with lower contents of chia).

PC2 was responsible for 17.11% of the data variance, and there was the separation of sample E on the upper region of the graph, which involves the 5 replicates of the central point (tests 5, 6, 7, 8 and 9). These formulations

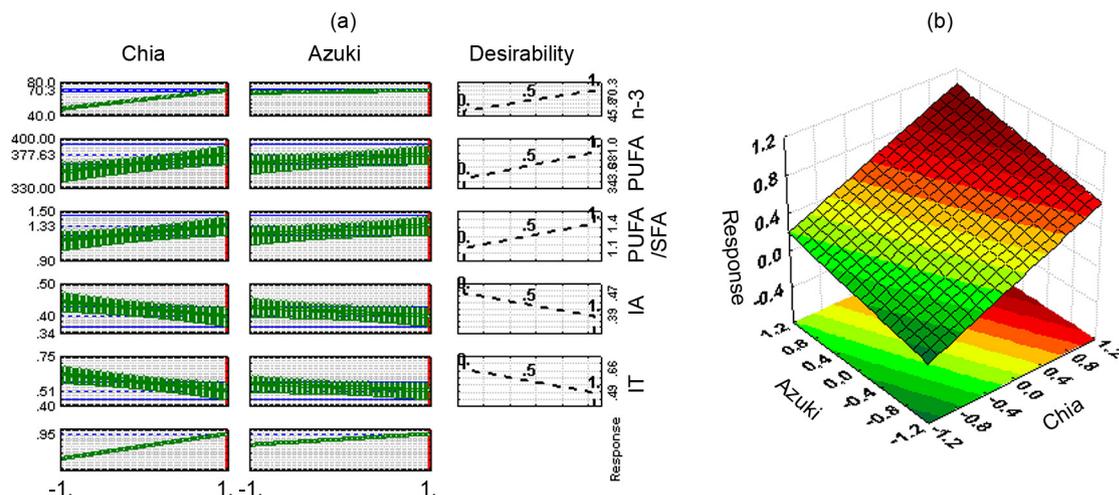


**Figure 2.** Principal components analysis for complete 2<sup>2</sup> experimental design with central point in quintuplicate. PC: principal component; A: test 1; B: test 2; C: test 3; D: test 4; E: tests 5, 6, 7, 8 and 9; n-3: sum of fatty acid from omega-3 series; PUFA: polyunsaturated fatty acids; SFA: total saturated fatty acids; IA: atherogenicity index; IT: thrombogenicity index.

present the intermediate values for n-3 series fatty acids, PUFA and PUFA / SFA ratio. Increased PUFA and n-3 in the cake formulations are associated with the high contents of alpha-linolenic fatty acid in the chia.<sup>8</sup>

Figure 3 shows the desirability function for the following constrictions: maximum value of fatty acids from n-3 series, total PUFA and PUFA / SFA ratio (equation 3); and minimum value of IA and IT indices (equation 4). The highest levels of chia and azuki flours were described as a point of major desirability (test 4). The higher concentrations of the factors studied were determinant for greater composition of fatty acid, such as higher contents of PUFA and fatty acids from n-3 series. The nutrient with the greatest increase was the alpha-linolenic fatty acid whose content in test 4 was 153.28% bigger than in test 1, corroborating with results found by Souza *et al.*<sup>30</sup>

According to the Institute of Medicine,<sup>33</sup> the contribution of alpha-linolenic fatty acid to the dietary reference intakes were determined for adults (1.6 g day<sup>-1</sup>), using a portion of 60 g of cake (equivalent to one slice). The values were 12.63, 13.94, 19.07, 20.46 and 16.70%, for formulations A, B, C, D and E, respectively. The greatest contribution was



**Figure 3.** (a) Conditions of restrictions and responses of the desirability function for complete  $2^2$  experimental design with central point in quintuplicate. n-3: sum of fatty acid from omega-3 series; PUFA: polyunsaturated fatty acids; SFA: total saturated fatty acids; IA: atherogenicity index; IT: thrombogenicity index; (b) response surface for the optimum region using the application of desirability functions.

presented by formulation D, which has the highest level of both factors chia and azuki flour.

Table 6 shows fatty acid composition of optimum experimental point by desirability function (formulation D) and of control product. Both samples presented the same fatty acids, however, there were some significant differences in contents of butyric acid (4:0), capric acid (10:0), lauric acid (12:0), heptadecanoic acid (17:0), oleic acid (18:1n-9), linoleic acid (18:2n-6) and arachidic acid (20:0). The main difference was observed for alpha-linolenic acid, which increased 188.03% in optimum formulation when compared to control cake. As a result, PUFA contents also showed an increase in the optimum formulation (18.16%). Furthermore, atherogenicity index and thrombogenicity index showed lower values for optimum point when compared to control cake (21.57% IA and 41.18% IT, respectively).

## Conclusions

Chemometric methods were very useful for improvement of fatty acids composition of gluten-free chocolate cake. Factor chia flour presented higher contribution for increasing n-3 series and polyunsaturated fatty acids, as well as nutritional indices. The models analyzed were significant and allowed obtaining the response surfaces. Principal components analyses distinguished samples with higher contents of chia flour. The formulation with higher contents of chia and azuki flour was considered as a point of greatest desirability. This formulation presented higher levels of n-3 fatty acid and PUFA, and lower indices of atherogenicity and thrombogenicity than control cake. The addition of grains with high nutritional value is a great

**Table 6.** Fatty acid quantification and *t*-test for comparison between optimum experimental point and control cake

Fatty acid	Composition / (mg kg <sup>-1</sup> of product)		<i>p</i> -Value
	Optimum experimental point	Control	
4:0	6.61 ± 0.17	4.94 ± 0.81	0.0515
6:0	1.52 ± 0.07	1.12 ± 0.11	0.0413
8:0	1.38 ± 0.06	1.06 ± 0.06	0.0139
10:0	2.53 ± 0.26	2.49 ± 0.13	0.8192
12:0	3.57 ± 0.40	3.71 ± 0.14	0.5734
14:0	22.88 ± 2.81	30.83 ± 1.38	0.0146
15:0	13.79 ± 1.16	18.93 ± 0.90	0.0013
16:0	156.20 ± 3.53	172.61 ± 3.24	0.0006
16:1n-9	5.78 ± 0.38	6.50 ± 0.17	0.0315
17:0	1.38 ± 0.13	1.28 ± 0.06	0.3798
18:0	65.16 ± 0.90	91.30 ± 3.82	0.0099
18:1n-9	239.69 ± 3.18	240.58 ± 2.88	0.3101
18:1n-7	9.90 ± 0.13	9.73 ± 0.08	0.2893
18:2n-6	307.26 ± 3.47	299.13 ± 7.48	0.0844
18:3n-6	3.45 ± 0.02	3.25 ± 0.14	0.0005
18:3n-3	70.25 ± 0.93	24.39 ± 1.38	0.0005
20:0	1.49 ± 0.05	1.42 ± 0.02	0.1892
22:0	5.15 ± 0.01	4.74 ± 0.07	0.0061
Sums and ratios of fatty acid			
n-3	70.25 ± 0.93	24.39 ± 1.38	0.0005
PUFA	386.11 ± 3.59	326.76 ± 7.60	0.0006
PUFA / SFA	1.37 ± 0.03	0.98 ± 0.03	0.0003
Indices of the quality of the lipid fraction			
IA	0.40 ± 0.03	0.51 ± 0.02	0.0017
IT	0.49 ± 0.02	0.84 ± 0.03	0.0007

Mean and standard deviation for optimum experimental point and control cakes; n-3: total fatty acids from omega-3 series; PUFA: total polyunsaturated fatty acids; SFA: total of saturated fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity.

alternative to increase nutrients contents in food products, especially bakery ones.

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