FORMULATION AND EVALUATION OF GLUTEN-FREE COOKIES FROM RICE AND GREEN BANANA FLOUR

Formulação e avaliação de biscoitos isentos de glúten a partir de farinhas de arroz e de banana verde

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ABSTRACT: Celiac disease (CD) is defined as an autoimmune pathology mediated by specific antibodies and is associated with genetic factors, which causes an inflammatory process of the intestinal mucosa in response to gluten ingestion. A study was conducted to evaluate some gluten-free cookie formulations using rice and green banana flour as raw material, and to evaluate the acceptance and chemical composition of the cookies elaborated. Three different cookie formulations were prepared to contain 100% rice flour, 50% rice flour and 50% green banana flour and 100% green banana flour. The following sensory attributes were evaluated: crispness, flavor, and overall impression, using the 9-point hedonic scale and purchase intention using the 5-point structured scale, to check the acceptance of cookies. The chemical composition of the formulation most accepted by sensory analysis was quantified. Sensory evaluation showed that all cookie formulations were accepted; however, the increase of the green banana flour content has resulted in lower acceptance, which may be due to the slightly bitter flavor that green banana flour gives the product. The most accepted cookie formulation had 15.78% protein, 0.80% ash, 4.31% moisture content, 12.35% lipid, and 66.77% carbohydrate. These nutritional characteristics are following Brazilian legislation. Therefore, all cookie formulations have marketing potential, which is very important, especially for people with gluten sensitivity.

Key words: Food intolerance. Autoimmune diseases.Celiac disease.

RESUMO: A doença celíaca (DC) é definida como uma patologia autoimune mediada por anticorpos específicos e está associada a fatores genéticos, que causam um processo inflamatório da mucosa intestinal em resposta à ingestão de glúten. Foi realizado um estudo para avaliar algumas formulações de biscoitos sem glúten usando farinha de arroz e banana verde como matéria-prima, e avaliar a aceitação e composição química dos biscoitos elaborados. Três formulações diferentes de biscoitos foram preparadas contendo 100% de farinha de arroz, 50% de farinha de arroz e 50% de farinha de banana verde e 100% de farinha de banana verde. Foram avaliados os seguintes atributos sensoriais: crocância, sabor e impressão geral, utilizando a escala hedônica de 9 pontos e intenção de compra utilizando a escala estruturada de 5 pontos, para verificar a aceitação dos biscoitos. Foi quantificada a composição química da formulação mais aceita pela análise sensorial. A avaliação sensorial mostrou que todas as formulações de biscoitos foram aceitas; entretanto, o aumento do teor da farinha de banana verde resultou em menor aceitação, o que pode ser devido ao sabor levemente amargo que a farinha de banana verde confere ao produto. A formulação de biscoito mais aceita tinha 15,78% de proteína, 0,80% de cinza, 4,31% de umidade, 12,35% de lipídio e 66,77% de carboidrato. Essas características nutricionais estão de acordo com a legislação brasileira. Portanto, todas as formulações de biscoitos têm potencial de comercialização, o que é muito importante, principalmente para pessoas com sensibilidade ao glúten.

Palavras-chave: Intolerância alimentar. Doenças autoimunes. Doençacelíaca.

INTRODUÇÃO

Celiac disease (CD) is a serious autoimmune disease that occurs in genetically predisposed people where the ingestion of gluten causes an inflammatory process of the intestinal mucosa (SILANO et al., 2016). About 1% of the world's population consists of celiac people (KELLY et al., 2015). In Europe, the disease affects about 0.5% to 1% of the population, whereas in Brazil celiac disease is not well established, but it is estimated to affect 1 in 681 people (NASCIMENTO; BARBOSA; TAKEITI, 2012). However, there is still a deficiency in its diagnosis and is thus considered a neglected disease, showing itself as a disease of low prevalence, mortality and incidence (PAULA; CRUCINSKY; BENATI, 2014)

Ingestion of gluten-containing foods may cause manifestations of hypersensitivity, leading to intolerance to this protein compound and because of malabsorption syndrome, which is common in celiac people. A person with the celiac disease begins to show signs such as malnutrition, fatigue, weight loss, diarrhea, bloating, flatulence and steatorrhea. However, diarrhea is the most common symptom of celiac disease. The treatment indicated for people with celiac disease is based on the removal of gluten from the diet (FASANO; CATASSI, 2012; GONÇALVES et al., 2013).

However, a gluten-free diet is a complex practice because the availability of gluten-free products in markets is limited, has high prices, and has lower sensory, technological, and nutritional quality. The food industry has difficulty producing quality gluten-free foods that please consumers (HERA; MARTINEZ; GOMÉZ, 2013).

Therefore, the elaboration of new food products with alternative, functional and gluten-free raw material is essential to facilitate the diet of celiac people, as well as to offer quality food. As an example of alternative raw material, have the use of rice flour and green banana flour in the production of gluten-free cookies (DEMIRKESEN et al., 2010).

Thus, the study was conducted to evaluate some gluten-free cookie formulations using rice flour (RF) and green banana flour (GBF) as raw material, and to evaluate the acceptance and chemical composition of the cookies elaborated.

MATERIAL E MÉTODOS

Elaboration of gluten-free cookies:

The samples of cookies were formulated in the Laboratory of Dietary Techniques of the Centro Universitário INTA (UNINTA), and all ingredients used in the preparation of cookies were purchased in commercial establishments in the city of Sobral, CE, Brazil. Three cookie formulations with different percentages of rice flour and green banana flour were elaborated (Table 1).

Each formulation resulted in 100 cookies with an average weight of 5 grams. Due to the rheological properties of green banana flour, which has an elastic to viscous character (TRIBESS, T. B, 2009), less green banana flour was used in biscuits of sample GBF than rice flour in sample RF (Table 1). Initially, the alternative raw materials were weighed, and then vegetable shortening, sugar, and egg yolks were added in a plastic bowl. The ingredients were manually mixed until homogeneous, then flour, raisins and baking powder were added.

The cookie dough was rolled and cut and then baked in an oven at 180 °C for 15 minutes. They were then cooled to room temperature and packaged in hermetically sealed 25-gram polyethylene containers and stored at room temperature. The elaboration flowchart was performed as proposed by Backi (2011).

Microbiological Analyzes:

Following the microbiological standards established by RDC No. 12/2001, the presence of *Salmonella* sp./25 mL of the sample, total and thermotolerant coliform counts (45 °C/g) and coagulase-positive Staphylococciwere analyzed (BRASIL, 2001). Five packages of each cookie formulation were used for microbiological analysis.

Salmonella sp./25 mL analysis followed the methodology described by the Food and Drug Administration (FDA, 2016). Total and thermotolerant coliform counts were performed according to the methodology described by the American Public Health Association (APHA, 2001). Coagulase-positive Staphylococci (CoPS) counts were performed by the surface plating method.

Standard Count in Plates of Coagulase-Positive Staphylococci (CoPS):

A 25-gram sample was taken, placed in a sterile container, and 225 mL of 0.85% sterile saline was added, dilution10^{-1}.0.1 mL of 0.85% sterile saline was inoculated into Petri dishes containing Baird-Parker Agar (BP) using the 10^{-1} dilution. With the aid of Drigalski's loop, the sample was spread evenly over the surface of the culture medium. The plates were incubated at 35 °C for 48 hours. Typical colonies (black, shiny, opaque ring and surrounded by a clear halo) were then counted and the results expressed in colony-forming units per gram of cookie (CFU/g).

Presence of *Salmonella*spp:

A sample of 25 grams of the cookie was taken, placed in a sterile container, and 225 mL of sterile lactose broth was added (10^{-1} dilution), homogenized for 2 minutes, and then the material was incubated for 24 hours at 35 °C, a pre-enrichment step. Subsequently, 1.0 mL of the pre-enriched dilution was transferred to test tubes containing 10 mL of Tetrationate broth and incubated for 48 ± 2 hours at 35 °C. After 48 hours, the broth was striated with the aid of a platinum loop in Petri dishes prepared with *Brilliant* Green Bile Agar (BGB), which were incubated for 35 hours at 35 °C. The plates were then examined for the presence of *Salmonella* colonies.

Quantification of Total and Thermotolerant Coliforms:

The most probable number of multiple tube technique (MPN/g) was used. A 1.0 mL sample of dilution 10^{-1} was transferred to a series of three Duhram tubes containing sterile lactose broth, which were incubated at 35 ± 1 °C for 48 ± 2 hours in a bacteriological oven. After incubation, the analysis of the tubes was performed, and the tubes that presented medium turbidity and gas production were considered positive.

Sensory Evaluation:

The sensory evaluation consisted of judging the quality of prepared cookies by a panel of 53 non-trained untrained female and male judges, aged between 18 and 65 years. The evaluation deals with analyzing the overall sensory quality of cookies as perceived by the sense of sight, taste, and touch. A hedonic scale rating test was used to measure the degree of pleasurable and un-pleasurable experience of cookies on a scale of 9 points from "dislike extremely" (score 1) to "like extremely" (score 9). The panellists were given an evaluation form which listed various sensory parameters (crispness, flavor, and overall acceptability) and score options with number rankings.For the purchase intention sensory attribute, a 5-point structure scale was used. The samples were rated based on the criteria: 1 - "certainly not buy", 3 - "maybe buy/maybe not buy", and 5 - "certainly buy," (DUTCOSKY, 2013).

Each evaluator received three samples of approximately 5.0 g each, randomly distributed on disposable napkins and identified with random three-digit codes, between one sample and another drinking water was provided at room temperature to clean the taste buds.

This study was submitted and approved by the Ethics and Research Committee (CEP) of the University Center INTA - UNINTA under the number CAAE: 96850118.9.0000.8133 and followed all the ethical principles that guide scientific research, according to the National Health Council (NHC) 466/2012 (BRAZIL, 2013). All participants in this research signed theInformed Consent Document.

Physicochemical Analysis:

The following parameters characterized the most accepted biscuit formulation by sensory evaluation: moisture content in an oven at 105 °C to constant weight, proteins by Kjeldahl method, lipids by direct extraction in Soxleht, ashes by incineration in muffle furnace at 550 °C and total carbohydrates were estimated by calculating the difference (IAL, 2005).

Statistical Analysis:

Data were analyzed using SISVAR software version 5.6 and means compared by Tukey test at a 5% significance level.

RESULTADOS E DISCUSSÃO

Microbiological evaluation

The three cookie formulations produced from rice flour (RF) and green banana flour (GBF) met the microbiological standards established by Brazilian legislation, since the amount of coagulase-positive Staphylococciwas< 25 CFU/g, *Salmonella* spp. was not detected, and the number of thermotolerant coliforms (45 °C) was < 3.0 MPN/g. According to Brasil (2001), cookies are suitable for consumption when they have the following standards: the absence of *Salmonella* spp. at 25 grams, maximum 5.0×10^2 CFU/g and 10^2 MPN/g for coagulase-positive *Staphylococcus* and thermotolerant coliforms (45 °C), respectively. These results indicate that there was a careful hygienic-sanitary control during all stages of the processing and handling of cookies, which ensured the sanity of the products and the food safety of the sensory team.

Sensory evaluation

From the average sensory scores, it can be verified that none of the studied cookie formulations were rejected, since all hedonic values are within the acceptance range of the hedonic scale, i.e., all samples received a score higher than 5 (Table 2). It is considered as rejection range on the hedonic scale, scores between 1 and 4, score 5 represents indecision and scores between 6 and 9 represent product acceptance (IAL, 2005).

The rice flour cookie was the most accepted in all the sensory parameters analyzed. This is because green banana flour (GBF) was not used in its preparation. According to Drenownoski and Carneros (2000), green banana flour gives the food product a bitter taste, which justifies the lower acceptance of cookies containing green banana flour (GBF and RF+GBF). In this formulation, flavor was the parameter with the best score, but in spite of that we cannot fail to mention the parameters of crispness and overall acceptability that obtained averages above 6.5, having also been accepted by the judges. This result is of paramount importance, since for cookies to be accepted and appreciated by the consumer, they need to have a crunchy consistency, the loss of crunchiness leads to softening, which causes non-consumption of this product (ROUDAUT et al., 2002; BRAVIN et al., 2006).

A similar result was reported by Wang; Zhanga and Mujumdarb (2012), who found that cookies with green banana flour in different concentrations had the lowest acceptance. Regarding the rice flour cracker, the parameter that had the highest hedonic value was the flavor, which is justified by the fact that the rice flour has a mild flavor, giving no unpleasant taste to the product (SIVARAMAKRISHNAN; SENGE; CHATTOPADHYAY, 2004).

The results of this research suggest that all cookie formulations have the potential to be marketed since they obtained hedonic scores higher than 6.5 for all sensory parameters evaluated. As reported by Dutcosky (2013), hedonic averages higher than 6.5 have acceptance higher than 70%. Studies show that for a product to have potential for commercialization, it must obtain a minimum of 70% acceptability in all analyzed sensory parameters (TEIXEIRA; MEINERT; BARBETTA, 1987), a fact that occurred with all cookie formulations in this study.

This study emphasizes the potentiality of commercialization of the cookies studied since all are gluten-free, and if they are commercialized can be consumed by celiac people. This is because there is no adequate variety of gluten-free products on the market, making it difficult for people with this food restriction. Regarding purchase intention, hedonic values indicate that the rice flour cookie had the best result for this attribute (Table 3).

The results indicate that all cookie formulations were accepted regarding the purchase intention parameter, since they had a score higher than 3.2, being judged as "probably buying" or "certainly buying" on the 5-point structured scale. However, it appears that the rice flour cookie had a higher score compared to the other two cookie formulations, which is related to the use of rice flour. This is because rice flour has a mild flavor, aroma, and texture (POLANCO et al., 1995). The use of green banana flour in cookie formulations may have interfered with the purchase intention of the judges.

Some studies report that crackers made with rice flour and proportions of 20% and 30% green banana flour, sensory

tasters judged the samples as "maybe buy/maybe not buy", which corresponds to score 3. However, when the proportion of green banana flour was reduced to 10%, the result was "possibly buy" (SACHINI, 2011). This suggests that the lower the proportion of green banana flour, the better the consumer acceptance. Similar results were evidenced in the present study, in which the rice flour cookie was more accepted when compared to cookies containing green banana flour.

Physicochemical evaluation

The rice flour cookie was the most accepted by the sensory evaluation, and the physicochemical parameters are shown in Table 4. The moisture content of cookies was lower than 5.0%, which is within the standard established by the AgênciaNacional de VigilânciaSanitária (ANVISA), which is 15% as the maximum percentage value for humidity in cookies and crackers (BRASIL, 2005). The low moisture content of the cookies may also have contributed to the absence of microbial growth in the studied samples, giving stability to the cookie.

The rice flour cookies proved to be a good source of protein, which can be justified high protein content of this flour (from 7% to 9%), being protein, the second component found in higher concentration in rice flour (SEVERO; MORAIS; RUIZ 2010). The protein content obtained in this research was higher than the value found in a study conducted with cookies formulated with 50% extruded rice flour, which presented protein content ranging from 6.36 to 7.56 g/100 g cookie (LACERDA et al., 2009).

Another fact that may have contributed to the high protein content of cookies was the use of eggs in the preparation of cookies. The protein content of spaghetti produced with rice flour was 31.39%, and the high protein content justified by the use of eggs in its formulation (SILVA; BELLANI; FERREIRA, 2017).

The ash content (or mineral content) is below 1%. However, it is following the legislation, which determines a maximum content of 3.0% (BRAZIL, 2005). The mineral content of rice flour is influenced by the cultivation conditions and also by the processing steps. Also, the highest concentration of minerals is found on the outside of grain as in bran, and in lower concentration in polished grain (ITANI et al., 2002). This justifies the low ash content of the cookies analyzed since rice flour is produced from polished rice (SOUZA et al., 2013).

The lipid content of rice flour cookies was lower than that found in cookies formulated with wheat flour substitution by 10% of jatoba flour, in which the lipid content was between 27.99% and 28.38% (SILVA et al ., 2001). However, this study showed lipid content obtained similar to that reported in the study conducted with cookies made with defatted Baru flour, which was 11.87% (PINELLI et al., 2015). It is noteworthy that the lipid content present in food may vary according to the ingredients used in its preparation (MARIANI et al., 2015).

The lipid content of rice flour crackers can be attributed to the use of Vegetable shortening in the formulation since rice flour has a low lipid content since the highest percentage of this nutrient is present in higher concentration in the aleurone and germ layer. These layers are lost during the obtaining of flour (MARCO; ROSSELL, 2008).

Regarding the carbohydrate content, the value obtained (Table 4) can be justified by the fact that rice flour has in its composition high carbohydrate content, about 80%, being represented basically by starch. Due to this high carbohydrate content, Brazilian legislation states that flour can be traded as rice starch (SEVERO, MORAES; RUIZ, 2010). It is advisable to add fiber sourced ingredients to reduce carbohydrate content in products (MARIANI et al., 2015). Fiber content was not determined in this study and was therefore counted as carbohydrate.

Table 1 -Cookie formulation with rice flour (RF) and green banana flour (GBF).

Sample	Rice flour (g)	Green banana flour (g)	Sugar (%)*	Vegetable shortening (%)*	Egg yolks (%)*	Raisins (%)*	Baking powder (%)*
RF	750	–	50	25	21	7.8	1
GBF	–	470	50	25	21	7.8	1
RF+GBF	275	275	50	25	21	7.8	1

*In relation to the total weight used (w:w). Source: The Authors (2019).

Table 2 – Hedonic scores for the crispness, flavor and overall acceptability characteristics of cookies produced from rice flour (RF) and green banana flour (GBF).

Attributes	RF	GBF	RF+GBF
Crispness	8.11[b]± 0.87*	6.79[a]± 1.83*	7.39[a]± 1.74*
Flavor	8.22[b]± 0.72*	6.71[a]± 1.82*	7.33[a]± 1.71*
Overall acceptability	8.03[b]± 0.83*	6.84[a]± 1.67*	7.30[a]± 1.71*

Mean followed by distinct letters on the line indicate significant difference by Tukey test (p <0.05). * Standard deviation of the mean.

Table 3 –Average hedonic score for the purchase intention attribute of the different cookie formulations produced from rice flour (RF) and green banana flour (GBF).

Samples	Purchase intention
RF	4.60[a]± 0.57*
GBF	3.73[b] ± 1.21*
RF+GBF	4.09[b]± 1.10*

Mean followed by distinct letters on the line indicate significant difference by Tukey test (p <0.05). * Standard deviation of the mean. Source: The Authors (2019).

Table4 – Chemical composition of cookie most accepted by sensory evaluation(cookie produced from rice flour (RF).

Physicochemical parameters	RF
Moisture content(%)	4.31 ± 0.25*
Ash (%)	0.80 ± 0.001*
Lipids (%)	12.35 ± 0.39*
Protein (%)	15.78 ± 2.69*
Carbohydrates (%)	66.77 ± 2.16*

* Standard deviation of the mean. Source: The Authors (2019).

CONCLUSÕES

The biscuits formulated in this study have shown potential for commercialization and thus increase the diversification of gluten-free products in the market. Besides, green banana flour and rice flour have proved to be good alternatives for formulating these foods instead of wheat flour. It is concluded that the use of a lower proportion of green banana flour in the cookie formulation results in better product acceptance in all evaluated sensory parameters; therefore, more studies should be carried out in order to mitigate the low acceptance of green banana flour in food products.

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