Influence of binary mixtures of cassava starch and rice flour on the chemical and sensory characteristics of gluten-free bread

Influência de misturas binárias de amido de mandioca e farinha de arroz nas características químicas e sensoriais de pão sem glúten

Influencia de mezclas binarias de almidón de mandioca y harina de arroz sobre las características químicas y sensoriales del pan sin gluten

Abstract

In this study, response surface methodology based on simplex-centroid design was used to optimize the gluten-free bread formulation with rice flour and cassava starch as independent variables. Bread formulations were evaluated by physicochemical analysis and descriptive sensory analysis encompassing appearance, structure, texture, and aroma parameters by a trained sensory panel. The five formulations composition showed statistical differences concerning aw, lipid and protein content. Carbohydrate was significantly correlated with specific volume and lipids with protein. Overall, rice flour's addition improved lipid and protein, whereas further rises in cassava starch allowed developing bread with higher specific volume and sensory scores. The optimum combinations of the variables to maximize scores of porosity, texture, elasticity, and protein content, should be obtained with 51.75% of rice flour and 48.25% of...
cassava starch. The use of the simplex-centroid design and the response desirability function in the optimization was useful for evaluating the influence and potential of the binary mixture of rice flour and cassava starch on the sensory quality and chemical characteristics of gluten-free bread. These research findings open the scope for further investigation of rice flour and cassava starch and their useful application in gluten-free bread processing.

**Keywords:** Celiac disease; Desirability function; Quality; Multivariate analysis.

### Resumen

En general, la adición de características químicas al pán sin gluten,><br>Moreover, consumer expectations of gluten-free products are better than their wheat-containing analogs (Conte et al., 2016; El Khoury et al., 2018; Prada et al., 2019). Global market data indicated that gluten-free product sales are forecasted to increase by a compound annual growth rate of 10.4% between 2015 to 2020 (Masih & Sharma, 2016). Furthermore, consumer expectations have urged the food industry to continuously adjust and improve the formulations and processing techniques used in gluten-free product manufacturing.

The sensory and technological attributes of gluten-free bread have been reported as the most judgemental variables considered for purchase decisions (Nascimento et al., 2014; Wang et al., 2017). In fact, many gluten-free products available on the market are of poor technological quality, exhibiting low volume, with a flat appearance, pale crust, crumbly texture, high

1. **Introduction**

Gluten is a major protein component of some cereals fundamental in the functional properties of flour and rheological properties of the dough in bakery application (Bender & Schönlechner, 2020; El Khoury et al., 2018; Kan et al., 2017). However, there has been an increase in the quantity of diagnosed patients with gluten-sensitive enteropathy and other gluten-related disorders (such as wheat allergies and non-celiac gluten sensitivity), as well as the number of consumers interested in wheat-free who believe that gluten-free products are better than their wheat-containing analogs (Conte et al., 2016; El Khoury et al., 2018; Prada et al., 2019). Global market data indicated that gluten-free product sales are forecasted to increase by a compound annual growth rate of 10.4% between 2015 to 2020 (Masih & Sharma, 2016). Furthermore, consumer expectations have urged the food industry to continuously adjust and improve the formulations and processing techniques used in gluten-free product manufacturing.

The sensory and technological attributes of gluten-free bread have been reported as the most judgemental variables considered for purchase decisions (Nascimento et al., 2014; Wang et al., 2017). In fact, many gluten-free products available on the market are of poor technological quality, exhibiting low volume, with a flat appearance, pale crust, crumbly texture, high
staling rate, bland flavour, besides considerable variation in the nutrient composition, with low protein and high-fat contents (Nascimento et al., 2014; Wang et al., 2017).

Rice flour has a bland taste, white color, hypoallergenic properties, low levels of protein and sodium, the absence of gliadin, and the presence of easily digested carbohydrates (Park et al., 2014; Wang et al., 2017). However, despite the numerous advantages, the lack of gluten protein impairs bread formulation with rice flour. The addition of polymeric hydrocolloid substances such as starch, xanthan gum, and guar gum can help mimic gluten properties (Park et al., 2014; Wang et al., 2017). Cassava starch has low cost and is naturally abundant, renewable, and generally considered a carbohydrate source, riboflavin, thiamin, and nicotinic acid (Nascimento et al., 2021; Wang et al., 2017; Zhu, 2015). When it is cooked, in aqueous dispersion, they produce high clarity and high viscosity pastes and present low gelatinization temperature and low tendency to retrogradation compared to cereal starches (Wang et al., 2017).

There are still problems with gluten-free products, such as their high prices, limited variety, availability, and low nutritional quality (Prada et al., 2019; Sungur, 2018). Consequently, a balance between nutritional value, sensory and technological properties is necessary to match consumers’ requests (Calle et al., 2020). Therefore, in the present work, the chemical and sensory attributes of gluten-free bread obtained from binary mixtures of rice flour with sweet manioc starch at different percentages were evaluated. A method to discriminate gluten-free bread mixtures using chemometrics tools was brought based on selected consumer-driven quality characteristics of bread.

2. Methodology

2.1 Materials

Commercial rice flour (protein 1%, carbohydrates 5%), cassava starch (protein 50%, carbohydrates 2%, fat 2% and sodium 1%), potato (Solanum tuberosum), guar and xanthan gum, soybean oil, refined sugar, mineral water, egg, salt, dry yeast, bread improver (powder mix of starch, soy flour, inactive dry yeast, hemicellulose and lipase enzyme, fatty acid mono and diglyceride emulsifier, alpha-amylase flour improver, antioxidant glucose oxidase) were obtained from a local supermarket (Curitiba, Brazil).

2.2 Bread elaboration

Rice flour and sweet manioc flour were mixed and homogenized through the simplex-centroid design containing seven different assays with three repetitions of the centroid point, as presented in Table 1 (binary mixtures). The flour mix represented 37.1% of the formulation. The other components such as cooked potato (33.0 g/100 g), bread improver (1.5 g/100 g), xanthan gum (1.0 g/100 g), gum (1.0 g/100 g), soybean oil (10 g/100 g), salt (1.8 g/100 g), baking powder (2.6 g/100 g), refined sugar (3.0 g/100 g), eggs (3.0 g/100 g) and water (6.0 w/w) were kept constant and mixed with the flours. For each formulation, samples were prepared firstly by suspending yeast and sugar in warm mineral water (40 °C) for 15 minutes at ambient temperature. After, dry ingredients were pre-mixed. Then, the cooked potato was introduced to dry ingredients. Afterward, oil, eggs, and a mix of water, yeast, and sugar were added. Fermented doughs were divided into 70 g portion and manually molded to French bread type proof by 30 min. Finally, loaves were baked at 200 °C for 20 min. After baking, gluten-free bread was allowed to cool at room temperature for two hours, packed in low-density polyethylene plastic bags, and stored at 20 °C ± 2.
Table 1. Simplex-centroid design with seven treatments to gluten-free French bread recipes.

| Assays | Independent variables (original % and coded) |
|--------|---------------------------------------------|
|        | Rice flour (X1) | Sweet manioc starch (X2) |
| F1     | 63.0 (1) | 37.0 (0) |
| F2     | 45.0 (0) | 55.0 (1) |
| F3     | 54.0 (0.5) | 46.0 (0.5) |
| F4     | 58.5 (0.75) | 41.5 (0.25) |
| F5     | 49.5 (0.25) | 50.5 (0.75) |

Source: Authors.

2.3 Bread characterization

Physicochemical parameters (moisture, protein, lipids, sodium, dietary fiber, ash, carbohydrate and energy) were determined according to the standard methods of the Association of Official Analytical Chemists - AOAC (2008). Water activity (aw) was determined using an electronic dew-point water activity meter AquaLab Series 3TE (Decagon, Washington), at 25 ± 0.02 ºC. The bread was weighed and the loaf volume was measured using a seed displacement method according to Ding, Peng, Li, and Yang (2019).

2.4 Sensory evaluation

The evaluation was done with ten trained voluntary candidates (men and women, aged 25 to 65 years) recruited from staff and graduate students of the food and nutrition security program at the Federal University of Parana preselected out of 25 candidates, according to their discriminating and reproducibility capacity according to ISO 8586-1(1993). Sensory analysis of the gluten-free French bread formulations was conducted using the Quantitative Descriptive Analysis, and the attributes were assessed using the references and discussed with the panel group. Fifteen sensory points related to appearance, aroma, taste, and texture of bread samples were applied. The intensity of these attributes of gluten-free French bread presents in the list was evaluated using a 9 cm unstructured scale, ranging from 0 (not perceived) to 9 (very intense). A quarter of each piece of bread (including crust and crumb) was displayed (in randomized order) on a plastic dish encoded with a three-digit number to judges in four repetitions. The acceptance test of the formulations was conducted in a session using 126 untrained volunteers using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely) to appearance, aroma, taste, texture, and overall quality (Costa et al., 2020).

Mineral water at room temperature was given to drink between each sample for palate cleansing. The assessment was realized in individual booths under white light at room temperature. The project was approved by the Research Ethics Committee (CEP) of the Federal University of Paraná, Brazil, under No. 1360.025/2007-02; CAAE: 0036.0.208.000-06.

2.5 Statistical analysis

RSM was applied to model the best sensory profile and properties of French bread gluten-free. A second-order polynomial model was used to analyze the experimental data. The generalized model applied in the analysis of RSM is presented in Equation 1.

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1) \]

Where \( Y \) is the predicted response, \( \beta_0 \) is constant, \( \beta_i \), \( \beta_{ii} \), and \( \beta_{ij} \) are the coefficients of linear, quadratic and interaction regression, respectively. \( X_i \) and \( X_j \) are independent variables.
ANOVA evaluated the statistical significance of the terms in the regression equations for each response. The terms with statistically insignificant results were excluded from the initial model and the experimental data were tested again only for the significant parameters ($p \leq 0.05$). We used the desirability function to maximize porosity, texture, elasticity, protein and minimize the lipid content proposed by Derringer and Suich (1980). All the statistical analyses were performed using STATISTICA software (StatSoft INC., version 10.0, USA). The results were submitted to analysis of variance (ANOVA), and in the case of significant differences, the averages were compared using Duncan's test ($p \leq 0.05$). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using STATISTICA software (StatSoft INC., version 10.0, USA) to describe the relationship between loaf formulation (n=5) and sensory analyses (n=16) according to Ávila et al. (2019).

3. Results and Discussion

Gluten-free bread formulations resulting were characterized regarding their proximate composition and specific volume. Formulations presented differences ($p \leq 0.05$) in water activity, lipids and protein content (Table 2). F1 and F4 exhibited the highest lipid and protein values, probably attributed to the rice flour (63% rice flour in F1 and 58.50% in F4). The baking process produced loaves with similar moisture content and water activity, indicating that the addition of xanthan and guar gum to the formulations affected the water removal. These adjuvants have a high hygroscopic capacity. This effect can be attributed to the hydroxyl groups in the hydrocolloid structure, allowing more water interactions through hydrogen bonding (Lorenzo et al., 2009; Sciarini et al., 2010).

Table 2. Different quality characteristics of gluten-free bread based on simplex-centroid design mixtures of rice flour and sweet manioc starch.

| Formulations | F1          | F2          | F3          | F4          | F5          |
|--------------|-------------|-------------|-------------|-------------|-------------|
| Moisture (g/100g) | 38.12 ± 1.37 | 38.12 ± 0.98 | 38.41 ± 0.55 | 38.45±0.31  | 38.66±0.46  |
| $a_w$*        | 0.950 ± 0.004$^b$ | 0.953 ± 0.002$^b$ | 0.951 ± 0.003$^b$ | 0.959±0.003$^a$ | 0.954±0.006$^{ab}$ |
| Ash (g/100g)  | 1.75 ± 0.10  | 1.73 ± 0.08  | 1.74 ± 0.10  | 1.79±0.06   | 1.75±0.06   |
| Lipids (g/100g) | 4.89 ± 0.50$^a$ | 3.53 ± 0.52$^c$ | 4.00 ± 0.35$^b$ | 4.49±0.60$^a$ | 3.78±0.26$^{bc}$ |
| Protein (g/100g) | 3.44 ± 0.24$^a$ | 2.92 ± 0.26$^c$ | 3.08 ± 0.17$^{bc}$ | 3.36±0.18$^{ab}$ | 2.78±0.52$^c$ |
| Fiber (g/100g) | 4.50 ± 0.82  | 4.92 ± 1.68  | 5.34 ± 0.63  | 4.62±0.39   | 5.61±0.82   |
| Carbohydrate (g/100g) | 47.30 ± 0.80  | 48.79 ± 2.07  | 47.43 ± 0.58  | 47.29±0.81  | 47.42±1.06  |
| Sodium (mg)   | 535.30 ± 20.90 | 523.13 ± 19.66 | 523.18 ± 23.76 | 497.91±60.29 | 522.99±38.40 |
| Total energy value (Kcal) | 246.95 ± 3.14 | 238.60 ± 12.86 | 238.03 ± 1.29 | 243.05±4.11 | 234.84±3.05 |
| Specific volume (cm$^3$/g) | 1.75 ± 0.09$^b$ | 1.87 ± 0.11$^a$ | 1.68 ± 0.06$^c$ | 1.70±0.07$^{bc}$ | 1.75±0.06$^b$ |

Mean ± standard deviation; n = 20 for specific volume; n = 5 for sodium; n = 3 for fiber and n = 9 for moisture, $a_w$, ash, lipids and protein. Values followed by different letters within a column denote significant differences. Source: Authors.

The lipid content varied between 3.53 to 4.89 g/100g, and this low lipid value is expected for loaves, according to USDA (2019). For nutrition to be claimed as a "source of dietary fibers," it must contain at least 3 g/100 g of total dietary fiber. Therefore, our formulations resulted in loaves with fiber content between 4.50 to 5.61 g/100g, which can be considered beneficial as dietary fiber sources (Pellegrini & Agostoni, 2015). The ash value found confirms that the formulations were made from refined flours with low mineral content. Using linear correlation of Pearson (Table 3), we observed that carbohydrate is significantly correlated ($P \leq 0.05$) with specific volume ($r = 0.90$), as well as lipids with protein ($r =0.93$ and $r = 0.89$, respectively), and lipids and protein with total energy ($r = 0.89$ and $r = 0.95$, respectively).
Specific volume is one of the most important visual characteristics of bread, and a high ratio of volume per weight is usually associated with proper aeration of the loaves (Conte et al., 2018). The specific volume ranged from 1.68 ± 0.06 to 1.87 ± 0.11 cm³/g and is significantly dependent on the recipe applied in line with previous findings. The F2 formulation, with the highest amount of sweet cassava starch, presented a higher specific volume than the other formulations. Starch helps in the formation of flexible gas cells that retain carbon dioxide during waterproofing and baking, thereby increasing the volume and texture of bread (Onyango et al., 2011). The variation of visual appearance from formulations is shown in Figure 1a.

**Figure 1.** a) The appearance of gluten-free bread, 1) formulation with 63% rice flour (RF) and 37% cassava starch (CS), 2) 45% RF and 55% CS, 3) 54% RF and 46% CS, 4) 58.5% RF and 41.5% CS, 5) 49.5% RF and 50.5% CS; b) Optimization of the rice flour and starch mixtures to maximize porosity, texture, elasticity, protein and minimize the lipid content in French bread gluten-free.
The mean values of the 15 sensory attributes for each sample of gluten-free bread are shown in Table 3. The following attributes varied significantly among formulations: the opening of the crust, crust color, yeast aroma, yeast taste, saltiness, and sweetness. These attributes were, therefore, useful in characterizing differences among loaves. All formulations were acceptable as they received scores greater than 6, ranging from 6.17 to 6.90 among all attributes. The highest appearance score was obtained for formulation F3, and the highest texture score was for formulation F1, while formulation F4 had a median score. Formulations F1 and F3 did not present high volume, nevertheless, they exhibited good appearance and taste.

Table 3. Mean intensity ratings of 15 descriptive attributes and acceptance test for gluten-free bread formulations evaluated by a trained sensory panel.

| Attribute               | F1     | F2     | F3     | F4     | F5     |
|-------------------------|--------|--------|--------|--------|--------|
| **Visual Appearance**   |        |        |        |        |        |
| Opening of the crust    | 5.47\textsuperscript{ab} | 5.60\textsuperscript{ab} | 5.82\textsuperscript{a} | 5.54\textsuperscript{ab} | 5.26\textsuperscript{b} |
| Symmetry                | 5.34   | 5.30   | 5.05   | 4.87   | 4.83   |
| Crust color             | 4.87\textsuperscript{a} | 4.70\textsuperscript{ab} | 4.80\textsuperscript{a} | 4.31\textsuperscript{b} | 4.80\textsuperscript{a} |
| Crumb color             | 3.87   | 4.09   | 4.10   | 3.86   | 3.95   |
| Porosity                | 4.34   | 4.55   | 4.46   | 4.34   | 4.55   |
| **Aroma**               |        |        |        |        |        |
| Characteristic          | 5.63   | 5.79   | 5.70   | 5.81   | 5.77   |
| Yeast                   | 3.12\textsuperscript{ab} | 3.37\textsuperscript{ab} | 3.60\textsuperscript{a} | 3.20\textsuperscript{ab} | 3.00\textsuperscript{b} |
| **Taste**               |        |        |        |        |        |
| Characteristic          | 5.98   | 5.87   | 5.96   | 5.88   | 6.04   |
| Yeast                   | 3.11\textsuperscript{a} | 3.04\textsuperscript{a} | 3.01\textsuperscript{a} | 2.72\textsuperscript{ab} | 2.51\textsuperscript{b} |
| Saltiness               | 3.40\textsuperscript{a} | 3.25\textsuperscript{ab} | 3.36\textsuperscript{ab} | 2.97\textsuperscript{b} | 3.02\textsuperscript{ab} |
| Sweetness               | 1.78\textsuperscript{b} | 1.94\textsuperscript{ab} | 1.72\textsuperscript{b} | 1.99\textsuperscript{ab} | 2.20\textsuperscript{a} |
| **Crust Texture**       |        |        |        |        |        |
| Crunchiness             | 4.10   | 3.95   | 4.17   | 4.30   | 4.22   |
| **Crumb Texture**       |        |        |        |        |        |
| Elasticity              | 4.52   | 4.83   | 4.64   | 4.52   | 4.84   |
| Softness                | 5.94   | 5.92   | 5.91   | 6.01   | 6.19   |
| Moistness               | 5.51   | 5.51   | 5.48   | 5.46   | 5.82   |
| **Acceptance test**     |        |        |        |        |        |
| Appearance              | 6.85\textsuperscript{ab} | 6.46\textsuperscript{c} | 6.89\textsuperscript{a} | 6.80\textsuperscript{ab} | 6.53\textsuperscript{bc} |
| Aroma                   | 6.68   | 6.45   | 6.65   | 6.58   | 6.52   |
| Taste                   | 6.86   | 6.82   | 6.88   | 6.62   | 6.83   |
| Texture                 | 6.74\textsuperscript{a} | 6.17\textsuperscript{b} | 6.52\textsuperscript{ab} | 6.52\textsuperscript{ab} | 6.41\textsuperscript{ab} |
| Global quality          | 6.85   | 6.62   | 6.90   | 6.83   | 6.64   |

Results represent the mean ± standard deviation (n=16). Different letters in the same line represent results with a statistical difference, according to the Duncan test. Source: Authors.

In order to verify the influence upon the binary mixtures, multiple regression models were used to evaluate the effects, and mathematical models were obtained to predict the best formulation (Table 4). The models proposed for the porosity, texture, elasticity, lipids and protein were significant (P<0.01), and residual analysis showed a normal distribution (P>0.05) that explain up more than 83% of all variance in data with adjusted $R^2>$0.77 (adjusted $R^2$ is a correction of $R^2$, taking into account the degrees of freedom involved in the total sum of squares and regression sum of squares). All the linear effects in the models contributed positively (P<0.05) in the loaves formulation (Table 4).
Table 4. Statistical properties of the model generated to the sensory profile and properties of French bread gluten-free.

| Properties      | Visual | Acceptance | Specific Volume | Lipids | Protein |
|-----------------|--------|------------|-----------------|--------|---------|
| R²              | 0.896  | 0.911      | 0.992           | 0.975  | 0.828   |
| R adj           | 0.861  | 0.882      | 0.983           | 0.967  | 0.770   |
| Model p-value   | 0.02   | 0.01       | <0.01           | <0.01  | 0.03    |
| RSME            | 0.002  | 0.005      | <0.001          | 0.009  | 0.018   |
| F-value         | 25.94  | 30.78      | 144.45          | 118.47 | 14.39   |

b-coefficients:
- (X₁) Rice Flour: 4.32, 6.72, 1.75, 4.82, 3.44
- (X₂) Cassava starch: 4.57, 6.22, 1.87, 3.45, 2.79

Source: Authors.

After modeling the characteristics of bread formulations, a simultaneous optimization procedure using the desirability function (D) was performed with the models for porosity, texture, elasticity, protein, and lipid to maximize the first values and minimize the lipid value (Figure 1b). The optimum range suggested by desirability is a formulation with 51.75% rice flour and 48.25% of starch. These optimization results corroborated the formulation that showed the highest "global acceptability" by the sensory judges. Formulation F3 with 54% of starch and 46% rice flour was the recipe closest to the optimized point.

Faggian et al. (2020) reported bread made with 100% rice flour showed the lowest values of specific volume and the highest elasticity and cohesiveness values, lower protein quantity than formulations containing bean flour. According to our results, it was postulated that the best gluten-free bread formulation, to display an improved volume, the best acceptation and elasticity, highest protein content, and lower lipids should be the combination of 51.75% of rice flour and 48.25% of starch. These results corroborate with Onyango et al. (2011) that evidenced that Sorghum bread containing 50% cassava starch had the best overall crumb properties and López et al. (2004) that present a bread composed of 45% rice flour, 35% corn starch and 20% cassava starch with best results of crumb, flavor and appearance.

Multiple factor analysis with the representation of sensory descriptors and the quality properties of gluten-free bread formulations revealed that the first two principal components explain up to 74.34% of the total variability (Figure 2a and b). Overall, the first PC explained up to 44.96% of the total variance, and the second PC explained 29.38% (Figure 2).
The first group (Figure 2a, b, right side) showed higher values of sweetness, yeast aroma, and taste, the opening of the crust, saltiness, and crust color. The second group (Figure 2a, b, left side) showed higher protein values, lipids, texture, and appearance and lower sweetness and water activity. Hierarchical cluster analysis draws connections between formulations, producing a dendrogram in which similar samples are grouped. This similarity is a function of the distance between the samples (Figure 2c and d). Two clusters can be observed in the dendrogram that separates the samples F2, F4, and F5 (Cluster 1) and the samples F1 and F3 (Cluster 1). These findings corroborated the results obtained by PCA.

4. Conclusion
This work provides meaningful information concerning the sensory characteristics of gluten-free bread elaborated
from rice flour mixed with starch and highlights that some attributes, as higher values of protein and lipids, driving the acceptability. Rice flour's addition improved lipid and protein content, whereas further rises in cassava starch allowed developing bread with higher specific volume and sensory scores. The mixed-use of rice flour and cassava starch is encouraged for formulations of gluten-free bread to optimize the sensory quality. The use of the simplex-centroid design and the desirability function for optimizing the mixture of rice flour and cassava starch resulted in the gluten-free bread with good sensory quality and volume improved, maximizing scores of porosity, texture, and elasticity. These approaches were successfully used for evaluated the potential and the influence of the binary mixtures on the chemical and sensory characteristics of gluten-free bread.

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