UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

APLICAÇÃO DE FERRAMENTAS QUIMIOMÉTRICAS NO DESENVOLVIMENTO E CARACTERIZAÇÃO DE PRODUTOS ALIMENTÍCIOS CONTENDO *Salvia hispanica*, L.

ALOISIO HENRIQUE PEREIRA DE SOUZA

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá como critério para obtenção do grau de Doutor em Ciência de Alimentos.

Orientador: Prof. Dr. Makoto Matsushita

Maringá

2015

Orientador

Prof. Dr. Makoto Matsushita

BIOGRAFIA

Aloisio Henrique Pereira de Souza, natural de Londrina, no estado do Paraná, Brasil. Possui graduação em Tecnologia de Alimentos pela Universidade Tecnológica Federal do Paraná – *Campus* Londrina (2009). Mestre em Ciência de Alimentos pela Universidade Estadual de Maringá em 2011. Atualmente professor do ensino básico, técnico e tecnológico lotado no Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso do Sul – *Campus* Coxim. Tem experiência em análises físico-químicas, microbiológicas, sensoriais e instrumentais de alimentos atuando principalmente nos seguintes temas: desenvolvimento e caracterização de alimentos com propriedades funcionais; química analítica de alimentos; métodos cromatográficos de separação; quimiometria aplicada ao desenvolvimento e caracterização de alimentos.

DEDICO

Aos meus queridos Pais, Aloisio e Jacqueline,
Aos meus irmãos Jéssica e José,
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com quem compartilho os meus momentos de alegria e de dificuldade.
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APRESENTAÇÃO

Está tese de doutorado está apresentada na forma de dois artigos científicos, em que o artigo 1 encontra-se publicado e artigo 2 será submetido.

ARTIGO 1: Souza, A. H. P.; Gohara, A. K.; Rotta, E. M.; Chaves, M. A.; Silva, C. M.; Dias, L. F.; Gomes, S. T. M.; Souza, N. E.; Matsushita, M. Effect of the addition of chia's by-product on the composition of fatty acids in hamburgers through chemometric methods. Journal of the Science of Food and Agriculture, v. 95, p. 928-935, 2015. Qualis-Capes B1 para Ciência de Alimentos.

ARTIGO 2: Souza, A. H. P.; Gohara, A. K.; Rotta, E. M.; Morais, D. R.; Joia, B. M.; Gomes, S. T. M.; Souza, N. E.; Matsushita, M. Aplicação de fatorial categórico no desenvolvimento de hambúrguer vegetal contendo chia como fonte de ômega 3: Estudo de otimização. Será submetido na revista "Journal of the Brazilian Chemical Society", com qualis-Capes B1 para Ciência de Alimentos.


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RESUMO GERAL

INTRODUÇÃO. Os hambúrgueres são comumente produzidos a partir de cortes bovinos de baixo custo comercial juntamente com tecidos de gordura tipicamente de origem subcutânea, o qual exerce influência direta e majoritária sobre a composição lipídica do produto final. Os lipídios também são de grande importância, pois são fonte de energia e ácidos graxos essenciais, além de atuarem no transporte de vitaminas lipossolúveis. No entanto, os lipídios de origem animal apresentam altos teores de colesterol e ácidos graxos saturados, os quais estão associados a diversos tipos de doenças cardiovasculares e coronarianas, além de risco de obesidade. Assim, a utilização de fontes vegetais de lipídios poderia resultar em produtos considerados mais saudáveis. Neste contexto, destaca-se o uso da chia (*Salvia hipanica*, L.) que é considerada um grão fonte do ácido graxo alfa linolênico. No desenvolvimento dos produtos alimentícios faz-se necessário a variação de diversos ingredientes e parâmetros no processamento. O planejamento fatorial é uma ferramenta dinâmica, que possibilita fazer um número reduzido de experimentos, avaliar várias variáveis simultaneamente, seus efeitos, maior confiabilidade nos resultados, em um processo interativo de adição ou retirada de ensaios no modelo, para viabilizar a seleção das principais variáveis e apresentação de modelos matemáticos com conclusões a partir de resultados qualitativos. Os planejamentos fatoriais categóricos possuem a característica de extrair informações sobre tipos de ingredientes, processos e parâmetros que não podem ser variados como nas variáveis numéricas.

OBJETIVOS. O objetivo desta tese foi a aplicação de métodos quimiométricos para investigar a influência dos fatores numéricos e categóricos no desenvolvimento, processamento de formulações de hambúrgueres bovinos e vegetais contendo coproduto ou farinha integral de chia sobre a composição de ácidos graxos, oxidação lipídica, proximal e características nutricionais.

MATERIAL E MÉTODOS.
ARTIGO 1: Um planejamento fatorial 2^2 completo (dois fatores em dois níveis) com duplicata foi realizado para investigar a influência dos fatores: % de proteína texturizada de soja (PTS) e farinha de chia parcialmente desengordurada (FDC) na substituição parcial da mistura de carne bovina e toucinho suíno em hambúrgueres. Foram feitas análises de composição em ácidos graxos, oxidação lipídica e proximal, sendo propostos modelos matemáticos com as respostas, análise de componentes principais e avaliação da função de desejabilidade.
ARTIGO 2: Aplicação de planejamento fatorial categórico no desenvolvimento de hambúrguer vegetal como fonte de ácido graxo ômega-3 e obter uma melhor formulação através da otimização do experimento. Para isso foi investigado a influência de duas fontes de ômega-3, emulsificantes/ligantes e o efeito do processamento sobre a composição dos ácidos graxos.

RESULTADOS E DISCUSSÃO.
ARTIGO 1: Os fatores % de PTS e FDC foram significativos, e o aumento dos valores nestes, contribuiu para melhorar a composição em ácidos graxos, proteína bruta e cinza. A análise de componentes principais distinguiram as amostras com maior teor de chia através do CP1 e CP2. Foram feitas algumas restrições nas respostas para a análise de desejabilidade. Nesta análise o nível superior de PTS e FDC foi caracterizado como o ponto ótimo de maior desejabilidade.

ARTIGOS 2: Todos os efeitos principais e de interação foram significativos. Na análise hierárquica houve a formação de dois grupos bem definidos, sendo um com chia e outro com a linhaça, ambos tendo a goma xantana/carboximetilcelulose e sem cocção. A composição em ácidos graxos nos ensaios foi igual e houve uma variação significativa entre as formulações. Nos modelos o efeito principal emulsificante e a interação com os três fatores avaliados apresentaram o maior percentual de contribuição. Nesta análise o produto contendo chia como fonte de ômega-3, a goma xantana/carboximetilcelulose como emulsificante no hambúrguer assado foi caracterizado como o ponto ótimo de maior desejabilidade.

CONCLUSÕES.

ARTIGO 1: O planejamento fatorial aplicado no hamburguer demonstrou que o aumento dos fatores estudados contribuiu para melhorar a composição em ácidos graxos, proteína bruta, cinza e a qualidade nutricional do produto. A análise de componentes principais distinguiram as amostras com maior teor de chia através do CP1 e CP2. Na análise de desejabilidade o nível superior de PTS e FDC foi caracterizado como o ponto ótimo não havendo a necessidade de fazer outro ponto experimental. A adição do coproduto de chia é uma alternativa para aumentar os teores de alfa linolênico e obter alimentos nutricionalmente balanceados.

ARTIGO 2: O uso de vegetais, com destaque para a chia é uma alternativa no desenvolvimento de um alimento nutricionalmente balanceado, tendo em vista a aplicação de várias ferramentas quimiométricas desde a seleção dos ingredientes, processamento e na caracterização do hambúrguer vegetal.

GENERAL ABSTRACT

INTRODUCTION. The burgers are commonly produced from low cost commercial beef cuts along with fat typically subcutaneous tissue origin, and which has a direct influence on the majority lipid composition of the final product. Lipids are also of great importance, as they are a source of energy and essential fatty acids, and act in the transport of fat-soluble vitamins. However, animal lipids have high levels of cholesterol and saturated fatty acids, which are associated with many types of cardiovascular and coronary heart disease, and obesity risk. Thus, the use of vegetable lipid sources could result in products which are considered healthier. In this context, it highlights the use of chia (Salvia hipanica, L.) is considered a grain source of alpha linolenic fatty acid. In the development of food products it is necessary to change the various ingredients and processing parameters. The factorial design is a dynamic tool, which enables to make a small number of experiments, to evaluate multiple variables simultaneously, their effects, more reliable results, in an interactive process of addition or removal tests on the model, to enable the selection of the main variables and presentation of mathematical models with findings from qualitative results. Categorical factorial designs have the characteristic extracting information about types of ingredients, processes and parameters that cannot be varied as in the numeric variables.

OBJECTIVES. The objective of this thesis was the application of chemometric methods to investigate the influence of numerical and categorical factors in the development of bovine burgers processing formulations and vegetables containing co-product or wholemeal chia on the fatty acid composition, lipid oxidation, proximal and features nutrition.

MATERIAL AND METHODS.
ARTICLE 1: A full factorial design 2^2 (two factors in two levels) in duplicate were performed to investigate the influence of factors:% of textured soybean protein (TSP) and partially defatted flour chia (FDC) in partial replacement of the beef mixture and pork bacon on burgers. Composition analyzes were made in fatty acids, lipid oxidation and proximal being proposed mathematical models with the answers, principal component analysis and evaluation of the desirability function.
ARTICLE 2: Factorial design application categorical in developing vegetable burger as fatty acid source of omega-3 and get a better formulation by optimizing the experiment. For this we investigated the influence of two sources of omega-3, emulsifiers / binders and the effect of processing on the composition of fatty acids.

RESULTS AND DISCUSSION.
ARTICLE 1: The percentage of PTS and FDC factors were significant, and the increase in these values, helped to improve the fatty acid composition, crude protein and gray. The principal component analysis distinguished the samples with greater chia content through CP1 and CP2. There have been some restrictions on the responses to the desirability analysis. In this analysis the higher level of PTS and FDC was characterized as the optimal point of highest desirability.
ARTICLE 2: All the main and interaction effects were significant. In the hierarchical analysis was the formation of two well-defined groups, one with creaky and one with flaxseed, both having xanthan / carboxymethylcellulose and without cooking gum. The fatty acid composition in the tests was equal and there was a significant variation between the formulations. In models the main effect emulsifier and interaction with the three evaluated factors showed the highest percentage of contribution. In this analysis the product containing

chia as a source of omega-3, xanthan gum / carboxymethylcellulose as an emulsifier in baked hamburger was characterized as the optimal point of highest desirability.

CONCLUSIONS.

ARTICLE 1: The experimental design used in the burger showed that the increase of the studied factors contributed to improving the fatty acid composition, protein, ash and the nutritional quality of the product. The principal component analysis distinguished the samples with greater chia content through CP1 and CP2. The desirability of analyzing the top-level PTS and FDC was characterized as the optimum point there is no need to do another experimental point. The addition of the coproduct chia is an alternative to increase the alpha linolenic content and more nutritionally balanced foods.

ARTICLE 2: The use of vegetables, especially the chia is an alternative in the development of a nutritionally balanced food, with a view to applying various chemometric tools from the selection of ingredients, processing and characterization of vegetal hamburger.

ATTECHMENT 1: Effect of the addition of chia's by-product on the composition of fatty acids in hamburgers through chemometric methods.

Research Article

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Effect of the addition of chia's by-product on the composition of fatty acids in hamburgers through chemometric methods

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Abstract

BACKGROUND: Hamburger is a meat-based food that is easy to prepare and is widely consumed. It can be enriched using different ingredients, such as chia's by-product, which is rich in omega-3. Chemometrics is a very interesting tool to assess the influence of ingredients in the composition of foods. A complete factorial design 2^2 (two factors in two levels) with duplicate was performed to investigate the influence of the factors (1) concentration of textured soy proteins (TSP) and (2) concentration of chia flour partially defatted (CFPD) as a partial replacement for the bovine meat and porcine fat mix in hamburgers.

RESULTS: The results of proximal composition, lipid oxidation, fatty acids sums, ratios, and nutritional indexes were used to propose statistical models. The factors TSP and CFPD were significant, and the increased values contributed to improve the composition in fatty acids, crude protein, and ash. Principal components analysis distinguished the samples with a higher content of chia. In desirability analysis, the highest level of TSP and CFPD was described as the optimal region, and it was not necessary to make another experimental point.

CONCLUSION: The addition of chia's by-product is an alternative to increase the α-linolenic contents and to obtain nutritionally balanced food.

Keywords: *Salvia hispanica* L; α-linolenic; response surface methodology; principal components analysis; desirability function

INTRODUCTION

Chia (*Salvia hispanica* L.) is an angiosperm plant from the Lamiaceae family, described as a tropical and sub-tropical grain, which was largely consumed in pre-Columbian America as well as the region that includes Mexico and Guatemala.[1,2]

According to Ixtaina *et al.*[3] the lipid fractions of this grain are considered a source of α-linolenic fatty acid (18:3*n*-3). Soy (*Glycine max* L. Merril) is considered by the US Food and Drug Administration[4] to be a food with a high nutritional value, containing a desirable balance of amino acids.

Hamburger is a food that is very easy to prepare, practical, and widely consumed. It can be defined as a meat industrialized product made with ground meat from butchery animals, with or without addition of adipose tissue and other ingredients, molded, and submitted to an appropriate technological process. Proteins and fats from vegetables and other animals may also be incorporated.[5]

Factorial design allows 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.[6] In principal components analysis (PCA), the standard recognition is made, the information is feasible, and there is a reduction in the data's dimensionality. The principal components (PCs) are orthogonal among each other and the explained variance decreases with increasing number of PCs.[7]

This goal of this study was to use chemometric methods to assess the influence of the factors textured soy protein percentage and addition of chia's by-product into hamburger formulations on the fatty acid composition, lipid oxidation, and proximal and nutritional characteristics.

EXPERIMENTAL

Sampling

Chia flour (*Salvia hispanica* L.) used in this research was partially defatted (CFPD), and is a by-product of the cold-pressing process for lipid extraction. This ingredient was obtained from Giroil Agroindustria Ltda. (Santo Angêlo, RS, Brazil). The textured soy protein (TSP) was acquired in granule shape. The sampling of CFPD

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Table 1. Factors and levels investigated in a complete experimental design, 2^2 in duplicate

Factor	Symbol	Unit	Type	Level −1	Level +1
Chia flour partially defatted	CFPD	g kg^{-1}	Numeric	80	120
Textured soy protein	TSP	g kg^{-1}	Numeric	80	120

and TSP consisted of two samples of 5 kg that were ground in a hammer mill and passed through a 14-mesh strainer for homogenization. The meat fraction was composed of two samples of 5 kg of Longissimus dorsi and 500 g of porcine fat (10:1, m/m). They were cut into pieces and milled in a meat miller. The meat, the porcine fat, TSP, and the other ingredients were acquired in the Maringá's shops, Paraná, Brazil.

Experimental design

A complete 2^2 planning (two factors at two levels) in duplicate was used to investigate the influence of the factors on the fatty acids and proximal composition of hamburgers. The two factors were the concentrations of TSP and CFPD (Table 1). All the experiments were randomized. The responses analyzed in this study were sums, proportions, and nutritional indexes of fatty acids, lipid oxidation, and proximal composition.

Formulation processing

All the ingredients were previously weighed individually. The meat and porcine fat mix (10:1, m/m), TSP and CFPD, in the respective percentage for each product, was homogenized (883.50 g kg^{-1} of the whole formulation). Water at 5 °C (80.00 g kg^{-1}); glutamate monosodium (5.00 g kg^{-1}); carrageenan gum (4.00 g kg^{-1}); oregano powder (0.40 g kg^{-1}); garlic powder (3.00 g kg^{-1}); and white pepper powder (0.50 g kg^{-1}) were added and mixed until the formation of a homogeneous paste. Afterwards, pressing and casting was performed in a manual hamburger maker with 11 cm diameter, obtaining hamburgers with liquid weight around 100 g (±0.05) per unit. Hamburger disks were individually wrapped into polyethylene bags and stored in a freezer at −18 °C for 24 h. Hamburgers were submitted to the firing process on a Teflon griddle at 300 °C for 3 min with two inversions of the product, without adding oil for frying.

Fatty acid composition

The first step to determine the fatty acid composition is the conversion of the lipids into fatty acid methyl esters (FAMEs), according to Hartman and Lago.[8] The FAMEs were separated using a CP-3380 gas chromatograph (Varian, Santa Clara, California, 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^{-1}, the make-up gases were nitrogen at 30 mL min^{-1} and synthetic air at 300 mL min^{-1}, and the flame gas was hydrogen at 30 mL min^{-1}; 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^{-1} and maintained for 5 min, and then increased from 185 °C to 225 °C at 10 °C min^{-1} and maintained for 10 min. The retention times were compared to those of standard methyl esters (Sigma, St Louis, MO, USA). The fatty acids were quantified using tricosanoic acid methyl ester

Table 2. Complete design 2^2 in duplicate and the obtained responses to the sums, proportions, fatty acids indexes, lipid oxidation, and proximal composition

	Independent variable, numeric level*		Response								
Test	x_1	x_2	SFA[a]	MUFA[a]	PUFA[a]	*n*-6[a]	*n*-3[a]	PUFA:SFA[a]	*n*-6:*n*-3[a]	IA	IT
1	80	80	4156.20	3667.16	1271.16	1027.69	243.78	0.31	4.22	5.78	15.72
2	80	80	4088.39	3691.06	1286.41	1038.77	247.64	0.31	4.19	5.59	15.66
3	120	80	3956.99	3515.59	1638.35	1107.85	530.50	0.41	2.09	5.28	15.94
4	120	80	3953.37	3526.28	1623.41	1099.34	524.07	0.41	2.10	5.34	15.97
5	80	120	4038.91	3536.76	1532.50	1206.54	325.96	0.38	3.70	4.39	12.38
6	80	120	4004.58	3516.51	1508.41	1181.55	326.86	0.38	3.61	4.30	12.04
7	120	120	3749.13	3305.23	1980.60	1263.26	717.34	0.53	1.76	3.16	9.65
8	120	120	3749.92	3335.65	1999.53	1275.63	723.90	0.53	1.76	3.15	9.69

	x_1	x_2	HH	Malondialdehyde[b]	Lipid[c]	Proteins[c]	Carbohydrates[c]	Ash[c]	Moisture[c]	Energy[c]
1	80	80	1.68	2.53	91.29	184.60	124.64	41.64	558.23	8612.09
2	80	80	1.78	2.53	93.07	186.22	118.29	41.40	561.03	8606.68
3	120	80	1.94	15.17	95.92	191.22	132.10	43.69	537.07	9029.11
4	120	80	1.93	15.17	96.17	191.56	131.48	43.70	537.09	9033.66
5	80	120	1.83	0.50	74.83	185.17	158.83	45.82	535.35	8580.65
6	80	120	1.82	0.50	72.89	186.73	159.10	45.78	535.50	8538.33
7	120	120	2.07	15.15	60.54	206.39	173.80	48.00	511.27	8648.25
8	120	120	2.10	12.62	61.37	201.39	178.67	47.57	511.00	8677.37

*Expressed according to Table 1.
[a]mg fatty acid kg^{-1} of food; [b]mg malondiadehyde kg^{-1} of food; [c]kJ kg^{-1} of food.
x_1, chia defatted flour; x_2, soy textured protein; SFA, total of saturated fatty acids; MUFA, total of monounsaturated fatty acids, except *trans* isomer; PUFA, total of polyunsaturated fatty acids, except *trans* isomer; *n*-6, summation of fatty acids from omega-6 series; *n*-3, summation of fatty acids from omega-3 series; IA, atherogenicity index; IT, thrombogenicity index; HH, hypocholesterolemic and hypercholesterolemic proportion.

Table 3. Mathematic equations, regression coefficients, *P*-values, and the *F* test of all applied responses to the response surface methodology

Parameter	Equation	R^2	*P*-value	*F*
SFA	$396.22 - 10.98x_1 - 7.66x_2 - 2.63x_1x_2$	0.981	<0.0001	416.53
MUFA	$351.18 - 9.11x_1 - 8.82x_2 - 1.20x_1x_2$	0.992	<0.0001	625.02
PUFA	$160.51 + 20.54x_1 + 15.02x_2 + 2.94x_1x_2$	0.999	<0.0001	345.01
n-6	$115.01 + 3.64x_1 + 8.17x_2 + 0.13x_1x_2$	0.992	<0.0001	295.01
n-3	$45.50 + 16.89x_1 + 6.85x_2 + 2.82x_1x_2$	0.999	<0.0001	363.86
PUFA:SFA	$0.41 + 0.06x_1 + 0.05x_2 + 0.01x_1x_2$	0.999	<0.0001	298.12
n-6:*n*-3	$2.93 - 1.00x_1 - 0.22x_2 - 0.05x_1x_2$	0.999	<0.0001	261.51
IA	$4.62 - 0.39x_1 - 0.87x_2 - 0.20x_1x_2$	0.997	<0.0001	168.34
IT	$13.38 - 0.57x_1 - 2.44x_2 - 0.70x_1x_2$	0.998	<0.0001	299.55
HH	$1.89 + 0.12x_1 + 0.06x_2 + 0.01x_1x_2$	0.964	<0.0001	142.25
Malondialdehyde	$8.02 + 6.50x_1 - 0.83x_2$	0.990	<0.0001	27.26
Total lipids	$8.08 - 0.23x_1 - 1.34x_2 - 0.42x_1x_2$	0.998	<0.0001	233.01
Crude proteins	$19.17 + 0.60x_1 + 0.33x_2 + 0.30x_1x_2$	0.967	<0.0001	279.29
Carbohydrates	$14.71 + 0.69x_1 + 2.05x_2 + 0.17x_1x_2$	0.992	<0.0001	152.28
Ash	$4.47 + 0.10x_1 + 0.21x_2 - 0.004x_1x_2$	0.997	<0.0001	721.7
Moisture	$53.58 - 1.17x_1 - 1.25x_2 - 0.04x_1x_2$	0.998	<0.0001	1523.71
Crude energy	$871.58 + 13.13x_1 - 10.46x_2 - 7.97x_1x_2$	0.995	<0.0001	1344.65

x_1, chia defatted flour; x_2, soy textured protein; R^2, regression factor; SFA, total of saturated fatty acids; MUFA, total of monounsaturated fatty acids, unless *trans* isomer; PUFA, total of polyunsaturated fatty acids, unless *trans* isomer; *n*-6, sum of fatty acids from omega-6 series; *n*-3, sum of fatty acids from omega-3 series; IA, atherogenicity index; IT, thrombogenicity index; HH, hypocholesterolemic and hypercholesterolemic proportion.

(Sigma) as an internal standard.[9] The peak areas were determined with Star 5.0 software (Varian). According to Joseph and Ackman[9] [Eqn (1)], correction factors of FAMEs for flame ionization detectors in individual fatty acids (FAs) were used and their concentrations expressed in mg FA per kg^{-1} 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 is the mass of fatty acid X (mg g^{-1} of sample); M_p is the mass of internal standard (mg); M_A is the mass of sample (g); A_X is the area of fatty acid X (mV.s); A_p is the area of the internal standard (mV.s); F_{CT} is the theoretical correction factor; and F_{CEA} is the 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^{-1}), considering the signal-noise rate relative to the background signal as 3 and 10, respectively.[10]

Nutritional quality index of lipid fraction

The atherogenicity index (IA) was determined as [(12:0 + (4 × 14:0) + 16:0)]/(MUFA + *n*-6 + *n*-3). The thrombogenicity index (IT) was determined as (14:0 + 16:0 + 18:0)/[(0.5 × MUFA) + (0.5 × *n*-6) + (3 × *n*-3) + (*n*-3/*n*-6)], according to the method given by Ulbricht and Southgate.[11] The hypocholesterolemic/hypercholesterolemic ratio (HH) was determined as (18:1 *n*-9 + 18:2 *n*-6 + 20:4 *n*-6 + 18:3 *n*-3 + 20:5 *n*-3 + 22:5 *n*-3 + 22:6 *n*-3)/(14:0 + 16:0), according to the method given by Santos-Silva *et al.*[12]

Lipid oxidation

The lipid oxidation assessment was based on the analysis of reactive substances to thiobarbituric acid (TBA), according to the methodology described in the Analytics Rules from the Adolfo Lutz Institute.[13]

Proximal composition

Moisture, ash, and crude protein contents were determined according to Cunniff,[14] adopting 6.25 as the total nitrogen conversion factor to crude protein. Total lipids were extracted according to the Bligh and Dyer methodology.[15] Carbohydrates were calculated by difference: [100 − (g kg^{-1} moisture + g kg^{-1} ash + g kg^{-1} crude protein + g kg^{-1} total lipids)].

Energetic value was determined by indirect calorimetry (calculus), which considered conversion factors for crude protein (4 cal g^{-1}), carbohydrates (4 cal g^{-1}), and total lipids (9 cal g^{-1}), as well as their contents, cal = [(4 * crude proteins) + (4 * carbohydrates) + (9 * total lipids)].[16] The results were expressed in kcal per kg^{-1} of food and then converted to joules through the factor 4.1868 J per 1 kcal and are shown as kJ kg^{-1} of food.

Statistical analysis

All the analyses were done in triplicate. Fatty acid composition was demonstrated by the general average of the experiments repetitions ($n = 6$, A: test 1 and 2; B: tests 3 and 4; C: tests 5 and 6; D: tests 7 and 8) and standard deviation following that proposed by Skoog *et al.*[17] with the analytical error propagation. Initially, the values of effects, interaction, and variance analysis (ANOVA) values were obtained. Afterwards, the whole variables had their normal and homogeneity of variance assessed through the combings. Then, the variance analysis (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 mathematical model used to adjust the data was:

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad (2)$$

where Y_i is the expected response, β_0 is the constant term, β_1, β_2 and β_{12} are the regression terms.[18]

The model's equations were arranged to a global response using a desirability function. This procedure involved a transformation

Table 4. Effects calculus and interaction for the complete factorial design 2^2

Response	Effect		
	x_1	x_2	x_1x_2
SFA	−21.97	−15.31	−5.26*
MUFA	−18.22	−17.65	−2.40
PUFA	41.08	30.04	5.88*
n-6	7.28	16.33	0.25
n-3	33.79	13.70	5.63
PUFA:SFA	0.13	0.09	0.03
n-6:*n*-3	−2.00	−0.44	0.11
IA	−0.78	−1.75	−0.41
IT	−1.14	−4.88	−1.40
HH	0.24	0.12	0.03*
Malondialdehyde	13.01	−1.66	–
Total lipids	−0.45	−2.67	−0.84
Crude protein	1.20	0.65	0.60
Carbohydrates	1.39	4.11	0.34*
Ash	0.21	0.42	−0.01*
Moisture	−2.34	−2.51	−0.09*
Crude energy	26.27	−20.92	−15.93

*These interactions effects are not significant ($P < 0.05$). x_1, chia defatted flour; x_2, soy textured protein; SFA, total of saturated fatty acids; MUFA, total of monounsaturated fatty acids, except *trans* isomer; PUFA, total of polyunsaturated fatty acids, unless *trans* isomer; *n*-6, sum of fatty acids from omega-6 series; *n*-3, sum of fatty acids from omega-3 series; IA, atherogenicity index; IT, thrombogenicity index; HH, hypocholesterolemic and hypercholesterolemic proportion.

of each response (Y_i) estimated for a desirable value (d_i), in which $0 \leq d_i \leq 1$.

If the objective or target T to the response Y_i is a maximum value then Eqn (3) applies:

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

If the objective or target to the response Y_i is a minimum value, then Eqn (4) applies:

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

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 desirability values (d_i) were arranged through a geometric average to form a global or general convenience (D). 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 property balance becomes more favorable.

Principal components analysis (PCA) consisted of using of the sums, proportions, and indexes of fatty acids, malondialdehyde, and proximal composition values (loadings). For this analysis, the averages of the eight tests were separated into groups (scores): A (tests 1 and 2), B (tests 3 and 4), C (tests 5 and 6), and D (tests 7 and 8). Averages were auto-scaled, so that the whole variables showed the same weight. In this way, PCA bi-dimensional graphics were obtained. All the statistical analyses were done using the software Statistica, version 8.0,[19] adopting the level of 5% significance level for rejection of the null hypothesis ($P < 0.05$).

RESULTS AND DISCUSSION

Table 2 presents the conditions of the complete factorial model 2^2, in duplicate, applied to the tests, as well as the values obtained for the sums, ratios, and indexes of fatty acids, lipid oxidation (malondialdehyde), proximal composition, and crude energy (indirect methods).

Each model's equation, such as their respective determination coefficients (R^2), significance (P-value), and F test, is listed in Table 3. Data that belongs to independent variables and the responses were analyzed to acquire the linear regression equations (Table 3), as well as each main effect value and the interaction among these effects (Table 4), and also the contribution percentages of these effects for the model through ANOVA.

Residual plots showed normality and variance homogeneity for all the responses, which were considered satisfactory. This shows that the models were significant and that there was no lack of fit. The values of P and the F test indicate that the models were highly significant (Table 4).

Interaction effects for the total saturated fatty acids (SFA, $P = 0.0507$), polyunsaturated fatty acids (PUFA, $P = 0.0995$), fatty acids from omega-6 series, except the *trans* isomers (*n*-6, $P = 0.7634$), hypocholesterolemic/hypercholesterolemic ratio (HH, $P = 0.3494$), total carbohydrates ($P = 0.1556$), ash ($P = 0.4784$), and moisture (0.2836) were non-significant and kept in the models to provide the 'flattening' in the models and their absence would compromise the R^2 value. The main effect to the malondialdehyde CFPD ($P = 0.0585$) and interactions ($P = 0.5917$) in the beginning were non-significant, and were removed from the interaction contribution and, in this case, the model remained highly adjusted and linear (Table 4 and Table 5).

High levels of both factors (12%, Table 4) contributed to lower values of *n*-6:*n*-3 ratios (Table 2).[20] An imbalance in *n*-6:*n*-3 ingestion is associated with myocardial infarction, hypercholesterolemia, increasing LDL and arterial pressure, atheroma formation, and dyslipidemia, among other diseases.[21] Crude energy obtained a bigger contribution mainly for the TSP effect and was positive. CFPC effects and interaction were negative, showing that the incorporation of these vegetables can reduce the hamburger's caloric value and promote a balanced diet through a food that easy and fast to prepare. In the ash content, the interaction was negative among the factors, but it was not significant ($P < 0.05$). According to Gohara *et al.*[22] the chia flour added contributes to a significant increase in percentages of all mineral in chocolate cakes.

Under the selected operation system, LOD and LOQ were estimated at 0.15 and 0.50 mg g^{-1} of total lipids, respectively. The contents of fatty acids were calculated for the final product and presented in Table 5. Fatty acid compositions of all formulations were similar (Table 5), with a decrease in the SFA, monounsaturated fatty acids (MUFA), and PUFA of long chain into the D product (tests 7 and 8). It was possible to identify and quantify the *trans* isomers of oleic fatty acid (18:1 *n*-9) and linolenic fatty acid (18:2 *n*-6) in all the formulations. The total content of *trans* fatty acid in all formulations were upper than proposed to Brasil.[23] This regulations considering 0.02 g *trans* fatty acid per 80 g with 'free *trans*

Table 5. Absolute quantification of fatty acids (as mg fatty acid kg^{-1} food) of the hamburger formulations

	Formulation			
Fatty acid	A	B	C	D
10:0	67.00 ± 3.11	60.20 ± 1.40	59.10 ± 14.42	35.63 ± 1.38
12:0	65.56 ± 5.49	59.78 ± 7.06	62.63 ± 12.13	39.54 ± 0.74
14:0	1491.10 ± 44.85	1416.60 ± 22.86	1173.10 ± 6.41	876.55 ± 5.28
14:1*n*-11	107.24 ± 0.21	104.10 ± 8.74	88.23 ± 4.51	65.04 ± 2.41
14:1*n*-9	183.02 ± 3.04	163.53 ± 6.22	140.75 ± 0.06	109.49 ± 4.93
14:1*n*-7	103.75 ± 3.04	101.78 ± 1.97	89.82 ± 0.63	69.08 ± 1.36
15:0	198.72 ± 1.06	188.07 ± 1.65	168.71 ± 1.07	120.92 ± 1.34
15:1*n*-9	128.38 ± 2.07	115.88 ± 1.52	99.73 ± 9.36	68.00 ± 0.40
15:1*n*-7	80.26 ± 4.15	81.00 ± 3.06	75.40 ± 12.11	49.29 ± 0.96
16:0	22148.62 ± 336.64	21 631.08 ± 108.21	17 162.52 ± 429.19	13 199.76 ± 72.46
16:1*n*-9	210.08 ± 23.59	216.05 ± 6.97	175.09 ± 3.71	124.08 ± 3.10
16:1*n*-7	1685.67 ± 25.27	1613.87 ± 36.13	1283.15 ± 22.57	971.65 ± 11.28
16:1*n*-5	239.13 ± 2.25	232.44 ± 6.97	195.15 ± 15.02	153.72 ± 13.24
17:0	507.04 ± 15.60	550.26 ± 21.16	427.93 ± 7.99	303.59 ± 1.20
17:1*n*-7	356.60 ± 9.07	383.61 ± 21.78	297.13 ± 7.73	218.46 ± 1.87
18:0	13372.64 ± 473.84	13 913.56 ± 64.11	10 519.46 ± 339.52	8167.92 ± 155.50
18:1*n*-9 t	647.03 ± 27.84	668.93 ± 9.64	527.15 ± 7.18	429.58 ± 12.56
18:1*n*-9	29324.20 ± 699.01	29 257.76 ± 44.12	22 402.79 ± 568.23	17 390.07 ± 298.63
18:1*n*-7	1 178.27 ± 21.87	1180.97 ± 33.48	904.42 ± 33.04	742.26 ± 6.44
18:1*n*-5	134.62 ± 13.67	172.27 ± 1.55	142.68 ± 24.86	99.31 ± 2.64
18:2*n*-6t[a]	72.05 ± 4.12	86.48 ± 14.75	74.80 ± 8.26	69.40 ± 0.31
18:2*n*-6	9425.07 ± 194.14	10 500.21 ± 41.93	8728.34 ± 286.58	7681.46 ± 127.42
20:0	144.98 ± 9.64	167.18 ± 1.86	132.82 ± 9.69	110.78 ± 3.70
18:3*n*-3	1825.00 ± 35.56	4614.18 ± 24.74	2073.93 ± 36.80	4134.31 ± 64.23
20:1*n*-9	183.43 ± 10.15	192.49 ± 8.22	154.34 ± 9.53	115.91 ± 1.12
20:2*n*-6	237.86 ± 15.64	256.40 ± 3.72	180.19 ± 2.47	150.27 ± 4.88
20:3*n*-3	180.13 ± 13.68	197.28 ± 4.91	133.12 ± 5.61	112.03 ± 2.47
22:2*n*-6	36.23 ± 4.75	34.25 ± 4.07	33.89 ± 3.86	21.90 ± 2.02
20:4*n*-6	63.35 ± 3.92	64.76 ± 0.87	58.23 ± 3.52	34.59 ± 1.70
20:5*n*-3	259.93 ± 6.99	252.71 ± 4.94	2033.78 ± 2.41	146.22 ± 3.80

[a] Sum of the *trans* isomers of linolenic fatty acids (18:2 *n*-6). A, tests 1 and 2; B, tests 3 and 4; C, tests 5 and 6; D, tests 7 and 8.

fat' in the portion of hamburger. This is due to the kind of thermic treatment used and to the surface on display to the oxygen associated with the susceptibility of these fatty acids to the oxidative process.

There are many lipid oxidation mechanisms that involve the formation of free radicals and/or reactive complexes due to excessive exposure to heat, oxygen, and matrices with high humidity. In this context, the formation of malondialdehyde is highlighted, it is an aldehyde with three carbon atoms, formed by reaction in an acidic environment (pH 1–2), and high temperature (≥100 °C), resulting in products from the decomposition of hydroperoxides. This complex comes from polyunsaturated fatty acid oxidation with at least three instaurations. Malonaldehyde determination by the reaction with thiobarbituric acid (TBA) can be influenced by the presence of acetaldehyde, sugars and Maillard reaction complexes into the sample.[24] B and D samples that obtained the higher contents of α-linolenic fatty acid (18:3 *n*-3), having chia as source and, at the higher level, obtained the highest malondialdehyde contents (Table 2). The smaller contents of fatty acids 20:4 *n*-6 and 20:5 *n*-3 into the formulation D (Table 5) were checked. This factor is associated with the higher level of TSP and CFPD that composes the biggest concentration of these vegetables, and the lipid degradation products' formation, such as the malondialdehyde (Table 2).

In principal components analysis (PCA, Fig. 1), PC1, which could explain 69.46% of the data variance, was able to distinguish

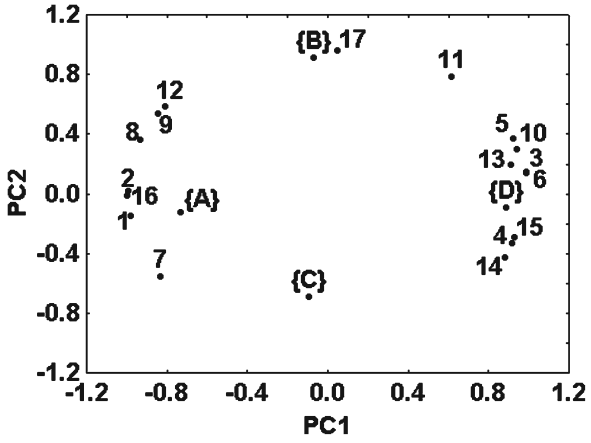

Figure 1. Principal components analysis for complete experimental design 2^2. PC, principal component. A: tests 1 and 2; B: tests 3 and 4; C: tests 5 and 6; D: tests 7 and 8. 1 – SFA: total saturated fatty acids; 2 – MUFA: total monounsaturated fatty acids, excpet *trans* isomers; 3 – PUFA: total polyunsaturated fatty acids, except *trans* isomers; 4 – *n*-6: sum of fatty acids from the omega-6 series; 5 – *n*-3: sum of fatty acids from the omega-3 series; 6 – PUFA:SFA; 7 – *n*-6:*n*-3; 8 – IA: atherogenicity index; 9 – IT: thrombogenicity index; 10 – HH: hypocholesterolemic and hypercholesterolemic proportion; 11 – malondialdehyde; 12 – total lipids; 13 – crude protein; 14 – carbohydrates per difference; 15 – ash; 16 – moisture; 17 – crude energy. Scores: A–D; loadings: 1–17.

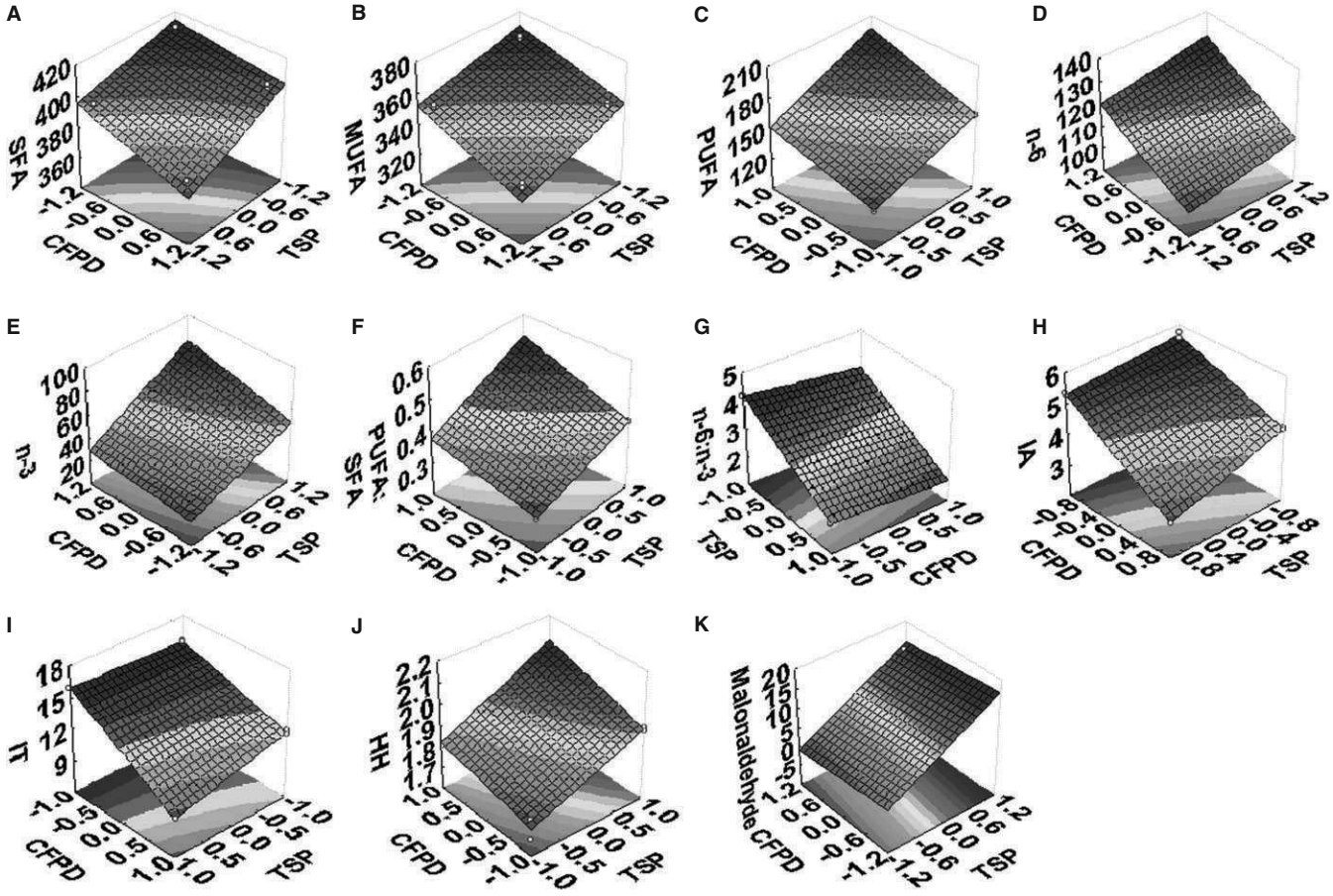

Figure 2. Response surfaces to sums, proportion, fatty acids indexes, and lipid oxidation. CFPD, chia flour partially defatted; TSP, textured soy protein; SFA; total saturated fatty acids; MUFA, total monounsaturated fatty acids, except the *trans* isomer; PUFA, polyunsaturated fatty acids, except *trans* isomers; *n*-6, sum of omega-6 fatty acid series; *n*-3, sum of omega-3 fatty acid series; IA, atherogenicity index; IT, thrombogenicity index; HH, hypocholesterolemic and hypercholesterolemic proportion.

the formulation with higher TSP and CFPD concentration. This occurred due to the loadings (Fig. 1), which shows the highest contents to the PUFA, *n*-6 and *n*-3 sums, proportion PUFA:SFA and HH, malondialdehyde, crude protein, total carbohydrates, and ash. These effects can be noted in the response surface models (Fig. 2A and C). The biggest HH proportions and PUFA:SFA (Table 2) are important due to the hypocholesterolemic effect and the prevalence of polyunsaturated fatty acids, which are involved with the smaller risk of cardiovascular diseases.[21] The bigger ash contraction is an indirect indicator of a higher content of minerals, and the ingestion of micronutrients is essential for biological systems maintenance, because they take part as cofactors in metabolic reactions.[25]

In the PC2 there was a positive contribution of PUFA, *n*-3, PUFA:SFA, IA, IT, HH, malondialdehyde, total lipids, and crude protein, causing the separation of sample B (Fig. 1). This principal component was responsible for 22.07% of the data variance and it is observed that the CPFD lipid fraction allowed improved atherogenicity and thrombogenicity indexes. These index decreases show a direct relation with the attenuated risk of coronary diseases. Increased PUFA and *n*-3 in the hamburger B is associated with the high contents of α-linolenic fatty acid in the chia.[3]

Figure 3 and Fig. 4 show the desirability function for the following constrictions: maximum value of PUFA:SFA ratio, HH index, crude protein and ash [Eqn (3)]; minimum value of *n*-6:*n*-3 ratio, IA and IT indexes and total lipids [Eqn (4)]. The highest levels of TSP and CFPD were described as a point of major desirability (tests 7 and 8). The higher concentrations of the factors studied were crucial for greater composition of fatty acid and raised contents of ash, crude protein and total lipids in the development of a healthier hamburger. The nutrient with the greatest increase was α-linolenic fatty acid whose content in tests 7 and 8 was 126.54% greater than in tests 1 and 2. Experiments with higher levels of TSP and CFPD were not conducted due to technological aspects, mainly the mass texture.

CONCLUSION

Factorial design improved the lipid fraction and the macronutrients in hamburgers showing that the factors with a higher percentage of textured soy protein and chia flour partially defatted were significant. The increases in these factors contributed positively to the composition of fatty acids, crude protein, ash, and the product's nutritional quality. Principal components analysis distinguished the samples with the highest chia content through PC1 and PC2. Constraints were maintained in the responses to the desirability analysis. In this analysis, higher levels of TSP and CFPD were described as a point of greatest desirability without the need to obtain another experimental point. The addition of chia's

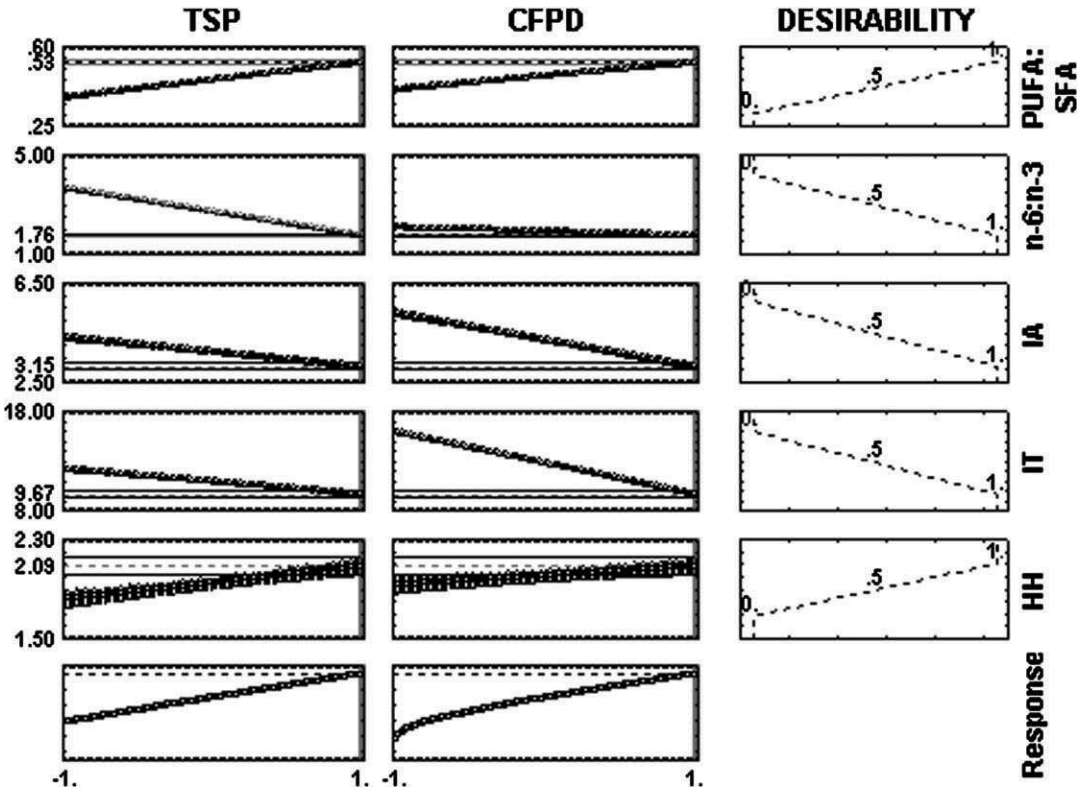

Figure 3. Chia flour partially defatted (CFPD) and textured soy protein influence (TSP) in the proportions of fatty acids in the hamburger formulations. SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids, except *trans* isomers; PUFA, total polyunsaturated fatty acids, except *trans* isomers; *n*-6, sum of fatty acids from the omega-6 series; *n*-3, sum of fatty acids from the omega-3 series; IA, atherogenicity index; IT, thrombogenicity index; HH, hypocholesterolemic and hypercholesterolemic proportion.

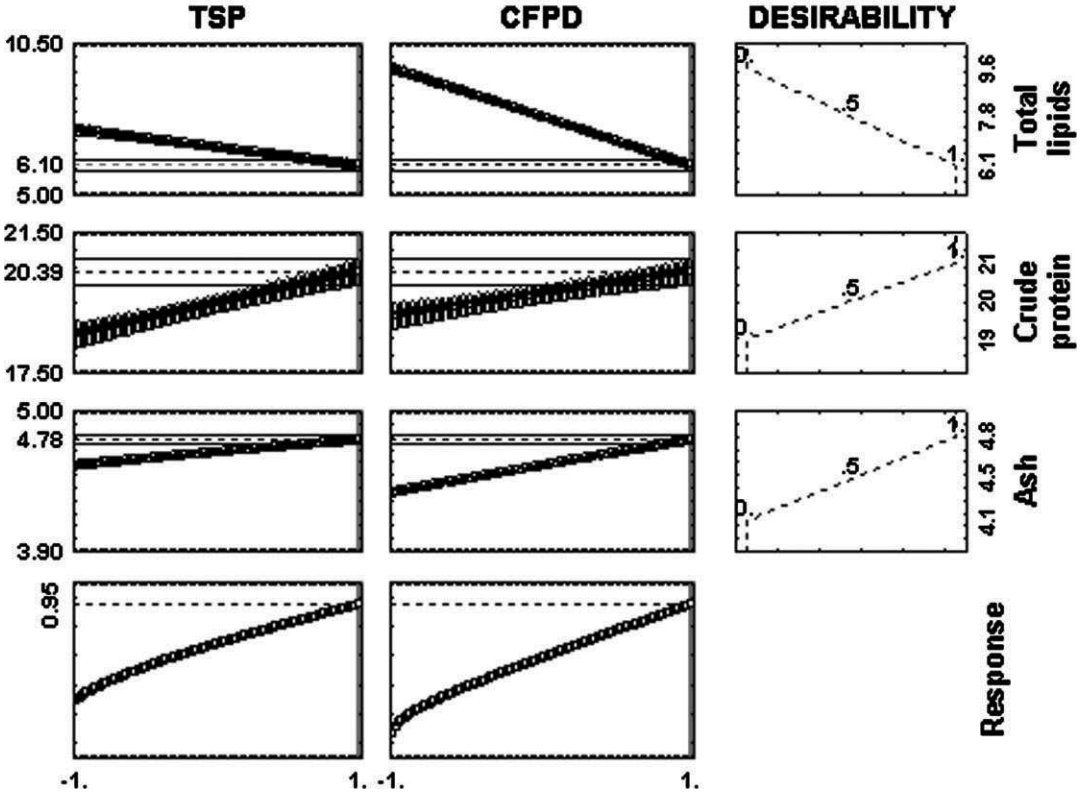

Figure 4. Chia flour partially defatted (CFPD) and textured soy protein influence (TSP) in the total lipid (TL) contents, crude protein (CP), and ash in the hamburger formulations.

by-product is an alternative method of increasing the α-linolenic contents and obtaining a nutritionally balanced food.

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Aplicação de fatorial categórico no desenvolvimento de hambúrguer vegetal contendo chia como fonte de ômega 3: Estudo de otimização

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Resumo:

O objetivo deste trabalho foi a aplicação de planejamento fatorial categórico no desenvolvimento de hambúrguer vegetal como fonte de ácido graxo ômega-3 e obter uma melhor formulação através da otimização do experimento. Um planejamento categórico do tipo 3x2 completo (três fatores em dois níveis) em duplicata foi realizado para investigar a influência da fonte de ômega-3, o tipo de emulsificante e o tratamento térmico na composição de ácidos graxos em hambúrguer vegetal. Todos os efeitos principais e de interação foram significativos. Na análise hierárquica houve a formação de dois grupos bem definidos, sendo um com chia e outro com a linhaça, ambos tendo a goma xantana/carboximetilcelulose e sem cocção. A composição em ácidos graxos nos ensaios foi igual e houve uma variação significativa entre as formulações. Nos modelos o efeito principal emulsificante e a interação com os três fatores avaliados apresentaram o maior percentual de contribuição. Nesta análise o produto contendo chia como fonte de ômega-3 e a goma xantana/carboximetilcelulose como emulsificante no hambúrguer assado foi caracterizado como o ponto ótimo de maior desejabilidade. O uso de chia e de ferramentas quimiométricas mostrou-se promissor como uma alternativa no desenvolvimento de alimento nutricionalmente balanceado.

Palavras-chave: *Salvia hispanica* L.; alfa linolênico; planejamento de experimentos; desejabilidade; dendograma.

Introdução

Os hambúrgueres são comumente produzidos a partir de cortes bovinos de baixo custo comercial juntamente com tecidos de gordura tipicamente de origem subcutânea, o qual exerce influência direta e majoritária sobre a composição lipídica do produto final [1]. Do

ponto de vista tecnológico, os lipídios auxiliam na estabilização de emulsões e melhoram a textura dos alimentos [2-4]. Os lipídios também são de grande importância, pois é fonte de energia e ácidos graxos essenciais, além de atuarem no transporte de vitaminas lipossolúveis [5]. No entanto, os lipídios de origem animal apresentam altos teores de colesterol e ácidos graxos saturados, os quais estão associados a diversos tipos de doenças cardiovasculares e coronarianas, além de risco de obesidade [6-8]. Assim, a utilização de fontes vegetais de lipídios poderia resultar em produtos considerados mais saudáveis [9]. Atualmente existem diversos estudos direcionados ao enriquecimento da qualidade nutricional de produtos cárneos, principalmente o aumento dos teores de ácidos graxos poli-insaturados, especialmente os da série ômega-3, devido ao seu efeito positivo na saúde humana [10-11], além de ser um atrativo do ponto de vista comercial [12]. Alguns estudos sugerem até a utilização de outros tecidos adiposos para aumentar os teores de ácidos graxos monoinsaturados de hambúrgueres [13] ou ainda a suplementação da dieta dos animais [1], sob alto custo e tempo de produção.

A obtenção de proteína de origem animal de alto valor biológico demanda custos elevadíssimos, assim, a produção de alimentos baseados em proteínas vegetais vem se tornando um grande atrativo do ponto de vista comercial e ganhando destaque na mesa dos consumidores. Para os produtos cárneos, a proteína de soja é a mais utilizada, pois apresenta características semelhantes à de origem animal, quando comparado a outras proteínas de origem vegetal. Além disso, ela apresenta outras propriedades como emulsificante, estabilizante e capacidade de retenção de água, característica bastante interessante, pois pode influenciar na textura do produto final [14]. Na realidade, a proteína de soja já vem sendo utilizada na substituição parcial da carne de hambúrgueres para diminuir custos, sendo que essa utilização é limitada pela legislação brasileira ao máximo de 7,5% [15].

Além dos aspectos relacionados à qualidade proteica e lipídica, os vegetais são importantes fontes de fibra, cujo consumo em quantidades suficientes podem estar associados à redução de doenças como distúrbios gastrintestinais, doenças cardíacas, obesidade, diabetes, hipertensão e câncer [16-17]. A substituição de matéria-prima animal por ingredientes de origem vegetal também constituem uma maneira de aumentar o consumo de vegetais, principalmente pelos jovens, já que estudos mostraram que esta classe, em espacial, não consomem a quantidade recomendada de frutas e vegetais que é de 5 ou mais porções por dia [18].

O planejamento fatorial é uma ferramenta dinâmica, que possibilita fazer um número reduzido de experimentos, avaliar várias variáveis simultaneamente, seus efeitos, maior confiabilidade nos resultados, em um processo interativo de adição ou retirada de ensaios no modelo, para viabilizar a seleção das principais variáveis e apresentação de modelos matemáticos com conclusões a partir de resultados qualitativos. Os planejamentos fatoriais categóricos possuem a característica de extrair informações sobre tipos de ingredientes, processos e parâmetros que não podem ser variados como nas variáveis numéricas [19].

O objetivo deste trabalho foi a aplicação de um planejamento fatorial categórico no desenvolvimento de hambúrguer vegetal como fonte de ácido graxo ômega-3 e obter uma melhor formulação através da otimização do experimento. Para isso foi investigado a influência de duas fontes de ômega-3, emulsificantes/ligantes e o efeito do processamento sobre a composição dos ácidos graxos.

Experimento

Amostragem

Os grãos de chia (*Salvia hispanica*, L.) e linhaça marrom (*Linum usitatissimum*, L) utilizadas neste estudo foram adquiridas da empresa Giroil Agroindustria Ltda. (St. Angelo-RS). A proteína texturizada de soja (PTS) foi adquirida na forma de grânulos. A amostragem dos grãos consistiu em dois lotes de 5 kg que foram moídos em moinho martelo e passadas numa peneira de 14 mesh para homogeneização. O trigo integral, orégano, alho desidratado e a pimenta calabresa desidratada, utilizados nas formulações foram moídos e determinados a sua granulometria, conforme descrito anteriormente. Esses ingredientes foram adquiridos no comércio da cidade de Maringá, Paraná, Brasil.

Delineamento experimental

Um planejamento categórico do tipo 3x2 completo (três fatores em dois níveis) em duplicata foi realizado para investigar a influência da fonte de ômega-3, o tipo de emulsificante e o tratamento térmico na composição de ácidos graxos no desenvolvimento de hambúrguer vegetal (Tabela 1). As respostas analisadas foram os somatórios, razões e índices nutricionais dos ácidos graxos.

Tabela 1. Fatores e níveis avaliados no planejamento experimental categórico 3x2 completo em duplicata

Fator	Tipo	Níveis	
		1	2
Fonte de n-3	Categórico	Chia	Linhaça
Emulsificante	Categórico	Goma xantana e carboximetilcelulose	Gema de ovo
Tratamento	Categórico	Cru	Assado

Processamento das formulações

Todos os ingredientes foram previamente pesados separadamente. A proteína texturizada de soja (13,95%) foi imersa em água a 25°C (50,00%) por duas horas. Posteriormente foram incorporados à PTS hidratada a respectiva quantidade (g kg^{-1}) dos demais ingredientes (Tabela 2) e misturados até a formação de uma massa homogênea. O agente emulsificante/ligante foi obtido através do planejamento categórico proposto (Tabela 1), que variou entre gema de ovo comercial pasteurizada ou o uso do gel contendo a goma xantana, carboximetilcelulose e água. O gel consistiu de uma mistura prévia dos sólidos com a água (~25°C), com posterior aquecimento até 60°C para formação do gel. Posteriormente procedeu-se com a prensagem e moldagem em hamburgueira manual de 11 cm de diâmetro, obtendo-se hambúrgueres com peso líquido de 100 g (± 0,05) a unidade. Os discos foram embalados individualmente em sacos de polietileno e armazenados em freezer sob a temperatura de -18 °C por 24 horas. Os hambúrgueres foram submetidos ao processo de cocção em chapa de teflon a 300ºC por 3 min com duas inversões do produto conforme Souza *et al*. [20].

Tabela 2. Formulações desenvolvidas para o hambúrguer vegetal através do planejamento categórico 3x2 completo

Experimentos (formulações)[1]				
Ingredientes[2]	1	2	3	4
PTS[3]	139,50	139,50	139,50	139,50
Água[4]	500,00	500,00	500,00	500,00
Trigo integral	99,00	99,00	99,00	99,00
Fontes do ácido graxo alfa linolênico utilizado no planejamento categórico[5]				
Chia	51,40	51,40	-	-

Linhaça	-	-	51,40	51,40
Óleo de soja	50,00	50,00	50,00	50,00
Sal	15,00	15,00	15,00	15,00
Orégano em pó	5,00	5,00	5,00	5,00
Alho em pó	5,00	5,00	5,00	5,00
Pimenta calabresa em pó	0,10	0,10	0,10	0,10
Colorau em pó	15,00	15,00	15,00	15,00
Agentes emulsificantes/ligantes utilizados no planejamento categórico[6]				
Gema de ovo	120,00	-	120,00	-
Água[7]	-	100,00	-	100,00
Goma xantana	-	10,00	-	10,00
Carboximetilcelulose	-	10,00	-	10,00

[1]Formulações desenvolvidas conforme o planejamento categórico, sem o tratamento térmico. [2]Expresso em g por Kg^{-1}. [3]Proteína texturizada de soja. [4]Água necessária para hidratar a proteína texturizada de soja e posteriormente as demais farinhas utilizadas. [5]Planejamento categórico com os níveis de farinhas fonte de ômega-3. [6]Planejamento categórico com os níveis dos agentes emulsificantes/ligantes. [7]Água necessária para hidratar a goma xantana e posterior incorporação da carboximetilcelulose.

Extração lipídica

Os lipídios totais foram extraídos a frio através da mistura de solventes contendo clorofórmio:metanol:água (2:2:1,8, v/v/v) de acordo com a metodologia proposta por Bligh e Dyer [21].

Composição em ácidos graxos

A composição em ácidos graxos consistiu na conversão dos lipídios totais em ésteres metílicos de ácidos graxos (EMAG), conforme o método de metilação descrito em Hartman e Lago [22]. Os EMAG foram separados em cromatógrafo a gás CP-3380 (Varian, EUA), equipado com detector de ionização de chama e coluna capilar de sílica fundida CP-7420 (100 m x 0,25 mm x 0,25 µm de cianopropril – 100% ligado). A taxa do fluxo dos gases usados foram 1,4 mL min^{-1} de H_2 como gás de arraste, 30 mL min^{-1} para N_2 de gás auxiliar, 30 e 300 mL min^{-1}, respectivamente, H_2 e ar sintético para a chama. A temperatura do injetor e detector foram de 235°C e a coluna foi programada a 165°C por 4 min, seguido de uma rampa de 4°C min^{-1} até 185°C por 5 min e a segunda rampa de 10°C min^{-1} até 225°C por 10 min. Procedeu-se com injeções de 2 µL para cada solução contendo os EMAG, para uma razão de divisão de 1:100. Essas condições foram utilizadas para produtos de origem vegetal conforme Souza *et al*. [23] e Silva *et al*. [24].

Os EMAG foram identificados através da comparação dos tempos de retenção, com os padrões da Sigma® (EUA). Para a quantificação dos ácidos graxos foi utilizado o tricosanoato de metila (23:0, Sigma®, EUA), como padrão interno, de acordo com Joseph e Ackman [25]. As áreas dos picos foram determinadas utilizando-se o software Star 5.0 (Varian, EUA). Conforme demonstrada na Equação 1 [25-26], foram utilizados os fatores de correção do detector de ionização de chama e conversão dos EMAG em ácidos graxos individuais e sua concentração expressa em mg AG por 100g de alimento.

$$M_x = \frac{A_X \cdot Mp \cdot F_{CT}}{A_P \cdot M_A \cdot F_{CEA}} \quad (1)$$

Em que:

M_X = Massa do ácido graxo X em mg g^{-1} de amostra.

M_P = Massa do padrão interno em miligramas.

M_A = Massa da amostra em gramas.

160 A_X = Área do ácido graxo X.

161 A_P = Área do padrão interno.

162 F_{CT} = Fator de correção teórico.

163 F_{CEA} = Fator de conversão éster metílico para ácido graxo.

164

165 Os limites de detecção (LD) e quantificação (LQ) foram estimados através da análise
166 em triplicata das diluições do éster metílico do ácido araquidônico como padrão (1,00
167 mg mL^{-1}), sendo considerado a razão sinal-ruído e multiplicado por 3 e 10 para
168 determinar os respectivos limites [27].

169

170 *Índices de qualidade nutricional da fração lipídica*

171 Foram determinados os índices de aterogenicidade (IA) = [(12:0 + (4 x 14:0) + 16:0)] /
172 (AGMI + n-6 + n-3), e de trombogenicidade (IT) = (14:0 + 16:0 + 18:0) / [(0,5 x
173 AGMI) + (0,5 x n-6) + (3 x n-3) + (n-3/n-6)], por Ulbricht *et al*. [28]. A razão
174 hipocolesterolêmica por hipercolesterolêmica (HH) = (18:1n-9 + 18:2n-6 + 20:4n-6 +
175 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3) / (14:0 +16:0), de acordo com Santos-Silva *et*
176 *al*. [29].

177

178 *Análises estatísticas e multivariadas*

179 Os pontos experimentais do planejamento fatorial categórico 3x2 foram realizados em
180 duplicata, sendo que para cada amostra, as análises foram feitas em triplicata. A
181 composição em ácidos graxos foi demonstrada com a média geral das repetições dos
182 experimentos (n= 6, A: ensaios 1 e 2; B: ensaios 3 e 4; C: ensaios 5 e 6; D: ensaios 7 e
183 8; E: ensaios 9 e10; F: ensaios 11 e 12; G: ensaios 13 e 14; H: ensaios 15 e 16).

Os valores dos efeitos principais, interações e análise de variância (ANOVA) foram obtidos primeiramente. Após, a normalidade e a homogeneidade de variância dos resíduos foram verificadas. Procedeu-se com a análise de variância (ANOVA entre os grupos) para todas as respostas investigadas (Tabela 3). A fim de verificar o efeito das variáveis independentes sobre as respostas, a metodologia de superfície de resposta foi aplicada. O modelo matemático básico para ajustar os dados foi (Equação 2):

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \qquad (2)$$

Em que: Y_i é a resposta esperada, β_0 é a constante, β_1, β_2, β_{11}, β_{22} e β_{12} são os termos de regressão [30].

Para obtenção de uma resposta global foram selecionadas algumas equações, com o intuito de proceder com a otimização do experimento. Através da função de desejabilidade buscou-se a transformação de cada variável resposta (Y_i) estimado para um valor desejável (d_i), em que $0 \leq d_i \leq 1$.

Se o objetivo ou alvo T para a resposta Y_i é um valor máximo (Equação 3):

$$d_i = \begin{cases} 0 & Y_i < L \\ \left(\dfrac{Y_i - L}{T - L}\right)^r & L \leq Y_i \leq T \\ 1 & Y_i > T \end{cases} \qquad (3)$$

Se o objetivo ou alvo T para a resposta Y_i é um valor mínimo (Equação 4):

$$d_i = \begin{cases} 1 & Y_i < T \\ \left(\dfrac{U - Y_i}{U - T}\right)^r & T \leq Y_i \leq U \\ 0 & Y_i > U \end{cases} \qquad (4)$$

Em que, L é o limite inferior e U é o superior.

A função de conveniência é linear quando o peso r é igual a 1. Caso seja escolhido $r > 1$ há mais ênfase no valor próximo ao alvo. Ao preferir $0 < r < 1$ este é menos importante.

Os valores individuais de desejabilidade (d_i) foram combinados através de uma média geométrica para formar uma conveniência global ou geral (D). Este valor único de D [0, 1] fornece a avaliação global da conveniência e os níveis de resposta combinados, e D irá aumentar à medida que o equilíbrio das propriedades torna-se mais favorável.

A análise hierárquica (cluster) consistiu na utilização dos somatórios, razões e índices de ácidos graxos. Para isso foram utilizadas as médias das repetições dos experimentos de 1 a 16. Utilizou-se o método hierárquico e foi usada a distância euclidiana com ligação completa. Desta forma, foi obtido um gráfico bidimensional. As análises dos modelos e otimização foram realizadas no programa software Statistica, versão 8.0 [31], e o teste de Tukey e análise hierárquica no programa Excel software, versão 2007, (Microsoft, EUA), através do suplemente Action, versão do R 3.0.2, (Portal Action, Brasil), sendo adotado o nível de 5% ($p < 0,05$) de significância para rejeição da hipótese de nulidade em todas as análises estatísticas.

Resultados e discussões

As condições do modelo fatorial categórico 3x2 completo (Tabela 3), em duplicata, aplicado no desenvolvimento e processamento do hambúrguer vegetal, e as respostas dos somatórios, razões e índices de ácidos graxos. Os limites de detecção e quantificação estimados para os ácidos graxos foram 0,15 e 0,50 mg g^{-1} de lipídios totais, respectivamente.

Os resíduos apresentaram distribuição aleatória, normalidade e homogeneidade na variância. Esses resultados mostraram que todos os fatores avaliados foram significativos e comprovados pelos valores do teste F (Tabela 4). Por se tratar de um planejamento contendo somente fatores categóricos não é possível avaliar a falta de ajuste. Verificou-se uma alta correlação dos valores previstos pelos valores observados

pelo modelo. Isso é comprovado pelo expressivo valor de $R^2_{calculado}$ que foi igual a 0.999 e a pouca diferença com o $R^2_{ajustado}$ (0.997 a 0.999) em todos os modelos avaliados (Tabela 5). Este fato permite explicar até 99% dos fenômenos ocorridos dentro da faixa de estudo investigada.

Tabela 3. Planejamento fatorial categórico 3x2 completo em duplicata e as respostas obtidas para os somatórios, razões e índices de ácidos graxos

Ensaios	Variáveis independentes			Respostas				
	Níveis							
	x_1	x_2	x_3	AGS	AGMI	AGPI	n-6	n-3
1	Chia	GX[1] e CMC[2]	Cru	450,62	1971,63	2023,33	1186,32	837,02
2	Chia	GX e CMC	Cru	451,34	1971,50	2018,20	1182,27	835,93
3	Linhaça	GX e CMC	Cru	547,17	2409,61	1943,60	1170,66	772,94
4	Linhaça	GX e CMC	Cru	550,56	2391,58	1935,54	1165,35	770,19
5	Chia	Gema de ovo	Cru	865,57	2876,65	2680,98	1604,75	1076,22
6	Chia	Gema de ovo	Cru	869,28	2885,55	2680,31	1605,04	1075,27
7	Linhaça	Gema de ovo	Cru	981,05	3366,38	2475,41	1582,95	892,46
8	Linhaça	Gema de ovo	Cru	990,49	3362,08	2477,27	1583,80	893,47
9	Chia	GX e CMC	Assado	464,97	2006,37	2076,92	1201,16	875,76
10	Chia	GX e CMC	Assado	459,94	1991,07	2061,30	1192,05	869,25
11	Linhaça	GX e CMC	Assado	742,60	2487,57	1844,97	1170,81	674,15
12	Linhaça	GX e CMC	Assado	753,84	2473,46	1861,39	1176,60	684,79
13	Chia	Gema de ovo	Assado	930,66	3143,27	2461,63	1655,97	805,66
14	Chia	Gema de ovo	Assado	920,81	3148,72	2462,03	1657,57	804,46
15	Linhaça	Gema de ovo	Assado	1009,18	3376,04	2532,52	1591,56	940,96

16	Linhaça	Gema de ovo	Assado	1010,76	3377,20	2536,52	1593,88	942,64

Ensaios	x_1	x_2	x_3	AGPI:AGS	n-6:n-3	IA	IT	HH
1	Chia	GX e CMC	Cru	4,49	1,42	0,07	0,20	13,67
2	Chia	GX e CMC	Cru	4,47	1,41	0,07	0,20	13,66
3	Linhaça	GX e CMC	Cru	3,55	1,51	0,07	0,23	13,40
4	Linhaça	GX e CMC	Cru	3,52	1,51	0,08	0,23	13,24
5	Chia	Gema de ovo	Cru	3,10	1,49	0,11	0,29	9,33
6	Chia	Gema de ovo	Cru	3,08	1,49	0,11	0,29	9,28
7	Linhaça	Gema de ovo	Cru	2,52	1,77	0,11	0,32	9,04
8	Linhaça	Gema de ovo	Cru	2,50	1,77	0,11	0,32	8,90
9	Chia	GX e CMC	Assado	4,47	1,37	0,07	0,20	13,55
10	Chia	GX e CMC	Assado	4,48	1,37	0,07	0,20	13,60
11	Linhaça	GX e CMC	Assado	2,48	1,74	0,11	0,32	8,92
12	Linhaça	GX e CMC	Assado	2,47	1,72	0,11	0,33	8,78
13	Chia	Gema de ovo	Assado	2,65	2,06	0,12	0,31	8,66
14	Chia	Gema de ovo	Assado	2,67	2,06	0,11	0,31	8,80
15	Linhaça	Gema de ovo	Assado	2,51	1,69	0,11	0,32	8,99
16	Linhaça	Gema de ovo	Assado	2,51	1,69	0,11	0,32	8,92

[1]Goma xantana. [2]Carboximetilcelulose. x_1: farinhas como fonte do ácido graxo alfa linolênico; x_2: agentes emulsificante/ligante; x_3: tratamento térmico aplicado. AGS: total de ácidos graxos saturados; AGMI: total de ácidos graxos monoinsaturados; AGPI: total de ácidos graxos poli-insaturados; n-6: somatório de ácidos graxos da série ômega 6; n-3: somatório de ácidos graxos da série ômega 3; IA: índice de aterogenicidade; IT: índice de trombogenicidade; HH: razão hipocolesterolêmica e hipercolesterolêmica.

Os coeficientes de regressão e os respectivos intervalos de confiança estão listados na Tabela 5. Os efeitos principais (x_1, x_2 e x_3), efeitos de interação de dois fatores (x_1x_2, x_1x_3 e x_2x_3) e efeito de interação de três fatores ($x_1x_2x_3$) podem ser obtidos através da divisão por dois dos respectivos coeficientes de regressão. Todos os efeitos principais e de interação foram significativos para todos os modelos.

Tabela 4. Resultados da ANOVA, teste-F das respostas avaliadas no planejamento categórico para o hambúrguer vegetal

	GL	AGS	AGMI	AGPI	n-6	n-3
x_1	1	3754,45	11901,44	1176,46	366,47	2169,55
x_2	1	27216,83	69171,27	32999,12	69268,16	7245,96
x_3	1	940,09	666,18	252,64	147,83	1813,35
x_1x_2	1	358,22	175,15	175,32	50,67	1054,17
x_1x_3	1	258,34	179,91	129,52	56,88	860,10
x_2x_3	1	179,89	130,63	96,17	43,97	645,33
$x_1x_2x_3$	1	538,34	420,21	1084,92	30,37	4704,50
Erro puro	8					
SQ total	15					

	GL	AGPI:AGS	n-6:n-3	IA	IT	HH
x_1	1	14794,42	1413,99	509,54	2261,68	1186,69
x_2	1	19297,57	10116,99	3388,42	4971,69	7969,10
x_3	1	2458,93	4445,16	570,33	918,77	1171,88
x_1x_2	1	5388,00	3031,39	445,63	749,10	1082,93
x_1x_3	1	424,49	1584,84	245,76	429,39	644,19
x_2x_3	1	435,40	1031,81	274,63	344,83	694,74

$x_1x_2x_3$	1	2398,39	8549,61	539,46	742,79	1075,63
Erro puro	8					
SQ total	15					

GL: graus de liberdade. SQ: somatório quadrático. x_1: farinhas como fonte do ácido graxo alfa linolênico; x_2: agentes emulsificante/ligante; x_3: tratamento térmico aplicado. AGS: total de ácidos graxos saturados; AGMI: total de ácidos graxos monoinsaturados; AGPI: total de ácidos graxos poli-insaturados; n-6: somatório de ácidos graxos da série ômega 6; n-3: somatório de ácidos graxos da série ômega 3; IA: índice de aterogenicidade; IT: índice de trombogenicidade; HH: razão hipocolesterolêmica e hipercolesterolêmica.

As percentagens de contribuição de cada efeito nos modelos foram extraídas da ANOVA (Tabela 6). O tipo de emulsificante apresentou a maior significância em todas as respostas. Isso se deve ao fato de a gema de ovo contribuir para a composição em ácidos graxos [23] na formulação do hambúrguer vegetal e a goma xantana com a carboximeltilcelulose na quantidade de carboidratos totais. As fontes de ômega-3 tiveram uma boa contribuição como efeito principal (x_2) e no efeito de interação com o tipo de emulsificante (x_1x_2). De acordo com Sargi *et al*. [33] a composição em ácidos graxos da chia e linhaça são muito semelhantes com destaque para os elevado teor no somatório de ácidos graxos poli-insaturados. O desenvolvimento de produtos alimentícios como barra alimentícia [34], granola [35], minipanetone [36] e biscoito [37] demonstrou uma melhora significativa nos teores de ácidos graxos poli-insaturados, ressalta-se o alfa-linolênico.

As superfícies de resposta para o somatório de ômega-3 estão listadas na Figura 1. O máximo de n-3 foi verificado através dos níveis inferiores dos fatores fonte de ômega-3

e do tratamento (Figura 1A e 1B), assim como o nível superior do tipo de emulsificante empregado (Figura 1A). O diagrama de Pareto (Figura 1C) permitiu analisar a contribuição dos efeitos do planejamento, destaca-se uma alta contribuição do efeito principal x_2 e da interação $x_1x_2x_3$, em que o uso da goma xantana/carboximetilcelulose e da farinha de chia favoreceram o aumento do ácido graxo alfa-linolênico.

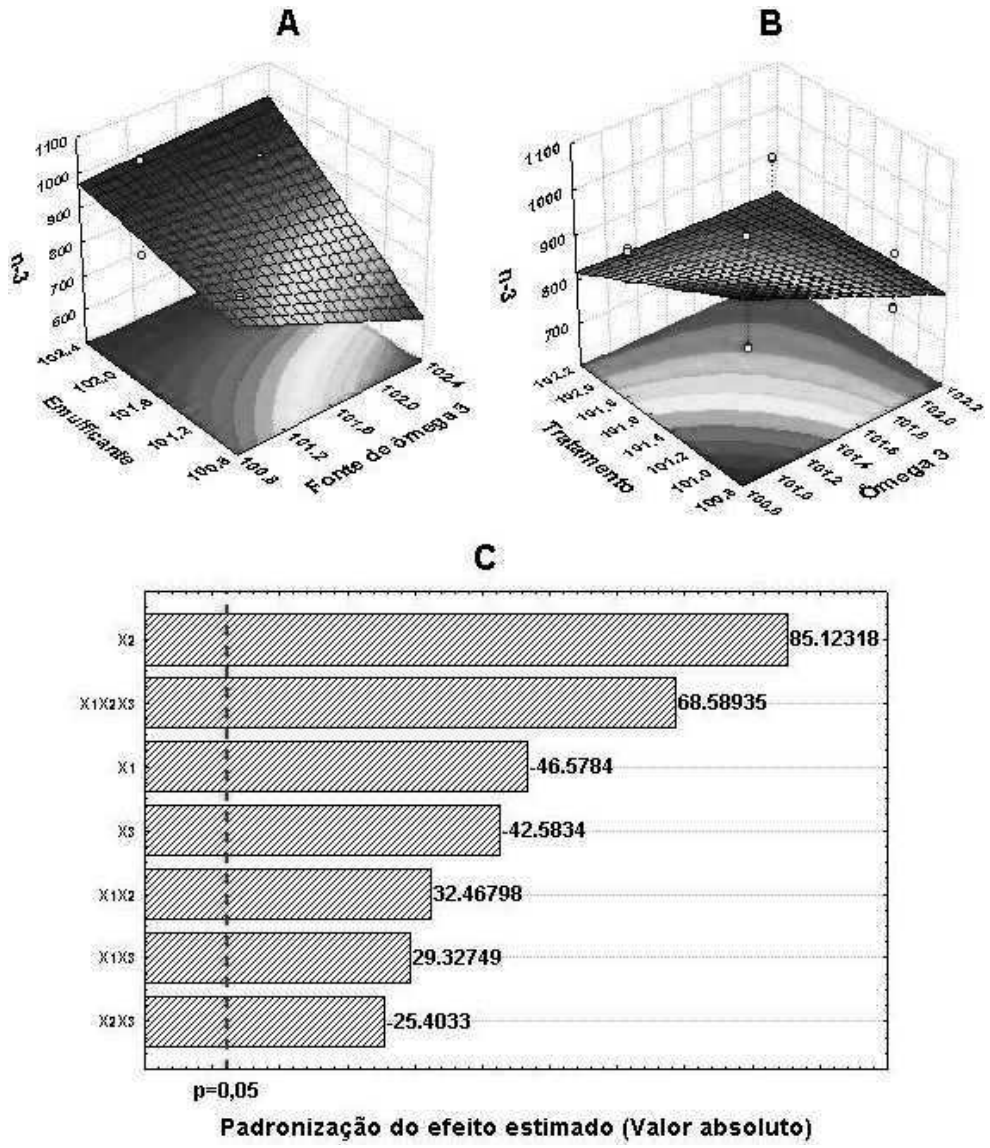

Figura 1. Superfícies de resposta (A e B) e diagrama de Pareto (C) contendo a contribuição e significância dos efeitos principais e de interações do planejamento categórico aplicado para o hambúrguer vegetal. A: superfície de resposta fonte de

283 ômega-3 *versus* emulsificante. B: superfície de resposta fonte de ômega-3 *versus*

284 tratamento. C: Diagrama de Pareto.

285 **Tabela 5.** Coeficientes de regressão, intervalo de confiança e coeficiente de determinação das respostas avaliadas no planejamento categórico do

286 hambúrguer vegetal

	AGS	AGMI	AGPI	n-6	n-3
Média	749,93	2702,42	2254,50	1395,05	859,45
	(747,17, 752,69)*	(2698,12, 2706,71)*	(2250,89, 2258,10)*	(1393,17, 1396,93)*	(857,57, 861,33)*
x_1	73,28	203,07	-53,59	-15,59	-38,00
	(70,52, 76,04)*	(198,78, 207,36)*	(-57,20, -49,99)*	(-17,47, -13,72)*	(-39,88, -36,12)*
x_2	197,30	489,57	283,84	214,39	69,44
	(194,54, 200,06)*	(485,28, 493,86)*	(280,24, 287,44)*	(212,52, 216,27)*	(67,56, 71,32)*
x_3	36,67	48,04	-24,84	9,90	-34,74
	(33,91, 39,43)*	(43,75, 52,34)*	(-28,44, -21,23)*	(8,03, 11,78)*	(-36,62, -32,86)*
x_1x_2	-22,63	-24,64	20,69	-5,80	26,49
	(-25,39, -19,88)*	(-28,93, -20,34)*	(17,09, 24,29)*	(-7,68, -3,92)*	(24,61, 28,37)*
x_1x_3	19,22	-24,97	17,78	-6,14	23,93
	(16,46, 21,98)*	(-29,26, -20,68)*	(14,18, 21,39)*	(-8,02, -4,26)*	(22,04, 25,81)*

x_2x_3	-16,04	21,28	-15,32	5,40	-20,72
	(-18,80, -13,28)*	(16,98, 25,57)*	(-18,93, -11,72)*	(3,52, 7,28)*	(-22,61, -18,84)*
$x_1x_2x_3$	-27,75	-38,16	51,47	-4,49	55,96
	(-30,51, -24,99)*	(-42,45, -33,87)*	(47,86, 55, 07)*	(-6,37, -2,61)*	(54,07, 57,84)*
R^2	0,9998	0,9999	0,9998	0,9999	0,9996
	AGPI:AGS	n-6:n-3	IA	IT	HH
Média	3,22	1,63	9,75E-2	2,75E-1	10,67
	(3,21, 3,23)*	(1,63, 1,63)*	(9,70E-2, 9,81E-2)*	(2,74E-1, 2,76E-1)*	(10,63, 10,71)*
x_1	-4,59E-1	4,61E-2	5,34E-3	2,33E-2	-6,49E-1
	(-4,68E-1, -4,50E-1)*	(4,32E-2, 4,89E-2)*	(4,80E-3, 5,89E-3)*	(2,21E-2, 2,44E-2)*	(-6,92E-1, -6,05E-1)*
x_2	-5,24E-1	1,23E-1	1,38E-2	3,45E2	-1,68
	(-5,33E-1, -5,16E-1)*	(1,20E-1, 1,26E-1)*	(1,32E-2, 1,43E-2)*	(3,34E-2, 3,56E-2)*	(-1,72, -1,64)*
x_3	-1,87E-1	8,17E-2	5,65E-3	1,48E-2	-6,45E-1
	(-1,96, -1,78)*	(7,88E-2, 8,45E-2)*	(5,11E-2, 6,20E-2)*	(1,37E-2, 1,60E-2)*	(-6,88E-1, -6,01E-1)*
x_1x_2	2,77E-1	-6,74E-2	-5,00E-3	-1,34E-2	6,20E-1

	(2,68E-1, 2,86E-1)*	(-7,03E-2, -6,46E-2)*	(-5,54E-3, -4,45E-3)*	(-1,45E-2, -1,23E-2)*	(5,76E-1, 6,63E-1)*
x_1x_3	-7,78E-2	-4,88E-2	3,71E-3	1,01E-2	-4,78E-1
	(-8,65, -6,91)*	(-5,16E-2, -4,59E-2)*	(3,17E-3, 4,26E-3)*	(9,01E-3, 1,13E-2)*	(-5,21E-1, -4,35E-1)*
x_2x_3	7,88E-2	3,93E-2	-3,92E-3	-9,08E-3	4,96E-1
	(7,00E-2, 8,75E-2)*	(3,65E-2, 4,22E-2)*	(-4,47E-3, -3,34E-3)*	(-1,02E-2, -7,96E-3)*	(4,53E-1, 5,40E-1)*
$x_1x_2x_3$	1,85E-1	-1,13E-2	-5,50E-3	-1,33E-2	6,18E-1
	(1,76E-1, 1,94E-2)*	(-1,16E-2, -1,10E-2)*	(-6,05E-3, -4,95E-3)*	(-1,45E-2, -1,22E-2)*	(5,74E-1, 6,61E-1)*
R^2	0,9998	0,9997	0,9987	0,9992	0,9994

*Intervalo de confiança dos coeficientes a 95% de confiança. R^2: coeficiente de determinação. x_1: farinhas como fonte do ácido graxo alfa linolênico; x_2: agentes emulsificante/ligante; x_3: tratamento térmico aplicado. AGS: total de ácidos graxos saturados; AGMI: total de ácidos graxos monoinsaturados; AGPI: total de ácidos graxos poli-insaturados; n-6: somatório de ácidos graxos da série ômega 6; n-3: somatório de ácidos graxos da série ômega 3; IA: índice de aterogenicidade; IT: índice de trombogenicidade; HH: razão hipocolesterolêmica e hipercolesterolêmica.

Os efeitos de interação x_1x_2, x_1x_3 e $x_1x_2x_3$ foram positivos e a interação x_2x_3 foi negativa para o n-3. Quando foi utilizada farinha de chia, com a goma xantana e a carboximetilcelulose como agente emulsificante e no produto ainda cru, houve um maior teor do ácido graxo alfa-linolênico, mas ao usar gema de ovo e após o processo de cocção houve uma tendência na sua redução (Tabela 5).

Ao avaliar as razões dos ácidos graxos (AGPS:AGS e HH, Tabela 3 e 5) foi verificada a prevalência os ácidos poli-insaturados, que pode ser evidenciado com todos os efeitos principais (x_1, x_2 e x_3) e a interação x_1x_3 negativa, bem como as interações de x_1x_2, x_2x_3 e $x_1x_2x_3$ positivas. Isso possibilitou verificar a contribuição dos lipídios proveniente da chia [33], goma xantana e carboximetilceluse e o hambúrguer cru. Desta forma os modelos AGPS:AGS e HH apresentaram melhores razões nos ensaios 1 e 2, que corroboram com os valores preconizados por Simopoulos [38] e Ratnayke e Galli [39]. Esses autores relatam que ao preferir alimentos com maiores níveis de ácidos graxos poli-insaturados isto pode diminuir o risco de inúmeras doenças e ser um fator a mais para o desenvolvimento de hábitos de vida saudável.

A razão de ácidos graxos n-6:n-3 apresentaram efeitos principais positivos e o de interações contendo todos os fatores investigados negativo (Tabela 5). Isto indicou que a adição de linhaça marrom, que contém maior teor de ácidos graxos da série n-6 e menor da série n-3 que a chia [33], a gema de ovo, que além do ácido graxo linoleico possui outros da família n-6 [32], e no produto assado obteve uma razão menos favorável ao ideal 1:1 [38]. O maior consumo de n-6 pode aumentar a resposta inflamatória no organismo e desencadear inúmeras doenças relacionadas [40].

Tabela 6. Resultados da ANOVA, somatório quadrático das respostas avaliadas no planejamento categórico para o hambúrguer vegetal

	GL	AGS	AGMI	AGPI	n-6	n-3
x_1	1	85915,74	659814,26	45955,57	3890,92	23102,56
x_2	1	622822,13	3834846,51	1289025,86	735439,85	77158,87
x_3	1	21512,67	36933,02	9868,72	1569,52	19309,48

x_1x_2	1	8197,32	9710,17	6848,59	537,94	11225,37
x_1x_3	1	5911,85	9974,36	5059,18	603,87	9158,83
x_2x_3	1	4116,44	7242,19	3756,51	466,82	6871,79
$x_1x_2x_3$	1	12319,19	23296,41	42379,74	322,49	50096,06
Erro puro	8	183,07	443,52	312,50	84,94	85,19
SQ total	15	760978,39	4582260,44	1403206,65	742916,36	197008,14

	GL	AGPI:AGS	n-6:n-3	IA	IT	HH
x_1	1	3,37	3,34E-2	4,57E-4	8,66E-3	6,73
x_2	1	4,40	2,43E-1	3,04E-3	1,90E-2	45,22
x_3	1	5,60E-1	1,07E-1	5,11E-3	3,52E-3	6,65
x_1x_2	1	1,23	7,29E-2	4,00E-3	2,87E-3	6,14
x_1x_3	1	9,67E-2	3,80E-3	2,20E-3	1,64E-3	3,66
x_2x_3	1	9,92E-2	2,48E-2	2,46E-3	1,32E-3	3,94
$x_1x_2x_3$	1	5,47E-1	2,05E-1	4,84E-3	2,84E-3	6,10
Erro puro	8	1,82E-3	1,92E-4	7,17E-6	3,06E-5	4,53E-2
SQ total	15	10,30	7,25E-1	5,36-3	3,99E-2	78,49

GL: graus de liberdade. SQ: somatório quadrático. x_1: farinhas como fonte do ácido graxo alfa linolênico; x_2: agentes emulsificante/ligante; x_3: tratamento térmico aplicado. AGS: total de ácidos graxos saturados; AGMI: total de ácidos graxos monoinsaturados; AGPI: total de ácidos graxos poli-insaturados; n-6: somatório de ácidos graxos da série ômega 6; n-3: somatório de ácidos graxos da série ômega 3; IA: índice de aterogenicidade; IT: índice de trombogenicidade; HH: razão hipocolesterolêmica e hipercolesterolêmica.

Os índices de ácidos graxos estão relacionados com os seguintes ácidos graxos saturados: láurico (12:0), miristico (14:0), palmítico (16:0) e esteárico (18:0). Os teores dos ácidos graxos esteárico (4,43%) e palmítico (126,14%) foram superiores aos encontrados para a chia [33]. Isso pode justificar

o comportamento dos modelos dos índices de aterogenicidade (IA) e trombogenicidade (IT) que apresentou efeito de interação $x_1x_2x_3$ negativo. Este resultado indicou que o uso da linhaça e a gema de ovo, este que além de contribuir com os ácidos graxos citados anteriormente, também favoreceu o aumento do ácido graxo mirístico (Tabela 7). Recomenda-se o menor valor possível para estes índices [28]. Os experimentos C, D, G e H que continham linhaça apresentaram os maiores índices de aterogenicidade, destaca-se o ensaio H que obteve os maiores IA e IT (Tabela 3). Nos estudos realizados por Fuchs *et al*. [41] com hambúrguer de peixe enriquecido com linhaça e Souza *et al*. [20] com adição de chia, esses índices foram superiores ao presente estudo, assim como em produtos constituídos por vegetais [34,42].

Tabela 7. Quantificação absoluta dos ácidos graxos nas formulações de hambúrgueres vegetais

Ácidos graxos[1]	Experimentos[2]							
	A	B	C	D	E	F	G	H
14:0	2,82^{d}±0,10	3,76^{d}±0,19	6,87^{b}±0,05	8,10^{a}±0,56	3,23^{d}±0,02	5,58^{c}±0,36	7,77^{ab}±0,31	7,61^{ab}±0,05
16:0	280,38^{g}±0,09	311,85^{e}±1,18	570,21^{c}±2,50	620,19^{ab}±5,85	287,24^{f}±2,17	466,93^{d}±5,32	611,01^{b}±5,73	629,36^{a}±3,77
16:1n-7	7,48^{d}±0,07	9,37^{d}±0,02	32,21^{b}±0,46	36,87^{a}±1,06	8,17^{d}±0,12	27,04^{c}±0,15	35,48^{a}±0,59	36,16^{a}±0,78
18:0	123,62^{f}±0,63	183,87^{e}±0,58	232,36^{d}±0,62	298,05^{c}±0,50	125,85^{f}±1,13	231,62^{d}±2,59	247,45^{b}±0,33	315,64^{a}±0,43
18:1n-9	1849,50^{f}±0,21	2264,46^{e}±12,09	2690,11^{d}±5,72	3158,76^{a}±4,03	1874,09^{f}±10,08	2326,87^{c}±8,66	2940,43^{b}±4,82	3167,62^{a}±0,05
18:1n-7	88,91^{e}±0,20	99,36^{d}±0,32	124,65^{c}±0,13	132,61^{b}±0,08	90,08^{d}±0,48	99,49^{d}±1,20	133,26^{b}±0,49	135,74^{a}±0,95
18:2n-6	1184,29^{de}±2,86	1168,01^{f}±3,76	1604,90^{b}±0,20	1583,38^{c}±0,60	1196,61^{d}±6,45	1173,71^{ef}±4,09	1656,77^{a}±1,13	1592,72^{bc}±1,64
20:0	44,15^{c}±0,11	49,38^{b}±0,45	57,99^{a}±0,55	59,43^{a}±0,76	46,13^{bc}±0,23	44,09^{c}±0,32	59,51^{a}±0,60	57,37^{a}±3,13
18:3n-3	836,48^{e}±0,77	771,56^{g}±1,94	1075,75^{a}±0,67	892,96^{c}±0,72	872,51^{d}±4,60	679,47^{h}±7,52	805,06^{f}±0,85	941,80^{b}±1,19
20:1n-9	25,66^{d}±0,03	27,40^{c}±0,32	34,12^{b}±0,24	35,99^{a}±0,15	26,37^{cd}±0,14	27,12^{cd}±0,26	36,82^{a}±0,12	37,09^{a}±0,95

Médias seguidas letras iguais na mesma linha não houve diferença significativa ($p<0,05$) pelo teste de Tukey. [1]Expresso em mg de AG por 100g^{-1} de alimento. [2]Experimentos obtidos a partir do planejamento fatorial categórico, em que A: ensaios 1 e 2; B: ensaios 3 e 4; C: ensaios 5 e 6; D: ensaios 7 e 8; E: ensaios 9 e 10; E: ensaios 11 e 12; F: ensaios 13 e 14; G: ensaios 15 e 16.

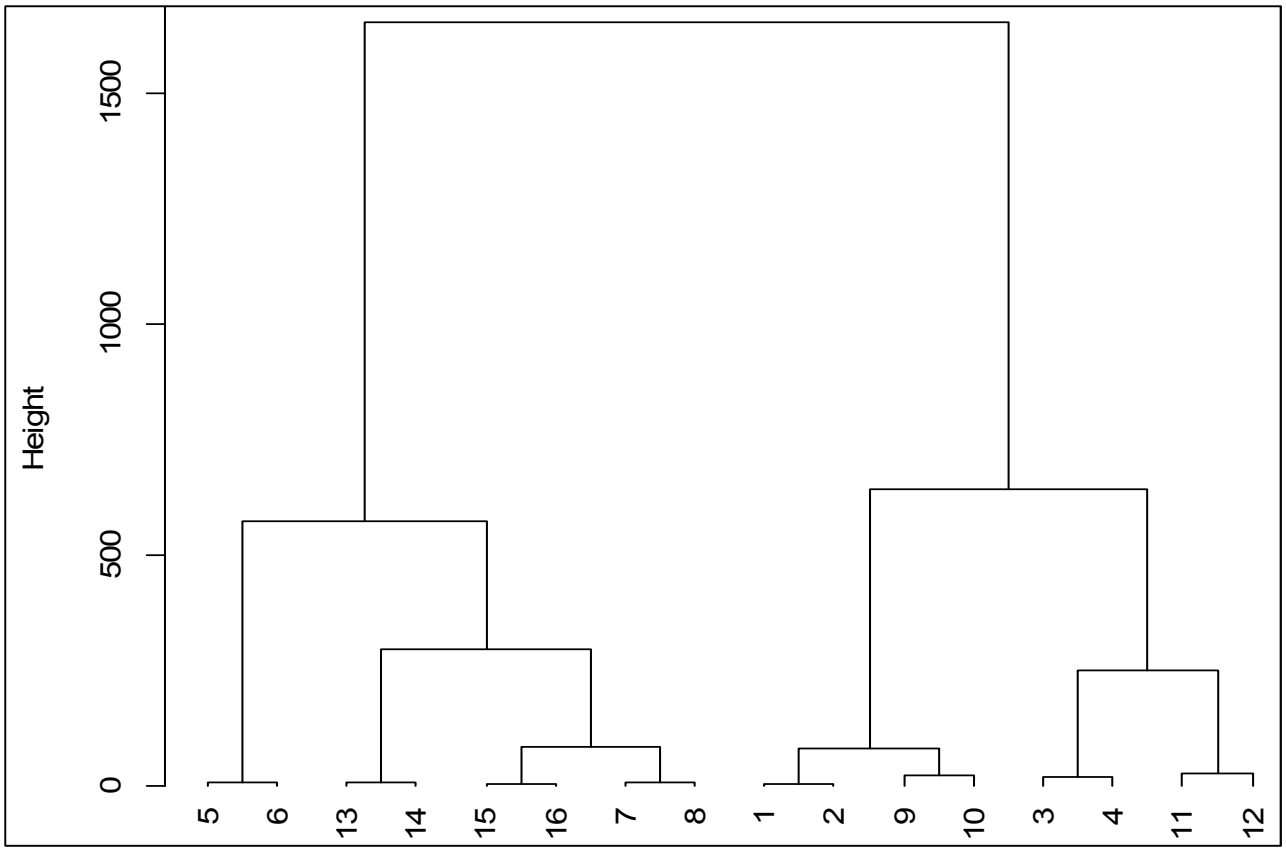

Figura 2. Análise hierárquica (cluster) no planejamento fatorial categórico para o hambúrguer vegetal. Os números de 1 a 16 são os experimentos (Tabela 3).

Na análise hierárquica (Figura 2) as amostras contendo chia e goma xantana/carboximetilcelulose para ambos os tratamentos (cru e assado) formaram um grupo (ensaios A e E). As formulações de hambúrgueres com e sem processo de cocção, com linhaça e goma xantana/carboximetilcelulose formaram um novo grupo (ensaios B e F), havendo ligação com o grupo anterior devido sua semelhança no tipo de emulsificante. Os produtos contendo gema de ovo se organizaram de forma que os ensaios apresentassem a seguinte ordem de ligação D=H<G<C.

A função de desejabilidade teve como objetivo obter a melhor formulação através do máximo teor do somatório de ácidos graxos ômega-3 e da razão hipocolesterolêmica/hipercolesterolêmica, e a menor razão dos ácidos graxos das séries ômega-6 por ômega-3, os índice de aterogenicidade e trombogenicidade. A formulação de hambúrguer com chia como fonte de ômega-3, goma xantana/carboximetilcelulose (emulsificante), no produto assado foi a resposta otimizada (Figura 3).

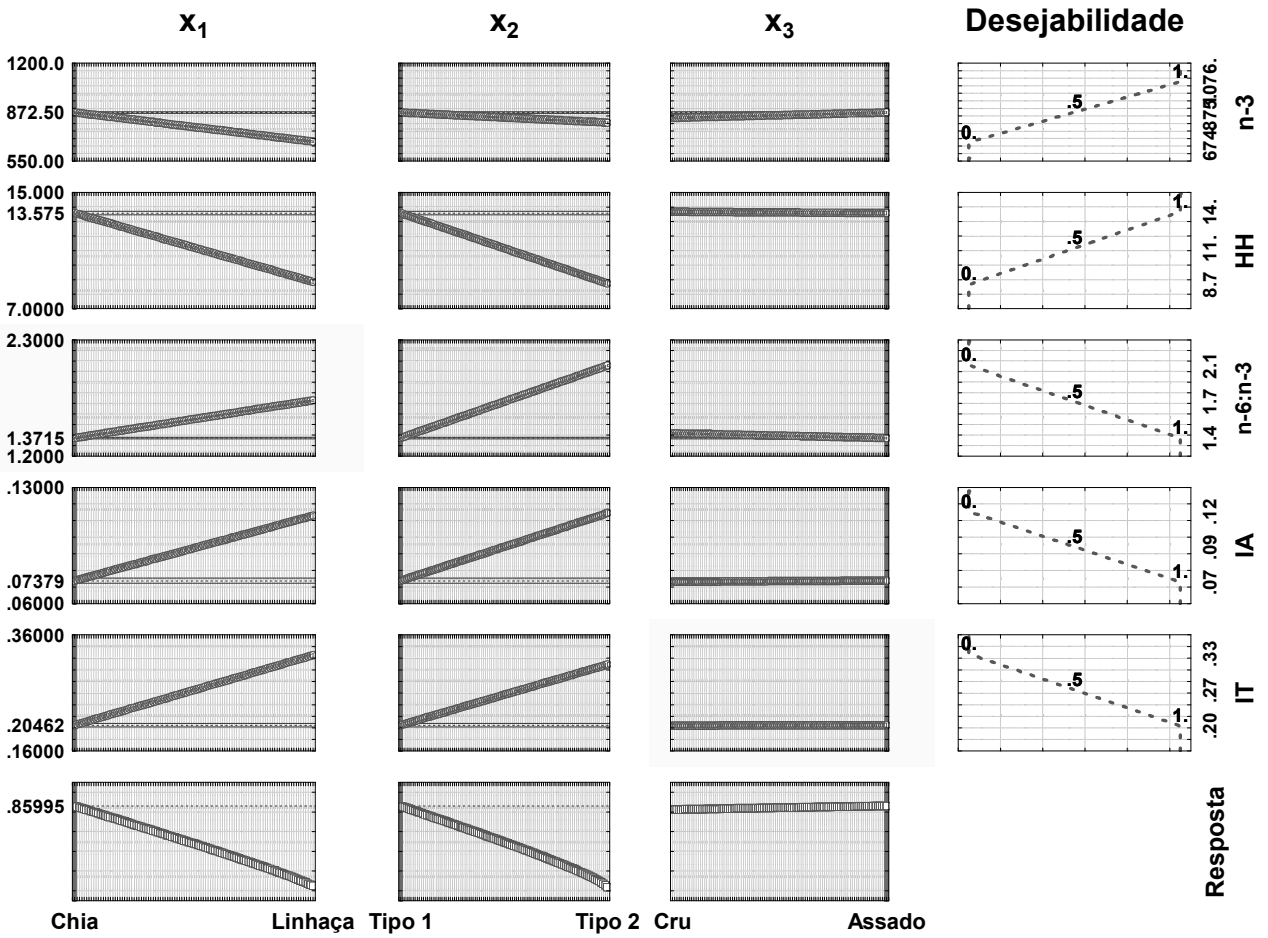

Figura 3. Gráfico da função de desejabilidade do planejamento categórico para o hambúrguer vegetal. x_1: farinhas como fonte do ácido graxo alfa linolênico; x_2: agentes emulsificante/ligante; x_3: tratamento térmico aplicado. n-6: somatório de ácidos graxos da série ômega 6; n-3: somatório de ácidos graxos da série ômega 3; IA: índice de aterogenicidade; IT: índice de trombogenicidade; HH: razão hipocolesterolêmica e hipercolesterolêmica. Tipo1: goma xantana e carboximetilcelulose. Tipo 2: gema de ovo. Linha vertical vermelha representa a conveniência global.

Conclusão

O planejamento fatorial categórico realizado para obter a melhor composição em ácidos graxos em hambúrguer vegetal demonstrou que todos os fatores investigados, efeitos principais e de interação foram significativos. As formulações contendo chia e goma xantana/carboximetilcelulose contribuiu positivamente para melhorar a composição e os índices de ácidos graxos no produto. Na análise hierárquica houve a formação de dois grupos bem definidos, sendo um com chia e outro com a

linhaça, ambos tendo a goma xantana/carboximetilcelulose e sem cocção. Foram feitas algumas restrições nas respostas para a análise de desejabilidade. O perfil em ácidos graxos nos ensaios foi igual e houve uma variação significativa entre as formulações. Nos modelos o efeito principal do tipo de emulsificante e de interação com os três fatores avaliados apresentaram o maior percentual de contribuição. Nesta análise o produto contendo chia como fonte de ômega-3, a goma xantana/carboximetilcelulose como emulsificante no hambúrguer assado foi caracterizado como o ponto ótimo de maior desejabilidade. O uso de vegetais, com destaque para a chia é uma alternativa no desenvolvimento de um alimento nutricionalmente balanceado, tendo em vista a aplicação de várias ferramentas quimiométricas desde a seleção dos ingredientes, processamento e na caracterização do hambúrguer vegetal.

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ANEXO 2: Normas da revista Journal of the Brazilian Chemical Society.

Forthcoming Papers
Current Issue
Past Issues
Statistics
Cover Gallery/ePUB
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JBCS Medal of Honor
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Instructions for Authors

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The **Journal of the Brazilian Chemical Society** (**JBCS**) embraces all aspects of chemistry except education, philosophy and history. It is a medium for reporting selected original and significant contributions to new chemical knowledge. The Journal publishes **Articles, Communications**, **Short Reports**, **Reviews**, **Accounts** and **Letters**.
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Authors should present their materials with the utmost conciseness and clarity. The **Introduction** should clearly and briefly identify, with relevant references, both the nature of the problem under investigation and its background. Extensive reviews of the literature cannot be accepted.
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It should contain RELEVANT and COMPLEMENTARY DATA to those presented in the manuscript. If new compounds are identified or characterized, **all spectra** should be included (see Section 8).
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Details:

First Page

- **Graphical Abstract (GA)** (see Section 5)

Second Page

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Jailson B. de Andrade,*,[a] Marta V. Andrade[b] and Heloisa L. C. Pinheiro[c]

Third Page

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Reagents and equipments
X-ray data

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3. Provstyanoi, M. V.; Logachev, E. V.; Kochergin, P. M.; Beilis, Y. I.; Izv. Vyssh. Uchebn. Zadev.; Khim. Khim. Tekhnol. **1976**, 19, 708. (CA 85:78051s).

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7. Kadin, S. B.; US pat. 4,730,004 **1988** (CA 110:P23729y).
8. Eberlin, M. N.; Mendes, M. A.; Sparrapan, R.; Kotiaho, T.; Br PI 9.604.468-3 **1999**.

- **Books**

9. Cotton, F. A.; Wilkinson, G.; Advanced Inorganic Chemistry, 5th ed.; Wiley: New York, USa, 1988.

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- **Software**

11. Sheldrick, G. M.; SHELXL-93; Program for Crystal Structure Refinement; University of Göttingen, Germany, 1993.

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- **Dissertation/Thesis:** do not use as bibliographic reference. Include only the articles that were produced from that research work.

8. Supplementary Information (SI)

This material will be available online in the **JBCS** Page as PDF file. It should contain relevant and complementary data to those presented in the manuscript. Their format can be: tables, graphs, spectra, films and so on.
Any synthesized or identified compound must be accompanied by the spectra used for such identification. This is especially important for Natural Products, Organic and Inorganic Chemistry manuscripts in which the characterization/identification techniques are part of the work.

8.1 Manuscripts including crystallographic data

Deposition of Crystallographic Data

Prior to the submission of the typescript including crystallographic data, the author(s) should deposit, in the relevant Data Center, the data corresponding to each structure to be reported.

Data for **organometallic**, **organic** and **coordination (Werner-type) compounds** should be sent to the Cambridge Crystallographic Data Center (CCDC) by e-mail, in CIF format. More information and a checklist of data items to be included in the deposit can be obtained from the CCDC homepage: http://www.ccdc.cam.ac.uk/.
Data for inorganic compounds should be sent to Fachinformationszentrum Karlsruhe (FIZ) by e-mail: crysdata@FIZ-Karlsruhe.de.

Deposition Codes

The Data Centers will provide deposition codes for each data set, which should be quoted in the typescript under a Supplementary Information heading before the Acknowledgements.
Standard text for CCDC:

Crystallographic data (excluding structure factors) for the structures in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC XXXXXX. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

Preparation of Crystallographic Material

When the manuscript is submitted, the following guidelines should be observed:
The Abstract should not contain crystal data, but a concise statement of the main features of the structural results.
The following crystallographic data should be given in a paragraph of a Table, in a concise format:

8.1.1 Color, habit and size of the crystal(s) used, behavior of the compound under the data collection conditions.

8.1.2 The chemical formula should correspond to the complete chemical unit encompassing the crystallographic symmetry, the formula weight, F(000), the absorption coefficient and the measured and calculated densities.

8.1.3 The unit cell parameters with esd's and the X-ray wavelength used.

8.1.4 The crystal system, space group and number of chemical units per cell.

8.1.5 Type of diffractometer used and method of data collection, total number of data collected, number of unique reflections, R(int) value, number of observed reflections with cut-off parameter, use or not of absorption correction, transmission factors.

8.1.6 The final results: R, wR, S and the number of parameters refined; treatment of hydrogen atoms; final peak and hole in the last difference map. Only refinements on F2 will be accepted.

Discussion of the Structure

It must include a labeled diagram of the structure, a list of relevant geometric parameters - interatomic bond distances and angles, torsion angles, hydrogen bond parameters, etc. Data of less important parts of the structure, such as ligand sub-groups (phenyl rings, etc.) should be omitted.

8.2 Manuscripts including NMR, IR, mass spectra, etc.

Whenever a compound is synthesized or identified (new or already known), it is imperative to send all spectral data (data and spectra) as Supplementary Information (SI) along with your submission, at the end of your doc file.

A brief mention to the existence of complementary data should be included in the Supplementary Information topic before the **Acknowledgments** section. Example:

Supplementary Information

Supplementary information (Figure S1-S4, Table S1) is available free of charge at http://jbcs.org.br as PDF file.

How to send this type of information:

Join all spectra in one SI file. Do not forget to add captions to each one of them, identifying each individual spectrum (e.g., Figure S1. ^{1}H NMR Spectrum of...; Figure S2. IR Spectrum of...; Figure S3. ^{13}C{^{1}H} Spectrum of...; Table S1. Data for...). If the spectra will be digitalized (scanned), choose options: black&white, without background and 300 dpi at least. Add this file to the end of your manuscript, which should then comprehend one single doc file, containing GA, text with tables and figures, and SI.

9. Procedure for Manuscript Submission

9.1 Manuscript to be Evaluated for the First Time

The **JBCS** submission offers only online submission. The submissions are made using the ScholarOne^{TR}-**JBCS** system by clicking the link "Submission online (ScholarOne)" at our website (http://mc04.manuscriptcentral.com/jbchs-scielo).

- All the authors must have their names introduced in the platform, so fill this part and inform the correct co-authors' e-mail addresses in the system.
- In the ScholarOne-JBCS system, all files need to be uploaded individually:

(i) Main manuscript: as full.doc, not as full.pdf and

(ii) Figures/Schemes (just the ones from the main document), including GA image: as jpg, tiff, opj, xls, etc (not as individual doc files or grouped in a doc file).

Figures built using Excel/Origin programs provide pictures higher quality in the final work (proof), so upload preferentially original xls/opj files.

• In the main document (full.doc): also keep tables/figures/schemes/equations and their legends as close as possible of their first citation.

9.2 Manuscript already Evaluated (Resubmission: Reject and Reject&Resubmission)

In cases that the manuscript has already received a decision from JBCS Editor like Reject and Reject&Resubmission some specific requirements are necessary:

(1) Main document: the modifications need to be highlighted with a different color guiding Editor/Reviewers with changes made in relation to the original version (do not use the track changes mode in MS Word).

(2) Be sure that the Response Letter, in the place of the cover letter, itemizes each comment addressed, as well as any changes made, of all Referee(s) and Editor (if so). Write a very convincing text explaining the points that were introduced/removed, new experiments that were used. Add, please: "Response Letter for ID JBCHS-201x-0xxx (previous ID): ..."

(3) Replace all the files that were modified uploading with the new files.

For Reject&Resubmission decision, Authors may access the previous ID (one that received the decision) in the Author Center at the JBCS-ScholarOne submission site (http://mc04.manuscriptcentral.com/jbchs-scielo at the link "Manuscripts with Decisions") and then in "create a resubmission" to resubmit the manuscript.
With the resubmission, the manuscript will receive a new ID. The use of this link will accelerate the evaluation since the system will keep all the decisions for the previous ID linked to the new ID.

All these actions for an already evaluated manuscript will expedite the assessment.

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