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

DESENVOLVIMENTO DE BARRAS ALIMENTÍCIAS E BISCOITOS ISENTOS DE GLÚTEN, CONTENDO *CHENOPODIUM QUINOA*, BRS PIABIRU, *AMARANTHUS CRUENTUS*, BRS ALEGRIA E *LINUM USITIASIMUM*, L.

LILIAN MARIA PAGAMUNICI

Maringá
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Tese apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do grau de Doutor em Ciência de Alimentos.

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BIOGRAFIA

Lilian Maria Pagamunici de Oliveira nasceu em 15 de maio de 1982, na cidade de Paranavaí - Paraná. Em 2007 concluiu o Curso Superior em Tecnologia em Alimentos, pela Universidade Tecnológica Federal do Paraná - Campus Campo Mourão. Em 2009 concluiu Mestrado em Ciências de Alimentos pela Universidade Estadual de Londrina. Tem experiência nas áreas de Ciência e Tecnologia de Alimentos, atuando principalmente nos seguintes temas: desenvolvimento de novos produtos, alimentos funcionais, análise proximal e sensorial de alimentos. Atua na cidade de Maringá, como docente na Faculdade Ingá (UNINGÁ) e como técnico de laboratório na Companhia de Saneamento do Paraná – SANEPAR.

DEDICO

Ao meu amado Rodrigo Alves de Oliveira,
Aos meus queridos Pais, Aníbal e Luciana,
As minhas irmãs Carol e Ana,
A todos que acreditaram neste sonho.

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À Deus, por estar presente em mim e em todo o universo.

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos, descritos a seguir:

1. **Autores:** Lilian M. Pagamunici, Aline K. Gohara, Aloisio H. P. Souza, Paulo R. S. Bittencourt, Alex S. Torquato, Weliton Batiston, Sandra T. *M. Gomes, Nilson E Souza, Jesuí V. Visentainerb Makoto Matsushita.* ***Título:*** *"Using Chemometric Techniques to Characterize Gluten-Free Cookies Containing the Whole Flour of a New Quinoa Cultivar".* **Situação:** Artigo publicado em dezembro de 2013, na Revista *Journal of the Brazilian Chemical Society,* com Qualificação B1 no Qualis – Capes, da área de Ciências de Alimentos (Anexos: 1 - carta de aceite; 2 - normas de publicação revista).

2. Autores: Lilian M Pagamunici, Aloisio H P Souza, Aline K Gohara, Nilson E Souza, Sandra T M Gomes, Makoto Matsushita. **Título**: *"Development, characterization and chemometric analysis of a gluten-free food bar containing whole flour from a new cultivar of Amaranth".* **Situação:** Artigo submetido em setembro de 2013 à Revista Ciência e Agrotecnologia, com Qualificação B2 no Qualis - CAPES, da área de Ciências de Alimentos. Atualmente encontra-se sob o status "under review" (Anexos: 3 - carta de submissão; 4 – normas de publicação revista).

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RESUMO GERAL

INTRODUÇÃO: A doença celíaca (CD), definida como a intolerância à proteína do glúten, surge a partir da resistência desta proteína às enzimas digestivas, o que desencadeia uma resposta inflamatória, em indivíduos geneticamente predispostos. O principal tratamento baseia-se na restrição do consumo de alimentos contendo esta fração protéica. Alimento que contém glúten, como aveia, cevada, centeio e trigo, causa inflamação nas vilosidades intestinais, com subseqüente atrofia e baixa absorção de nutrientes em indivíduos afetados. A CD é uma das doenças genéticas mais freqüentes da humanidade, afetando 0,5% a 1,0% da população mundial. No Brasil, estudos realizados em bancos de sangue, indicam a prevalência da DC em 1:276 a 1:681 nos doadores. A maioria dos produtos industrializados apresenta glúten em sua composição, dificultando a dieta dos portadores desta doença, a qual se torna monótona e restrita. Ainda, estes produtos, geralmente apresentam alta quantidade de carboidratos simples e de gorduras saturadas. O desenvolvimento de alimentos para celíacos e com bom aporte nutricional vem de encontro às necessidades desta classe de consumidores. A farinha de arroz é uma alternativa para substituição da farinha de trigo no processamento de produtos de panificação como o biscoito sem glúten, pois não contém esta fração protéica, porém em seu perfil de aminoácidos verifica-se deficiência em lisina. Na maioria das barras de cereais disponíveis no mercado, o ingrediente principal é a aveia e o teor de gorduras saturadas nestas barras é elevado. Uma alternativa para a produção de biscoitos e barras alimentícias para celíacos, com bom aporte nutricional, é a utilização de ingredientes sem glúten e com nutrientes essenciais. Neste contexto, destaca-se, a quinoa, o amaranto e a linhaça. A quinoa e o Amaranto são pseudocereais (sementes que apresentam similaridade em composição proximal com os cereais) originados das regiões Andinas, principalmente do Chile. Destacam-se pela presença de proteínas de alta qualidade e composição mineral. O amaranto apresenta em sua composição proximal, aproximadamente, 12-13% de proteínas; 67-69% de carboidratos; 10-13% de fibras; 5-6% de lipídios e 2-3% de minerais. A Quinoa apresenta 14-15% de proteínas; 55-64% de carboidratos; 9-11% de fibras; 6-10% de lipídios e 3-4% de minerais. Encontram-se também, na constituição destes dois pseudocereais, as saponinas. Estas atuam como inseticida e agente antimicrobiano natural para a planta, mas quando ingerido em altas concentrações no organismo humano apresenta efeito tóxico. Com intuito de adaptar as culturas de quinoa e amaranto ao clima brasileiro para o cultivo das espécies no País, a Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), desenvolveu culturas de quinoa e amaranto, geneticamente modificadas, resultando nas espécies *Chenopodium quinoa* BRS Piabiru, *Amaranthus cruentus* BRS Alegria sem saponinas. A linhaça (*Linum usitiasimum*, L.) destaca-se, pelo alto teor de Omega-3, como uma alternativa para melhorar o perfil lipídico de produtos sem glúten, pois apresenta 14-22% de ácido oléico; 15-17% de ácido linoléico e 52-60% de ácido alfa-linolênico em relação ao seu conteúdo total de lipídios (44%). Sendo assim, o desenvolvimento de produtos prontos para o consumo, como biscoito e barra alimentícia sem glúten, com bom aporte nutricional, vem de encontro às necessidades do público consumidor celíaco. Além da composição nutricional, deve-se levar em consideração, no desenvolvimento de barras alimentícias e biscoitos, a aceitação sensorial dos mesmos. A influência dos ingredientes utilizados, sobre as características sensoriais, nutricionais e tecnológicas, pode ser detectada com a utilização de ferramentas quimiométricas, pois as mesmas permitem o reconhecimento de padrões, coleta de informações, e uma redução da dimensionalidade dos dados, bem como a organização dos mesmos em uma estrutura mais simples, de fácil entendimento. A análise de componentes

principais é baseada na realização e comparações lineares das variáveis originais. Os componentes principais (PC) são mutuamente ortogonais e explica a diminuição da variância com o aumento no número de PC.

OBJETIVO: O propósito deste trabalho foi desenvolver barra alimentícia e biscoito isentos de glúten, utilizando *Chenopodium quinoa* BRS Piabiru, *Amaranthus. cruentus* BRS Alegria, como fonte de proteínas e minerais, e *L. usitiassimum,* L. como fonte de ácido alfa-linolênico, caracterizando-os quanto às características nutricionais, tecnológicas e sensoriais.

MATERIAL E MÉTODOS: Os pseudocereais (quinoa e amaranto) foram doados pela EMBRAPA e os demais ingredientes foram adquiridos no comércio local de Maringá – PR. Para o desenvolvimento da barra alimentícia, variaram-se os teores de amaranto e flocos de arroz. A quantidade dos demais ingredientes não variou entre as formulações. As barras foram formadas pela junção de duas fases: a sólida (pseudocereal, oleaginosas, frutas) e a ligante (xarope). Foram desenvolvidas três formulações de fase sólida (A, B, C) contendo 1% de flocos de milho, 2% de banana *in natura* e 4% de linhaça cada uma. Além destes ingredientes, as formulações A, B e C, continham respectivamente, 30%, 36% e 42% de amaranto; 13%, 7% e 1% de flocos de arroz. A fase ligante das Barras A, B e C continha a mesma composição: 10% de açúcar mascavo, 1% de óleo de canola, 20% de xarope de glicose, 10% de mel, 3% de açúcar invertido e 6% de água. Na produção das barras, os ingredientes da fase sólida foram homogeneizados e adicionados ao xarope ligante previamente aquecido até atingir concentração de 85 a 89° Brix. As duas fases foram misturadas até completa interação. As três massas formadas (A, B e C) foram prensadas, laminadas e cortadas em barras de 25g, nas dimensões de 9 x 3 x 1,5 cm. Cada unidade teve um dos lados banhado em chocolate. Na produção do biscoito, três formulações foram desenvolvidas usando farinha de quinoa como substituinte parcial da farinha de arroz em diferentes níveis. Os três biscoitos (I, II e III) continham 3% de açúcar mascavo, 3% de açúcar refinado, 12% de mel, 5% de manteiga, 5% de gema de ovo, 5% de farinha de linhaça, 1% de bicarbonato de sódio, 7% de água, 9% de gotas de chocolate, 2% de cacau em pó, 8% de castanha do Brasil, 1% de aroma de nozes. Além destes ingredientes, as formulações I, II e III, continham respectivamente, 6%, 10% e 14% de farinha de quinoa; 33%, 29% e 25% de farinha de arroz. No processamento dos biscoitos, os ingredientes secos (exceto as farinhas) foram misturados para formar uma porção uniforme de cada formulação. A cada porção adicionou-se a manteiga e homogeneizou por 4 minutos. Adicionaram-se então as farinhas de quinoa e arroz e homogeneizou por mais 1 minuto. As massas formadas (I, II e III) foram laminadas a espessura de 7 mm e cortadas em forma circular com diâmetro de 6 cm. Os biscoitos foram assados a 180°C por 20 minutos. As barras e biscoitos produzidos foram avaliados quanto: presença de glúten (ensaio imuenzimático – ELISA); composição proximal (umidade e cinzas – método gravimétrico, proteínas – método Kjeldahl, lipídios extração a frio – método Bligh-dyer, carboidratos por diferença); valor calórico (método direto - bomba calorimétrica, método indireto – cálculo); atividade de água (aqualab), cor (metodologia tritimulus L*, a*, b*); composição em ácidos graxos (cromatografia em fase gasosa); composição mineral (espectrofotometria por absorção atômica); índice nutricional da composição lipídica (Aterogenicidade, trombogenicidade, razão entre ácidos graxos hipocolesterolêmicos por hipercolesterolêmicos), condições higiênico-sanitárias (análises microbiológicas); avaliação sensorial (aceitabilidade, preferência e intenção de compra); porcentagem do valor diário recomendado de minerais em diferentes faixas etárias e sexos; influência dos componentes principais nas características determinantes dos produtos.

RESULTADOS E DISCUSSÃO: Frações de glúten não foram detectadas nas barras alimentícias e nos biscoitos. Na composição proximal dos produtos destacaram-se, principalmente, os teores de proteínas e cinzas. O uso de fontes promissoras de proteína é requerido, pois em geral, as farinhas utilizadas no processamento de produtos para celíacos apresentam elevada concentração de carboidratos e baixo teor de proteínas. Na composição de ácidos graxos destacam-se os linoléico (n-6) e o alfa-linolênico (n-3), por serem considerados estritamente essenciais. As razões entre n-6:n-3 variaram nas formulações das barras e biscoitos entre 1,44:1 a 2,50:1 e 3,08:1 a 4,38:1, respectivamente, e as razões entre ácidos graxos poli-insaturados e saturados estiveram entre 0,45 a 0,55 e 0,85 a 0,92, respectivamente. As barras e os biscoitos apresentaram bom aporte mineral, destacando-se, de maneira geral, os conteúdos de cálcio, potássio, magnésio, fósforo, zinco e ferro. A cor das barras tendeu para vermelho menos intenso e amarelo escuro. Nos biscoitos, a coloração tendeu para o marrom escuro. Com relação às condições higiênico-sanitárias, esta foi satisfatória, devido à ausência de quantidades significativas dos micro-organismos. Quanto à análise sensorial, todos os produtos apresentaram boa aceitação, não houve preferência entre as formulações de barras e de biscoitos e todos apresentaram alta intenção de compra. Análise multivariada dos biscoitos e barras permitiu a seleção de componentes principais (PC1, PC2 e PC3) que explicaram a variância dos dados obtidos nas caracterizações analíticas destes produtos.

CONCLUSÃO: A utilização de quinoa, amaranto e linhaça permitiu o desenvolvimento de formulações de barras alimentícias e biscoitos para pessoas com doença celíaca com bom aporte nutricional e aceitação sensorial, destacando-se teores protéicos, perfil mineral e qualidade lipídica. A utilização de linhaça como fonte de Omega-3 contribuiu para boas razões entre n-6:n-3 e diminuição do conteúdo de ácidos graxos saturados nas barras e biscoitos. A análise multivariada permitiu uma melhor caracterização e diferenciação dos produtos desenvolvidos destacando o efeito da adição dos pseudocereais, na qualidade nutricional e sensorial dos mesmos.

GENERAL ABSTRACT

INTRODUCTION: Celiac disease (CD) is the intolerance to gluten protein, which presents resistance to the digestive enzymes and triggers an inflammatory response in genetically predisposed individuals. The main treatment is based on restricting the consumption of foods containing this protein fraction. Food containing gluten, such as oats, barley, rye and wheat, causes inflammation in the intestinal villi, followed by atrophy and low absorption of nutrients in affected individuals. CD is one of the most frequent genetic diseases of humanity, affecting 0.5% to 1.0% of world population. In Brazil, studies performed in blood banks screening, indicate the prevalence of CD in 1:276 to 1:681 of donors. Most of industrial products have gluten in their composition, making the diet of patients with this disease very difficult, which becomes monotonous and restricted. Besides, these products usually have high amounts of simple carbohydrates and saturated fats. The development of food for celiac patients with good nutritional contribution corresponds to the needs of this class of consumers. Rice flour is an alternative to replace wheat flour in bakery products processing such as gluten free cookie, because it does not contain this protein fraction, but the amino acid profile is deficient in lysine. In most cereal bars available in the market, the main ingredient is oats and contents of saturated fat are high in these bars. An alternative for the production of cookies and food bars for celiac with good nutritional support is the use of gluten-free ingredients with essential nutrients. In this context, quinoa, amaranth and flaxseed are emphasized. Quinoa and amaranth are pseudocereals (grains with similar proximate composition of cereals) originated from Andean regions, mainly from Chile. The grains are highlighted due to high quality proteins and mineral composition. Amaranth presents approximately 12-13% protein, 67-69% carbohydrates, 10-13% fiber, 5-6% fat and 2-3% minerals. Quinoa has 14-15% protein, 55-64% carbohydrates, 9-11% fiber, 6-10% fat and 3-4% minerals. The constitution of these two pseudocereals also presents saponins, which act as natural antimicrobial agent and insecticide for the plant, but in high concentrations in the human body they become toxic. In order to adapt crops of quinoa and amaranth to the Brazilian climate for cultivation of the species in the country, the Brazilian Agricultural Research Corporation (EMBRAPA) developed crops of quinoa and amaranth, genetically modified, resulting in the species *Chenopodium quinoa* BRS Piabiru and *Amaranthus cruentus* BRS Alegria without saponins. Flaxseed stands out for its high content of Omega-3, considerate as an alternative to improve the lipid profile of gluten-free products because the grain has 14-22% oleic acid, 15-17% linoleic acid and 52-60% alpha-linolenic acid in relation to its total lipids (44%). Therefore, the development of products ready for consumption such as gluten free cookie and food bar, with good nutritional finds the needs of the celiac consuming public. Besides the nutritional composition, another topic to be taken into consideration in the development of food bars and cookies is sensory acceptance. The influence of ingredients on the sensory, nutritional and technological characteristics can be detected with the use of chemometric tools, because they allow the recognition of patterns, information gathering and a reduction of data dimensionality, as well as the organization of them in a more simple and easy to understand structure. The principal component analysis is based on achievement and linear comparisons of the original variables. The principal components (PCs) are mutually orthogonal and explain the decrease in variance with increase in number of PCs.

OBJECTIVES: The purpose of this study was the development of gluten-free cookies and food bar, containing *Chenopodium quinoa* BRS Piabiru and *Amaranthus cruentus* BRS Alegria as source of protein and minerals, and *L. usitiassimum,* L. as a source of alpha-

linolenic acid, as well as the assessment of nutritional, sensorial and technological characteristics.

MATERIAL AND METHODS: The pseudocereals (quinoa and amaranth) were donated by EMBRAPA and the other ingredients were purchased in local shops in Maringá - PR. For food bar development, the contents of amaranth and rice flakes were varied. The amount of other ingredients was the same for the formulations. The bars were formed by mixing two phases: solid (pseudocereal, oilseeds, fruits) and binder (syrup). Three formulations of solid phase were developed (A, B, C) containing 1% corn flakes, 2% banana and 4% flaxseed in each one. In addition to these ingredients, the formulations A, B and C contained respectively 30%, 36% and 42% amaranth, 13%, 7% and 1% rice flakes. The binder phase of the bars A, B and C contained the same composition: 10% brown sugar, 1% canola oil, 20% of glucose syrup, 10% honey, 3% invert sugar and 6% water. In the production of bars, the ingredients of the solid phase were homogenized and added to the syrup preheated to achieve concentration from 85 to 89° Brix. The two phases were mixed until complete interaction. The three masses formed (A, B and C) were pressed, rolled and cut into bars of 25g, with dimensions of 9 cm x 3 cm x 1.5 cm. Each unit has one side covered with chocolate. In cookie production, three formulations were developed using quinoa flour as a partial replacement of rice flour at different levels. The three cookies (I, II, III) contained 3% brown sugar, 3% white sugar, 12% honey, 5% butter, 5% egg yolk, 5% flaxseed meal, 1% sodium bicarbonate, 7% water, 9% chocolate chips, 2% cocoa powder, 8% Brazil nuts, 1% aroma of nuts. In addition to these ingredients, the formulations I, II and III contained, respectively, 6%, 10% and 14% quinoa flour, 33%, 29% and 25% rice flour. In the processing of cookies, dry ingredients (except the flour) were mixed to form a uniform portion of each formulation. Butter was added in each portion and homogenized for 4 minutes. The quinoa and rice flours were added and homogenized for 1 minute. The masses formed (I, II and III) were laminated to a thickness of 7 mm and cut into circular shape with a diameter of 6 cm. The cookies were baked at 180° C for 20 minutes. The food bars and cookies produced were evaluated in relation to the presence of gluten (immunoenzymatic assay - ELISA); proximate composition (moisture and ash - Gravimetric method, proteins - Kjeldahl method, lipid - Bligh & Dyer method, carbohydrate by difference); calorie value (direct method – calorimetric bomb, indirect method - calculation); water activity (Aqualab), color (tritimulus methodology L*, a*, b*), fatty acid composition (gas phase and chromatography); mineral composition (atomic absorption spectrophotometry); nutritional index of lipid composition (atherogenicity, thrombogenicity, ratio of hypocholesterolemic / hypercholesterolemic fatty acids), sanitary conditions (microbiological); sensory evaluation (acceptability, preference and purchase intent); percentage of the daily recommended intake of minerals in different ages and genders; influence of principal components in determining characteristics of the products.

RESULTS AND DISCUSSION: Gluten fractions were not detected in food bars and cookies. In the proximal composition of products, protein and ash content were highlighted. The use of promising sources of protein is required, because the most of kinds of flour used in products for celiac patients have high carbohydrate concentration and low protein content. In fatty acid composition, the linoleic (n-6) and alpha-linolenic acid (n-3) were highlighted because they are considered strictly essential. The ratios n-6:n-3 in food bar and cookie formulations ranged from 1.44:1 to 2.50:1 and from 3.08:1 to 4.38:1, respectively, and the ratios of polyunsaturated and saturated fatty acids ranged from 0.45 to 0.55 and from 0.85 to 0.92, respectively. Food bars and cookies presented good mineral contents, especially the concentrations of calcium, potassium, magnesium, phosphorus, zinc and iron. The color of the bars tended to low intense red and dark yellow. Cookies color tended to dark brown. The

sanitary conditions was satisfactory, there was absence of significant quantities of micro-organisms. In sensory analysis all products showed good acceptance, no preference for the formulations of bars and cookies and all formulations showed high purchase intent. Multivariate analysis of cookies and food bars allowed the selection of principal components (PC1, PC2 and PC3) which explained the variance of data from the analytical characterizations of these products.

CONCLUSION: Use of quinoa, amaranth and flaxseed enabled the development of formulations of food bars and cookies for people with celiac disease. These products presented good nutritional characteristics and sensory acceptance, especially protein content, mineral profile and lipid quality. The use of flaxseed as a source of Omega-3 contributed to good ratios of n-6:n-3 and reduced the content of saturated fatty acids in food bars and cookies. Multivariate analysis allowed better characterization and differentiation of products developed, highlighting the effect of adition of pseudocereals in nutritional and sensory qualities of the products.

ARTIGO 1

TÍTULO: Using chemometric techniques to characterize gluten-free cookies containing the whole flour of a new Quinoa cultivar

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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 °C 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 °C 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 °C 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 °C 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.

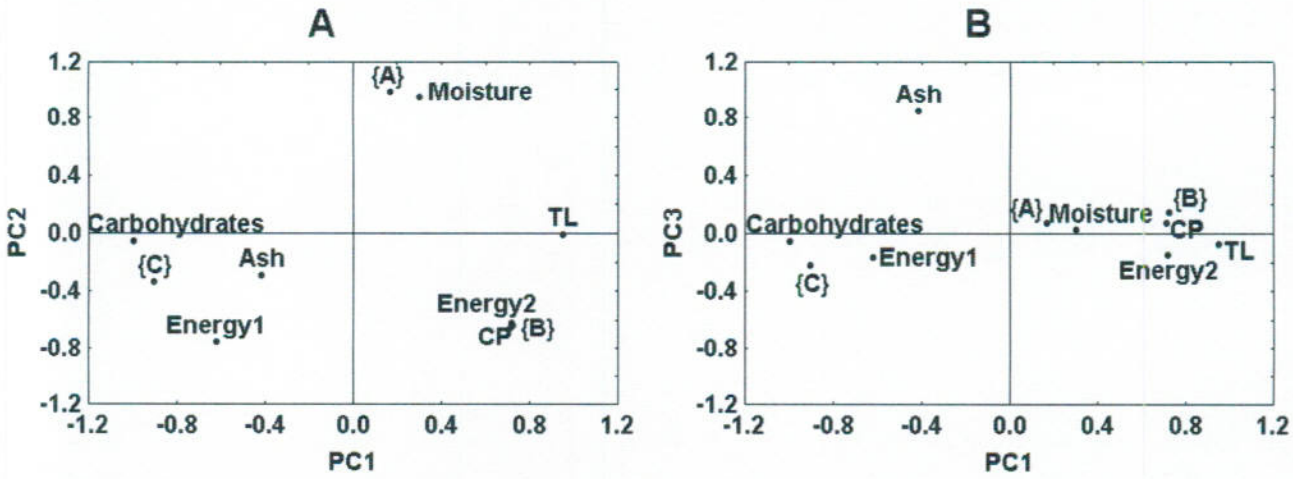

Figure 1. Principal component analysis of the proximal composition of cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC1 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Analyses (Parameters/Loadings): Ash, Carbohydrates, Energy1, Energy2, Moisture, CP, TL. CP: crude protein;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

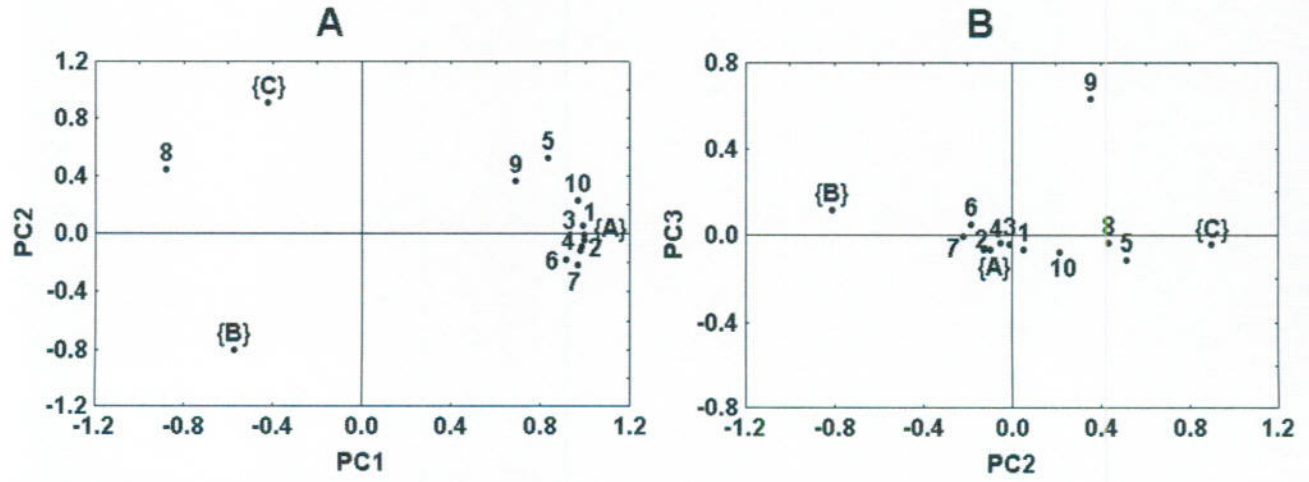

Figure 2. Principal component analysis of fatty acids: sums, ratios, and nutritional index of the cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC2 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Analyses (Parameters/Loadings): 1 = SFA (total saturated fatty acids); 2 = MUFA (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

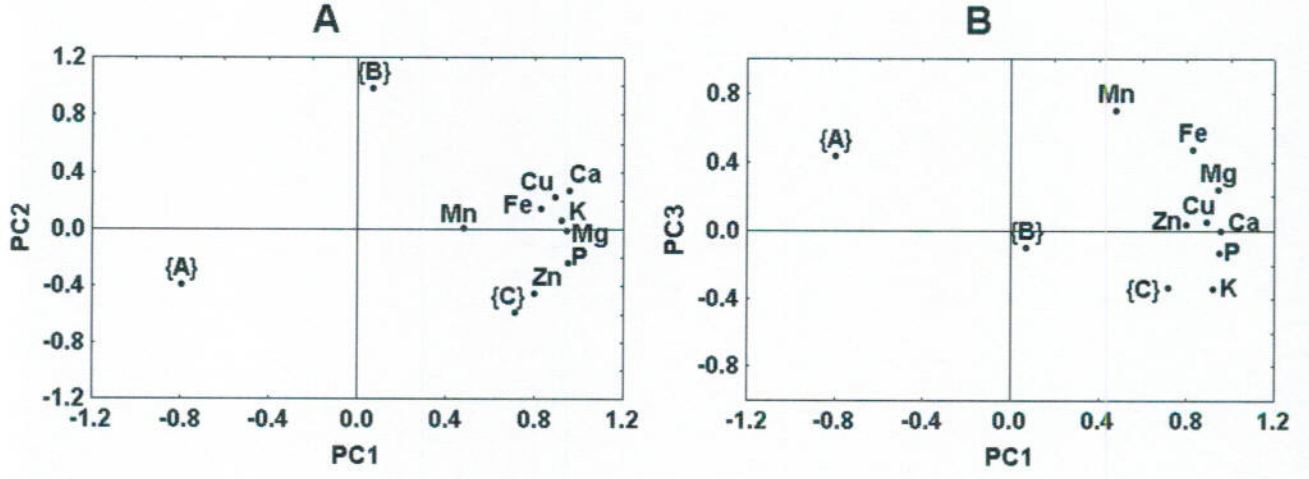

Figure 3. Principal component analysis of minerals quantification in the cookie formulations. PC: principal component. (A) PC1 × PC2; (B) PC1 × PC3. Formulations (Samples/Scores): {A}, {B} and {C}. Minerals (Parameters/Loadings): Ca, Cu, Fe, Mg, Mn, K, P and Zn.

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.

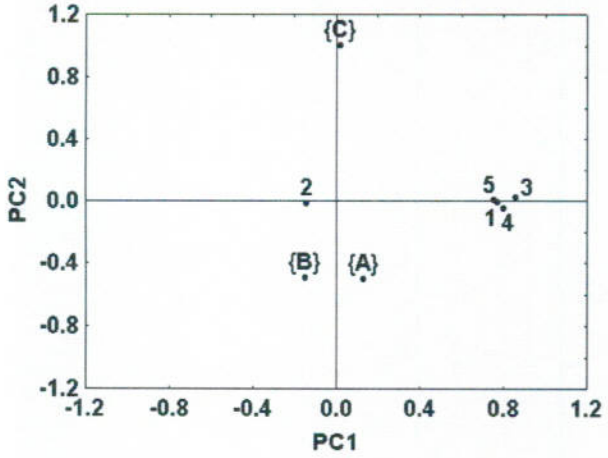

Figure 4. Principal component analysis of sensory attributes in the cookie formulations. PC: principal component; 1: Appearance; 2: Flavor; 3: Texture; 4: Crispness; 5: Overall acceptance.

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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ARTIGO 2

TÍTULO: Development, characterization and chemometric analysis of a gluten-free food bar from a new cultivar of Amaranth

REVISTA: *Ciência e Agrotecnologia*

DEVELOPMENT, CHARACTERIZATION AND CHEMOMETRIC ANALYSIS OF A GLUTEN-FREE FOOD BAR CONTAINING A NEW CULTIVAR OF AMARANTH

DESENVOLVIMENTO, CARACTERIZAÇÃO E ANÁLISE QUIMIOMÉTRICA DE BARRA ALIMENTÍCIA SEM GLÚTEN CONTENDO UM NOVO CULTIVAR DE AMARANTO

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ABSTRACT

Food bars are consumed heavily, especially because of their practicality; however they cannot be ingested by celiac patients and present low contents of essential nutrients. The goal of this study was the development and physical-chemical, nutritional and sensory evaluation of a gluten-free food bar containing amaranth and linseed. Gluten fractions were not detected in the food bar formulations. Crude protein and total lipid contents ranged from 68.32 to 76.60 and 74.56 to 82.06 g kg^{-1} of food, respectively. The polyunsaturated/saturated and n-6:n-3 fatty acid ratios ranged from 0.45:1 to 0.55:1 and 1.44:1 to 2.50:1, respectively. Calcium, magnesium, copper, iron, manganese and zinc were the principal minerals. Application of multivariate analysis enabled sample B to be distinguished according to its mineral and alfa-linolenic acid content. All food bar formulations had good sensory acceptance and high purchase intent.

Index terms: Principal component analysis, pseudo-cereals, linseed, fatty acids.

RESUMO

Barras alimentícias são muito consumidas, especialmente por sua praticidade; no entanto apresentam baixos teores de nutrientes essenciais e, em sua maioria, não podem ser ingeridas por pessoas celíacas. O objetivo deste estudo foi o desenvolvimento, avaliação físico-química, nutricional e sensorial de barras alimentícias sem glúten contendo amaranto e linhaça. As barras alimentícias formuladas não apresentaram frações de glúten. Os teores de proteína bruta e lipídios totais variaram entre 68,32-76,60 e 74,56-82,06 g kg^{-1} de alimento, respectivamente. As razões entre ácidos graxos poli-insaturados/saturados e n-6:n-3 variaram de 0,45:1 para 0,55:1 e 1,44:1 para 2,50:1, respectivamente. Os principais minerais foram o cálcio, magnésio, cobre, ferro, manganês e zinco. Aplicação de análise multivariada permitiu

diferenciar a amostra B das demais em relação ao seu conteúdo mineral e teor de ácido alfa-linolênico. Todas as barras formuladas tiveram boa aceitação sensorial e intenção de compra elevada.

Termos para indexação: Análise de componentes principais, pseudocereal, linhaça, ácido graxo.

INTRODUCTION

Celiac disease, intolerance to the intake of gluten protein, arises from its resistance to digestive enzymes and affects genetically predisposed individuals by triggering an inflammatory response (Arendt *et al.*, 2008). The amaranth (*Amaranthus* spp.) from the Andean region is classified as a pseudo-cereal. Levels of 12.2–13.8% crude protein, 67.4–69.2% carbohydrate, 9.7–12.9% dietary fiber, 5.0–6.3% total lipids and 2.5–3.4% minerals, respectively, have been reported for amaranth (Arendt et al., 2008; Gutiérrez et al., 2010). The linseed *(Linum usitatissimum* L.), high levels of crude fiber and total lipids, 8.3 and 43.9%, respectively, are also found in linseed (Gutiérrez *et al.*, 2010). Cultivar *Amaranthus cruentus* BRS Alegria was genetically modified for central-western Brazil climate conditions and to remove saponins while still maintaining its chemical composition (Spehar & Santos, 2002; Spehar *et al.*, 2003). Food bars or cereal bars normally have high contents of soluble and insoluble fibers, lipids and carbohydrates, but low protein content, thus being an energy source (Mahanna & Lee, 2010). The goal of this paper was the development, quimiometric analysis, physico-chemical, sensory and nutritional assessment of gluten-free food bars containing amaranth and linseed.

MATERIAL AND METHODS

Sampling and formulations

The grains of *A. cruentus* BRS Alegria were provided by EMBRAPA. The other ingredients were purchased from local shops in Maringá, Paraná state. The food bar formulations (Table 1) consisted of a binder phase (syrup) and a solid phase (grains, nuts and raisins). The binder phase was heated under stirring and the soluble sugar content was measured until it reached 85–89°Brix on a digital refractometer (Leica Microsystems Inc., USA). The phases were mixed and pressed to obtain a form with dimensions 90 mm X 30 mm X 15 mm weighing 25 g. These bars were then covered with melted chocolate (0.1–0.2mm thickness).

Table 1 – Food bar formulations

Solid phase	Formulations		
	A	B	C
Amaranth*	300.00	360.00	420.00
Rice flakes*	130.00	70.00	10.00
Cornflakes*	10.00	10.00	10.00
Linseed flour*	40.00	40.00	40.00
Banana*	20.00	20.00	20.00
Binder phase	(formulations A, B, C)		
Brown sugar* 100.00, Canola oil* 10.00, Glucose syrup* 200.00, Honey* 100.00, Invert sugar* 30.00, Water* 60.00.			

*Ingredients in g kg^{-1} of product.

Fatty acid composition, mineral quantification and chemical analysis

The moisture, ash and crude protein contents were determined according to Cunniff (1998) using a factor of 5.80 (Arendt *et al.*, 2008) to convert the percentage of nitrogen into crude protein content. The total lipids were determined according to Bligh & Dyer (1959). The carbohydrate content was calculated by difference (Faithfull, 2002).

To determine the fatty acid composition, the lipids were converted into fatty acid methyl esters (FAME) and methylated according to Hartman & Lago (1973). The FAME were separated in a 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 1.4 mL min^{-1} carrier gas hydrogen, 30 mL min^{-1} make-up gas nitrogen, 300 mL min^{-1} synthetic air and 30 mL min^{-1} flame gas hydrogen; the sample was injected in a split ratio of 1:100. The injector and detector temperatures were both 235 °C. The column temperature was maintained at 165 °C for 4 min, then increased 4 °C min^{-1} to 185 °C and maintained for 5 min, then raised to 225 °C at 10 °C min^{-1} and maintained for another 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 & Ackman (1992). The peak areas were determined with Star 5.0 software (Varian, USA) and the concentrations were expressed in mg kg^{-1} of food.

For 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 an atomic absorption spectrophotometer AA240FS (Varian, USA) as mg of mineral *per* kg of product using standard solutions and analytical curves.

Indices of the nutritional quality of lipids and gluten test

The gluten fractions in the final products were determined using a commercial ELISA kit (Enzyme-linked immunosorbent assay)-R5 Ridascreen® Gliadin (R-Biopharm, Germany), a Sunrise spectrophotometer (Tecan, Switzerland) at 450 nm, and Rida-Win software (R-Biofarm, 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 greater than 2.00 mg gluten 100 g^{-1} of food, as recommended by the Codex Food Commission (1983).

A better approach to the nutritional evaluation of fat is utilization of indices based on the functional effects of fatty acid composition. These indices were available as the index of atherogenicity (IA) = [(12:0 + (4 x 14:0) + 16:0)] / (ΣMUFA + Σn-6 +Σn-3), and index of thrombogenicity (IT) = (14:0 + 16:0 + 18:0) / [(0.5 x ΣMUFA) + (0.5 x Σn-6) + (3 x Σn-3) + (Σn-3/Σn-6)], by Ulbricht & Southgate (1991), 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.* (2002).

Sensory analysis

A group of 80 untrained volunteer panelists and potential consumers of the products developed participated in the sensory analysis, which consisted of 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 food bar 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, where

nine was the maximum score on the hedonic scale. The lowest IA value for considering the products 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 purchase intent test was determined using a 5-point scale (1 = definitely would not buy and 5 = definitely would buy) (Lawless & Heymann, 2010).

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.

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 Tukey's *post hoc* and Student "t" tests to compare direct and indirect calorimetric measurements. Friedman's test was used only for the Preference Ordering test, according to Lawless & Heymann (2010). The multivariate analysis was performed by applying Principal Component Analysis (PCA). The average of three individual batches was used with respect to the 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 food bar formulations developed, corroborating studies that have shown the absence of gluten in other varieties of the same species of grains (Alvarez-Jubete *et al.*, 2010). The

results of the physical-chemical analyses are shown in Table 2. The crude protein content showed significant differences ($p < 0.05$) because it increased progressively and proportionally with increasing grain concentrations in the food bar formulations, corroborating studies accomplished by Enriquez *et al.* (2003).

Table 2 – Proximal composition of food bar formulations

Parameters	Formulations		
	A	B	C
Moisture (g kg^{-1})	95.78^{a}±0.15	89.40^{b}±0.65	88.40^{b}±1.11
Ash (g kg^{-1})	13.37^{c}±0.26	13.52^{b}±0.40	13.71^{a}±0.62
Crude protein (g kg^{-1})	68.32^{b}±0.91	68.69^{b}±0.80	76.60^{a}±0.49
Total lipids (g kg^{-1})	79.58^{a}±3.12	82.06^{a}±2.96	74.56^{b}±0.30
Carbohydrates [1] (g kg^{-1})	742.95^{a}±14.80	746.33^{a}±14.74	746.73^{a}±14.37

Means followed by the same letters in rows do not differ by Tukey's test ($p < 0.05$). [1]Carbohydrates determined by difference.

According to Brasil (1998), the food bars can be considered a "source of protein" because they presented 10% of the DRI (Recomended Daily Intake) for adults (50 g day^{-1}) in 100 g of product. According to Gutierrez *et al.* (2010), linseed has a mineral content of 2.66%, while pseudo-cereals have approximately 3% (Arendt *et al.*, 2008), which contributed to the high mineral content of the products developed. There was a difference ($p < 0.05$) in total lipids between the samples. The food bar formulations presented proximal composition and percent energy similar to those of Freitas & Moretti (2006) in a study developing a cereal bar, with 10.71, 2.20, 15.31, 5.64 and 60.97% moisture, ash, crude protein, total lipids and carbohydrates, respectively.

Table 3 presents thrombogenicity and atherogenicity indices. The major ratios HH and PUFA:SFA (Table 3) are important due to their hypocholesterolemic effect and the

prevalence of polyunsaturated fatty acids are related to lowered risk of cardiovascular disease (Ratnayake & Galli, 2009). The saturated fatty acid contents of food bars A, B and C were 4.02, 3.87 and 3.63%, respectively (Table 3). Excessive consumption of lipids 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 food bars ranged from 1.4:1 to 2.5:1, which is near the ideal value of 1:1 (Simopoulos, 2011).

Table 3 – Absolute quantification of fatty acids in food bar formulations

Fatty Acid	Formulations		
(mg kg^{-1})	A	B	C
10:0	824.45^{a}±4.29	793.52^{b}±4.20	745.60^{c}±1.61
12:0	17746.34^{b}±4.23	17833.28^{a}±4.68	17040.69^{c}±0.53
14:0	6998.27^{a}±4.12	6789.64^{b}±3.98	6436.02^{c}±0.45
16:0	8706.05^{a}±4.40	7767.80^{b}±3.68	7049.65^{c}±0.73
16:1n-7	107.43^{a}±5.87	49.24^{c}±5.43	70.09^{b}±1.67
18:0	5572.19^{a}±4.21	5119.72^{b}±3.95	4676.40^{c}±0.49
18:1n-9	15528.45^{b}±4.16	15913.90^{a}±3.80	13926.32^{c}±1.02
18:2n-6	13030.43^{a}±4.17	12660.30^{b}±3.77	11119.13^{c}±1.02
18:3n-3	5203.87^{c}±4.10	8655.69^{a}±3.84	7723.67^{b}±0.87
20:0	237.58^{a}±4.63	225.67^{a}±8.38	205.04^{b}±7.55
24:0	144.19^{a}±5.21	141.14^{a}±5.37	123.77^{b}±4.35
	Sums and ratios of fatty acids		
SFA	40229.07^{a}±362.38	38670.77^{b}±669.39	36277.17^{c}±103.43
MUFA	15635.88^{a}±204.09	15963.14^{a}±209.89	13996.41^{b}±87.02
PUFA	18234.30^{b}±1480.08	21315.99^{a}±190.69	18842.80^{a}±162.11
n-6	13030.43^{a}±173.38	12660.30^{b}±143.66	11119.13^{b}±140.63
n-3	5203.87^{c}±50.28	8655.69^{a}±124.96	7723.67^{b}±79.84

PUFA/SFA	0.45^{b}±0.01	0.55^{a}±0.02	0.52^{a}±0.01
n-6:n-3	2.50^{a}±0.01	1.46^{b}±0.02	1.44^{b}±0.01
	Indices of the nutritional quality of lipids		
IA	1.61^{b}±0.02	2.51^{a}±0.07	2.61^{a}±0.02
IT	0.71^{a}±0.01	0.49^{c}±0.01	0.50^{b}±0.01
HH	2.14^{c}±0.01	2.53^{a}±0.04	2.44^{b}±0.01

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, IA: Index of atherogenicity, IT: Index of thrombogenicity, HH: Hypocholesterolemic/hypercholesterolemic fatty acid ratio.

As shown in Table 4, the major mineral component was Mg and Ca. The first plays a vital role in a wide range of biochemical and physiological processes and the presence of calcium in the diet contributes to increasing the bioavailability and absorption of Mg, Mn and Zn. The content of the trace mineral Zn in the samples varied significantly ($p < 0.05$). These minerals are essential for the maintenance of biological systems because they participate as cofactors in metabolic reactions (Hathcock, 2004).

Table 4 – Mineral composition of food bar formulations

Mineral (mg kg^{-1} of sample)	Formulations		
	A	B	C
Ca	2606.97^{a}±86.76	2648.20^{a}±85.33	2380.11^{b}±47.71
Cu	27.46^{a}±0.13	26.85^{a}±2.74	23.74^{b}±0.96
Fe	115.61^{b}±5.91	127.48^{a}±5.77	124.43^{a}±10.98
Mg	2801.95^{b}±521.35	3001.86^{a}±128.22	2594.19^{c}±170.89
Mn	38.77^{b}±3.73	41.05^{a}±0.90	40.04^{a}±0.52
Zn	53.43^{b}±6.57	67.64^{a}±1.67	54.43^{b}±1.73

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

The acceptance test results are shown in Table 5. The attributes flavor and acceptance ranged from slightly liked to moderately liked with a significant difference ($p < 0.05$). The food bars were considered well accepted when the acceptance rate was above 70%, as proposed by Lawless & Heymann (2010). The formulations showed no differences ($p < 0.05$) in preference ordering by the Friedman's test. As to the purchase intent results, ranging from probably buy to surely buy, the consumption potentials of the gluten-free food bars were above 85% for all the formulations

Table 5 – Means and standard deviation of acceptance test attributes of food bar formulations

Formulations	Attributes					
	Appearance	Flavor	Texture	Crispness	Overall Acceptance	I.A.[1] (%)
A	$7.64^{a}\pm1.10$	$7.38^{a}\pm1.43$	$6.92^{a}\pm1.56$	$7.07^{a}\pm1.67$	$7.38^{a}\pm1.53$	82.00^{a}
B	$7.34^{a}\pm1.40$	$6.88^{b}\pm1.43$	$6.86^{a}\pm1.51$	$6.68^{a}\pm1.62$	$7.08^{b}\pm1.76$	78.67^{b}
C	$7.46^{a}\pm1.36$	$6.83^{b}\pm1.72$	$6.61^{a}\pm1.78$	$6.86^{a}\pm1.86$	$6.91^{b}\pm1.81$	76.78^{b}

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.

Figures 1, 2 and 3 show the principal component analysis (PCA). The NIPALNS algorithm enabled selection of PC1, PC2 and PC3, which were significant ($p < 0.05$) and explained 99.67, 89.81 and 99.87% of the data variance, respectively, in fatty acids/nutritional indices, mineral and sensory attributes. Figure 1A on PC1 enabled formulation A to be distinguished due to the positive contributions of SFA, MUFA, n-6, n-6:n-3 and IT. By analyzing PC2 and PC3 (Figures 1A and 1B), formulation C obtained

positive contributions in the sums of SFA, MUFA and n-6, and in the PUFA:SFA ratio, which was different from the others.

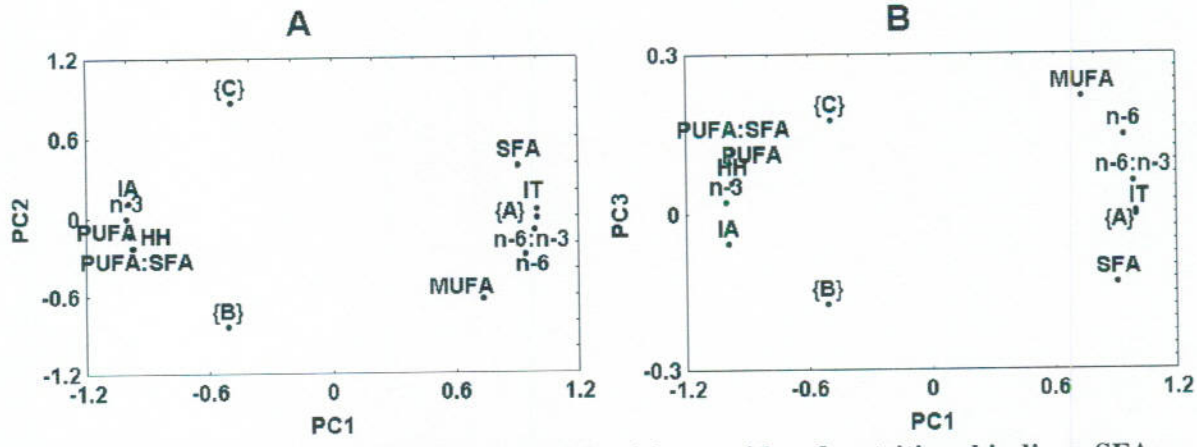

Figure 1 – Principal component (PC) analysis of fatty acid and nutritional indices. SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids, PUFA: total polyunsaturated fatty acids, n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids, IA: Index of atherogenicity, IT: Index of thrombogenicity, HH: Hypocholesterolemic/hypercholesterolemic fatty acid ratio.

As shown in Figure 2A, all minerals made a positive contribution in PC1, but only copper and iron presented positive contributions in PC3 (Figure 2B). The minerals Ca and Cu in PC2 (Figure 2A) were responsible for the differentiation of formulation A.

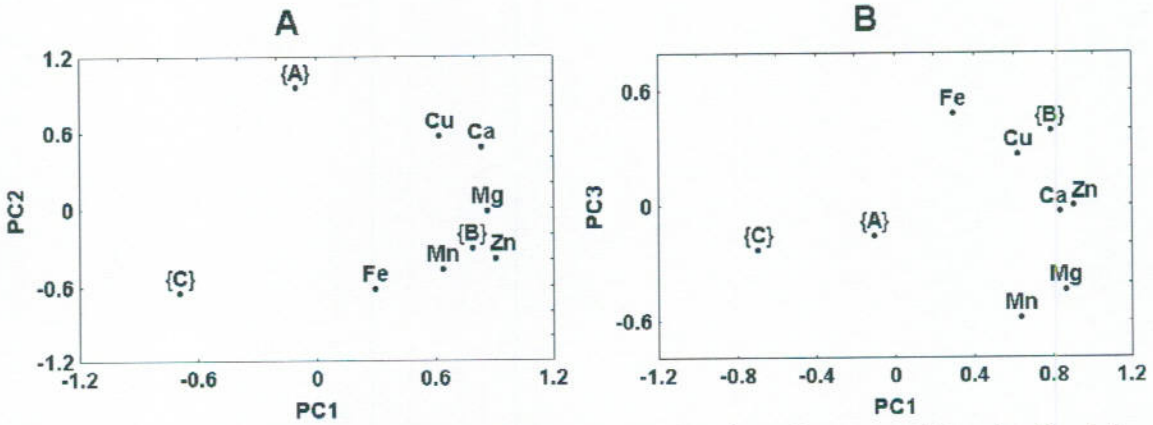

Figure 2 – Principal component (PC) analysis of mineral composition in food bar formulations.

In a multivariate analysis of all sensory attributes, all of them were highly significant in PC1, as shown in Figure 3A, distinguishing sample A, which recorded the highest scores for all attributes (Table 5). In PC2 and PC3 (Figures 3A and 3B), respectively, texture and flavor attributes made positive contributions to samples B and A.

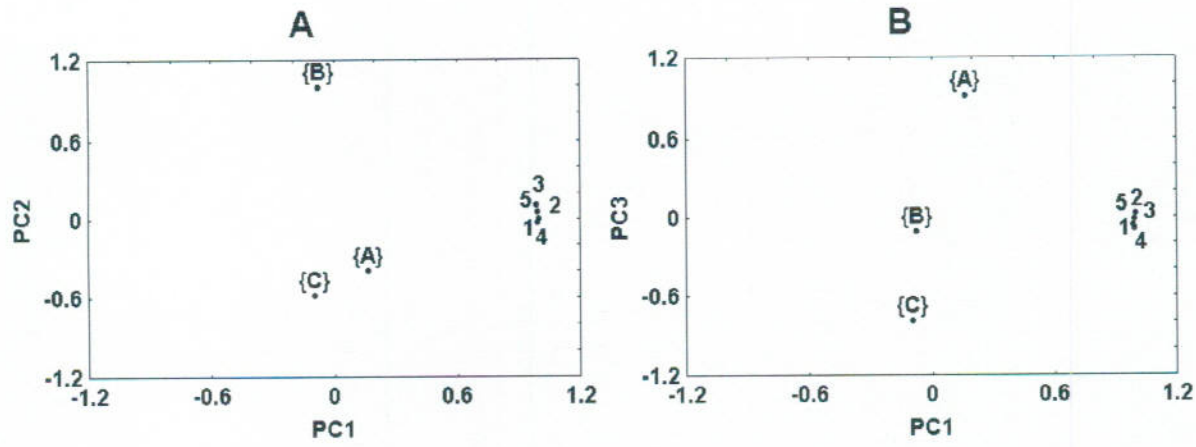

Figure 3 – Principal component (PC) analysis of sensory analysis in food bar formulations. 1 Appearance, 2 Flavor, 3 Texture, 4 Crispness and 5 Overall acceptance.

Although there was no significant difference ($p < 0.05$) in some of the parameters analyzed, the principal component analysis allowed apparently equal samples to be distinguished according to loadings (studied parameters) and scores (samples).

CONCLUSION

The use of naturally gluten-free ingredients allowed the development of food bar formulations for celiac disease patients. Promising grains like amaranth and linseed contributed to increasing the protein, lipid and mineral contents in the products. The evaluation of the nutritional indices in the lipid fraction verified their anti-atherogenic, anti-thrombogenic and hypocholesterolemic effects and good ratios for PUFA:SFA and n-6:n-3, corroborating other studies. All formulations are good sources of minerals. With respect to

sensory analysis, there was no preference for a specific formulation and the purchase intent indices were high. Application of multivariate analysis allowed sample B to be distinguished due to the contributions of alpha-linolenic acid and mineral content to the weights of its constituents.

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ANEXOS

ANEXO 1

ACEITE PARA PUBLICAÇÃO
Journal of the Brazilian Chemical Society

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31-Oct-2013

Dear Dr. Matsushita:

It is a pleasure to accept your manuscript entitled "Using Chemometric Techniques to Characterize Gluten-Free Cookies Containing the Whole Flour of a New Quinoa Cultivar" in its current form for publication in the Journal of the Brazilian Chemical Society. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Journal of the Brazilian Chemical Society, we look forward to your continued contributions to the Journal.

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ANEXO 2

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Descriptions of experiments should be given in sufficient details to enable other researchers to repeat them. The degree of purity of materials should be given, as well as all quantities used. Descriptions of established procedures are unnecessary. Standard techniques and methods used throughout the work should be stated at the beginning of the section in a **Materials and/or Methods** subsection, in the **Experimental** section. Apparatus should be described only if it is non-standard. Commercially available instruments should be referred to by their suppliers and models.

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4.1 Graphs and Figures (also see Section 2)

Lines and Lettering: Lines should be black and of an adequate and even thickness. Solid, broke, dotted and dot-dash lines should be used in graphics. Particular care should be taken to ensure that the lines in a spectrum are of adequate thickness.

Lettering should not be smaller than 7 pt (Times New Roman) and lines not thinner than 0.5 pt. Lettering and lines should be of uniform density throughout the figures.

Labeled atoms in ORTEP (or any other) diagrams should have atom numbers in parenthesis, e.g., Fe(1), C(44).

Symbols representing physical quantities should be given in italics, e.g., *J* (Hz), δ (ppm), *m/z*, etc.

Units should be expressed in the appropriate form, e.g., g cm^{-3} or mol L^{-1}, rather than g/cm^3 or mol/L (see Section 4.5)

Graphs

- **Scales:** graphs should have only the minimum necessary scale divisions marked by numerals.
- **Axis labels** should use SI units, separated from quantities (see details in the green book http://old.iupac.org/reports/1993/homann/index.html):

For graphs, use slashes in X and Y axes to separate axes names from units. For example: 2θ / degree; Temperature / °C; time / min; Size range / mm; Wavenumber / cm^{-1}. Use parentheses only to group a set of units, e.g., Concentration / (mol L^{-1}) ; 10^3 $(T/K)^{-1}$, etc.

Pay close attention to the way decimal values are expressed in English. Employ dots instead of commas.

Figures must have a high quality in order to be well reproduced. Use at least a 900 dpi resolution. If necessary, resize to a smaller size to get higher quality.

Curves should be labeled (a), (b), (c) etc. and further information be given in the figure legend/caption.

Data Points must be shown sufficiently large to be distinguishable. Whenever possible, they should be marked with the following symbols (use alternated full and open symbols):

●, ○, ■, □, ▲, Δ, ♦, ◇

Graphs/Figures should be pasted from their original files (Origin, ChemDraw, Corel etc.) and have an excellent quality. If you have to digitalize (scan) the figures (photos, for instance), choose the following scan options: black & white (B&W), no background and minimum of 300 dpi. If you wish them to be published online in color, send both the colored and B&W versions to the Editorial Office, matching the captions of the figures to accommodate the alternatives.

For computer-generated artwork, background or shadings should be avoided.

4.2 Structural Formulae

Figures, schemes and structures should be drawn to fit single or double-column widths. They should look proportional in case they are reduced.

Structures should be numbered with bold Arabic numerals, e.g.,**1**, **2**.

All chemical structures included in the manuscript should be drawn using the same letter type (Times New Roman or Arial), size of cyclic groups, size and thickness of chemical bonds, and, the most important, authors should use the same standard throughout the work, including all figures, schemes, etc.

The following organic group abbreviations may be used: Me, Et, ^{n}Pr, ^{n}Bu, ^{s}Bu, ^{t}Bu, Ph, CO^2R, CO^2H, ^{i}PrOH.

One variable univalent substituent is indicated by R. When more than one independent variable general substituent is present, R^1, R^2, R^3, etc. should be used.

A variable metal may be indicated by M and variable ligands by L^1, L^2, L^3 or L1, L2, L3, etc.

4.3 Photographs

Photographs should be highly contrasted, positive and not mounted.

When necessary, the scale should be drawn on the photograph itself and not below.

Color prints are rarely reproduced satisfactorily in black and white. Original B&W photographs are preferred to report experimental results, such as electron micrographs or to illustrate special equipment adaptations.

4.4 Colored Illustrations

Online Version

From 2010 onwards, the publication of colored illustrations will be totally free of charge in the ONLINE version of the Journal.

Printed Version

Black & White (B&W) illustrations are free of charge. If color figures are presented in your Manuscript (Ms), they will automatically be converted into black-and-white (except GA). Color prints rarely reproduce satisfactorily in black and white. Thus, pay attention so that no information is missed because of the conversion. If the authors want to have colored illustrations on the printed version, they will be asked to pay for their cost: the current fee is 250 USD for all figures (remember that in the online version, they are free of charge).

4.5 Tables, Data and Units

Tables

Format your table to give straightforward information to the reader. Do not use shades or bold lettering. Indicate any extra information as a footnote with letters, e.g., a, b, c, etc. For examples, see any "PDF" files in: http://jbcs.sbq.org.br/forthcoming_papers.asp.

Data

For negative numbers, ions and equations in text and tables use – (negative symbol) instead of - (hyphen). Examples: Cl^-, –0.40, $y = ax - b$.

Units

Use International System Units (SI), e.g., m, s, kg, Pa, mol L^{-1}, etc, separated from quantities with a blank space. Example: 300 K, not 300K. See: http://old.iupac.org/reports/1993/homann/index.html.

Note: Molar (M) is no longer a valid concentration expression for IUPAC; it is suggested mol L^{-1} or mol dm^{-3}, but be consistent throughout your manuscript.

For examples, see any "PDF" files in: http://jbcs.sbq.org.br/forthcoming_papers.asp

5. Graphical Abstract (GA) and Text for GA

Concerning the ***JBCS*** Table of Contents, it is expected from authors careful with their **Graphical Abstract** (GA) proposition.

This way, the figure should summarize the content of the manuscript in a concise, pictorial form, designed to capture the attention of a wide readership. The author should present a new figure, using as an idea a key structure, a reaction, an equation, a concept, a graphic, a theorem, etc. It should use colors as much as possible and have an artistic and imaginative idea. Short movies are also welcome (as supplementary information (SI)). It is not acceptable photos of commercial equipment in GA or in the text of the manuscripts.

Pay Attention: the image should have a 900 dpi resolution (*.tiff / *.jpg or any other image file that can be edited and be 8 cm wide and 4 cm high). Along with the GA figure, insert a short explanatory text about it below (three lines at the most).

Take a look at our recent publications whose Table of Contents presents Graphical Abstracts (http://jbcs.sbq.org.br). Therefore, be smart to advertise your manuscript: send a beautiful and appealing graphical image.

6. Equations

When writing equations, use the Word editing equation option or any other equation editor. Equation cannot be added in the main text as image format.

7. Reference Citation rules

- **Reference numbers**

Reference numbers in the **text** should be typed consecutively as superscripts after punctuation, without parentheses or brackets. Examples:

sodium salicylate,[1-3]
Nishide *et al.*,[4]
by reduction of chromic acid.[4-8,12]

The cited literature should be listed on a separate page (double-spaced) in the same order it appears in the text.

- **Journal Titles**

Journal title abbreviations are those defined in the Chemical Abstracts Service Source Index (see http://www.cas.org/content/references/corejournals). If an authoritative abbreviation for a Journal cannot be located or if the abbreviation is not obvious, the full Journal title should be cited.

- **Style Rules for Year, Volume and Page**

#. Author, A. C.; Author B.; Author C. F.; *Abbreviation of the Journal* **Year**, *Volume*, Page.

1. Author, A. C.; Author, B.; Author, C. F.; *J. Braz. Chem. Soc.* **2010**, *21*, 77.

- Author initials should be separated from each other, e.g., Author, A. C.;
- Use semi-colons to separate different author's names. No "and" is necessary in any case.
- *Journal Abbreviations* should come inItalics: *J. Braz. Chem. Soc.*
- **Years** - bold font: **2010**
- *Volume* - Italic style: *21*
- Page - only the initial page, followed by dot: 77.

Examples:

2. Varma, R. S.; Singh, A. P.; *J. Indian Chem. Soc.* **1990**, *67*, 518.

In case the journal is not easily accessible, the best choice is to quote its Chemical Abstracts number, as follows

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).*

Pay attention to the connection words in the names, as for instance: da Silva, M. A. or Silva, M. da, as follows:

4. Pinto, A. C.; de Andrade, J. B.; *Quim. Nova* **1999**, *22*, 448.

- **Composite References**

They should be used whenever possible, rather than a series of individual references, without letters (a),

(b), (c), etc. Use only a semi-colon to separate them. The style for composite references is as follows:

5. Varela, H.; Torresi, R. M.; *J. Electrochem. Soc.* **2000**, *147*, 665; Lemos, T. L. G.; Andrade, C. H. S.; Guimarães, A. M.; Wolter-Filho, W.; Braz-Filho, R.; *J. Braz. Chem. Soc.* **1996**, *7*, 123; Ângelo, A. C. D.; de Souza, A.; Morgon, N. H.; Sambrano, J. R.; *Quim. Nova* **2001**, *24*, 473.

- **Patents**

They should be identified in the following form. Whenever possible, Chemical Abstracts numbers should be quoted in parentheses:

6. Hashiba, I.; Ando, Y.; Kawakami, I.; Sakota, R.; Nagano, K.; Mori, T.; *Jpn. Kokai Tokkyo Koho 79 73,771* **1979**. *(CA 91:P193174v)*
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.

Chapter in a book: only the main title should be given, with the chapter author's name and the editor´s name after the title (this in italic):

10. Regitz, M. In *Multiple Bonds and Low Coordination in Phosphorus Chemistry*; Regitz, M.; Scherer, O. J., eds.; Georg Thieme Verlag: Stuttgart, Germany, 1990, ch. 2.

- **Software**

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

- **Web Pages**

12. http://www.sbq.org.br/jbcs, accessed in June 2013.

- **Unpublished material Reference**

For material **accepted** for publication: in this case, the DOI number should be provided by the authors.

13. Magalhães, U. H.; *J. Braz. Chem. Soc.*, DOI xx.

For other reference examples, see "PDF" files in: http://jbcs.sbq.org.br/forthcoming_papers.asp

- **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 PDF 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 PDF file, containing GA, text with tables and figures, and SI.

9. Procedure for Manuscript Submission

- Manuscript Submission

  The ***JBCS*** submission offers **only** online submission.

- Submissions after July 8, 2013 must be made using the ScholarOne^{TR} system by clicking the corresponding link on our website http://jbcs.sbq.org.br/.

  In the ScholarOne platform, it is necessary to upload:

  1. Main document (full.doc), including all figures, tables and their legends, added just after the first citation.
  2. All original figure files, including GA image, these in jpg, tiff, opj, xls, etc., need to be uploaded SEPARATELY.

  For example: see that if you have 10 figures, you need to upload the 10 original files (opj, xls, tiff, etc.) AND the main document (full.doc with figures added inside).

  - In case that the figure is an image, this needs to be in high resolution.
  - Please, do not send the figures inserted in a .doc file, send all the original files (opj, xls, tiff, etc.). This will accelerate the evaluation of your manuscript and the publishing process, in case it is accepted for publication.

  3. For works with SI section, add the SI section in the end of the full.doc file AND also upload the figure files SEPARATELY.

- For manuscripts submitted before July 8, 2013, send your Revised Version (V2, V3, etc)] using the OLD SYSTEM (http://jbcs.sbq.org.br/). Final Version is mandatory only for submission before July 8, 2013, in the old system.

  Being your manuscript (evaluated as a PDF file in the old system) accepted, the ***JBCS*** needs your revised final open files (as .doc, .tiff, .xls, for the main manuscript and figures) to produce the proof. So, the follow the steps:

  1. Once logged in, choose the Author folder;
  2. Click on Manuscript Title;

3. Click on Send the Final Version

Text and Tables: Prepare individual files with text and tables. Use Word for Windows and save as *.doc. For other operating systems (Macintosh and Unix), save the file in *.rtf (Rich Text Format).

Graphics, Figures, Schemes: send them in the original program FILES:

Structures *.cdx (ChemDraw or ISIS-DRAW);

Graphics *.opj/org (Origin); *.xls/xlsx (Excel);

Others *.cdr (CorelDraw);

It is important that the Graphics, Figures, Schemes files are editable to allow any minor mistake correction.

Photos and Similar Images: only if necessary, scan them with a minimum of 900 dpi resolution as black&white drawing. Save them as *.tiff. Otherwise, save as *.eps; *.wmf .For Macintosh, only *.tiff is acceptable.

How to Name the Files: label the original files with the manuscript number and the corresponding description:

1. 559-09_full.doc (or*.rtf) containing text, tables, equations, etc. pasting figures and schemes at the end;
2. figure1.opj (or *.xls; *cdr; etc);
3. scheme2.cdx (or *.cwg; etc), etc.

Any problems with your file, the ***JBCS*** staff will contact you by e-mail.

How to Send your Files:

1. Access your Author's ***JBCS*** homepage with your login and password.
2. In the section Here to Send Final Version Files, click into manuscripts Title Link.
3. There you will find the link Send Final Version Files to upload the files, one by one. Alternatively you can zip all files in one, but be absolutely sure to have all necessary files into the zipped one.
4. After uploading all files, check the links online. The system works as a virtual disk and does not close, allowing you to upload extra files if requested or if you noted any missing one.

10. Galley Proofs - GP

The ***JBCS*** Journal Publishing Staff will contact you in the near future regarding your manuscript page proofs (GP).

The proofs are provided for the correction of printing errors only, i.e.,the proof correction should not be used for language or content improvement. If considered excessive, the change costs will be charged to the author(s).

Corrected galley proofs should be returned as soon as possible (within 72 h or in 3 business days).

Your manuscript will be published on the web only after you approve your page proofs.

ANEXO 3

CARTA DE SUBMISSÃO ARTIGO
Revista *Ciência e Agrotecnologia*

De: Renato Paiva <suporte.aplicacao@scielo.org>
Para: Dr. Makoto Matsushita <mmakoto@uem.br>
Enviadas: Mon, 16 Sep 2013 07:26:43 -0300 (BRT)
Assunto: [C&A] Agradecimento pela Submissão

Dr. Makoto Matsushita,

Comunicamos o recebimento do manuscrito intitulado "DESENVOLVIMENTO, CARACTERIZAÇÃO E ANÁLISE QUIMIOMÉTRICA DE BARRA ALIMENTÍCIA SEM GLÚTEN CONTENDO FARINHA DE UM NOVO CULTIVAR DE AMARANTO" submetido a Ciência e Agrotecnologia. Pela interface de usuário do sistema, utilizado para a submissão, será possível acompanhar o progresso do documento dentro do processo editorial, bastando logar no sistema localizado em:

URL do Manuscrito:
submission.scielo.br/index.php/cagro/author/submission/123799
Login: mmakoto

Em virtude do grande número de manuscritos recebidos exceder a capacidade de publicação bimestral da revista, estes são analisados pela Comissão Editorial, antes de serem submetidos à assessoria científica. Por esse mesmo motivo, a revista não aceita pedido de reconsideração.
Nessa análise, considera-se o escopo; apresentação do artigo segundo as normas da revista; formulação do objetivo de forma clara; clareza da redação; fundamentação teórica; atualização da revisão da literatura; coerência e precisão da metodologia; discussão dos fatos observados em relação aos descritos na literatura; resultados com contribuição significativa; qualidade das tabelas e figuras; originalidade e consistência das conclusões.
Após essa análise, é aplicado o critério da relevância comparativa aos manuscritos que atendam aos requisitos de qualidade. Segundo esse critério, somente os manuscritos que apresentam contribuição mais significativa para o avanço do conhecimento científico são avaliados pela assessoria científica.

Atenciosamente,
Secretaria Administrativa
Ciência e Agrotecnologia

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ANEXO 4

NORMAS PARA PUBLICAÇÃO ARTIGOS
Revista *Ciência e Agrotecnologia*

NORMAS PARA PUBLICAÇÃO DE ARTIGOS CIENTÍFICOS

1. Os conceitos e afirmações contidos nos artigos serão de inteira responsabilidade do(s) autor(es).

2. A Revista "Ciência e Agrotecnologia", editada bimestralmente pela Editora da Universidade Federal de Lavras (Editora UFLA), publica artigos científicos nas áreas de "Ciências Agrárias, Ciência e Tecnologia de Alimentos, Economia e Administração do Agronegócio, Engenharia Rural, Medicina Veterinária e Zootecnia", elaborados por membros da comunidade científica nacional e internacional. É condição fundamental que os artigos submetidos à apreciação da "Revista Ciência e Agrotecnologia" não tenham sido e nem serão publicados simultaneamente em outro lugar. Com a aceitação do artigo para publicação, os editores adquirem amplos e exclusivos direitos sobre o artigo para todas as línguas e países. A publicação de artigos dependerá da observância das Normas Editoriais, dos pareceres do Corpo Editorial e da Comissão *ad hoc*. Todos os pareceres têm caráter sigiloso e imparcial e, tanto os autores, quanto os membros do Corpo Editorial e/ou Comissão *ad hoc* não obtêm informações identificadoras entre si.

3. **Custo para publicação**: O custo da publicação é de R$30,00 (trinta reais) por página editorada (página impressa no formato final) até seis páginas e R$60,00 (sessenta reais) por página adicional. No encaminhamento inicial, efetuar o pagamento de R$80,00 (oitenta reais), **não reembolsável**, valor esse a ser descontado no custo final do artigo editorado (formato final). Por ocasião da submissão, deverá ser encaminhado o comprovante de depósito ou transferência bancária a favor de FUNDECC/Editora, Banco do Brasil, agência 0364-6, conta corrente 58.382-0. **O comprovante de depósito ou transferência bancária deve ser anexado no campo "Transferência de Documentos Suplementares".**

4. Os artigos submetidos para publicação deverão ser encaminhados via **eletrônica** (www.editora.ufla.br), editados em **língua inglesa** e usar somente nomenclaturas oficiais e abreviaturas consagradas. O trabalho deverá ser digitado no processador de texto Microsoft Word for Windows (versão 98, 2000, 2003 ou XP), tamanho A4 (21cm x 29,7cm), espaço duplo entre linhas, fonte: Times New Roman, tamanho: 12, observada uma margem de 2,5 cm para o lado esquerdo e de 2,5 cm para o direito, 2,5 cm para margem superior e inferior, 2,5 cm para o cabeçalho e 2,5 cm para o rodapé. Cada trabalho deverá ter no **máximo 16 páginas** e junto do mesmo deverá ser encaminhado ofício dirigido ao Diretor da Editora UFLA, solicitando a publicação do artigo. Esse ofício deverá ser assinado por todos os autores, constar nome dos autores sem abreviação, a titulação e o endereço profissional completo (rua, nº, bairro, caixa postal, cep, cidade, estado) telefone e email de todos; **ao submeter o artigo, o ofício deverá ser anexado no campo "Transferência de Documentos Suplementares".** Qualquer inclusão, exclusão ou alteração na ordem dos autores deverá ser notificada mediante ofício assinado por todos os autores (inclusive do autor excluído).

5. O **artigo científico** deverá conter os seguintes tópicos: a) **TÍTULO** (em letras maiúsculas) **em inglês e português**, escrito de maneira clara, concisa e completa, sem abreviaturas e palavras supérfluas. Recomenda-se começar pelo termo que represente o aspecto mais importante do trabalho, com os demais termos em ordem decrescente de importância; b) **NOME(S) DO(S) AUTOR(ES)** listados no lado direito, um debaixo do outro, **sendo o máximo de 6** (seis); c) **ABSTRACT** não deve ultrapassar **250** (duzentos e cinquenta) palavras e estar em um único parágrafo. **Deve conter pelo menos, breve introdução, objetivo e resultados**; d) **INDEX TERMS** contendo entre 3 (três) e 5 (cinco) palavras-chave em inglês que identifiquem o conteúdo do artigo, diferentes daquelas constantes no título e separadas por vírgula; e) **RESUMO** (tradução para o português do abstract); f) **TERMOS PARA INDEXAÇÃO** (tradução para o português do index terms); g) **INTRODUCTION** (incluindo a revisão de literatura e objetivo); h) **MATERIAL AND METHODS**; i) **RESULTS AND DISCUSSION** (podendo conter tabelas e figuras); j) **CONCLUSION**; k) **ACKNOWLEDGEMENTS** (opcional); l) **REFERENCES (sem citações de teses e dissertações)**.

6. **RODAPÉ**: Deve constar formação, titulação, instituição de vínculo empregatício, contendo endereço comercial completo (rua, número, bairro, Cx. P., CEP, cidade, estado) e e-mail do autor correspondente. Os demais autores devem informar a formação, titulação e instituição de vínculo empregatício.

7. **AGRADECIMENTOS (acknowledgements)**: ao fim do texto e, antes das Referências Bibliográficas, poderão vir os agradecimentos a pessoas ou instituições. O estilo, também aqui, deve ser sóbrio e claro, indicando as razões pelas quais se fazem os agradecimentos.

8. **TABELAS E QUADROS**: deverão ser feitos no Word e inseridos após citação dos mesmos dentro do próprio texto, salvo em doc.

9. **CASO O ARTIGO CONTENHA FOTOGRAFIAS, GRÁFICOS, FIGURAS, SÍMBOLOS E FÓRMULAS, ESSAS DEVERÃO OBEDECER ÀS SEGUINTES NORMAS:**

9.1 **Fotografias** podem ser **coloridas ou em preto e branco**, nítidas e com contraste, inseridas no texto, após a citação das mesmas, **salvas em extensão "TIFF" ou "JPEG" com resolução de 300 dpi.** Na versão impressa da revista, as fotografias sairão em **preto e branco**.

9.2 **Figuras** podem ser **coloridas ou em preto e branco**, nítidas e com contraste, inseridas no texto, após a citação das mesmas, **salvas em extensão "TIFF" ou "JPEG" com resolução de 300 dpi.** As figuras deverão ser elaboradas com letra **Times New Roman, tamanho 10, sem negrito; sem caixa de textos e agrupadas.** Na versão impressa da revista, as figuras sairão em **preto e branco**.

9.3 **Gráficos** deverão ser inseridos no texto após a citação dos mesmos. Esses deverão ser elaborados preferencialmente em Excel, com letra Times New Roman, tamanho 10, **sem negrito, salvos em extensão XLS e transformados em TIFF ou JPG,** com resolução de 300 dpi.

9.4 **Símbolos e Fórmulas Químicas** deverão ser feitas em processador que possibilite a formatação para o programa **Page Maker** (ex: MathType, Equation), sem perda de suas

formas originais.

10. **REFERÊNCIAS BIBLIOGRÁFICAS**: a partir do Volume 18, Número 1 de 1994, a normalização das referências bibliográficas é baseada na NBR6023/2002 da ABNT.

A exatidão das referências constantes da listagem e a correta citação no texto são de responsabilidade do(s)autor(es) do artigo.

Orientações gerais:

- Devem-se apresentar todos os autores do documento científico (fonte);
- O nome do periódico deve ser descrito por extenso, não deve ser abreviado;
- Em todas as referências deve-se apresentar o local de publicação (cidade), a ser descrito no lugar adequado para cada tipo de documento;
- As referências devem ser ordenadas alfabeticamente e "alinhadas à margem esquerda", conforme NBR6023/2002 (ABNT, 2002, p.3).
- Deve-se deixar espaçamento simples nas entrelinhas e duplo entre as referências.

EXEMPLIFICAÇÃO (TIPOS MAIS COMUNS):

ARTIGO DE PERIÓDICO:

DINIZ, E.R.; SANTOS, R.H.S.; URQUIAGA, S.S.; PETERNELLI, L.A.; BARRELLA, T.P.; FREITAS, G.B. de. Crescimento e produção de brócolis em sistema orgânico em função de doses de composto. **Ciência e Agrotecnologia**, Lavras, v.32, n.5, p.1428-1434, set./out. 2008.

LIVRO:

a) Livro no todo:

FERREIRA, D.F. **Estatística multivariada**. Lavras: UFLA, 2008. 672p.

b) Parte de livro com autoria específica:

BERGEN, W.G.; MERKEL, R.A. Protein accretion. In: PEARSON, A.M.; DUTSON, T.R. **Growth regulation in farm animals**: advances in meat research. London: Elsevier Science, 1991. v.7, p.169-202.

c) Parte de livro sem autoria específica:

JUNQUEIRA, L.C.; CARNEIRO, J. Tecido muscular. In: _____. **Histologia básica**. 11.ed. Rio de Janeiro: Guanabara Koogan, 2008. 524p.

DISSERTAÇÃO E TESE:

Não utilizar citações de dissertações e teses.

TRABALHOS DE CONGRESSO E OUTROS EVENTOS:

Não utilizar citações de trabalhos de congressos e outros eventos.

DOCUMENTOS ELETRÔNICOS:

As obras consultadas *online* são referenciadas conforme normas específicas para cada tipo de documento, **acrescidas de informações sobre o endereço eletrônico apresentado entre braquetes (< >), precedido da expressão "Disponível em:" e da data de acesso ao documento, precedida da expressão "Acesso em:"**.

Nota: "Não se recomenda referenciar material eletrônico de curta duração nas redes" (ABNT, NBR6023/2000, p. 4). Segundo padrões internacionais, a divisão de endereço eletrônico, no fim da linha, deve ocorrer sempre após barra (/).

a) Livro no todo
TAKAHASHI, T. (Coord.). **Tecnologia em foco**. Brasília, DF: Socinfo/MCT, 2000. Disponível em: <http// www.socinfo.org.br>. Acesso em: 22 ago. 2000.

b) Parte de livro
TAKAHASHI, T. Mercado, trabalho e oportunidades. In: _____. **Sociedade da informação no Brasil**: livro verde. Brasília, DF: Socinfo/MCT, 2000. cap.2. Disponível em: <http://www.socinfo.gov.br>. Acesso em: 22 ago. 2000.
c) Artigo de periódico (acesso online):
JASPER, S.P.; BIAGGIONI, M.A.M.; RIBEIRO, J.P. Avaliação do desempenho de um sistema de secagem projetado para os pequenos produtores rurais. **Ciência e Agrotecnologia**, Lavras, v.32, n.4, p.1055-1061, jul./ago. 2008. Disponível em: <http://www.editora.ufla.br/revista/32_4/(04)%20Artigo%204193.pdf>. Acesso em: 25 nov. 2008.

CITAÇÃO: PELO SISTEMA ALFABÉTICO (AUTOR-DATA) (baseado na ABNT, NBR10520/2002)

Dois autores - Silva & Leão (2008) ou (Silva & Leão, 2008).
Três ou mais autores - Ribeiro et al. (2008) ou (Ribeiro et al., 2008).
Obs.: Quando forem citados dois autores de uma mesma obra deve-se separá-los pelo sinal & (comercial). Se houver
mais de uma citação no mesmo texto, deve-se apresentar os autores em ordem cronológica crescente, por exemplo: Souza
(2004), Pereira (2006), Araújo (2007) e Nunes Júnior (2008); ou: (Souza, 2004; Pereira, 2006; Araújo, 2007; Nunes Júnior,
2008).

11. **Processo para publicação de artigos**: O artigo submetido para publicação, será encaminhado ao Conselho Editorial, para que seja inicialmente avaliado quanto à relevância comparativa a outros manuscritos da área de conhecimento submetidos para publicação. Apresentando relevância comparativa, o artigo é avaliado por consultores 'ad hoc' para emitirem seus pareceres. Aprovado por consultores e, caso necessário, o artigo é enviado ao autor correspondente para correções e/ou sugestões. Caso as correções não sejam retornadas à revista no prazo solicitado, a tramitação do artigo será automaticamente cancelada. O não atendimento as solicitações dos consultores sem justificativas também leva ao cancelamento automático do artigo. Após a aprovação das correções, o artigo é revisto quanto a Nomenclatura Científica, Inglês, Referências Bibliográficas e Português, sendo então encaminhado para editoração e publicação.

ANEXO 5

PARECER N° 692/2010

Comitê de Ética em Pesquisa Envolvendo Seres Humanos

Universidade Estadual de Maringá
Pró-Reitoria de Pesquisa e Pós-Graduação
Comitê Permanente de Ética em Pesquisa Envolvendo Seres Humanos

CAAE Nº. 0433.0.093.000-10 **PARECER Nº. 692 /2010**

Pesquisador(a) Responsável: Makoto Matsushita

Centro/Departamento: CCE/Departamento de Química

Título do projeto: Desenvolvimento e caracterização de alimentos, isento de glúten, contendo quino A (Chenopodium quinoa, BRS Piabiru) e/ou amaranto (Amaranthus cruentus, BRS Alegria)

Avaliação do Protocolo de Pesquisa:

A pesquisa pretende desenvolver, avaliar e analisar alimentos prontos para o consumo humano, isentos de glúten, elaborados à base de quinoa e amaranto, tendo em vista seus aspectos nutricionais, tecnológicos e sensoriais. Para tanto, pretende aplicar o Teste de Aceitação em 80 sujeitos provadores voluntários, não treinados e possíveis consumidores dos produtos avaliados.

Apresenta cronograma compatível com o desenvolvimento da pesquisa. O orçamento envolve gastos na ordem de R$ 251,00, sob a responsabilidade dopesquisador.

O Termo de Consentimento Livre e Esclarecido atende às disposições da Resolução 196/1996-CNS.

Ante o exposto, o COPEP é de parecer favorável à realização da presente pesquisa. SMJ, é o parecer.

SITUAÇÃO: **APROVADO**

CONEP: (X) para registro ( ) para análise e parecer Data: **19/11/2010**

Relatório Final para Comitê: ( ) Não (X) Sim Data: **1/03/2012**

O protocolo foi apreciado de acordo com a Resolução nº. 196/96 e complementares do CNS/MS, na 207ª reunião do COPEP em 19/11/2010.

Profa. Dra. Ieda Harumi Higarashi
Presidente do COPEP