Production of prebiotic gluten-free bread with red rice flour and different microbial transglutaminase concentrations: modeling, sensory and multivariate data analysis

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Abstract The aim of this study was to develop gluten-free bread formulated with red rice flour and microbial transglutaminase and prebiotic (inulin). First, the physicochemical analysis of minerals present in red rice flour was performed. Response surface methodology was used to analyze the effects of microbial transglutaminase (MTgase) [0.5; 1.0 and 1.5%] in combination with fermentation time (FT) [60; 80 and 100 min] on the quality parameters of gluten-free bread. Acceptance test was used to evaluate the sensory characteristics of breads together with multivariate analysis of data. The addition of MTgase increased bread volume, hardness and chewiness. However, the cohesiveness and springiness of all breads remained unaffected. The formulation (1.0% MTgase and 80 min FT) presented the best sensory attributes through PCA (principal component analysis) and greater acceptance. Overall, red rice flour, prebiotic and MTgase are promisingly useful ingredients for the production of gluten-free quality bread.

Keywords Microbial transglutaminase · Gluten replacer · Response surface methodology · Inulin · Sensory quality

Introduction

Among gluten-free foods, bread stands out (Jnawali et al. 2016). The development and/or improvement of gluten-free bread is a challenge for food technology area because of the unique role of gluten in yeast-leavened baked

products and in the bread-making process (Monthe et al. 2019). Gluten-free breads have, as basic ingredients, rice, cassava, corn, soybean flour and potato starch (Nagash et al. 2017). Some studies have suggested the use of other ingredients, such as, different cereals, or gums, enzymes, hydrocolloids, which provide viscoelastic properties to the dough that improve overall appearance, sensory properties, technological properties and shelf life (Gulsum et al. 2016).

There are many special rice cultivars that contain black, red and brown pigments (Shao et al. 2014). Red rice or “arroz da terra”, is a spontaneous form of *Oryza sativa* that has attracted consumers’ interest due to its taste, texture, nutritional value and high iron and zinc levels. Its red pigment is due to the presence of proanthocyanin which improves digestibility and has antioxidant action that can be beneficial for the being beneficial for the cardiovascular system (Li et al. 2016). In addition, red rice is a relevant diet component of several states of northeastern Brazil (Paraíba, Pernambuco, Ceará, Bahia, Alagoas), and since red rice has great economic and social importance, it was chosen as the main raw material in this study (Pereira 2004).

Red rice flour is a promising cereal ingredient for the preparation of gluten-free products replacing wheat flour due to its advantages of being natural, hypoallergenic and with bland taste. Since rice generally contains a small amount of prolamin, it is necessary to use appropriate additives such as gums, emulsifiers, and dairy products to increase the consistency or viscosity of the system (Das and Bhattacharya 2019).

An alternative to produce rice flour bread is the use of microbial transglutaminase, a low-cost enzyme of wide application in the food industry (Aaron and Torsten 2019). In the baking area, microbial transglutaminase causes the formation of covalent bonds among protein fractions,

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leading to an improvement in the viscoelastic properties, which has positive effect on the rheological behavior of dough. These bonds cause these proteins to retain air during fermentation, a role similar to that of gluten (Mohammadi et al. 2015).

The growing consumer demand for food products that are tasty and nutritious but also provide health benefits have encouraged, but also that provide health benefits have encouraged studies on prebiotics. The wide use of inulin in the food sector is based on its techno-functional attributes. Inulin is of great interest for the development of healthy products because it concurrently responds to a range of consumer requirements: it is fiber-enriched, prebiotic, low fat and low sugar (Stephen et al. 2017).

Inulin are the most widely studied functional ingredients in gluten-free breads (GFB) affecting beneficially its physical and sensory qualities and extending a shelf life (Capriles and Areas 2013). However, since the proteins of gluten-free rice flours are generally unable to retain gases during fermentation and baking therefore, the enzymes were often applied to improve the quality of gluten-free breads by promoting protein networks and elastic-like behaviour by protein cross-linking. The most commonly use enzymes in GFB production are the protein-connecting enzymes (microbial transglutaminase—TG) (Ziobro et al. 2016; Marco and Rosell 2008; Lazaridou et al. 2007).

In this context, the aim of this work was to study the effect of the interaction of red rice flour and the microbial transglutaminase enzyme in the production of prebiotic gluten-free breads, in order to contribute to the development of new products with high nutritional quality for gluten-intolerant individuals, generating an alternative for greater use of red rice in human nutrition.

Materials and methods

Materials

Red rice *Oryza sativa* L. (manufacturer Patoense-initial water content of approximately 11% w.b.) obtained from a local market of Campina Grande, PB, was used.

The raw materials used in the production of breads were: red rice flour, cassava starch (Yoki); water, sugar (União), dry yeast (Fleischmann) [*Saccharomyces cerevisiae*] flour improver (Zeas; containing corn starch, stearyl lactylate, ascorbic acid and amylase), apple vinegar (Minhoto), salt (Sosal), powdered milk (Nestle), egg (Só Ovos), canola oil (Soya), antimold (Adnor), inulin (Orafiti HPX Sweet mix-Beneo) and MTgase (Ajinomoto).

Flour preparation

Rice samples were submitted to grinding operation in batch process (10 g), in a laboratory mill (Tecnal, Brazil), with knives set to 1 mm, using 10 Mesh sieve. After grinding, rice flour with 11 g 100 g⁻¹ moisture content (wet basis) was packed in polyethylene airtight packaging, kept at room temperature of 25 °C ± 3.0 °C.

Flour characterization

Physicochemical characterization and determination of the red rice flour mineral composition

Moisture content was measured by AACC 44-16 and crude protein measurement was carried out by AACC 46-30 using correction index of 5.95 for nitrogen-to-protein conversion.

Fat content was determined using Soxhlet extractor (Velp Scientifica, Monza-Brianza, Italy) on 5 g of ground sample and diethyl ether as solvent. Red rice flour was also analyzed for starch (AOAC method 996.11) and total fiber (AOAC method 2009.01).

Water activity was measured by direct method at 25 °C through the Aqualab 3T Decagon Devices analyzer and fixed mineral residue (ashes) by total incineration of the organic matter in muffle furnace at 550 °C, as described in the official AOAC International method (2000).

Red rice flour color was evaluated using CR 300 digital colorimeter (Minolta, New Jersey, USA), determined according to the CIE-L* a* b* system (Commission Internationale L'Eclairage), according to methodology of Altamirano-Fortoul and Rosell (2011).

The caloric value was calculated using the Atwater and Woods (1896) coefficients, whose carbohydrates produce 4.0 kcal g⁻¹, lipids 9.0 kcal g⁻¹ and protein 4.0 kcal g⁻¹ protein.

Minerals were quantified by energy dispersive X-ray fluorescence, according to Gusmão et al. (2016) using ashes obtained from red rice flour. The fluorescent X-ray spectrometer used was EDX-720 model (Shimadzu, Japan).

Experimental design and gluten-free bread manufacturing

Bread without wheat flour with characteristics similar to traditionally produced sliced bread was produced by different combinations in the formulation of amylaceous base composed of 50% red rice flour and 50% cassava starch, totalizing 100% of farinaceous base.

Water (45%), canola oil (7.5%), egg (1.0%), inulin (3.0%), dry yeast (3.0%), sugar (5.8%), and salt (1.15%), powdered milk (11.5%), anti mold (0.3%), flour improver

(1.15%), apple vinegar (3.8%) concentrations were kept constant for all the seven gluten-free bread samples.

Gluten-free bread with red rice flour was produced using a 2^2 factorial design, with 4 factorial points (levels ± 1) and three central points (level 0), totaling 7 experiments, with MTgase (0.5, 1.0 and 1.5%) and fermentation time (60, 80 and 100 min) as experimental factors. A 95% confidence level was used. The ranges used in the work for microbial transglutaminase content and fermentation time were based on preliminary experiments and also on the literature on bread production (Scarnato et al. 2017; Mohammadi et al. 2015). Transglutaminase (MTgase) enzyme (E.C. 2.3.2.13-Activa STG-M, 20 and 34 U g⁻¹) was obtained from Ajinomoto, Brazil.

Ingredients were added based on starch and flour content. The enzyme MTgase was mixed to the dry flour (red rice flour and cassava starch) for 2 min; then the other ingredients were mixed in industrial mixer (Perfecta®) at medium speed for 10 min until dough formation. Dough portions of 600 g were weighed in loaf tins (170 mm, 7 mm, 6 mm). Tins were placed in chamber at 30 °C and 80% relative humidity for 60, 80 and 100 min, according to the experimental design. After fermentation, doughs were baked in an electric modular oven for 40 min at 190 °C. Breads were removed from moulds after a 60-min cooling period and weighed. They were then placed in polyethylene plastic bags and stored at 20 °C until analysis. Bread volume and texture were evaluated 24 h after baking. All breads were produced twice.

Gluten-free bread quality

The specific volume of breads was determined according to AACC methodology (AACC 2000). Specific volume was calculated using the volume/weight ratio, and results were expressed as cm³ g⁻¹.

For pH and acidity, 10 g of each sample were homogenized with 90 mL of distilled water (pH 6.5), and pH was determined using potentiometer model 0400 (Quimis, São Paulo, Brazil). Then, the suspension was titrated with 0.1 N NaOH solution to pH 8.5. Titratable acidity was expressed in mL of 0.1 N NaOH consumed by 10 g of bread (AOAC method 22.058).

Bread crumb samples were measured for color in the L*, a*, b* system using Minolta colorimeter CR-300 (Konica Minolta Business Technologies, Inc., Langenhagen/Hannover, Germany).

Crumb texture profile analysis was performed using TA.XT2i texture analyzer (Stable Micro Systems Ltd., Surrey, UK) according to AACC (2000) Approved Method 74-09. Samples were compressed in dual cycle using 36 mm cylinder probe with maximum compression of 40% at crosshead speed of 1.7 mm s⁻¹. Crumb hardness,

cohesiveness, springiness, and chewiness were calculated from a force x distance graph using the Texture Expert for Windows version 1 software. Analysis was performed for at least six replicates for each treatment and the average values were reported.

Sensory analysis

Sensory analysis of breads was carried out by a panel of 60 panelists, both male and female. Sensory attributes included appearance, color, aroma, taste, texture (softness) and overall acceptability. A nine-point hedonic scale was used to evaluate each sensory attribute of bread formulations; panelists scored on a scale from 1 (disliked extremely) to 9 (liked extremely). Each sample was served as slices at the same time, 2 h after baking. Samples were coded using random three-digit numbers and evaluated in individual booths at room temperature. Sensory profiles of the optimized gluten-free rice starch bread were assessed, and comparisons were made with reference wheat bread. As recommended by Lazaridou et al. (2007), gluten-free breads are considered acceptable if their mean scores for overall acceptability are above 5 (neither liked nor disliked).

The test was carried out with prior approval from the Ethics Committee for Research with human beings (CAAE-54102116.6.0000.5182), to meet the ethical and scientific requirements of Resolution 466/2012 of the National Health Council. Judges were aware of the research objectives, according to the Cleared and Informed Consent Form.

Statistical analysis

Data obtained in the experimental design were analyzed according to response surface methodology using the Statistica software 5.0 (StatSoft, Inc., Tulsa, OK, USA); For each response obtained, Analysis of Variance was performed through linear regression to verify the influence of factors on the values obtained and to verify if there were significant differences ($p < 0.05$) among treatments. In cases where there was statistically significant difference, response surfaces were generated in order to visualize the optimization range.

The acceptance test results were submitted to analysis of variance (ANOVA) and Tukey's test, considering 5% significance level. For a better understanding of the sensory characteristics of data obtained, the Principal Component Analysis (PCA) multivariate technique was applied, using the STATISTICA version 5.0 software. Through PCA, the outputs are projected onto a new coordinate system (eigen space), which is constructed by the eigenvector (main components).

Results and discussion

Red rice flour composition

The results of the physicochemical, physical and mineral composition characterization of red rice flour are shown in Table 1. The properties of red rice flour are very important for making gluten-free bread. The physicochemical composition of flour shows that it is a basically starchy food, with considerable carbohydrate, protein and fiber values, and low lipid and ash values. Severo et al. (2010) found the following values for the proximal composition of red rice flour: 7.34 g 100 g⁻¹ protein, 0.6 g 100 g⁻¹ lipid and 0.69 g 100 g⁻¹ ash; Sompong et al. (2011) obtained 7.56 g 100 g⁻¹ protein, 0.32 g 100 g⁻¹ lipid, 0.27 g 100 g⁻¹ ash and 4.5 g 100 g⁻¹ fiber; in this work, protein, ash, lipid and fiber values obtained were higher.

Frank et al. (2012) attributed to red rice flour higher protein contents when compared to white rice flour. Higher protein content in flour will be able to form bonds with greater starch content, thereby increasing the water holding capacity of the flour. High protein content improves the nutritional and textural profile of product prepared from such flour (Rosniyana and Hazila 2013). Wu et al. (2019) found values of 4.0–4.4 g 100 g⁻¹ of crude protein for white rice flour prepared by different milling method. Puppo et al. (2005) reported protein values of 10.9 g 100 g⁻¹ for wheat flour. Although wheat flour has a higher protein value, the value found in this study for red rice flour

is coming, and considering the justification for the production of gluten-free bread with the use of microbial transglutaminase, red rice flour has a satisfactory protein value.

Thus, the substitution of wheat flour for red rice flour in bakery products is justified by: (1) for the possibility of improvement in nutritional and sensorial characteristics in the final product; (2) due to the cost of red rice flour, which is a byproduct of the processing of rice, it is much lower than that of wheat flour. (3) because Brazil is practically self-sufficient in rice production, while wheat is largely imported; (4) increase the demand for gluten-free products for the population.

Model fitting

According to the experimental design, Table 2 presents the results of response: color (L*, + a* and + b*), water content, pH, acidity and specific volume and texture parameters for breads formulated with red rice flour, microbial transglutaminase enzyme and prebiotic (inulin). For water content, color (L*, a* and b*), elasticity and cohesiveness attributes, it was not possible to establish significant models, that is, experimental data did not fit the model (1st order), which indicates that, despite variations in enzyme concentrations and fermentation time, these did not influence the characteristics described, obtaining a uniform product for all study treatments. Values of pH, acidity, specific volume, firmness and chewiness variables fitted the 1st order model.

The experimental factors and response fitted the 1st order response surface model and tested for lack of fit on the regression model. The analysis of variance regarding the effects of MTgase and fermentation time of gluten-free red rice breads on the response variables is shown in Table 3. The statistical analysis indicated that the models were adequate because they have satisfactory R² values and the F value calculated was greater than the tabulated F value (9.28) and model significance.

Figure 1 presents the graphs of response surfaces generated based on the response variable: pH, acidity, specific volume, hardness and chewiness; the coded models (Response = $\beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_1x_2$) are represented in Eqs. 1, 2, 3, 4 and 5, respectively, with statistically significant coefficients in bold.

$$\text{pH} = 6.085 - 0.14 \text{ MTgase} - 0.32 \text{ FT} + 0.15 \text{ MTgase.FT} \quad (1)$$

$$\text{Acidity} = 3.93 - 0.11 \text{ MTgase} + 0.08 \text{ FT} - 0.10 \text{ MTgase.FT} \quad (2)$$

Table 1 Physicochemical, physical characterization and mineral composition of red rice flour

Analysis	Mean value $\pm$ standard deviation
Moisture content (g 100 g ⁻¹)	11.0 $\pm$ 0.01
Aw (water activity)	0.56 $\pm$ 0.01
Ash (g 100 g ⁻¹)	1.07 $\pm$ 0.05
Protein (g 100 g ⁻¹)	8.00 $\pm$ 0.01
Fat (g 100 g ⁻¹)	0.95 $\pm$ 0.02
Carbohydrate (Starch) (g 100 g ⁻¹)	76.30 $\pm$ 0.01
Total Fiber (g 100 g ⁻¹)	8.80 $\pm$ 0.01
Calorific value (kcal 100 g ⁻¹)	345.75 $\pm$ 0.02
L*	74.96 $\pm$ 0.05
a*	3.99 $\pm$ 0.04
b*	11.48 $\pm$ 0.07
Potassium (mg 100 g ⁻¹)	465.24 $\pm$ 0.10
Phosphorus (mg 100 g ⁻¹)	248.50 $\pm$ 0.25
Calcium (mg 100 g ⁻¹)	68.97 $\pm$ 0.17
Iron (mg 100 g ⁻¹)	9.95 $\pm$ 0.12
Zinc (mg 100 g ⁻¹)	24.44 $\pm$ 0.19
Manganese (mg 100 g ⁻¹)	5.24 $\pm$ 0.14

Table 2 Results of the dependent variables of the experimental planning for gluten-free bread formulation

Exp	MTgase (%)	FT (min)	Moisture content (g 100 g ⁻¹)	Crumb color parameters		pH	Acidity (g 100 g ⁻¹)	Specific volume (cm ³ g ⁻¹)	Texture parameters		
				L*	+ a*	+ b*			Springiness (–)	Cohesiveness (–)	Hardness (N)
F1	0.5	60	26.86 ± 0.49	62.03 ± 0.06	7.43 ± 0.02	16.09 ± 0.21	6.69 ± 0.11	2.58 ± 0.62	0.999 ± 0.01	0.597 ± 0.05	4.704 ± 1.05
F2	1.5	60	28.64 ± 0.55	60.76 ± 0.18	7.42 ± 0.06	15.74 ± 0.33	6.12 ± 0.06	3.18 ± 0.08	0.999 ± 0.01	0.573 ± 0.04	6.719 ± 1.55
F3	0.5	100	27.28 ± 0.09	59.19 ± 0.09	6.83 ± 0.17	14.11 ± 0.45	5.76 ± 0.03	3.06 ± 0.29	0.999 ± 0.02	0.653 ± 0.06	4.475 ± 1.23
F4	1.5	100	27.98 ± 1.53	58.95 ± 0.01	7.55 ± 0.05	15.07 ± 0.08	5.77 ± 0.00	3.18 ± 0.36	0.999 ± 0.01	0.697 ± 0.06	6.687 ± 1.31
F5	1.0	80	29.03 ± 0.62	61.63 ± 2.46	7.07 ± 0.83	18.47 ± 0.98	5.44 ± 0.01	3.66 ± 0.20	0.999 ± 0.01	0.786 ± 0.07	5.484 ± 1.33
F6	1.0	80	31.89 ± 0.67	60.29 ± 0.45	8.42 ± 0.29	18.97 ± 1.38	5.47 ± 0.02	3.72 ± 0.19	0.999 ± 0.02	0.806 ± 0.02	5.491 ± 1.45
F7	1.0	80	31.35 ± 0.03	62.70 ± 1.30	7.18 ± 0.60	18.31 ± 1.57	5.40 ± 0.01	3.80 ± 0.15	0.999 ± 0.01	0.807 ± 0.02	5.414 ± 1.54

MTgase microbial transglutaminase enzyme; FT fermentation time

*Triplicate mean (± standard deviation)

$$\text{Specific volume} = 3.00 + 0.18 \text{ MTgase} + 0.12 \text{ FT} - 0.12 \text{ MTgase.FT} \quad (3)$$

$$\text{Hardness} = 5.57 + 1.06 \text{ MTgase} - 0.07 \text{ FT} + 0.04 \text{ MTgase.FT} \quad (4)$$

$$\text{Chewiness} = 3.92 + 0.69 \text{ MTgase} + 0.23 \text{ FT} + 0.17 \text{ MTgase.FT} \quad (5)$$

Fermentation consists of the production of carbon dioxide and production of acids, helping in mass growth, which is an important stage for the development of breads and essential to achieve adequate size, flavor and texture. According to Marti et al. (2014), the optimal conditions for yeast development during fermentation of fresh dough are established with pH values around 5.0, and with the analysis of pH results (Fig. 1a), it was possible to show that fermentation time of 80 min was sufficient to obtain values close to standards.

By analyzing the response surface (Fig. 1b), it was observed that only experimental factors fermentation time had significant effect, directly proportional to response acidity, that is, increasing the fermentation time, greater acidity in breads is obtained. This may be justified due to the higher acid production over the fermentation time. The pH and acidity of breads are related to the type and concentrations of microorganisms responsible for fermentation, to the Fixed Mineral Residue (FMR), flour content, fermentation time, temperature and salt concentration in the dough (Scarnato et al. 2017).

The specific volume of gluten-free breads can vary greatly depending on the type of flour, additives and process used and shows relationship between solid content and air fraction in the roasted dough. Breads with lower specific volume present unpleasant appearance to the consumer and are associated with high water content, failure of beating and cooking, poor aeration, difficult chewing, improper taste and low conservation (Esteller and Lannes 2005).

By analyzing the response surface (Fig. 1c), it was observed that the addition of MTgase was positive for this response, providing higher specific volume values as the addition of enzyme increased within the optimum range. The activity of MTgase leads to the binding of glutamine and lysine, resulting in bread with higher specific volume (Chen and Han 2011). However, previous studies have indicated that large amounts of MTgase in bread formulations impair its growth, making dough hard, thus causing greater resistance (Mohammadi et al. 2015).

Figure 1d shows an increase in the hardness of breads, and the greatest values were represented by the addition of 1.5% MTgase. Results are consistent with those found by Storck et al. (2013), in which at this concentration, the enzyme had strong influence on the hardness of breads made with rice flour, causing substantial increase in this

Table 3 Analysis of variance on the effect of experimental factors on the response

Source	Degree of freedom	Sequential sum of squares				
		pH	Acidity	Specif volume	Texture parameters	
					Hardness (N)	Chewiness (J)
Regression	3	1.209	0.802	1.092	4.494	1.209
Residual error	3	0.087	0.080	0.067	0.061	0.087
Lack to fit	1	0.084	0.078	0.058	0.058	0.084
Pure error	2	0.002	0.001	0.010	0.004	0.002
Total	6	1.295	0.883	0.160	4.555	1.295
R-Sq (%)	—	93.31	90.88	94.18	98.66	93.31
F value (calculated)	—	13.43	9.97	16.54	73.43	23.44
p value	—	0.0012	0.019	0.049	0.0007	0.006
F value (tabulated) _{0,95,3,3}				9.28

Fig. 1 Response surfaces for responses **a** pH; **b** acidity; **c** specific volume; **d** hardness; **e** chewiness

parameter using 1.5% enzyme. Similarly, in a study conducted by Gujral and Rosell (2004), greater dough consistency was found with increased transglutaminase concentration. This increase indicates that more water is being bound to the dough structure by modified proteins.

Marco and Rosell (2008) found that the addition of transglutaminase promoted an increase in the dough hardness, which can be explained by the increase in the molecular weight of proteins resulting from cross-linking.

Analyzing Fig. 1e, it could be observed that the MTgase concentration increased the chewiness of the analyzed breads. Gluten-free bread samples added with enzymes required greater chewing effort than gluten bread samples and results indicate greater force in the swallowing process, which can lead to product with low acceptance (Pongjaruvat et al. 2014). Cornejo and Rosell (2015) found chewiness values ranging from 8.97 to 20.26 J for gluten-free breads formulated with rice flour of several grain varieties.

Sensory and multivariate analysis of data

The mean acceptance values of gluten-free breads with red rice flour, microbial transglutaminase enzyme and prebiotic (inulin) in relation to sensory attributes appearance, crumb color, aroma, taste, softness and overall impression are shown in Table 4.

It was observed that all gluten-free breads obtained good sensory acceptance. The average values of most attributes ranged from 5.7 to 7.2, equivalent to hedonic terms “liked slightly” to “liked moderately”. It is noteworthy that score 5 (neither liked nor disliked) was considered the cutoff point. Most judges assigned values equal to or greater than 6 for all samples (Table 4). As for the softness attribute, F1 (0.5% MTgase and 60 min TF), F2 (1.5% MTgase and 60 min TF) and F3 formulations (0.5% MTgase and 100 min TF) obtained mean score below 6.0, but without statistically significant difference compared to the other samples. This aspect needs to be improved in gluten-free breads, as softer products are preferred by consumers who generally relate softness to fresh products.

In a qualitative study performed with individuals diagnosed with celiac disease in Florianópolis-SC (Brazil), when asking about the texture of gluten-free breads, this

attribute received the highest number of negative comments. All participants, especially those most recently diagnosed, described the product as kneaded, elastic, hard, heavy, dry and pasty. There was general agreement among participants that the ideal gluten-free bread should have crunchy crust and soft crumb (Do Nascimento et al. 2017).

Regarding crumb color and aroma attributes, formulations were very similar to each other, with no statistically significant difference ($p < 0.05$), which were attributes that received the highest scores by judges, that is, all samples had good acceptability. In the sensory evaluation, the following comment were made on the aroma and color of gluten-free breads made with red rice flour, microbial transglutaminase enzyme: “I did not feel any difference in flavor and the color was also very similar. Overall, all attributes were above expectations”.

The mean scores obtained for the flavor attribute demonstrated that F2 (1.5% MTgase and 60 min TF) and F3 (0.5% MTgase and 100 min TF) formulations were the least accepted (study extremes), significantly differing from F1 (0.5% MTgase and 60 min TF), F4 (1.5% MTgase and 100 min TF) and CP formulations (1.0% MTgase and 80 min TF), which obtained better results; however, when analysis was based on the hedonic scale, difference was observed between score 6 (liked slightly) and score 7 (liked moderately), so that it is possible to affirm that there was greater preference for the flavor of CP formulation, which obtained average score of 7.0 in this attribute, a fact confirmed by the concordance coefficient of 38.57% (Table 4).

Overall impression reflects the overall evaluation of all attributes analyzed; thus, the CP formulation (1.0% MTgase and 80 min TF) obtained the highest score (6.8) statistically differing ($p < 0.05$) from the other samples that obtained mean scores of 6.4. This result can be compared to the overall preference among samples, where the

Table 4 Sensory analysis of gluten-free breads with red rice flour, microbial transglutaminase enzyme and prebiotic (inulin)

Samples	Appearance	Color	Aroma	Taste	Texture (softness)	Overall acceptability	Index of acceptability (%)
F1	6.8 ^a	7.1 ^a	6.9 ^a	6.8 ^a	5.8 ^a	6.4 ^a	72.59
F2	6.8 ^a	7.2 ^a	6.7 ^a	6.3 ^b	5.8 ^a	6.4 ^a	72.57
F3	6.5 ^{ab}	6.9 ^a	6.9 ^a	6.3 ^b	5.7 ^a	6.4 ^a	72.65
F4	6.2 ^b	6.7 ^a	6.6 ^a	6.6 ^a	6.1 ^a	6.4 ^a	71.19
CP	6.7 ^a	7.2 ^a	7.0 ^a	7.0 ^a	6.3 ^a	6.8 ^b	75.17
Dms	0.64	0.55	0.51	0.68	0.73	0.52	
M.G	6.6	7.0	6.8	6.5	5.9	6.5	
C.V	19.45	15.72	14.85	14.85	20.95	17.65	

Means with different letters in the same column differ significantly ($p \leq 0.05$)

F1 = 0.5% MTgase e 60 min FT; F2 = 1.5% MTgase e 60 min FT; F3 = 0.5% MTgase e 100 min FT; F4 = 1.5% MTgase e 100 min FT; CP = 1.0% MTgase e 80 min FT. CP = central point (experiments F5, F6 e F7)

MTgase microbial transglutaminase enzyme; FT fermentation time; dms significant difference; M.G average overall; C.V coefficient of variation in %

F1=0,5% MTgase e 60 min FT; F2= 1,5% MTgase e 60 min FT; F3= 0,5% MTgase e 100 min FT; F4= 1,5% MTgase e 100 min FT; CP= 1,0% MTgase e 80 min FT.

PC = central point (experiments F5, F6 e F7).

Fig. 2 Two-dimensional of principal components analysis of the sensory attributes of gluten-free bread with red rice flour, microbial transglutaminase enzyme and prebiotic (inulin)

center point formulation was the one with the highest preference by judges (34%), followed by F1 (22%) and F2 formulations (22%). Formulations with the highest fermentation times (F3 and F4) were the least preferred, with 14% and 8%, respectively.

The Acceptability Index (AI) was calculated based on the average scores given by evaluators, in which the CP formulation (1.0% MTgase and 80 min TF) had the highest acceptability index (75.17%), but the other formulations presented values above 70%.

Principal component analysis (PCA) is a widely used multivariate statistical method, which can transform original variables into a set of new orthogonal variables, so that most information is contained in the first few components with the largest variance.

In the PCA chart (Fig. 2), each bread sample is represented by a point, and each point corresponds to the mean value assigned by the sensory team. Similar samples occupy regions near the graph and are characterized by vectors (attributes) that are closest to them. Samples presented sensory characteristics different from each other since they are located in different quadrants.

Most variations that occurred among samples were explained by Principal Component 1, but when evaluated and associated to Principal Component 2, it was observed that both components explained 86.14% of the information

contained in the mean values of sensory variables, that is, the variability among samples could be well explained using only these two axes.

It was observed that CP formulation (1.0% MTase and 80 min TF) was discriminated from the other formulations regarding flavor, softness and overall impression while F1 formulation (0.5% MTgase and 60 min TF) was discriminated by appearance, aroma and crumb color. F2 (1.5% MTase and 60 min TF), F3 (0.5% MTgase and 100 min TF) and F4 formulations (1.5% MTgase and 100 min TF) were not exposed by any of the sensory attributes, since both samples are distant from attributes in the vector space and in opposite quadrants, which indicates negative correlation with respect to sensory attributes. Although not discriminated by any sensory attributes, these formulations had good overall quality.

Conclusion

Red rice flour can be used as raw material in the bakery industry due to its nutritional quality. The addition of MTgase and the elevation of its level increased specific volume, firmness and chewiness of breads and to cover the greatest number of optimized attributes, it was possible to maintain the microbial transglutaminase enzyme levels

between 0.5 and 1.0% with fermentation time of 60–80 (min). All formulations were sensorially accepted, with averages above 5.0, but softness attribute needs to be improved in gluten-free breads, since softer products are preferred by consumers, and CP formulation (1.0% MTgase and 80 min TF) was the best represented by sensory attributes (flavor, softness and overall impression) in the multivariate analysis, a fact confirmed by the higher scores obtained in all these attributes, presenting greater acceptance and 75.17% preference among judges. These results show that the use of red rice flour, microbial transglutaminase enzyme and prebiotic in gluten-free bread formulations is an option for the celiac public and other consumers who seek for products with better physical and sensory quality.

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