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Article

Using Chemometric Techniques to Characterize Gluten-Free Cookies Containing the Whole Flour of a New Quinoa Cultivar

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A doença celíaca é definida como a intolerância às proteínas do glúten presente em certos cereais usados na produção de alimentos. Três formulações de biscoitos sem glúten, contendo *Linum usitatissimum* L. e diferentes concentrações de *Chenopodium quinoa* BRS Piabiru, foram desenvolvidos e avaliados em relação as caracterísiticas fisico-químicas, nutricionais e sensoriais. Não foi detectado glúten nos biscoitos formulados. O conteúdo de proteína bruta e lipídios totais variaram 85,58 a 97,55 e 121,69 a 166,19 g por kg de amostra, respectivamente. A variação da razão entre os ácidos graxos n-6:n-3 e poliinsaturados/saturados foi de 0.85:1 a 0.92:1 e 3.08:1 a 4.38:1, respectivamente. A Formulação C apresentou melhores teores de ácido alfa-linolênico, índices nutricionais da fração lipídica e conteúdo mineral por porção, com excelentes características sensoriais. A análise multivariada destacou o efeito da concentração de quinoa nas qualidades nutricionais e sensoriais do produto.

Celiac disease is defined as intolerance to the gluten proteins present in certain cereals used to prepare foodstuffs. We developed and performed physico-chemical, sensory, and nutritional assessments of three formulations of gluten-free cookies containing *Linum usitatissimum* L. and different levels of whole *Chenopodium quinoa* BRS Piabiru flour. No gluten was detected in the prepared cookie formulations. The crude protein and total lipid contents ranged from 85.58 to 97.55 and 121.69 to 166.19 g *per* kg of sample, respectively. The polyunsaturated/saturated and n-6:n-3 fatty acid ratios ranged from 0.85:1 to 0.92:1 and 3.08:1 to 4.38:1, respectively. Formulation C had the best alpha-linolenic acid content, lipid fraction nutritional indices and mineral content per portion, with excellent sensory characteristics. Multivariate analysis highlighted the effect of the concentration of quinoa on the nutritional and sensory qualities of the product.

Keywords: pseudo-cereal, linseed, fatty acids, minerals, principal component analysis

Introduction

Celiac disease (CD), defined as the intolerance to gluten protein, arises from the resistance of the protein to digestive enzymes, which triggers an inflammatory response in genetically predisposed individuals. Gluten-rich foods such as oat, barley, rye, and wheat cause inflammation in the small intestine villi, with subsequent atrophy and low absorption of nutrients in affected individuals. CD is one of the most frequent genetic disorders of humankind, affecting 0.5% to 1% of the general population.[1] In Brazil, screening studies carried out at blood banks indicated that the prevalence ranged from 1:681 to 1:276 donors.[2] There are fewer gluten-free products available than foods containing gluten.[3]

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The development of gluten-free foods requires ingredients with high nutritional value, such as quinoa (*Chenopodium quinoa* Willd) and linseed (*Linum usitatissimum* L.). Quinoa, from the Andean region, is classified as a pseudo-cereal, while linseed is an oilseed native to western Asia and the Mediterranean. Quinoa is composed of 55.1-63.9% carbohydrate, 8.8-11.1% dietary fiber, 5.8-10.3% total lipids, 3.0-3.3% minerals, and 14.5-14.8% crude protein.[3,4] Crude protein fractions are important because they are directly related to the essential amino acid composition of this pseudo-cereal.[5-7] High levels of crude fiber and total lipids - 8.3 and 43.9%, respectively - have been found in linseed.[4] Linseed is distinct from the pseudo-cereals due to its lipid fractions of 14.5-22.2%, 15.1-17.4%, and 51.8-60.4% for oleic (18:1 n-9), linoleic (18:2 n-6), and alpha-linolenic (18:3 n-3) acid, respectively, while quinoa contains 0.6-3.8%, 23.6-26.5%, and 35.3-48.1%, respectively.[4,8]

C. quinoa Willd. and other native varieties have a bitter taste due to the presence of saponins and water-soluble and thermolabile compounds, which are toxic in high doses *in vivo* but serve as efficient insecticides and anti-microbial agents for the plant.[9] The cultivar *C. quinoa* BRS Piabiru was genetically modified for the climate conditions of central-western Brazil and to remove saponins while maintaining its chemical composition in a study conducted by the Brazilian Agricultural Research Corporation (EMBRAPA), Cerrados facility, Brasília, DF, Brazil.[10]

Multivariate analysis enables the extraction of more information than univariate analysis. This chemometric tool permits pattern recognition, information gathering, and a reduction of data dimensionality, as well as the organization of the data in a simpler structure that is easier to understand. Principal component analysis (PCA) is based on performing linear comparisons of the original variables. The principal components (PC) are mutually orthogonal and explain variance decreases with an increase in PC number.[11]

Bakery products are among the most commonly consumed foods,[12] mainly because of their convenience and excellent sensory quality. The development of cookies rich in essential compounds such as amino acids, minerals, fibers, and fatty acids that are also free of anti-nutritional factors is necessary, particularly due to the dietary restrictions of celiac disease patients. The goal of this study was the development and physico-chemical, sensory, and nutritional assessment of gluten-free cookies containing the whole flour of *C. quinoa* BRS Piabiru as a source of protein and minerals and *L. usitatissimum* L. as a source of alpha-linolenic acid, using chemometric analytic techniques.

Experimental

Sampling and formulations

The grain of *C. quinoa* BRS Piabiru used in the development of the cookie formulations was provided by EMBRAPA. The other ingredients were purchased from local shops in Maringá, Paraná state. Samples of quinoa and linseed were taken from 60 kg bags of grain. The linseed was coarsely ground.

Three formulations of cookie (A, B and C) were developed using quinoa flour to partially substitute rice flour in different levels. The ingredients of cookies were accurately weighed and mixed to yield a uniform mixture for each formulation (A, B, and C) (Table 1). The butter and dry ingredients were mixed at low speed using a KitchenAid mixer (St. Joseph, MI, USA) for 3 min and scraped down after each minute. The mass was then mixed for 1 min and scraped down every 20 s. Finally, the mixture of flours was added, and the dough was mixed at low speed for 1 min, with scraping every 20 s. After the mixing was complete, the dough was removed and flattened with a rolling pin to the desired thickness of 7 mm (6 cm in diameter). The cookie formulations were then baked at 180 ºC for 20 min. Three replicates were prepared for each formulation (n = 3).

Table 1. Cookie formulations

Ingredients in g *per* kg of product	Formulation		
	A	B	C
Quinoa flour	60.00	100.00	140.00
Rice flour	330.00	290.00	250.00
Brown sugar	30.00	30.00	30.00
Refined sugar	30.00	30.00	30.00
Honey	120.00	120.00	120.00
Butter	50.00	50.00	50.00
Egg yolk	50.00	50.00	50.00
Linseed flour	50.00	50.00	50.00
Sodium carbonate	10.00	10.00	10.00
Water	70.00	70.00	70.00
Chocolate drops	90.00	90.00	90.00
Cacao powder (70%)	20.00	20.00	20.00
Brazil nut	80.00	80.00	80.00
Nut flavor	10.00	10.00	10.00

Gluten test

The gluten fractions in grains of quinoa, linseed, rice, corn flakes, and in the final products were determined using a commercial enzyme-linked immunosorbent assay (ELISA)

Ridascreen® Gliadin kit R5 (R-Biopharm, Germany), a Sunrise spectrophotometer (Tecan, Switzerland) at 450 nm, and Rida-Win software (R-Biopharm, Germany). The limits of detection and quantification of the method were 1.50 ng gliadin mL^{-1} or 3.00 ng gluten mL^{-1}, and 2.50 ng gliadin mL^{-1} or 5.00 ng gluten mL^{-1}, respectively, with a sensitivity > 2.00 mg gluten *per* 100 g of food, as recommended by the Codex Food Commission.[13] The gluten fractions were extracted with a 60% (v/v) ethanol solution and a reagent cocktail.

Chemical and instrumental analysis

The moisture, ash, and crude protein contents were determined according to Cunniff[14] using a factor of 5.80 to convert the percentage of nitrogen into crude protein content.[3] The total lipids were determined according to Bligh and Dyer.[15] The total carbohydrate content was calculated as the remaining weight.[16]

The caloric value was determined through direct (instrumental) and indirect (calculation) calorimetry. For the instrumental method, the samples were milled and dried at 105 ºC for 4 h. The crude energy was determined in a 1261 Automatic Isoperibol (Parr, USA) oxygen bomb calorimeter. In the indirect method, conversion factors were used for each product component: 4 kcal for carbohydrates and crude protein and 9 kcal for lipids.[17] The results were obtained in kcal of food, converted into Joules using a factor of 4.1868 J to 1 cal.

The water activity was analyzed using AquaLab 4TE (Decagon, USA) at 25 ºC with an infrared detector. The color of the product was determined by Tristimulus L*a*b* colorimetry: 'L' (whiteness, 100 = white, 0 = black), 'a' (+, red; –, green) and 'b' (+, yellow; –, blue), using a CR-400 (Konica Minolta, Japan) colorimeter. The rate of color change was calculated with the equation (ΔE): $\Delta E = (a^2 + b^2 + L^2)^{1/2}$.

Fatty acid composition and mineral quantification

To determine the fatty acid composition, the lipids were converted into fatty acid methyl esters (FAME) and methylated according to Hartman and Lago.[18] The FAME were separated using a CP-3380 gas chromatograph (Varian, 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 ºC at 10 °C min^{-1} and maintained for 10 min. The retention times were compared to those of standard methyl esters (Sigma, USA). The fatty acids were quantified using tricosanoic acid methyl ester (Sigma, USA) as an internal standard, according to Joseph and Ackman.[19] The peak areas were determined with Star 5.0 software (Varian, USA), and the concentrations were expressed as mg *per* kg of food.

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

Indices of the nutritional quality of lipids

A better approach to the nutritional evaluation of fat is the utilization of indices based on the functional effects of fatty acid composition. These indices are the index of atherogenicity (IA) = [(12:0 + (4 × 14:0) + 16:0)] / (ΣMUFA + Σn-6 + Σn-3) and the index of thrombogenicity (IT) = (14:0 + 16:0 + 18:0) / [(0.5 × ΣMUFA) + (0.5 × Σn-6) + (3 × Σn-3) + (Σn-3 / Σn-6)], as defined by Ulbricht *et al*.,[20] as well as the hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) = (18:1n-9 + 18:2n-6 + 20:4n-6 + 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3) / (14:0 + 16:0), according to Santos-Silva *et al*..[21]

Microbiological characterization

Food safety and product contamination by *Bacillus cereus*, thermotolerant coliforms, coagulase-positive staphylococcus, and *Salmonella sp.* after processing were determined as proposed by Vanderzant and Splittstoesser and Brazil before sensory analysis was performed.[22,23]

Sensory analysis

A group of 80 non-trained volunteer panelists and potential consumers of the developed products participated in the sensory analysis, which consisted of acceptance testing, preference ordering, and intent-to-purchase of the developed formulations. In the acceptance test, the appearance, flavor, texture, crispness, and overall acceptance of the food were assessed using a nine-point hedonic scale (1 = extremely disliked to 9 = extremely liked). The samples were presented in random complete blocks for comparison. The index of

acceptability (IA) of the products was calculated as (global aspect grade × 100%) / 9, where 9 was the maximum score on the hedonic scale. The lowest IA value for considering the products as well accepted by the consumers was 70%. The ordering test assessed the preference for each formulation; the results were obtained by summing the order values of each sample. The intent-to-purchase was determined using a five-point scale (1 = would definitely not buy and 5 = would definitely buy).[24]

Calculation of the dietary reference intake

The Dietary Reference Intake (DRI) is an estimate of the percentage of daily nutrient requirements according to age and gender as established by the Institute of Medicine for individuals aged over 12 months.[25,26] The DRIs for Ca, Cu, Fe, K, Mg, Mn, P, and Zn were determined as the mean amounts in 30 g portions, as proposed by Brazil as an appropriate serving size for cookies.[27]

Ethical aspects

The sensory testing in this study was approved by the Standing Committee on Ethics in Research Involving Human Beings of Maringá State University, CAAE File No. 0433.0.093.000-10. All panelists signed a free and informed consent form prior to their participation in the sensory analysis.

Statistical analysis

Fatty acid composition and mineral, instrumental, and physico-chemical analyses were carried out in triplicate. The Pearson correlation analysis was applied to compare the direct and indirect methods for energy determination. The Friedman test was used only for the preference-ordering test, according to Lawless and Heymann.[24] Multivariate analysis was performed by applying principal component analysis (PCA). The average of the three individual batches was used with respect to the proximal composition, direct and indirect energy methods, sums, ratios and indices of fatty acids, mineral composition, and sensory attributes. The averages were autoscaled using the NIPALS algorithm. The statistical software SAS, version 7.0, was used with a 5% ($p < 0.05$) significance level to select principal components.

Results and Discussion

Gluten fractions were not detected by the ELISA test in either the grains or the gluten-free cookie formulations, corroborating previous studies that have shown the absence of gluten in other varieties of the same species of grains used in this study.[28]

The results of the physico-chemical and instrumental analyses are shown in Table 2. Principal component analysis allowed the selection of PC1, PC2, and PC3, which explained 96.63% of the data variance in the proximal composition and crude energy (Table 3). The levels of total lipids, protein, and instrumental crude energy made a large contribution to the formation of PC1, accounting for the characterization of formulations A and B. The use of quinoa in the formulations mainly increased the protein fraction in products intended for celiac patients, consistent with a study by Enriquez *et al*..[29] Generally, gluten-free products present a high carbohydrate concentration and a low protein content. Segura and Rosell reported products with up to 92% carbohydrates.[30] The cookies developed in this study are promising products for celiac disease patients due to their reduced carbohydrate content and increased protein content.

In PC1 of Figure 1A, only formulation A showed a significant contribution from the moisture content. The ash content was responsible for distinguishing samples A and B of the C in PC3 (Figure 1B and Table 3). According to Gutierrez *et al*.,[4] linseed has a mineral content of 2.66%, while those of pseudo-cereals are ca. 2.5%;[30-33] which contributes to the high mineral content of the products.

The indirect method of determination of crude energy yielded negative results for all significant PCs ($p < 0.05$, Table 3). This may have occurred due to the larger error associated with estimates made by the indirect method because the instrumental method is able to determine the energy provided by other compounds present in food. In the Pearson correlation analysis, there was a strong positive ($r = 0.8533$) and significant correlation ($p = 0.0034$) between the direct and indirect methods. The color variation (ΔE) showed that all the products tended towards dark brown.

Formulation A had the highest contribution from PC1 with respect to the sums, ratios, and indices of fatty acids (Tables 3 and 4; Figure 2A), except for the IA. In PC2 (Table 3, Figure 2B), the batches of cookie C differed from the others with respect to the content of alpha-linolenic acid and the nutritional indices of the lipid fraction (Table 4). The PUFA:SFA ratio and IT were responsible for the formation of PC3 (Table 3, Figure 2B), which characterized sample B.

The classes of fatty acids and their relationship to the proper functioning of the body may be described using nutritional indices and ratios.[20,21,33,34] The indices of atherogenicity (IA) and thrombogenicity (IT) relate the presence of lauric (12:0), myristic (14:0), palmitic

(16:0), and stearic (18:0) fatty acids with the occurrence of coronary disease when compared with the effects of monounsaturated fatty acids, especially oleic acid (18:1 n-9) and the omega-3 and -6 series. Ulbricht *et al.*[20] found higher IA and IT values in coconut oil, emphasizing the direct relationship between a lower ratio and an attenuated risk of

Table 2. Proximal composition, crude energy, water activity and color of cookie formulations

Parameter	Formulation		
	A	B	C
Moisture / (g kg^{-1})	149.00 ± 0.05	152.40 ± 0.40	167.79 ± 1.38
Ash / (g kg^{-1})	20.04 ± 0.10	20.76 ± 0.04	21.32 ± 0.31
Crude protein / (g kg^{-1})	85.58 ± 1.83	97.55 ± 2.19	94.49 ± 2.19
Total lipids / (g kg^{-1})	166.19 ± 0.47	121.69 ± 1.81	125.36 ± 2.71
Carbohydrates[a] / (g kg^{-1})	579.18 ± 1.26	607.61 ± 0.16	591.04 ± 1.37
Crude energy[b] / (kJ kg^{-1})	17395.33 ± 4.12	16394.83 ± 7.74	16204.52 ± 11.10
Crude energy[c] / (kJ kg^{-1})	1711.10 ± 0.00	1750.52 ± 0.00	1734.62 ± 0.00
A_a[d]	0.43 ± 0.00	0.44 ± 0.00	0.47 ± 0.00
L*	18.45 ± 0.15	21.53 ± 2.05	32.02 ± 2.95
a*	4.72 ± 0.06	5.41 ± 0.60	8.29 ± 0.59
b*	7.20 ± 0.07	8.10 ± 0.53	10.31 ± 0.41
ΔE[e]	20.36 ± 0.02	23.64 ± 0.18	34.65 ± 0.15

[a]Carboydrates determined by difference; [b](instrumental) and [c]indirect (calculated) methods; [d]water activity; [e]rate of color variation.

Table 3. Eigen analysis of the correlation matrix loadings of the significant principal components (PC) for the proximal composition, sums, ratios and index of fatty acids, minerals, and sensory attributes

Proximal composition												
	Eigenvalues	Total variance / %	Moisture	Ash	CP	TL	Carbohydrates	Energy1[a]	Energy2[b]			
PC1	4.9384	49.3844	0.3073	–0.4135	0.7142	0.9537	–0.9912	–0.6192	0.7231			
PC2	3.8717	38.7171	0.9439	–0.2917	–0.6639	–0.0159	–0.0546	–0.7594	–0.6247			
PC3	0.8531	8.5315	0.0193	0.8460	0.0699	–0.0730	–0.0549	–0.1673	–0.1490			
Fatty acid: sums, ratios and nutritional index												
	Eigenvalues	Total variance / %	SFA	MUFA	PUFA	n-6	n-3	PUFA:SFA	n-6:n-3	IA	IT	HH
PC1	10.1188	77.8367	0.9933	0.9848	0.9988	0.9978	0.8372	0.9187	0.9732	–0.8801	0.6922	0.9689
PC2	2.2170	17.0536	0.0530	–0.1224	–0.0081	–0.0470	0.5180	–0.1829	–0.2164	0.4372	0.3546	0.2202
PC3	0.4496	3.4581	–0.0677	–0.0685	–0.0445	–0.0385	–0.1179	0.0463	–0.0072	–0.0365	0.6276	–0.0775
Minerals												
	Eigenvalues	Total variance / %	Ca	Cu	Fe	K	Mg	Mn	P	Zn		
PC1	7.0916	64.4694	0.9577	0.8934	0.8313	0.9249	0.9464	0.4833	0.9524	0.8008		
PC2	1.8692	16.9927	0.2687	0.2252	0.1416	0.0626	–0.0169	0.0073	–0.2389	–0.4508		
PC3	1.2177	11.0703	0.0008	0.0513	0.4723	–0.3413	0.2400	0.6993	–0.1294	0.0346		
Sensory attributes												
	Eigenvalues	Total variance / %	Appearance	Flavor	Texture	Crispness	Overall acceptance					
PC1	2.6136	32.6699	0.7753	–0.1436	0.8590	0.8025	0.7553					
PC2	1.5033	18.7917	–0.0115	–0.0173	0.0241	–0.0529	0.0026					

[a]Direct (instrumental) method; [b]indirect (calculated) method. CP: crude protein; TL: total lipids; SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids; n-3: total omega-3 fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity; HH: hypocholesterolemic/hypercholesterolemic fatty acid ratio.

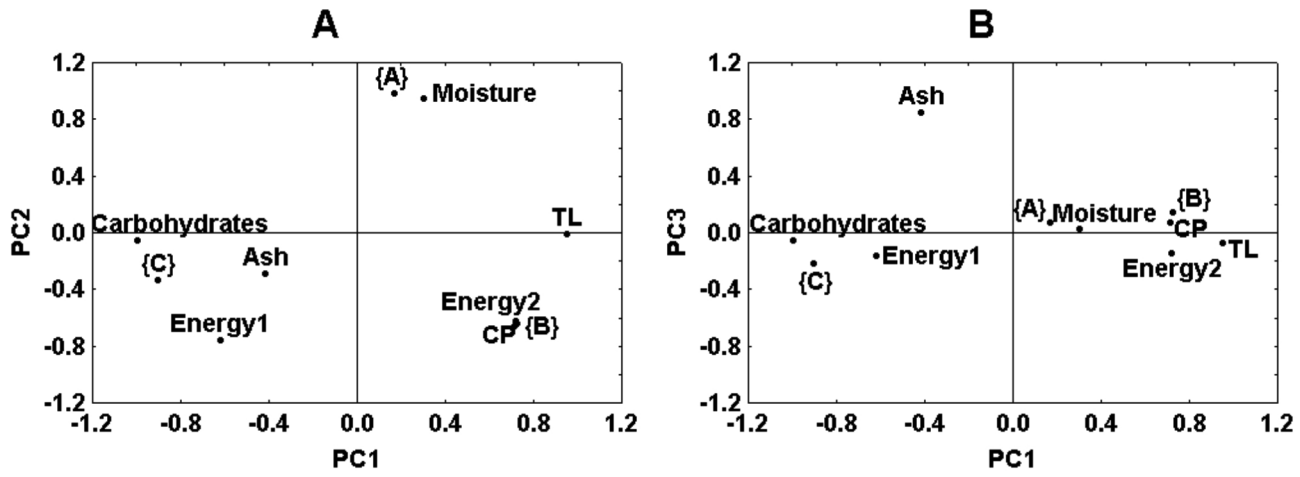

Figure 1. Principal component analysis of the proximal composition of cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC1 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Analyses (Parameters/Loadings): Ash, Carbohydrates, Energy1, Energy2, Moisture, CP, TL. CP: crude protein; TL: total lipid; Energy1: direct method (instrumental); Energy2: indirect method (calculated).

Table 4. Absolute fatty acid quantification of cookie formulations

Fatty acid	Formulation		
	A	B	C
Fatty acid content / (g kg^{-1})			
10:0	0.90 ± 0.03	0.91 ± 0.02	0.96 ± 0.03
12:0	2.16 ± 0.04	1.91 ± 0.04	2.49 ± 0.07
14:0	4.30 ± 0.08	3.83 ± 0.08	4.39 ± 0.13
16:0	29.30 ± 0.21	22.72 ± 0.06	23.61 ± 0.20
16:1n-7	0.99 ± 0.01	0.85 ± 0.01	0.90 ± 0.01
18:0	18.10 ± 0.03	11.58 ± 0.11	11.64 ± 0.05
18:1n-9	46.54 ± 1.12	35.58 ± 0.40	35.09 ± 0.24
18:2n-6	41.47 ± 0.02	26.99 ± 1.19	27.74 ± 0.27
18:3n-3	9.47 ± 0.16	8.29 ± 0.08	9.00 ± 0.10
20:0	0.35 ± 0.01	0.26 ± 0.01	0.29 ± 0.01
Sums and ratios of fatty acids			
SFA	55.15 ± 0.23	41.19 ± 0.16	43.38 ± 0.26
MUFA	47.53 ± 1.12	36.43 ± 0.40	35.99 ± 0.24
PUFA	50.94 ± 0.16	35.28 ± 1.19	36.74 ± 0.29
n-6	41.47 ± 0.02	26.99 ± 1.19	27.74 ± 0.27
n-3	9.47 ± 0.16	8.29 ± 0.08	9.00 ± 0.10
PUFA:SFA	0.92 ± 0.01	0.86 ± 0.04	0.85 ± 0.01
n-6:n-3	4.38 ± 0.02	3.26 ± 0.05	3.08 ± 0.02
Indices of the nutritional quality of the lipid			
IA	0.49 ± 0.01	0.56 ± 0.01	0.60 ± 0.01
IT	0.71 ± 0.01	0.67 ± 0.01	0.69 ± 0.01
HH	2.90 ± 0.03	2.40 ± 0.01	2.56 ± 0.05

SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids; n-3: total omega-3 fatty acids; IA: index of atherogenicity; IT: index of thrombogenicity; HH: hypocholesterolemic/hypercholesterolemic fatty acid ratio.

coronary disease. The major ratios of HH and PUFA:SFA (Table 3) are important due to their hypocholesterolemic effects, and the prevalence of polyunsaturated fatty acids is associated with a lower risk of cardiovascular disease.[33]

According to the Institute of Medicine,[35] saturated fatty acids must be avoided in a balanced diet. The saturated fatty acid contents of cookies A, B, and C were 5.51%, 4.12%, and 4.34%, respectively. The polyunsaturated fatty acids:saturated fatty acids (PUFA:SFA) ratio of the samples was approximately 0.9:1. The consumption of PUFA is recommended because the excessive consumption of SFA is associated with an increased risk of cardiovascular disease.[35] According to Simopoulos,[34] the excessive consumption of lipids, trans fatty acids, and an unbalanced n-6:n-3 ratio are related to a higher frequency of myocardial infarction, hypercholesterolemia, increased low density lipoprotein (LDL) cholesterol, increased blood pressure, atheroma, lipid disorders, and other disorders. These formulations did not contain *trans* fatty acids. The n-6:n-3 ratio of the cookies ranged from 3.08:1 to 4.38:1, which is close to the ideal value of 1:1.[34] Stroher *et al.*[36] analyzed many types and brands of cookies and found significant *trans* fatty acid contents in all samples, although they reported that the quantity of *trans* fatty acids has been decreasing.

As shown in Table 5, the major mineral components were K, Mg, and P. These minerals play a vital role in a wide range of biochemical and physiological processes. In the multivariate analysis, these micronutrients had the largest contribution (Table 3) in PC1 (Figure 3A); the other minerals (Ca, Cu, Fe, and Zn) also contributed significantly ($p < 0.05$) to this principal component. Sample C was best described by the effect of the incorporation of minerals in PC1 (Table 3). This is due to the higher correlation of

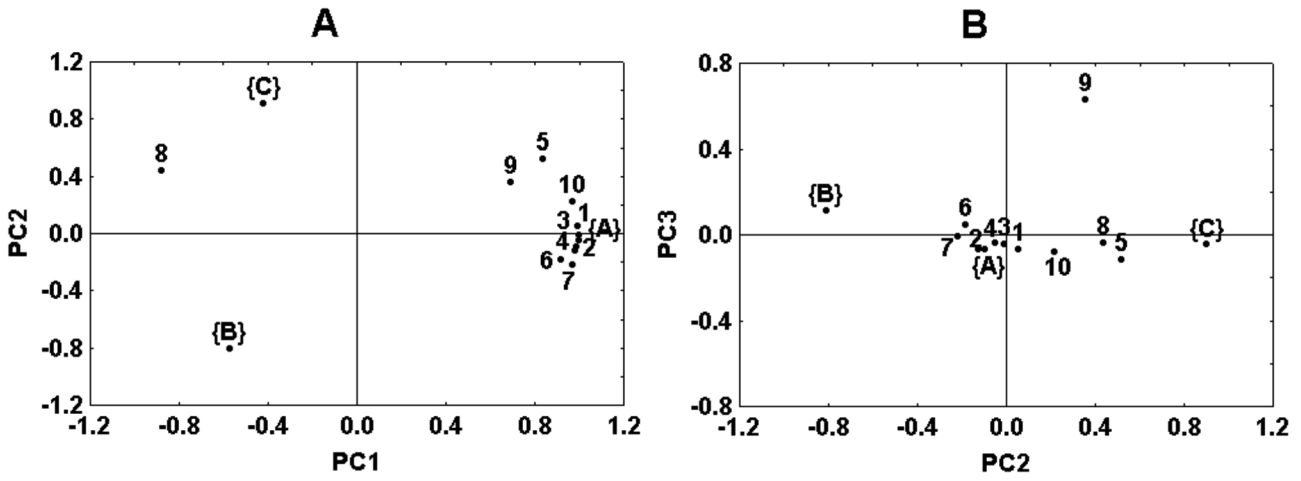

Figure 2. Principal component analysis of fatty acids: sums, ratios, and nutritional index of the cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC2 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Analyses (Parameters/Loadings): 1 = SFA (total saturated fatty acids); 2 = MUFA (total monounsaturated fatty acids); 3 = PUFA (total polyunsaturated fatty acids); 4 = n-6 (total omega-6 fatty acids); 5 = n-3 (total omega-3 fatty acids); 6 = PUFA:SFA; 7 = n-6:n-3; 8 = IA (index of atherogenicity); 9 = IT (index of thrombogenicity); 10 = HH (hypocholesterolemic/hypercholesterolemic fatty acid ratio).

the matrix sample with PC1 (0.7185) relative to sample B (0.0753). Repo-Carrasco-Valencia reported that quinoa presents excellent *in vitro* digestibility values for calcium, iron, and zinc.[32] These minerals are essential for the maintenance of biological systems because they are cofactors in metabolic reactions.[37] PC2 and PC3 (Table 3, Figures 3A and 3B) distinguished cookies B and A, respectively, with respect to the contents of Ca, Cu, and Mn for sample B, and Fe, Mg, and Mn for sample A.

Table 6 presents the nutritional contributions of the cookie formulations for different age groups,[25,26] based on the value per portion set forth by Brazilian standards.[27] The intake of trace minerals from the cookies reached values above 10% of the DRI. Cu and Mg contents were almost twice the DRI in some age groups, but this amount is not

Table 5. Mineral composition of cookie formulations

Mineral / (g *per* kg of sample)	Formulation		
	A	B	C
Ca	2.41 ± 0.05	2.57 ± 0.08	2.73 ± 0.06
Cu	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Fe	0.13 ± 0.02	0.15 ± 0.01	0.16 ± 0.03
K	4.27 ± 0.10	6.05 ± 0.13	6.52 ± 0.17
Mg	2.63 ± 0.21	2.87 ± 0.35	3.08 ± 0.11
Mn	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
P	2.54 ± 0.12	2.73 ± 0.49	3.11 ± 0.77
Zn	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.01

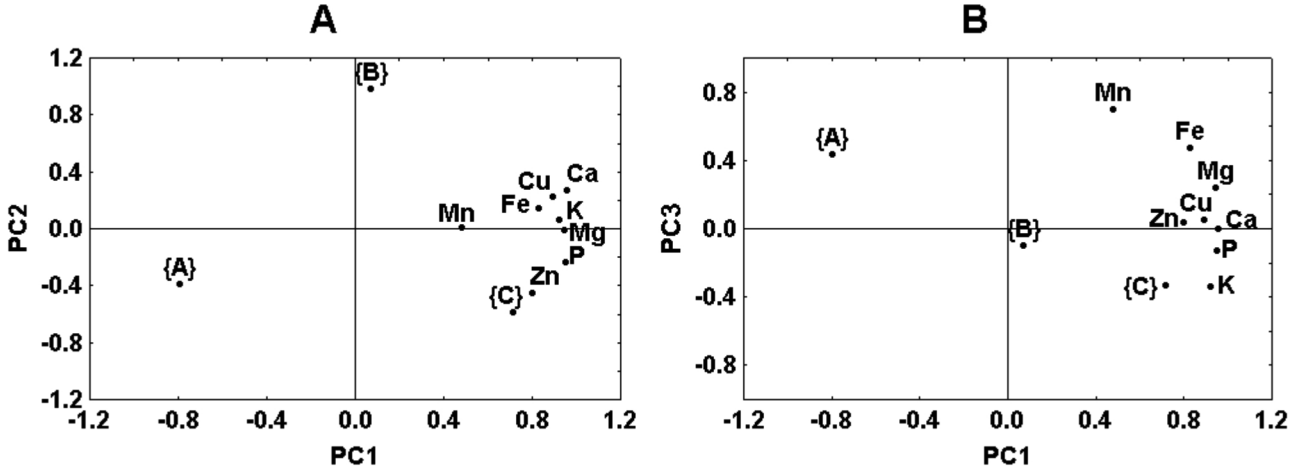

Figure 3. Principal component analysis of minerals quantification in the cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC1 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Minerals (Parameters/Loadings): Ca, Cu, Fe, Mg, Mn, K, P and Zn.

Table 6. Ca, Cu, Fe, K, Mg, Mn, P, and Zn contents in a 30 g food cookie as percentages of Dietary Reference Intake (DRI) by age and gender

Age group / years	Ca / %			Cu / %			Fe / %			K / %			Mg / %			Mn / %			P / %			Zn / %		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Children																								
1-3	10	11	11	209	236	243	4	4	4	4	6	6	230	105	113	97	100	103	16	17	19	53	54	68
4-8	7	8	8	162	183	188	4	4	4	3	4	5	141	65	69	78	80	82	15	16	18	32	33	41
Men																								
9-13	5	6	6	102	115	118	3	3	3	3	4	4	77	35	38	61	63	65	6	6	7	20	20	25
14-18	5	6	6	80	90	93	2	2	3	3	4	4	45	21	22	53	55	56	6	6	7	15	15	18
19-30	7	8	8	79	89	92	2	2	3	3	4	4	46	21	23	51	52	53	11	11	13	15	15	18
31-50	7	8	8	79	89	92	2	2	3	3	4	4	44	20	21	51	52	53	11	11	13	15	15	18
51-70	7	8	8	79	89	92	2	2	3	3	4	4	44	20	21	51	52	53	11	11	13	15	15	18
> 70	6	7	7	79	89	92	2	2	3	3	4	4	44	20	21	51	52	53	11	11	13	15	15	18
Women																								
9-13	5	6	6	102	115	118	3	3	3	3	4	4	77	35	38	73	75	77	6	6	7	20	20	25
14-18	5	6	6	80	90	93	2	2	3	3	4	4	51	23	25	73	75	77	6	6	7	18	18	23
19-30	7	8	8	79	89	92	2	2	3	3	4	4	59	27	29	65	67	68	11	11	13	20	20	25
31-50	7	8	8	79	89	92	2	2	3	3	4	4	57	26	28	65	67	68	11	11	13	20	20	25
51-70	6	7	7	79	89	92	2	2	3	3	4	4	57	26	28	65	67	68	11	11	13	20	20	25
> 70	6	7	7	79	89	92	2	2	3	3	4	4	57	26	28	65	67	68	11	11	13	20	20	25
Pregnant																								
14-18	5	6	6	71	80	83	2	2	2	3	4	4	46	21	23	58	60	62	6	6	7	13	14	17
19-30	7	8	8	71	80	83	2	2	2	3	4	4	53	24	26	58	60	62	11	11	13	15	15	18
31-50	7	8	8	71	80	83	2	2	2	3	4	4	51	23	25	58	60	62	11	11	13	15	15	18
Lactating																								
14-18	5	6	6	55	62	63	1	1	1	3	3	4	51	23	25	45	46	47	6	6	7	12	13	16
19-30	7	8	8	55	62	63	1	1	1	3	3	4	59	27	29	45	46	47	11	11	13	13	14	17
31-50	7	8	8	55	62	63	1	1	1	3	3	4	57	26	28	45	46	47	11	11	13	13	14	17

toxic because it is lower than the tolerable daily intake level.[25,26]

Because of the high contents of Cu, Mg, Mn, and Zn, i.e., over 15% of each mineral per portion,[38] the formulations can be considered good sources of these minerals. The consumption of foods rich in minerals may reduce the risk of coronary heart disease, anemia, osteoporosis, and prostate cancer by boosting the immune system.[37]

The cookie formulations presented low water activity, which contributed to the inhibition of microbial growth and the absence of *Bacillus cereus*, thermotolerant coliforms, coagulase-positive staphylococcus, and *Salmonella sp.*, indicating appropriate sanitary conditions according to Brazilian standards.[23]

The sensory analysis (Table 7) was performed by a team of volunteer panelists, who reported liking cookies and familiarity with the consumption of this product. The sensory attributes ranged from slightly liked to moderately liked for all the samples. PC1 in products A and C (Table 3, Figure 4) showed high contributions from appearance,

Table 7. Means and standard deviations of the acceptance test attributes of the cookie formulations

Formulation	Attributes				
	Appearance	Flavor	Texture	Crispness	Overall acceptance
A	6.38 ± 1.71	6.39 ± 1.99	6.58 ± 1.85	6.72 ± 1.72	6.32 ± 1.99
B	6.19 ± 1.63	6.17 ± 1.91	6.26 ± 1.84	6.39 ± 1.09	6.34 ± 1.79
C	6.29 ± 1.62	6.22 ± 1.97	6.55 ± 1.78	6.43 ± 1.77	6.38 ± 1.92

n = 80 panelists.

crispness, texture, and overall acceptance. PC2 (Figure 4) was not significantly different ($p < 0.05$) but highlighted the texture and overall acceptance attributes of the other formulations. The cookies were considered well accepted because the acceptance rate was above 70%, the cut-off proposed by Lawless and Heymann.[24] The formulations showed no difference ($p < 0.05$) in preference ordering as determined by the Friedman test.

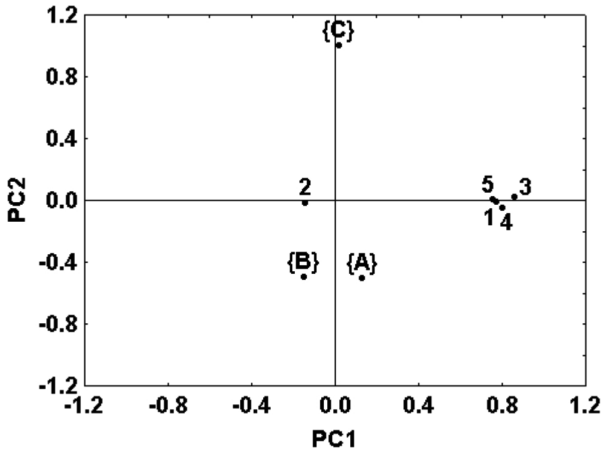

Figure 4. Principal component analysis of sensory attributes in the cookie formulations. PC: principal component; 1: Appearance; 2: Flavor; 3: Texture; 4: Crispness; 5: Overall acceptance.

The intent-to-purchase results, which ranged from "will probably buy" to "will surely buy", indicated that the consumption potentials of gluten-free cookies A, B, and C were 59%, 40%, and 58%, respectively.

Conclusion

The use of naturally gluten-free ingredients allows the development of cookie formulations suitable for celiac disease patients. In this study, promising grains such as quinoa and linseed contributed to an increase the protein, lipid, and mineral contents of the products. The percentage of SFA was below 4-5.5%. The n-6:n-3 ratio of the formulations was close to the values recommended in other studies. The Cu, Mg, Mn, P, and Zn contents were above 10% of the DRI. Formulation C presented the best alpha-linolenic acid content, nutritional indices in the lipid fraction and mineral content per portion, as well as excellent sensory characteristics. The formulations presented good hygienic/sanitary quality and good acceptance for the studied attributes. There was no preference for a specific formulation, and the purchase intent indices were considered high. Multivariate analysis allowed for the better characterization and distinction of the developed products and highlighted the effect of a higher concentration of quinoa on the nutritional and sensory qualities of the product.

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