Using Response Surface Methodology to evaluate the effect of pequi flour, and pulp and by-product on sweet bread development

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ABSTRACT. In this work, the effect of wheat flour and water replacement by pequi pulp and flour on the bread development and preparation enriched with this fruit was studied. Two experimental designs were used for two independent variables, the first evaluating the wheat flour partial replacement by pequi husk flour (x₁) and pequi pulp flour (x₂). The second design evaluated the wheat flour and water partial replacement by pequi husk flour (x₃) and pequi pulp (x₄), respectively. At the same time, a control test was conducted (without the addition of pequi flour and pulp) for comparison. The evaluated dependent variables of the bread quality characteristics were: dough volume; expansion rate; specific volume and density; texture profile and gluten content. It was possible to verify that only the gluten content was influenced by the replacement of wheat flour by pequi husk flour, whereas, only the specific volume was influenced by the replacement of water by pequi pulp. In general, the best replacement range was obtained with the formulation using between 0.75 to 2.5% pequi husk flour; up to 20% pequi pulp flour and between 5 and 55% of pequi pulp.

Keywords: Caryocar brasiliense Camb.; bakery technology; new product development; quality; food processing; substitution of ingredients.

Introduction

The Brazilian Cerrado stands out for the biodiversity of fruit species that have great potential for food use. Among these species, we highlight the ‘pequizeiro’ (Caryocar brasiliense Camb.), family Caryocaraceae, widely spread throughout the Brazilian territory, mainly in the states of Minas Gerais, Goiás, Mato Grosso, Tocantins, Bahia and Ceará. This tree produces typical fruit which is considered by locals as gold of the Cerrado (Rodrigues, Vilas Boas, Paula, & Alcântara, 2009; Rodrigues, Paula, Pinto & Vilas Boas, 2015).

Referring to the anatomical aspects, pequi is considered a drupe fruit, globose, consisting of the husk that usually involves one to three pyrenes. The husk consists of a fine gray-green exocarp and a white or light yellow external mesocarp. Pyrenees are formed by the internal mesocarp, called pulp, with a golden yellow color much appreciated in culinary, the spinous endocarp and the seed. Pyrenees are formed by the internal mesocarp, called pulp, with a golden yellow color much appreciated in culinary, the spinous endocarp and the seed (Vilas Boas et al., 2012; Leão, Franca, Oliveira, Bastos, & Coimbra, 2017; Rodrigues et al., 2009, 2015; Rodrigues, Vilas Boas, Paula, Pinto, & Piccoli 2011).

Predominantly the pequi internal mesocarp and eventually its seed are used, although its husk (exocarp + external mesocarp), which is discarded in culinary preparations, makes up about 80% of the fruit and has high exploitable functional and nutritional potential. One way to add value to pequi would be its full use, including its husk, in human food (Leão et al., 2017; Santos et al., 2018). Thus, stimulating the use of pequi husk and pulp in the formulation of well-known and consumed foods – such as bakery products – can be a way of spreading and increasing the consumption of this exotic fruit throughout the world.

Bread is a staple food and is related to human life, both as food and as an economic, political, religious and cultural symbol. The product is obtained by cooking, under technologically appropriate conditions, a fermented dough prepared with wheat flour and/ or other flours naturally containing gluten-forming proteins with addition of water and may also contain other ingredients (Agência Nacional de Vigilância Sanitária [ANVISA], 2005; Bock, Wrigley, & Walker, 2016).
The nutritional and functional enrichment of bread, one of the most consumed foods in the world, with pequi, can be an effective way to spread the use of this fruit with great potential. However, it is unknown and unexplored throughout the world, except for some locations in Brazil, where it is consumed in foods that involve rice and chicken. Moreover, adding value to pequi can contribute to the conservation of the Cerrado biome, whose biodiversity has been decreasing due to its unsustainable exploitation, by replacing native species with pastures and monocultures.

Partial substitution of wheat flour and water by other types of flour and pulp, respectively, becomes a viable alternative as long as the substitution level does not negatively affect the quality attributes of breads. According to Rahaie, Gharibzahedi, Razavi and Jafari (2014), the main changes in the quality of bread produced with substitution of other types of flour, involve the decrease in volume, gluten network weakening and changes in bread crumb texture.

The adequacy of experimental planning, aiming to predict the influence of certain variables on the performance of a given process, is fundamental for the determination of optimal replacement ranges. Among the several types of planning, the factorial systems stand out because they allow to evaluate two or more variables simultaneously, from a small number of experimental trials (Rodrigues & Iemma, 2014).

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques for constructing empirical models. This method is used to estimate, observe and optimize a response (output) influenced by several independent variables (inputs). The main applications includes: i) selection of independent variables through an experimental design according to the selected matrix; ii) mathematical-statistical treatment of the experimental results by adjusting a polynomial function; iii) evaluation of the adequacy of the model and iv) obtaining optimal values for each study (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Ghellab, Mu, Li, & Han, 2019).

Hence, the purpose of this study was to develop breads with the use of pequi husk and pulp flours, in partial replacement of wheat flour, as well as pequi pulp, in partial replacement of water and to obtain an ideal concentration range for each substituent using the response surface methodology aiming to develop a bakery product with technological qualities compared to a conventional sweet bread.

### Material and methods

#### Obtaining the raw material

The pequi fruit was obtained in the city of Montes Claros, Minas Gerais state, Brazil and transported to the Fruit and Vegetable Postharvest Laboratory of the Federal University of Lavras, Lavras – Minas Gerais, Brazil, where it was used to obtain the husk (exocarp + outer mesocarp) flour, pulp (inner mesocarp) flour and pulp. The fruit were selected and prewashed in running water to remove coarse dirt, sanitized in a solution containing 100 ppm sodium hypochlorite for 15 minutes, split in halves and the pits (pyrenes) were separated from the husk. Husks and pits were placed in polyethylene plastic bags and stored at -18ºC, until processing. The pulp was separated from the spinous endocarp and seed using a household grater. The husk and pulp of pequi fruit were then subjected to a steam bleaching process for 12 minutes to inhibit the darkening enzymes. Part of the bleached pulp was vacuum-sealed and frozen. The other part of bleached pulp and the bleached husk were dried in an oven at 65ºC for 24 hours. Subsequently, to obtain the flour, the dried material was ground in a knife mill, sieved using a No. 9 Mesh sieve and homogenized to obtain a uniformly granular product. The flours were vacuum-sealed and stored at room temperature until bread processing.

#### Bread processing

The control breads were made from the following basic formulation (percentages of backer): wheat flour (100%), water (45%), crystal sugar (15%), eggs (15%), milk powder (6%), soybean oil (5%), yeast (2.5%) and salt (2%). The enriched breads presented the same formulation, except for the partial replacement of wheat flour, by husk and pequi pulp flours, and water by pequi pulp.

The direct fermentation method was used, where all dried ingredients were mixed: wheat flour, crystal sugar, eggs, yeast and milk powder. Once homogenized, the wet ingredients were added: water and oil. Finally, salt was added until the development of the gluten or ‘veil point’. The batter was mixed in a Wallita® mixer at top speed for five minutes. Subsequently, the dough was weighed, divided, rounded and shaped in the G.Painz® bread curler and brought to the Klimaquip® fermentation chamber (30ºC and 90% RH) for 90
minutes. Soon after, the fermented dough was baked in a semi-industrial Practical Technicook® electric oven at 150°C for 20 minutes.

**Technological assessment of dough and bread**

**Dough volume**

Portions of dough of approximately 15 g were placed in 100 mL beakers to measure the dough volumes during the fermentation process which was conducted in a fermentation chamber set at 30°C and 90% RH for 90 minutes. To calculate the volume produced (ΔV), the difference between the final volume produced and the initial volume of the dough in the beakers was used (Equation 1). Measurements for each assay were performed in quadruplicate and results expressed in mL (Zambelli et al., 2017).

\[
\Delta V = V_f - V_i
\]

(1)

**Expansion index**

To calculate the expansion index an adapted Zambelli et al. (2017) methodology was used. The dough rolls modeled using the G.Painz® modeler were arranged in a form and measurements were taken of the diameter and height using a millimeter ruler and a digital caliper. The bread expansion index (EI) was calculated using Equation (2) and the results expressed in cm².

\[
\text{Expansion index (EI)} = \left( \frac{D_p + H_P}{2} \right) \left( \frac{D_M + H_M}{2} \right)
\]

(2)

where: \(D_p\) and \(H_P\) = Bread diameter and height after baking (cm); \(D_M\) and \(H_M\) = Diameter and height of molded doughs (cm).

**Specific volume and density**

The specific volume of breads was measured according to method No. 72-10, described by (American Association of Cereal Chemists [AACC], 2000), by filling a known volume container with millet seeds. The specific volume was calculated by dividing the volume of bread displaced (mL) by its dough (g) and the results expressed in mL g⁻¹. Density was calculated by the inverse relationship between displaced volume (mL) and baked sample weight (g) and results expressed in g mL⁻¹.

**Texture profile**

Texture parameters were determined according to Garzón, Rosell, Malvar and Revilla (2017) in a texture analyzer (TA – XT2i model, Stable Micro Systems, UK) using a texture profile analysis (TPA) double compression test. The bread slices, 1 cm, were compressed to 50% of original height at a test speed of 2 mm s⁻¹ and compression distance of 5.0 mm; interval between cycles of 10 s using a 36 mm cylindrical probe. For the test two slices of bread were placed overlapping and analysis of each test was carried out in quadruplicate. From the TPA curve, the variables analyzed were: hardness, springiness, cohesiveness, chewiness and resiliency.

**Moist gluten content**

The percentage of moist gluten was determined by washing the samples with distilled water, according to methodology No. 38-10, described by AACC (2000). The analysis was performed in quadruplicate from a formulation of wheat flour 20 g.

**Production of pequi sweet breads using the response surface methodology**

The methodology of an experimental Central Composite Rotational Design (CCRD) for two variables was used to determine the optimal conditions for the development of pequi bread with technical parameters similar to those of conventional sweet bread (control).

Two experimental designs were conducted. The first experimental design was performed to evaluate the effect of two independent variables: pequi husk flour (0 to 5%) and pequi pulp flour (10 to 20%). The second
experimental design was performed to also evaluate the effect of two independent variables: pequi husk flour (0 to 5%) and pequi pulp (0 to 40%).

The levels of pequi pulp and pequi pulp flour were determined from preliminary tests. The complete design consisted of 11 experiments including four factor (-1 and +1 levels) four axial points (+α levels) and three central points (Table 1). The response variables were considered the technological parameters to evaluate the quality of dough and bread.

Table 1. Coded levels of the two variables used to determine the optimal substitution level of pequi flour and pequi pulp used in the preparation of sweet bread from the central composite planning.

| Variables          | 1st Experimental Planning | Levels    |
|--------------------|---------------------------|-----------|
| Pequi husk flour   | -α: 0                     | -1: 0.73  |
| Pequi pulp flour   | 10                        | 11.46     |
|                    | 0                         | 2.5       |
|                    | +1: 4.27                  |           |
|                    | +α: 5.00                  |           |
| Pequi pulp         | 0                         | 5.86      |
| Pequi pulp         | 0                         | 20        |
|                    | +1: 34.14                 |           |
|                    | +α: 40.0                  |           |

Notes: ±α value corresponds to 1.41.

To evaluate the effects of the variables on the quality parameters of dough and bread, multivariate regression analysis was used, given by Equation (3).

\[ y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \]  

where, \( y \) = dependent variable; \( \beta_0 \) is the interception term, \( \beta_1 \) and \( \beta_2 \) are the linear coefficients; \( \beta_{11} \) and \( \beta_{22} \) are the quadratic coefficients; \( \beta_{12} \) refers to the interaction between the coefficients and \( x_1 \) and \( x_2 \) are the coded variables. The coefficient of determination \( R^2 (R^2 > 0.7) \) and the F test (\( F_{cal} > F_{tab} \)) were used to verify the quality of the second order model of the fit equation.

According to Rodrigues and Iemma (2014), after obtaining the models adjusted according to the second-order multivariate regression, the response surfaces and contour curves were determined, while the validation of the models was performed by repeating the test that most closely approximates the value obtained by the control, the test being performed in quadruplicate. The predicted parameters related to the technological analyzes were compared, by a means test (Tukey at 5% probability) with the experimental data obtained.

**Statistical analysis**

Statistical analyzes were performed using Statistica® version 8.0 (Stat. Soft. Inc., Tulsa, USA). The Tukey test was applied to the results of the Central Composite Rotational Design (CCRD) matrix for comparative effect between the tests and standard formulation, as well as in the validation model. To perform this test, Minitab® version 19 software (Minitab Inc., State College, Pennsylvania, USA) was used.

**Results and discussions**

**Experimental planning 1**

Experimental responses to the effect of partial substitution of wheat flour by pequi husk and pulp flours on the technological properties of dough and bread are shown in Table 2.

The elaboration of the standard bread was used as a reference to determine the ideal substitution ranges, that is, the closer the enriched bread results were to the control breads, the closer to the ideal the substitution levels were considered.

According to Table 2, the results obtained at the central points showed good repeatability. In accord to the Tukey test (p < 0.05), in a general way, all evaluated technological variables were affected by the formulation substitutions. However, from the analysis of the effect estimation (data not shown), the independent variables used in the bread formulations, under the conditions studied in the tests, had no significant effect in relation to the response variables. Thus, it was also not possible to establish mathematical models in function of these
variables, considering the p-value (> 0.05) and the coefficient of determination (R² < 0.70), except for the dough moist gluten content.

Table 2. CCRD with real and coded values and all responses and Tukey test to compare with control bread.

| Essay | Pequi husk flour (%) | Pequi pulp flour (%) | Moist volume (mL) | Equation Index | Specific volume (mL.g⁻¹) | Density (g.f) | Hardness (g.f) | Springiness (g.f) | Cohesiveness (g.sec) | Chewiness | Resilience | Moist gluten content (%) |
|-------|----------------------|----------------------|-------------------|----------------|------------------------|-------------|--------------|-----------------|---------------------|-----------|-----------|--------------------------|
| 1     | -1 (0.73)            | -1 (0.45)            | 30.00 ± 5.235     | 0.43 ± 0.017   | 5.02 ± 0.207           | 0.23 ± 0.002 | 110.87 ± 36.244 | 0.82 ± 0.002     | 0.72 ± 0.002       | 732.5 ± 140.67 | 0.32 ± 0.02 | 17.98 ± 1.81   |
| 2     | +1 (4.27)            | -1 (0.45)            | 22.00 ± 1.135     | 0.40 ± 0.014   | 2.44 ± 0.195           | 0.41 ± 0.003 | 129.66 ± 16.198 | 0.90 ± 0.007     | 0.72 ± 0.003       | 840.02 ± 85.77 | 0.33 ± 0.02 | 5.28 ± 0.02   |
| 3     | -1 (0.75)            | +1 (0.55)            | 25.50 ± 1.295     | 0.37 ± 0.015   | 2.42 ± 0.122           | 0.41 ± 0.003 | 115.73 ± 14.375 | 0.88 ± 0.002     | 0.71 ± 0.004       | 695.17 ± 76.72 | 0.31 ± 0.01 | 16.05 ± 0.04   |
| 4     | +1 (4.27)            | +1 (0.55)            | 22.75 ± 3.301     | 0.38 ± 0.016   | 1.76 ± 0.003           | 0.57 ± 0.002 | 132.60 ± 22.435 | 0.84 ± 0.016     | 0.67 ± 0.004       | 736.83 ± 104.44 | 0.28 ± 0.01 | 2.18 ± 1.50   |
| 5     | -1.41 (0)            | 0 (15)               | 25.00 ± 2.166     | 0.40 ± 0.013   | 2.25 ± 0.25            | 0.65 ± 0.050 | 141.82 ± 12.165 | 0.89 ± 0.048     | 0.67 ± 0.004       | 677.82 ± 98.98  | 0.27 ± 0.01 | 13.28 ± 0.73  |
| 6     | +1.41 (5)            | 0 (15)               | 26.00 ± 4.766     | 0.39 ± 0.011   | 2.07 ± 0.126           | 0.49 ± 0.010 | 132.59 ± 13.849 | 0.85 ± 0.040     | 0.67 ± 0.004       | 772.14 ± 97.53  | 0.28 ± 0.01 | 2.51 ± 2.45   |
| 7     | 0 (2.5)              | -1.41 (10)           | 27.50 ± 3.325     | 0.40 ± 0.009   | 2.25 ± 0.070           | 0.44 ± 0.001 | 107.30 ± 60.02  | 0.92 ± 0.036     | 0.67 ± 0.004       | 665.25 ± 28.64  | 0.29 ± 0.03 | 16.62 ± 0.28   |
| 8     | 0 (2.5)              | +1.41 (20)           | 23.75 ± 1.711     | 0.38 ± 0.005   | 2.09 ± 0.080           | 0.48 ± 0.012 | 108.54 ± 16.775 | 0.85 ± 0.016     | 0.67 ± 0.004       | 497.63 ± 519.49 | 0.28 ± 0.01 | 18.38 ± 0.54   |
| 9     | 0 (2.5)              | 0 (15)               | 25.00 ± 1.15      | 0.39 ± 0.011   | 2.09 ± 0.070           | 0.48 ± 0.012 | 132.05 ± 22.444 | 0.81 ± 0.016     | 0.68 ± 0.004       | 612.99 ± 121.59 | 0.28 ± 0.01 | 14.77 ± 0.00   |
| 10    | 0 (2.5)              | 0 (15)               | 22.88 ± 1.13      | 0.39 ± 0.013   | 2.00 ± 0.056           | 0.50 ± 0.005 | 124.28 ± 96.965 | 0.86 ± 0.008     | 0.69 ± 0.004       | 649.20 ± 79.45  | 0.29 ± 0.02 | 16.30 ± 1.73  |
| 11    | 0 (2.5)              | 0 (15)               | 21.63 ± 2.98      | 0.38 ± 0.006   | 2.01 ± 0.136           | 0.50 ± 0.005 | 107.48 ± 40.955 | 0.91 ± 0.030     | 0.66 ± 0.005       | 664.01 ± 252.05 | 0.27 ± 0.02 | 17.82 ± 0.26  |

Notes: Values are mean (n = 4) ± standard deviation (SD), including standard formulation. Different letters in the same column present significant differences at the level of 5% of significance (p < 0.05).

It was possible to verify a variation of 2.18% to 18.38% in moist gluten content of dough among the tests and, therefore, a significant effect (p < 0.05) of the pequi husk flour variable (x₁), making it possible to establish a mathematical model (for use with coded variables) within the conditions studied (Equation 4). Model repair was adopted to obtain good normality of the function.

\[
Moist\ gluten = 16.17882 - 5.22264x1 - 4.69838x1^2; \ R^2 = 89.56\% \tag{4}
\]

By regression analysis, a negative effect can be observed in the linear and quadratic term of the pequi husk flour variable, indicating that the lowest substitution level of variable x₁ provides the highest moist gluten percentage during the dough expression process. It is possible to verify that the gluten content presented very varied responses (Table 2), whereas the control presented values within the required standards (> 26%) to obtain a good quality bread dough (Instituto de Ciência e Tecnologia de Alimentos [ICTA], 2013). Therefore, this is an expected result, and it can be inferred that the gluten content is inversely proportional to the level of substitution of wheat flour by pequi husk flour, since it has no such viscoelastic protein formation properties.

Based on the regression analysis, the model for the moisture content of the control bread was verified by analysis of variance (ANOVA) (Table 3).

Table 3. Regression ANOVA for moist gluten content response.

| Source of variation | SS        | df | MS       | F calculated | F tabulated (5%; 2; 8) |
|---------------------|-----------|----|----------|--------------|------------------------|
| Regression          | 554.66    | 2  | 177.33   | 34.31        | 4.46                   |
| Residue             | 41.54     | 8  | 5.16     |              |                        |
| Total               | 596.01    | 10 |          |              |                        |

Notes: 56 Sum of squares; 46 Degrees of freedom; 66 Mean square.

According to Table 3, the analysis of variance (ANOVA) results revealed that the model for moist gluten content was significant (p < 0.05), according to the analysis of the F test (F_{calc} > F_{tab}) and a good coefficient of determination (R² = 0.89). The variation in the moist gluten content of the sweet breads, as a function of the pequi husk and pulp flours concentrations used is shown in Figure 1.

From the generated surface (Figure 1), it appears that the range between 0.75 and 2.5% replacement of wheat flour by pequi husk flour is ideal for obtaining a product that most resembles the pattern. The ideal replacement range for pequi pulp flour (x₂) is up to 20%, since no significant effects were found for this variable.

According to Coelho and Salas-Mellado (2015), Turfani, Narducci, Durazzo, Galli and Carcea (2017) and Yamsaengsung, Schoenelechner and Berghofer (2010) the higher the proportion of flour replacement by that from different vegetable sources, the higher the negative effects on the technological attributes of dough and bread quality, mainly due to the weakening of the gluten.
Figure 1. Contour curve of moist gluten content, as a function of variables: pequi husk flour and pequi pulp flour. Legend: Moist gluten: 0 to 16%. Average results obtained from the variable responses obtained from the different experimental tests.

According to Leão, Botelho, Oliveira and Franca (2018), the pequi exocarp + mesocarp flour composition has a high total dietary fiber content corresponding to approximately 45 g 100 g⁻¹, which contributes to the decrease in gluten content. According to Borges, Pirozi, Chaves, Germani and Paula (2011a), Vilhalva et al. (2011), Foste et al. (2014) and Tsatsaragkou, Gounaropoulos and Mandala (2014), the higher the fiber content present in the composition of flours from different plant sources, the greater the effect on viscoelastic protein formation, due to the high water absorption capacity.

Demirkesen, Mert, Sumnu and Sahin (2010) report, in their studies using chestnut flour in gluten-free formulation, that the water absorption process occurs from the interaction between the hydroxyl groups present in the fiber structure with the water used as an ingredient for bread formulation.

For the moist gluten content, the factorial tests (1 and 3), the axial tests (7 and 8) and the tests carried out at the central point (9, 10 and 11), do not differ statistically from each other, through the test of means. Their average values were those that most approached the standard formulation (> 26%) (Table 2). Model validation was performed by repeating a quadruplicate test (test 8) under the conditions of highest percentage of moist gluten content. Thus, the predicted moist gluten content was then compared with a Tukey test at 5% significance with the data resulting from the experiment (Table 4).

Table 4. The levels of variables used for the experimental validation test of the model.

| Pequi husk flour (x₁) | Pequi pulp flour (x₂) | Predicted result | Experimental result | Error (%) |
|-----------------------|-----------------------|------------------|---------------------|-----------|
| 0 (2.5)               | +1.41 (20)            | 16.17            | 18.49 ± 1.39        | 12.54     |

Notes: The results are presented as mean (n = 4) ± SD. The results with the same letters are not significantly different as determined by Tukey’s test, with p < 0.05.

As shown in Table 4, the relative error for the moist gluten content was low. This indicates that the results obtained in the validation test were satisfactory in relation to the response variable and the obtained model was validated (Equation 4).

Experimental planning 2

Experimental responses of the effect of partial replacement of wheat flour by pequi husk flour and water by pequi pulp in the preparation of sweet breads on technological variables studied are shown in Table 5.

According to analysis of the effect estimation (data not shown), pequi husk flour and pequi pulp did not affect the dependent variables studied (p-value > 0.05; R² < 0.70), except for specific volume of breads. Hence, it was possible establish a mathematical model only for that variable specific volume.

It was found that the values obtained for the specific volume ranged from 2.23 mL g⁻¹ to 4.21 mL g⁻¹ (Table 5). The lack of use of improvers justifies the low values for the specific volume found in this study. These results are similar to those found by Gandra, Del Bianchi, Godoy, Queiroz and Steel (2008) who obtained values of...
3.77 mL g⁻¹ and 4.40 mL g⁻¹ in breads enriched with fiber and added with lipases and monoglycerides and by Katina, Salmenkallio-Marttila, Partanen, Forssell and Autio (2006) who reported a value of 4.1 mL g⁻¹ for the specific volume of high-fiber bread added of enzyme mixture.

**Table 5.** CCRD with real and coded values for pequi husk flour (x₁) and pequi pulp (x₂), all responses and Tukey test to compare with control bread.

| Essay | Pequi husk flour (x₁) | Pequi pulp (x₂) | Mass volume (mL) | Expansion index | Specific volume (mL g⁻¹) | Density (g mL⁻¹) | Hardness (g) | Springiness (g) | Cohesiveness (g sec⁻¹) | Chewiness | Resilience |
|-------|----------------------|----------------|-----------------|----------------|----------------------|----------------|-------------|----------------|----------------------|-----------|-----------|
| 1     | -1 (0.73)            | -1 (0.86)      | 26.25 ± 1.26    | 0.46 ± 0.01    | 3.00 ± 0.13          | 0.33 ± 0.01    | 117.47 ± 111.74 | 0.96 ± 0.02 | 0.73 ± 0.02          | 819.85 ± 100.34 | 0.34 ± 0.01 |
| 2     | -1 (0.47)            | -1 (0.86)      | 25.25 ± 2.99    | 0.42 ± 0.01    | 2.58 ± 0.04          | 0.39 ± 0.01    | 1297.35 ± 196.32 | 0.93 ± 0.04 | 0.73 ± 0.09          | 881.39 ± 164.08 | 0.34 ± 0.01 |
| 3     | -1 (0.73)            | +1 (0.18)      | 21.00 ± 1.85    | 0.41 ± 0.01    | 2.36 ± 0.12          | 0.43 ± 0.02    | 1679.45 ± 191.36 | 0.92 ± 0.02 | 0.72 ± 0.09          | 1085.41 ± 126.55 | 0.32 ± 0.03 |
| 4     | -1 (0.47)            | +1 (0.18)      | 20.25 ± 0.96    | 0.40 ± 0.01    | 2.25 ± 0.19          | 0.43 ± 0.04    | 1428.31 ± 230.54 | 0.90 ± 0.02 | 0.71 ± 0.05          | 801.84 ± 185.62 | 0.30 ± 0.03 |
| 5     | -1.41 (5)            | 0 (20)         | 29.92 ± 0.19    | 0.46 ± 0.01    | 3.88 ± 0.08          | 0.26 ± 0.03    | 1446.21 ± 294.95 | 0.91 ± 0.05 | 0.73 ± 0.02          | 1005.47 ± 191.10 | 0.29 ± 0.04 |
| 6     | -1.41 (5)            | 0 (20)         | 26.38 ± 1.80    | 0.42 ± 0.01    | 3.82 ± 0.11          | 0.26 ± 0.01    | 1355.26 ± 142.27 | 0.94 ± 0.03 | 0.74 ± 0.09          | 923.56 ± 62.70  | 0.35 ± 0.02 |
| 7     | 0 (2.5)              | -1.41 (0)      | 26.75 ± 1.50    | 0.45 ± 0.01    | 2.64 ± 0.16          | 0.38 ± 0.02    | 1946.25 ± 598.57 | 0.95 ± 0.05 | 0.70 ± 0.01          | 1252.74 ± 325.92 | 0.32 ± 0.02 |
| 8     | 0 (2.5)              | -1.41 (40)     | 20.00 ± 0.00    | 0.45 ± 0.01    | 2.43 ± 0.11          | 0.41 ± 0.02    | 1380.70 ± 172.00 | 0.90 ± 0.05 | 0.72 ± 0.01          | 895.34 ± 105.25  | 0.30 ± 0.01 |
| 9     | 0 (2.5)              | 0 (20)         | 24.00 ± 0.82    | 0.44 ± 0.05    | 4.21 ± 0.11          | 0.24 ± 0.01    | 1841.51 ± 171.01 | 0.95 ± 0.03 | 0.71 ± 0.02          | 1216.44 ± 137.39 | 0.31 ± 0.01 |
| 10    | 0 (2.5)              | 0 (20)         | 24.00 ± 1.41    | 0.42 ± 0.01    | 4.17 ± 0.08          | 0.24 ± 0.00    | 1619.50 ± 180.96 | 0.89 ± 0.02 | 0.70 ± 0.01          | 1005.38 ± 151.53 | 0.32 ± 0.01 |
| 11    | 0 (2.5)              | 0 (20)         | 27.25 ± 0.96    | 0.45 ± 0.01    | 4.16 ± 0.09          | 0.24 ± 0.01    | 1573.95 ± 275.14 | 0.93 ± 0.02 | 0.70 ± 0.01          | 1021.96 ± 196.30 | 0.31 ± 0.01 |

Notes: Values are mean (n = 4) ± standard deviation (SD), including standard formulation. Different letters in the same column present significant differences at the level of 5% of significance (p < 0.05).

From the results through the design matrix (Table 5) it was possible to estimate a significant effect (p < 0.05) only for the pequi pulp variable (x₂), making it possible to establish a reparametrized mathematical model, under the studied conditions (Equation 5).

\[
\text{Specific volume} = 3.871765 - 0.888676x_2^2; \quad R^2 = 72.87\%
\]

According to Equation 4, the partial replacement of water by pequi pulp has a negative effect on the specific volume of breads, indicating that the lower the level of substitution, the higher the specific volume. The results showed, through the adequacy of the model, that the pequi pulp variable was the main factor that affected the specific bread volume. Miñarro, Albanell, Aguilar, Guamas and Capellas (2012) show that the specific volume of bread is considered one of the most important criteria in assessing the quality of the baking process, by providing quantitative measures of cooking performance and to exercise strong influence on consumer preference.

According to Table 5, extreme substitution levels (< 5% and > 35%) determined the lowest specific volume values – around 2.70 mL g⁻¹ – however, the results are close to the standard formulation (3.01 mL g⁻¹). This observation suggests that, due to the increase in substitution between the flours and fruit pulps used for the enrichment of bread, there was a significant reduction in the specific volume. Such observation is based on the high fiber content present in flour and pulps.

Several studies emphasize the nutritional value of pequi pulp, because the fruit is highly calorific (250 kcal 100 g⁻¹ to 350 kcal 100 g⁻¹), rich in lipids (27 g 100 g⁻¹ to 35 g 100 g⁻¹) and proteins (3 g 100 g⁻¹ to 6 g 100 g⁻¹). It is also a source of carotenoids (7.25 mg 100 g⁻¹ to 14.80 mg 100 g⁻¹) and vitamin C (50 mg 100 g⁻¹ to 105 mg 100 g⁻¹), besides having a high fiber content (6.8 g 100 g⁻¹ to 10.02 g 100 g⁻¹) (Alves, Fernandes, Sousa, Naves, & Naves, 2014; Arruda, Cruz, & Almeida, 2012; Vilas Boas et al., 2012; Gonçalves, Vilas Boas, Resende, Machado, & Vilas Boas, 2010, 2011; Ribeiro et al., 2012; Rodrigues et al., 2009, 2015).

From analyzes on the proximal composition of pequi pulp (data not shown), the pulp presented a high fiber content, corresponding to 5.21 g 100 g⁻¹. The result obtained is very close to results found in the literature. Thus, it is predictable that the amount of fiber present in pequi pulp will have a negative effect on the specific volume of breads (< 2.70 mL g⁻¹, Table 5).

According to Borges et al. (2011a), Vilhalva et al. (2011), Föste et al. (2014), Tsatsaragkou et al. (2014) and Stoll, Flöres and Thys (2015) in the case of breads, the presence of fiber may promote volume reduction due to the high absorption capacity of part of the water available for gluten formation and the lower tolerance to the fermentation process.

The substitution of up to 20% of the water by pequi pulp helped in the incorporation of ingredients and retention of gases produced during the fermentation process, resulting in higher dough extensibility and consequently higher bread volume (Table 5). Such observation occurs due to the characteristics of pequi pulp, especially its high lipid content.
According to Borges, Pirozi, Paula, Ramos and Chaves (2011b), the enrichment of salt bread with whole-grain having high fat concentration contributes to the formation of complexes with the forming of gluten proteins during the dough mixing process while improving the rheological characteristics and functional gluten properties. This positively influences the gas retention capacity in the dough and bread volume. Therefore, dough expansion is predictable at some degree of substitution (up to 20%), which results in a higher specific volume of the bread (> 4.10 mL g⁻¹, Table 5).

Based on the regression analysis, the validity of the model for specific volume was verified by analysis of variance (ANOVA) (Table 6).

| Source of variation | SS  | df | MS  | F calculated | F tabulated (5%; 1; 9) |
|---------------------|-----|----|-----|--------------|------------------------|
| Regression          | 4.88| 1  | 4.88| 24.17        | 5.12                   |
| Residue             | 1.81| 9  | 0.20|              |                        |
| Total               | 6.69| 10 |     |              |                        |

Notes: SS Sum of squares; df Degrees of freedom; MS Mean square.

The result of analysis of variance (ANOVA) revealed that the model obtained for specific volume was significant (p < 0.05), according to the analysis of the F test (F_{calc} < F_{tab}), with the determination coefficient R² > 0.70. As it is presented in Figure 2 shows the variation in the specific volume of sweet breads, as a function of the replacement levels of wheat flour by pequi husk flour and water by pequi pulp.

Figure 2. Contour curve of specific volume as a function of variables: pequi husk flour and pequi pulp. Legend: Specific volume: 2.2 to 3.8 mL g⁻¹. Average results obtained from the variable responses obtained from the different experimental tests.

The effects and optimum levels of independent variables were determined by plotting the contour curves. Figure 2 represents the specific volume of pequi sweet breads due to the partial replacement of water by pequi pulp. In this case, in order to obtain a product that resembles the standard, one alternative would be the replacement of 5.9 or 34% of pequi pulp and 5% of pequi husk flour to make better use of pequi waste.

Model validation was performed using the conditions for obtaining the largest specific volume of breads from the central point tests performed in quadruplicate. Thus, the validation of results related to the predicted specific volume was then compared to the standard (Tukey test at 5% significance level), with the results observed experimentally (Table 7).

As shown in Table 7, comparing predicted and observed experimental values, the results of the validation test were satisfactory with respect to variable response and the model obtained (Equation 5), since the relative error was low.
Table 7. The levels of variables used for the experimental validation test of the model.

| Pequi husk flour ($x_1$) | Pequi pulp ($x_2$) | Predicted result | Experimental result | Error (%) |
|--------------------------|-------------------|------------------|---------------------|-----------|
| 0 (2.5)                  | 0 (20)            | 3.87*            | 4.17 ± 0.08*        | 7.19      |

Notes: The results are presented as mean (n = 4) ± SD. The results with the same letters are not significantly different as determined by Tukey’s test, with $p < 0.05$.

Conclusion

The partial replacement of wheat flour by pequi husk and pulp flour do not affect the variables analyzed in dough and bread, except the moist gluten of dough - the lower the level of replacement of wheat flour by pequi husk flour, the higher the percentage of moist gluten of dough.

The partial replacement of water by pequi pulp do not affect the variables analyzed in dough and bread, except the specific volume of breads - the lower the level of replacement of water by pulp, the greater the specific volume of breads.

The ideal replacement ranges for obtaining pequi sweet breads with the same technological quality compared to the standard formulation were obtained from the formulations using between 0.75 to 2.5% pequi husk flour; up to 20% pequi pulp flour and between 5 and 35% pequi pulp.

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