Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v36i1.19195

# Development, characterization and chemometric analysis of gluten-free granolas containing whole flour of pseudo-cereals new cultivars

**Aloisio Henrique Pereira de Souza[1], Aline Kirie Gohara[1], Lilian Maria Pagamunici[1], Jesuí Vergílio Visentainer[1,2], Nilson Evelázio de Souza[2,3] and Makoto Matsushita[1,2*]**

*[1]Centro de Ciências Agrárias, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [2]Departamento de Química, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [3]Universidade Tecnológica Federal do Paraná, Londrina, Paraná, Brasil. *Author for correspondence. E-mail: mmakoto@uem.br*

**ABSTRACT.** The goal of this study was the development, quimiometric analysis, physical-chemical, microbiological, nutritional, and sensory evaluation of gluten-free granolas containing quinoa, amaranth and linseed. Gluten fractions were not detected in the granola formulations prepared. The crude protein and total lipids contents ranged from 86.72 to 97.49 and 97.84 to 134.03 g $kg^{-1}$ of food, respectively. The polyunsaturated/saturated and n-6: n-3 fatty acid ratios were 3:1. Calcium was the major mineral and the contents of trace minerals copper, iron, magnesium, manganese and zinc were over 10% of the dietary reference intake values. The granola color tended to light brown. The absence of *Bacillus cereus*, thermotolerant coliforms, coagulase positive staphylococcus, and *Salmonella* sp. ensured the product safety. All the granola formulations had good sensory acceptance and high purchase intent. The gluten-free granola formulations had good physical-chemical, sensory and nutritional quality. The use of chemometric analysis enabled to distinguish the samples with respect to their fatty acid composition, minerals content and sensory aspects.

**Keywords:** principal components analysis, amaranth, quinoa, linseed, gluten-free foods.

## Desenvolvimento, caracterização e análise quimiométrica de granolas isentas de glúten, contendo farinha integral de novas cultivares de pseudocereais

**RESUMO.** O presente estudo teve como objetivo o desenvolvimento, as análises quimiométrica, físico-química, microbiológica, nutricional e sensorial de granolas isentas de glúten, contendo quinoa, amaranto e linhaça. As frações de glúten não foram detectadas nas formulações de granolas. Os teores de proteína bruta e lipídios totais foram 86,72 a 97,49 e 97,84 a 134,03 g $kg^{-1}$ de alimento, respectivamente. As razões dos ácidos graxos poli-insaturados:saturados e n-6: n:3 foram de 3:1. O cálcio foi o mineral majoritário e os minerais cobre, ferro, magnésio, manganês e zinco estiveram acima de 10% para o valor de ingestão dietética de referência. A cor da granola tendeu para o marrom claro. Foram ausentes *Bacillus cereus*, coliformes termotolerantes, estafilococos coagulase positive e *Salmonella* sp., sendo atestada a sanidade dos produtos. Todas as granolas apresentaram boas características sensoriais e alta intenção de compra. As formulações de granolas isentas de glúten tiveram boas características físico-químicas, sensoriais e nutricionais. Pela análise quimiométrica foi possível distinguir as amostras com relação à sua composição em ácidos graxos, minerais e aspectos sensoriais.

**Palavras-chave:** análise de componentes principais, amaranto, quinoa, linhaça, alimentos sem glúten.

## Introduction

Gluten-rich foods such as oat, barley, rye, and wheat cause an inflammatory process in the small intestine villi, with subsequent atrophy and low absorption of nutrients in affected individuals (FASANO et al., 2008). The development of gluten-free foods requires ingredients with high nutritional value, such as amaranth, quinoa, and linseed. Amaranth and quinoa have similar compositions, mainly in terms of protein and ash contents (ARENDT; BELLO, 2008; GUTIÉRREZ et al., 2010). High levels of crude fiber and total lipids, 8.3 and 43.9%, respectively, have been found in linseed (GUTIÉRREZ et al., 2010).

Linseed is distinct from pseudo-cereals for its lipid fractions of 14.5-22.2% (oleic; 18:1 n-9), 15.1-17.4% (linoleic; 18:2 n-6), and 51.8-60.4%, (alpha-linolenic; 18:3 n-3) fatty acids, while quinoa and amaranth present 0.6-3.8, 23.6-26.5 and 35.3-48.1%, respectively (RYAN et al., 2007).

Cultivars *Chenopodium quinoa* BRS Piabiru and *Amaranthus cruentus* BRS Alegria were genetically modified for the central-western Brazil climate conditions and to remove saponins, because related are toxic compound in vivo but are efficient insecticides and anti-microbial agents for the plant, while still maintaining their chemical composition (SPEHAR; SANTOS, 2002; SPEHAR et al., 2003) in a study conducted by the Brazilian Agricultural Research Corporation - Cerrados facility, Brasília, Distrito Federal, Brazil (Embrapa).

The development of foods 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 sufferers. The goal of this paper was the development, quimiometric analysis, physico-chemical, sensory and nutritional assessment of gluten-free granolas containing *C. quinoa* BRS Piabiru and *A. cruentus* BRS Alegria as sources of protein and minerals, and *L. usitiassimum* L. as a source of alpha-linolenic acid.

## Material and methods

### Sampling and formulations

The grains of *A. cruentus* BRS Alegria and *C. quinoa* BRS Piabiru used in the development of the granola formulations were provided by Embrapa. The other ingredients were purchased from local shops in Maringá, Paraná State. Samples of amaranth, quinoa and linseed were taken from 60-kg grain bags. Linseed was ground coarse.

The granola formulations consisted of a binder phase (syrup) and a solid phase (grains, nuts and raisins). The phases were mixed and dried in a conventional oven at 200°C for 30 minutes. Table 1 shows the composition of formulations A, B and C. All formulations were prepared in three replicates.

**Table 1.** Granola formulations.

| Ingredients in g $kg^{-1}$ of product | Formulations | | |
|---|---|---|---|
| | A | B | C |
| Binder phase | | | |
| Brown sugar | 60.00 | 60.00 | 60.00 |
| Refined sugar | 60.00 | 60.00 | 60.00 |
| Honey | 150.00 | 150.00 | 150.00 |
| Invert sugar | 40.00 | 40.00 | 40.00 |
| Canola oil | 10.00 | 10.00 | 10.00 |
| Water | 30.00 | 30.00 | 30.00 |
| Solid phase | | | |
| Amaranth | 80.00 | 120.00 | 160.00 |
| Cashew nut | 30.00 | 30.00 | 30.00 |
| Brazil nut | 30.00 | 30.00 | 30.00 |
| Quinoa | 80.00 | 120.00 | 160.00 |
| Rice flakes | 120.00 | 100.00 | 80.00 |
| Cornflakes | 210.00 | 150.00 | 90.00 |
| Linseed flour | 50.00 | 50.00 | 50.00 |
| Sunflower seeds | 20.00 | 20.00 | 20.00 |
| Raisins | 30.00 | 30.00 | 30.00 |

### Gluten test

The gluten fractions in grains of amaranth, quinoa, linseed, rice and corn flakes, and in the final products were determined using a commercial ELISA kit (Enzyme-linked immunosorbent assay)-R5 Ridascreen® Gliadin (R-Biopharm, Germany. The gluten fractions were extracted with a 60% (v $v^{-1}$) ethanol solution and reagent *cocktail*®.

### Chemical and instrumental analysis

The moisture, ash and crude protein contents were determined according to Cunniff (1998) using factor 5.80 (ARENDT; BELLO, 2008) to convert the percentage of nitrogen into crude protein content. The total lipids were determined according to Bligh and Dyer (1959). The Nifext fractions (nitrogen free extract fraction) were calculated by difference.

The caloric value was determined through direct (instrumental) and indirect (calculation) calorimetry. For the instrumental method, the samples were milled and dried at 105ºC for 4h. 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 by Holands et al. (1994). The results were obtained in cal $g^{-1}$ of food and converted to Joule, using the factor 4.1868 J to 1 cal and expressed in kJ $kg^{-1}$ of product.

The water activity was analyzed using AquaLab 4TE (Decagon, USA) at 25ºC with an infrared detector. The color of the products was determined by Tristimulus L*a*b* colorimetry, ‘L’ (whiteness, 100 = white, 0 = black), ‘a’ (+, red; -, green) and 'b' (+, yellow; -, blue), in a CR-400 (Konica Minolta, Japan) colorimeter. The rate of color change was calculated with the equation (ΔE): $\Delta E = (a^2 + b^2 + L^2)^{1/2}$. The mechanical properties of granolas were obtained in a TA HD Plus (Stable, United Kingdom) universal texturometer with a 5.00 kg load cell, an acrylic cylinder with 35.00 mm diameter. A compression speed of 0.80 mm $s^{-1}$ was applied until 50% of the initial sample height was reached. This analysis was done in quintuplicate. Parameters associated with texture, force and deformation were obtained from the curve of maximum force peak *versus* height and expressed as hardness.

### 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 (1973). The FAME were separated in gas chromatograph CP-3380 (Varian, USA) fitted with a flame ionization detector and a CP 7420-select Fame fused-silica capillary column (100 m x 0.25 mm x 0.25 μm cyanopropyl). The gas flows were carrier gas hydrogen 1.4 mL $min.^{-1}$, make-up gas nitrogen 30 mL $min.^{-1}$, synthetic air 300 mL $min.^{-1}$ and flame gas hydrogen 30mL $min.^{-1}$; the sample was injected in 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 at 4°C $min.^{-1}$ to 185°C and maintained for 5 min., then raised from 185°C at 10°C $min.^{-1}$ to 225ºC and maintained for 10 min.

The retention times were compared to those of standard methyl esters (Sigma, USA). The fatty acids were identified using tricosanoic acid methyl ester (Sigma, USA) as an internal standard, following Joseph and Ackman (1992). The peak areas were determined with software Star 5.0 (Varian, USA) and the concentrations were expressed in mg $kg^{-1}$ of food.

In the mineral composition analysis, the samples were digested by the dry method (AOAC, 1995) and Ca, Cu, Fe, Mg, Mn and Zn were quantified in atomic absorption spectrophotometer AA240FS (Varian, USA) using standard solutions and analytical curves.

### Microbiological characterization

Food safety and product contamination after processing according to Brasil (2001) by *Bacillus cereus*, thermotolerant coliforms, coagulase positive staphylococcus and *Salmonella* sp. were determined as proposed by Vanderzant and Splittstoesser (1992) before sensory analysis.

### Sensory analysis

A group of 80 non-trained volunteer panelists and potential consumers of the products developed participated in the sensory analysis, which consisted in acceptance testing, preference ordering and intent of purchase of the formulations developed. In the acceptance test, appearance, flavor, texture, crispness and overall acceptance of the granolas were assessed using a 9-point hedonic scale (1 = extremely dislike to 9 = extremely like). The samples were presented in random complete blocks for comparison. The index of acceptability (IA) of the products was calculated as (global aspect grade x 100%) / 9. The ordering test assessed the preference for each formulation; the results were obtained by summing the order values of each sample. The purchase intent test was determined using a 5-point scale (1 = definitely would not buy and 5 = definitely would buy) (LAWLESS; HEYMANN, 2010).

### Ethical aspects

This study was approved by the Ethics Committee 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.

### Calculation of the dietary reference intake

The Dietary Reference Intake (DRI) is a percent estimate of the daily nutrient requirements per age and gender established by the Institute of Medicine (2001, 2011) for individuals aged over 12 months. The DRI of Ca, Cu, Fe, Mg, Mn and Zn were determined as the mean amounts in 30-g portions, as proposed by Brasil (2003) for cereal bars.

### Statistical analysis

Fatty acid composition, mineral, instrumental and physical-chemical analyses were carried out in triplicate. The results were submitted to variance analysis (ANOVA) and the means were compared using the Tukey's post hoc test. The Friedman test was used only for the Preference Ordering test, according to Lawless and Heymann (2010). Pearson's correlation coefficients were calculated according to Meullenet et al. (1998) for both instrumental (hardness) and sensory (texture and crispness) results; the means of the response variables were ln (natural logarithm) transformed. The multivariate analysis was performed by applying Principal Component Analysis (PCA). The average of the three individual batches was used with respect to sums and ratios of fatty acids, mineral composition and sensory attributes. The averages were autoscaled using NIPALS algorithm. The statistical software SAS, version 7.0, was used with a 5% ($p < 0.05$) significance level for rejection of the null hypothesis.

## Results and discussion

Gluten fractions were not detected by the ELISA test in either the grains or the gluten-free granola formulations developed.

The results of the physical-chemical and instrumental analyses are shown in Table 2. The crude protein content increased progressively and proportionally with the increase of grain concentrations in the granola formulations, corroborating with studies accomplished by Enriquez et al. (2003).

**Table 2.** Proximal composition, crude energy, water activity, hardness and color of granola formulations.

| Parameters | Formulations | | |
|---|---|---|---|
| | A | B | C |
| Moisture (g $kg^{-1}$) | $82.39^{a}\pm0.54$ | $71.91^{c}\pm2.08$ | $74.91^{b}\pm1.75$ |
| Ash (g $kg^{-1}$) | $17.40^{a}\pm0.38$ | $18.14^{a}\pm0.33$ | $15.89^{b}\pm0.52$ |
| Crude protein (g $kg^{-1}$) | $86.72^{b}\pm2.94$ | $92.62^{a}\pm1.62$ | $97.49^{a}\pm2.12$ |
| Total lipids (g $kg^{-1}$) | $97.84^{c}\pm1.46$ | $134.03^{a}\pm2.68$ | $122.72^{b}\pm3.02$ |
| Carbohydrates[1](g $kg^{-1}$) | $715.65^{a}\pm3.35$ | $683.30^{b}\pm3.77$ | $688.99^{b}\pm4.12$ |
| Crude energy[2] (kJ $kg^{-1}$) | $18305.50^{b}\pm0.00$ | $19244.20^{a}\pm0.00$ | $17722.30^{c}\pm0.00$ |
| Crude energy[3] (kJ $kg^{-1}$) | $17124.18^{c}\pm0.00$ | $18044.90^{a}\pm0.00$ | $17795.57^{b}\pm0.00$ |
| $A_a$[4] | $0.43^{c}\pm0.00$ | $0.44^{b}\pm0.00$ | $0.47^{a}\pm0.00$ |
| Hardness | $24.06^{a}\pm2.04$ | $24.91^{a}\pm3.77$ | $19.64^{b}\pm0.45$ |
| L | $33.37^{b}\pm1.04$ | $40.20^{a}\pm0.87$ | $39.55^{a}\pm0.49$ |
| A | $4.80^{b}\pm0.27$ | $5.01^{ab}\pm0.11$ | $5.21^{a}\pm0.04$ |
| B | $13.61^{b}\pm0.55$ | $15.92^{a}\pm0.13$ | $16.00^{a}\pm0.12$ |
| ΔE[5] | $36.36^{b}\pm1.21$ | $43.53^{a}\pm0.95$ | $42.98^{a}\pm0.51$ |

Means followed by the same letters in rows do not differ by Tukey's test ($p < 0.05$). [1]Carbohydrates determined by difference. [2]Direct (instrumental) and [3]indirect (calculated) methods. [4]Water activity. [5]Rate of color variation.

Generally, gluten-free products present a high carbohydrate concentration and a low protein content. Segura and Rosell (2011) reported on products with up to 92% carbohydrates.

According to Gutiérrez et al. (2010), linseed has a mineral content of 2.66%, while pseudocereals have approximately 3% (ARENDT; BELLO, 2008), which contributed to a high mineral content of the granolas developed.

There was difference ($p > 0.05$) in total lipids between the samples. The granola formulations presented proximal composition and percent energy similar to those of commercial granolas (GRANADA et al., 2003). The direct and indirect methods provided similar crude energy values.

The increase in the grain concentrations in the granolas did not affect formulation color. The color variation (ΔE) presented no difference ($p < 0.05$) to samples B and C. The color tended to light brown.

Fatty acids contents are presented in Table 3. According to the Institute of Medicine (2002/2005), saturated fatty acids must be avoided in a balanced diet. According to Simopoulos (2011), 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 cases, hypercholesterolemia, increased low density lipoprotein (LDL) cholesterol and blood pressure, atheroma, lipid disorders and other disorders. The n-6: n-3 ratio of the granolas ranged from 2.6: 1 to 3: 1, which is near the ideal value 1:1. Industrialized products like cookies (STROHER et al., 2012) commonly present *trans* fatty acids, which was not found in this study.

As shown in Table 4, the major mineral component was Ca. Its presence in the diet contributes to increase the bioavailability and absorption of Mg, Mn and Zn. These minerals are essential for the maintenance of biological systems because they participate as cofactors in metabolic reactions (HATHCOCK, 2004).

**Table 3.** Fatty acid absolute quantification of granola formulations.

| Fatty Acid | Formulations | | |
|---|---|---|---|
| (mg $kg^{-1}$) | A | B | C |
| 14:0 | $94.67^{c}\pm8.36$ | $116.05^{b}\pm5.48$ | $195.64^{a}\pm2.02$ |
| 16:0 | $9209.86^{a}\pm553.00$ | $12963.44^{a}\pm422.33$ | $11156.73^{a}\pm131.03$ |
| 16:1n-7 | $187.45^{c}\pm41.80$ | $234.83^{a}\pm4.79$ | $208.61^{b}\pm12.76$ |
| 18:0 | $5190.44^{b}\pm260.81$ | $6836.64^{a}\pm504.01$ | $6288.92^{a}\pm171.77$ |
| 18:1n-9 | $33498.58^{b}\pm1047.95$ | $4542.90^{a}\pm698.24$ | $42617.95^{a}\pm420.38$ |
| 18:2n-6 | $31470.26^{b}\pm1709.39$ | $43120.86^{a}\pm933.27$ | $37568.17^{a}\pm693.46$ |
| 18:3n-3 | $11981.10^{b}\pm38.84$ | $14616.21^{a}\pm1074.73$ | $14519.26^{a}\pm459.25$ |
| 20:0 | $133.18^{b}\pm12.58$ | $195.75^{a}\pm10.95$ | $223.08^{a}\pm11.01$ |
| 22:0 | $297.06^{b}\pm16.64$ | $391.75^{a}\pm9.30$ | $359.65^{b}\pm15.41$ |
| 22:1n-9 | $219.32^{c}\pm13.02$ | $334.66^{a}\pm26.17$ | $278.56^{b}\pm1.17$ |
| 24:0 | $212.65^{c}\pm18.69$ | $313.42^{a}\pm28.09$ | $240.44^{b}\pm13.21$ |
| Sums and ratios of fatty acids | | | |
| SFA | $15137.86^{a}\pm612.22$ | $20817.05^{a}\pm657.66$ | $18464.46^{a}\pm451.44$ |
| MUFA | $33905.35^{b}\pm1048.08$ | $5112.39^{a}\pm698.12$ | $43105.12^{a}\pm420.01$ |
| PUFA | $43451.36^{b}\pm1709.44$ | $57737.07^{a}\pm1095.95$ | $52087.43^{a}\pm831.22$ |
| n-6 | $31470.26^{b}\pm1709.39$ | $43120.86^{a}\pm933.27$ | $37568.17^{a}\pm693.46$ |
| n-3 | $11981.10^{b}\pm38.84$ | $14616.21^{a}\pm1074.73$ | $14519.26^{a}\pm459.25$ |
| PUFA/SFA | $2.87^{a}\pm0.08$ | $2.77^{b}\pm0.05$ | $2.82^{a}\pm0.04$ |
| n-6:n-3 | $2.63^{b}\pm0.05$ | $2.95^{a}\pm0.06$ | $2.59^{c}\pm0.04$ |

Means followed by the same letters in rows do not differ by Tukey's test ($p < 0.05$). SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids, PUFA: total polyunsaturated fatty acids, n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids.

**Table 4.** Mineral composition of granola formulations.

| Mineral (mg $kg^{-1}$ of sample) | Formulations | | |
|---|---|---|---|
| | A | B | C |
| Ca | $1918.06^{b}\pm4.49$ | $1987.97^{b}\pm191.13$ | $2144.38^{a}\pm83.75$ |
| Cu | $22.26^{b}\pm1.93$ | $33.59^{a}\pm2.90$ | $26.02^{b}\pm3.03$ |
| Fe | $77.18^{b}\pm0.21$ | $87.42^{a}\pm5.87$ | $86.66^{a}\pm0.42$ |
| Mg | $2148.67^{b}\pm150.07$ | $2156.93^{b}\pm35.77$ | $2554.04^{a}\pm31.90$ |
| Mn | $16.88^{c}\pm1.43$ | $23.97^{b}\pm1.10$ | $34.73^{a}\pm0.13$ |
| Zn | $54.26^{b}\pm2.91$ | $42.43^{c}\pm2.25$ | $78.76^{a}\pm2.83$ |

Means followed by the same letters in rows do not differ by Tukey's test ($p < 0.05$).

The granola formulations presented low water activity, which contributed to inhibit microbial growth and the absence of *B. cereus*, thermotolerant coliforms, coagulase positive staphylococcus and *Salmonella* sp., characterizing appropriate sanitary conditions by Brasil (2001).

Sensory analysis was performed by a team of volunteer panelists who reported liking granolas and to be used to consuming this product (Table 5). The granolas were considered well accepted when the acceptance rate was above 70%, as proposed by Lawless and Heymann (2010). All formulations had no difference ($p < 0.05$) in the preference ordering by the Friedman Test.

The Pearson's coefficient between sensory texture and instrumental hardness showed little positive correlation for samples A and C, respectively, $r = 0.12$ and $r = 0.03$ ($p < 0.05$). On the other hand, granola B had $r = -0.10$

($p < 0.05$), corresponding to little negative correlation. Pearson's coefficients for crispness and instrumental hardness showed little positive correlation for formulation A ($r = 0.21$, $p < 0.05$) and little negative correlation for B ($r = -0.06$, $p < 0.05$) and C ($r = -0.04$, $p < 0.05$). Although Pearson's coefficient indicated little correlation between instrumental and sensory attributes, the granola formulations had great sensory acceptance by potential consumers, which shows that both analyses are required to characterize the products.

As to the purchase intent results, ranging from probably buy to surely buy, the consumption potentials of gluten-free granolas A, B and C were 70, 64 and 68%, respectively.

Table 6 presents the nutritional contribution (INSTITUTE OF MEDICINE, 2001, 2011) of granola formulations for different age groups, based on the value per portion set forth by Brasil (2003). Cu contributed almost three times the DRI, an amount which is still not toxic as it is lower than the tolerable daily intake level (INSTITUTE OF MEDICINE, 2001, 2011).

Figure 1 shows principal component analysis (PCA). The NIPALS algorithm enabled to select PC1 and PC2, which were significant ($p > 0.05$) and explained 61.40% of data variance. PC1 allowed to distinguish formulation C, due to the positive contribution of the minerals composition, sensory attributes, sums of n-3 and MUFA, and the ratio: SFA: PUFA. By analyzing PC1 versus PC2 (Figure 1), formulation B obtained positive contribution in sums of n-6 and SFA, and the ratio n-6: n-3 which was different from the others. Although there was no significant difference ($p < 0.05$) for some of the parameters analyzed, principal component analysis allows to distinguish samples apparently equal using loadings (parameters studied) and scores (samples).

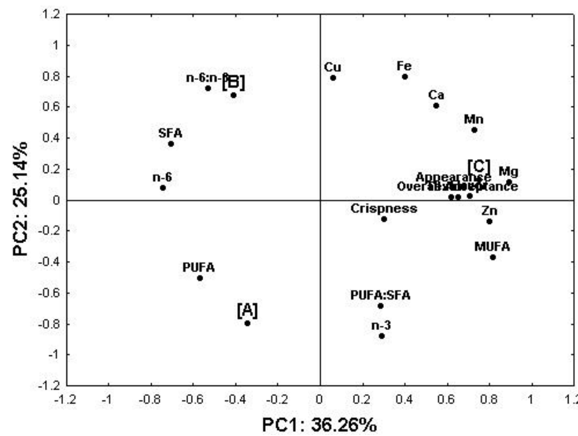


**Figure 1.** Principal components analysis of fatty acid, mineral composition and sensory analysis in granola formulations. Granola formulations [A], [B] e [C].

**Table 5.** Means and standard deviation of acceptance test attributes of granola formulations.

| Formulations | Attributes | | | | | |
|---|---|---|---|---|---|---|
| | Appearance | Flavor | Texture | Crispness | Overall Acceptance | I.A.[1] |
| A | 7.03[a]±1.81 | 7.39[a]±1.63 | 7.34[a]±1.66 | 7.28[a]±1.78 | 7.40[a]±1.71 | 82.22% |
| B | 6.98[a]±1.61 | 7.01[a]±1.76 | 6.95[a]±1.83 | 7.26[a]±1.73 | 7.03[a]±1.67 | 78.06% |
| C | 7.19[a]±1.46 | 7.74[a]±1.52 | 7.34[a]±1.64 | 7.36[a]±1.55 | 7.51[a]±1.69 | 83.47% |

Means followed by the same letters in columns do not differ by Tukey's test ($p < 0.05$). [1]I.A. = index of product acceptability; n = 80 panelists.

**Table 6.** Ca, Cu, Fe, Mg, Mn and Zn contents in 30-g granola portions as percentages of Dietary Reference Intake (DRI) per age and gender.

| Age group | Ca | | | Cu | | | Fe | | | Mg | | | Mn | | | Zn | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (years) | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C |
| Children | | | | | | | | | | | | | | | | | | |
| 1-3 | 8.22 | 8.52 | 9.19 | 196.76 | 296.47 | 229.41 | 33.09 | 37.46 | 37.16 | 80.58 | 80.89 | 95.62 | 42.25 | 60.00 | 86.75 | 54.30 | 42.40 | 78.80 |
| 4-8 | 5.75 | 5.96 | 6.43 | 152.05 | 229.09 | 177.27 | 23.16 | 26.22 | 26.01 | 49.58 | 49.58 | 58.94 | 33.80 | 48.00 | 69.40 | 32.58 | 25.44 | 47.28 |
| Men | | | | | | | | | | | | | | | | | | |
| 9-13 | 4.43 | 4.59 | 4.95 | 95.57 | 144.00 | 111.43 | 28.95 | 32.78 | 32.51 | 26.86 | 26.96 | 31.93 | 26.68 | 37.89 | 54.79 | 20.36 | 15.90 | 29.55 |
| 14-18 | 4.43 | 4.59 | 4.95 | 75.17 | 113.26 | 87.40 | 21.05 | 23.84 | 23.65 | 15.72 | 15.78 | 18.69 | 23.05 | 32.73 | 47.32 | 14.81 | 11.56 | 21.49 |
| 19-50 | 5.75 | 5.96 | 6.43 | 74.33 | 112.00 | 86.67 | 28.95 | 32.78 | 32.51 | 15.35 | 15.41 | 18.24 | 22.04 | 31.30 | 45.26 | 14.81 | 11.56 | 21.49 |
| 51-70 | 5.75 | 5.96 | 6.43 | 74.33 | 112.00 | 68.67 | 28.95 | 32.78 | 32.51 | 15.35 | 15.41 | 18.24 | 22.04 | 31.30 | 45.26 | 14.81 | 11.56 | 21.49 |
| >70 | 4.80 | 4.97 | 5.36 | 74.33 | 112.00 | 68.67 | 28.95 | 32.78 | 32.51 | 15.35 | 15.41 | 18.24 | 22.04 | 31.30 | 45.26 | 14.81 | 11.56 | 21.49 |
| Women | | | | | | | | | | | | | | | | | | |
| 9-13 | 4.43 | 4.59 | 4.95 | 95.57 | 144.00 | 88.29 | 28.95 | 32.78 | 32.51 | 26.86 | 26.96 | 31.93 | 31.69 | 45.00 | 65.06 | 20.36 | 15.90 | 29.55 |
| 14-18 | 4.43 | 4.59 | 4.95 | 75.17 | 113.26 | 69.44 | 15.44 | 17.48 | 17.34 | 17.91 | 17.98 | 21.28 | 31.69 | 45.00 | 65.06 | 18.10 | 14.13 | 26.27 |
| 19-50 | 5.75 | 5.96 | 6.43 | 74.33 | 112.00 | 68.67 | 12.87 | 14.57 | 14.45 | 20.14 | 20.22 | 23.94 | 28.17 | 40.00 | 57.83 | 20.36 | 15.90 | 29.55 |
| >50 | 4.80 | 4.97 | 5.36 | 74.33 | 112.00 | 68.67 | 28.95 | 32.78 | 32.51 | 20.14 | 20.22 | 23.94 | 28.17 | 40.00 | 57.83 | 20.36 | 15.90 | 29.55 |
| Pregnant | | | | | | | | | | | | | | | | | | |
| 14-18 | 4.43 | 4.59 | 4.95 | 66.90 | 100.80 | 78.00 | 8.58 | 9.71 | 9.63 | 16.42 | 16.18 | 19.16 | 25.35 | 36.00 | 37.05 | 13.58 | 10.60 | 19.70 |
| 19-50 | 5.75 | 5.96 | 6.43 | 66.90 | 100.80 | 78.00 | 8.58 | 9.71 | 9.63 | 17.91 | 19.98 | 21.28 | 25.35 | 36.00 | 37.05 | 14.81 | 11.56 | 21.49 |
| Lactating | | | | | | | | | | | | | | | | | | |
| 14-18 | 4.43 | 4.59 | 4.95 | 51.46 | 77.54 | 60.00 | 23.16 | 26.22 | 26.01 | 17.91 | 17.98 | 21.28 | 19.50 | 27.69 | 28.50 | 12.53 | 9.78 | 18.18 |
| 19-50 | 5.75 | 5.96 | 6.43 | 51.46 | 77.54 | 60.00 | 25.73 | 29.13 | 28.90 | 20.79 | 20.22 | 23.94 | 19.50 | 27.69 | 28.50 | 13.58 | 10.60 | 19.70 |

## Conclusion

The use of naturally gluten-free ingredients allowed the development of granola formulations for celiac disease patients. Promising grains like quinoa, amaranth and linseed contributed to increase the protein, lipid and mineral contents in the products. Formulation C presented the best alpha-linolenic acid and mineral contents per portion, but all the formulations are sources of Cu, Fe, Mn and Zn. The formulations presented good hygienic-sanitary conditions and had a good acceptance for the attributes studied. The use of chemometric analysis enabled to distinguish the samples with respect to their fatty acid composition, minerals content and sensory aspects.

## Acknowledgements

The authors would like to thank Capes, CNPq, Araucaria Foundation for the financial support and the institutions: Federal Technologic University of Parana – Medianeira Facility, Faculty Inga, Embrapa, Complex of Research Support Centers (Comcap/State University of Maringa) for the availability of resources and technology for the development of this research.

## References

AOAC-Association of Official Analytical Chemists. **Official Methods of Analysis of the Association of Official Anaytical Chemists**. Arlington: AOAC, 1995. (Chapter 50, p. 14, method 985.35).

ARENDT, E. K.; BELLO, F. D. **Gluten-free cereal products and beverages**. San Diego: Elsevier, 2008.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry Physiology**, v. 8, n. 37, p. 911-917, 1959.

BRASIL. **Resolução - RDC no. 12**. Regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial da República Federativa do Brasil, Brasília, 2001.

BRASIL. **Resolução - RDC no. 359**. Regulamento técnico de porções de alimentos embalados para fins de rotulagem nutricional. Diário Oficial da República Federativa do Brasil, Brasília, 2003.

CUNNIFF, P. A. **Official methods of analysis of AOAC International**. 16th ed. Arlington: AOAC International, 1998. (CD-ROM)

ENRIQUEZ, N.; PELTZER, M.; RAIMUNDI, A.; TOSE, V.; POLLIO, M. L. Characterization of wheat and quinoa flour blends in relation to their breadmaking quality. **Journal of the Argentine Chemical Society**, v. 91, n. 4/6, p. 47-54, 2003.

FASANO, A.; ARAYA, M.; BHATNAGAR, S.; CAMERON, D.; CATASSI, C.; DIRKS, M.; MEARIN, M. L.; ORTIGOSA, L.; PHILIPS, A. Federation of international societies of pediatric gastroenterology, hepatology, and nutrition consensus report on celiac disease. **Journal of Pediatric Gastroenterology and Nutrition**, v. 47, n. 2, p. 214-219, 2008.

GRANADA, G.; ROSA, V.; ZAMBIAZI, R.; KOETZ, P. Characterization of commercial granolas. **Ciência e Tecnologia de Alimentos**, v. 23, n. 1, p. 87-91, 2003.

GUTIÉRREZ, C.; RUBILAR, M.; JARA, C.; VERDUGO, M.; SINEIRO, J.; SHENE, C. Flaxseed and flaxseed cake as a source of compounds for food industry. **Journal of Soil Science and Plant Nutrition**, v. 10, n. 4, p. 454-463, 2010.

HARTMAN, L.; LAGO, R. C. A. Rapid preparation of fatty acid methyl esters from lipids. **Laboratory Practice**, v. 22, n. 6, p. 475-477, 1973.

HATHCOCK, J. N. **Vitamin and mineral safety**. 2nd ed. Washington, DC.: Council for Responsible Nutrition, 2004.

HOLANDS, B.; WELCH, A. A.; UNWIN, I. D.; BUSS, D. H.; PAUL, A. A.; SOUTHGATE, D. A. T. **MacCance and winddowson's**: The composition of foods. 5th ed. Cambridge: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, 1994.

INSTITUTE OF MEDICINE. **Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, manganese, molybdenum, nickel, silicon, vanadium, and zinc**. Washington, D.C.: National Academy Press, 2001.

INSTITUTE OF MEDICINE. **Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids**. Washington, D.C.: National Academy Press, 2002/2005.

INSTITUTE OF MEDICINE. **Dietary Reference Intakes for calcium and vitamin D**. Washington, D.C.: National Academy Press, 2011.

JOSEPH, J. D.; ACKMAN, R. Capillary column gas chromatographic method for analysis of encapsulated fish oils and fish oil ethyl esters: collaborative study. **Journal of American Oil Chemists' Society**, v. 75, n. 3, p. 488-506, 1992.

LAWLESS, H. T.; HEYMANN, H. **Sensory Evaluation of Food:** Principles and practices. 2nd ed. Berlin: Springer, 2010.

MEULLENET, J.; LYON, B. G.; CARPENTER, J. A.; LYON, C. E. Relationship between sensory and instrumental texture profile attributes. **Journal of Sensory Studies**, v. 13, n. 1, p. 77-93, 1998.

RYAN, E.; GALVIN, K.; O'CONNOR, T. P.; MAGUIRE, A. R. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. **Plant Foods for Human Nutrition**, v. 62, n. 3, p. 85-91, 2007.

SEGURA, M. E. M.; ROSELL, C. M. Chemical composition and starch digestibility of different gluten-free breads. **Plant Foods for Human Nutrition**, v. 66, p. 224-230, 2011.

SIMOPOULOS, A. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. **Molecular and Neurobiology**, v. 44, n. 2, p. 203-215, 2011.

SPEHAR, C. R.; SANTOS, R. L. B. Quinoa BRS Piabiru: alternative for diversification of cropping systems. **Brazilian Journal of Agricultural Research**, v. 37, n. 6, p. 889-893, 2002.

SPEHAR, C. R.; TEIXEIRA, D. L.; CABEZAS, W. A. R. L.; ERASMO, E. A. L. Amaranth BRS Alegria: alternative for diversification of croppings systems. **Brazilian Journal of Agricultural Research**, v. 38, n. 5, p. 659-663, 2003.

STROHER, G. L.; RODRIGUES, A. C.; GOHARA, A. K.; VISENTAINER, J. V.; MATSUSHITA, M.; SOUZA, N. E. Fatty acid quantification in different types of cookies with emphasis on *trans* fatty acids. **Acta Scientiarum. Technology**, v. 34, n. 1, p. 105-110, 2012.

VANDERZANT, C.; SPLITTSTOESSER, D. F. Compendium of Methods for the Microbiological Examination of Foods. **American Public Health Association** – APHA. 3rd ed. Washington, D.C.: Edwards Brothers Ann Arbor, 1992.

*Received on November 19, 2012.*

*Accepted on January 22, 2013.*