High protein, gluten-free cookies made with rice, beans and avocado

Galletas libre de gluten con alto contenido proteico a base de arroz, frijol y aguacate

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ABSTRACT

Cookies are versatile foods that can supply specific needs and can be used as food vehicles to increase the intake of various nutrients. The objective was to create a gluten-free butter cookie based on rice flour (Oryza sativa). A central composite design $2^2$ was used for analyzing the substitution of rice flour for bean flour (Phaseolus vulgaris, L.) (0-60%) and the substitution of butter for avocado puree (Persea americana) (0-100%). Response Surface Methodology were used to analyze the data with a significance of 10% ($p<0.1$) and a minimum $R^2$ of 0.6. Variables analyzed were protein content (%), fat content (%), spread ratio, weight loss (%), water activity, moisture content (%), and ash content (%). Increases in the percentage of substitution of bean flour and avocado puree increased the amount of protein, ash, and moisture significantly. Fat content increased significantly following the decrease in avocado substitution. The minimum weight loss was obtained on intermediate values of bean flour. Water activity and spread ratio were not affected by changes in the variables measured. Three different formulas were obtained for the optimization: 46% bean flour and 86% of avocado puree, a formulation with an appropriate moisture range by reducing the lipid oxidation (59% bean flour and 82% avocado puree), and a formulation with 100% avocado (40% bean flour and 100% avocado puree). Sensory evaluation results of optimized treatments indicated that the formula with 46% bean flour and 86% avocado puree presented the highest global acceptance. Results from this study showcase the possibility of producing gluten-free cookies with good protein content.

Keywords: Avocado; Biscuits; Central composite design; Gluten-free cookies; Protein; Response surface methodology; Rice.
INTRODUCTION

The search for gluten-free products emerges from the interest in reducing the risk of celiac disease, wheat allergy, and gluten sensitivity. Around 1% of the world’s population suffers from celiac disease, and the number of patients increases annually. It is estimated that only 20% of celiacs are diagnosed. In addition, in countries like Finland and Sweden, the data is estimated at up to 3%, and in Western Sahara, these levels can be between 5-6%. Celiac disease is an autoimmune disorder manifested as an allergic response of the gut surface to gluten. It arises from an interaction of genetic, immune, and environmental factors that affect an individual’s life. The symptoms are minor intestine inflammations and lesions, which can result from diarrhea and a general reduction of nutrient absorption rates after ingesting gluten. Also, consumers search for product ingredients to provide healthier food alternatives, such as those marketed as gluten-free. Therefore, the demand for gluten-free products has been steadily increasing worldwide. Products like gluten-free pastries, cookies, and biscuits can be designed to gain access to this expanding food market.

Wheat flour used in traditional baked products can be replaced by gluten-free flour made of rice and beans. The Food and Agriculture Office (FAO) of the United Nations recommends combining cereal and legumes as a vegetable source to complete amino acid intake. The combination of cereal and legumes is beneficial for human health because of the amino acid profile that results from the mixture. Cereals are lysine deficient (31 ± 10 mg/g of protein) and thus should be complemented with lysine-rich legumes (64 ± 10 mg/g of protein). In contrast, cereals are rich in sulfur-containing amino acids (37 ± 5 mg/g of protein), whereas legumes are deficient in these amino acids levels (25 ± 3 mg/g). Moreover, total protein content is different too. Rice contains around 7.2 g/100 g (protein/product), while beans can have high content like 20 g/100g (protein/product). Therefore, combining cereals and legumes can increase food’s nutritional value.

Fat is critical to provide texture and flavor. Butter is one of the main ingredients in cookie formulation. It can be considered as a source of saturated fatty acids (51.4 g/100 g of product), monounsaturated (21 g/100 g of product), and polyunsaturated (1.5 g/100 g of product) fatty acids. Avocados (Persea Americana) can substitute fat in pastry and bakery products like cookies. Indeed, the avocado puree can create aired crumbs that provide similar desired texture attributes and generate food products with a reduced fat content. Avocados fatty acids’ profile quantified by solvent extraction is 18.0 ± 0.2% of palmitic acid (C 16:0); 6.2 ± 0.1% of palmitoleic acid (C 16: 1); and 59.0 ± 0.6% of oleic acid (C 18: 1). Therefore, the percentage of avocado’s fat is mainly 2.1% saturated, 9.8% monounsaturated, and 1.8% polyunsaturated fatty acids.

Therefore, the objective of this study was to analyze the influence of bean flour and avocado puree on the physical, chemical, and sensory properties of gluten-free butter cookies.

MATERIALS AND METHODS

Materials. Red beans (Phaseolus vulgaris, L. spp) were pulverized using an electric highspeed grinder. The avocado puree was made from Persea Americana (cultivar Fuerte). The avocados were obtained in the local market of Quito, Ecuador. Avocados were selected using a rapid and non-destructive method by gently squeezing them. If the avocado was soft (indicating mesocarp oil content as a sign of ripening), the fruit was cut in half and tasted. If the fruit did not taste watery or rubbery, seed and pulp were extracted separately, and pulp was smashed manually until obtaining a homogeneous paste.

Cookies elaboration. Cookies’ base formulation was 100% flour, 50% margarine, 20% of butter, 50% sugar, 25% eggs, 5% honey, and 1.5% vanilla extract. Fat (margarine, butter, and/or avocado) was mixed with sugar. The batter was whipped with a hand dough mixer Global Brand (BGMCR50012/250 W) for one minute at low speed and

RESUMEN

Las galletas son alimentos versátiles que pueden suplir necesidades específicas y ser utilizados como vehículos para aumentar la ingesta de distintos nutrientes. El objetivo fue elaborar galletas de mantequilla libres de gluten a base de harina de arroz. Se realizó un diseño central compuesto donde se estudió la sustitución parcial de harina de arroz por harina de frijol (Phaseolus vulgaris, L.) (0-60%) y la sustitución de la mantequilla por puré de aguacate (Persea americana) (0-100%). Los resultados fueron analizados por la Metodología de Superficie de Respuesta con un nivel de significancia del 10% (p<0.1) y con un mínimo $R^2$ de 0.6. Las variables de respuesta fueron: proteína (%), grasa (%), diámetro/altura, pérdida de peso (%), humedad (%) y cenizas (%). A valores altos de las dos variables estudiadas la cantidad de proteína, de cenizas y de humedad aumentó significativamente. La cantidad de grasa se incrementó al utilizar bajos niveles de puré de aguacate. Los menores valores de pérdida de peso se obtuvieron en niveles intermedios de harina de frijol. La relación diámetro/altura no fue afectada por las variables estudiadas. Al optimizar se obtuvieron 3 formulaciones diferentes: 46% de harina de frijol y 86% puré de aguacate; 59% de frijol y 82% de puré de aguacate y otra (no estadística) 40% de frijol y 100% de puré de aguacate. Los resultados del análisis sensorial de las muestras optimizadas indicaron que la formulación con mayor aceptación global fue la elaborada con 46% de harina de frijol y 86% de puré de aguacate, concluyendo que es posible elaborar una galleta libre de gluten y con una adecuada cantidad de proteína.

Palabras clave: Aguacate; Diseño central compuesto; Frijol; Galletería; Gluten; Leguminosa; Superficie de respuesta.
three minutes at medium speed. Then, liquid ingredients (egg, vanilla extract, and honey) were incorporated. The batter was mixed for one minute at low speed and three minutes at medium speed. Solid ingredients (rice and bean flour) were added. Finally, the batter was mixed for two minutes at low speed.

The batter was put in a plastic pastry bag with a 3 cm diameter metal nozzle. Cookies were deposited over a greased metal mold. A two-digit analytical scale (max. 3.100 g and min. 0.5 g; error 0.1 g) was used to guarantee standardized weights. The cookies were baked at 165 °C for five minutes in an electric convection (Práctica, Brazil) oven. Baked cookies were cooled at room temperature for 30 minutes and stored at room temperature in sealed polypropylene bags without light before analysis.

**Experimental design.** A central composite design (CCD) was run to study the partial substitution of rice flour with red bean flour (0%-60%) and butter substitution with avocado puree (0%-100%). Eleven trials (treatments) were conducted: 4 for the factorial design, 4 for the axial points, and 3 replicates at the central point to estimate error. The used a was ± 1.4142 to ensure the model’s rotatability (Table 1). Results were analyzed using Response Surface Methodology generating mathematical models with 10% significance and an R² of a minimum of 0.60 (Table 2).

**Cookies characterization**

**Proximal analysis:** Protein (method 46-12.01), total fat (method 30-25.01), moisture (method 44-17.01), and ash (method 08-01.01) content were measured following the AACCi.

**Spread ratio and Weight loss:** The diameter and the height were measured from eight randomly chosen cookies with a Vernier caliper. The diameter (cm) was divided by the height (cm), and an average from the eight measurements was obtained.

The batter was weighed before baking with a two digits analytical scale. Baked cookies were weighed after chilling at room temperature, and weight loss was calculated (Equation 1)

\[
\text{Weight loss} = \frac{(\text{Weight batter-weight cookie})}{\text{Weight batter}} \times 100 \% \quad (1)
\]

**Optimization:** Software Design Expert 10 was used to optimize treatments. Levels of importance (1–5, being 1 the least and 5 the most important) and goals (maximize, in range, minimize) were established for each response variable (Table 3).

**Sensory evaluation:** Treatments with the highest desirability were chosen for sensory evaluation. An affective test (overall acceptance, flavor, hardness, and appearance) was carried out with 64 randomly selected students from Universidad San Francisco de Quito (in age from 18 to 24; 36 women and 28 men) at the sensory evaluation laboratory of Universidad San Francisco de Quito. Samples were evaluated by consumers using a hedonic scale of 7 points (1: dislike a lot - 4: neither like nor dislike - 7: like a lot). Tested samples were shown simultaneously to panelists but randomly distributed on white foam – dishes at room temperature using three digits codified numbers. Selected panelists were asked to test the samples from left to right, drink water for palate cleansing, and wait thirty seconds between samples. Results were submitted to a two-way analysis of variance (ANOVA) (P-Test) and Tukey test for mean differences at a 5% significance level using Minitab Software 2018.

**RESULTS**

**Proximal analysis.** Moisture content results varied between 3.2 ± 0.03 and 5.9 ± 0.05% (Table 1). The mathematical model (Table 2) and the surface response (Figure 1a) show the influence of avocado puree and bean flour on the moisture content of cookies (p<0.1). The results showed that the bean flour replacement affects moisture content more drastically than avocado puree replacement. Consequently, the response surface (Figure 1a) and the mathematical model showed that the final product’s moisture content increased with a high amount of the two studied factors (avocado puree and bean flour) and decreased when both factors tend to low replacements.

The increased replacement of bean flour and avocado puree resulted in higher protein content of cookies (Table 2 and Figure 1b). Results support that bean flour content has a positive and linear effect on protein content in cookies.

Related to the fat content, avocado puree significantly affects cookies’ fat content and has a linear and negative effect on the mathematical model. Bean flour also affects biscuits’ fat content and has a quadratic effect on the model, as observed in table 2 and Figure 1c. Fat substitution with avocado puree is more drastic than bean flour replacement. Therefore, results show a drastic decrease in cookies’ fat content while increasing avocado puree.

Bean flour, avocado puree, and the interaction of both factors significantly influence ash content. Bean flour replacement affected ash content positively (Table 2). The mathematical model shows that both treatments’ interactions negatively influenced ash content (Figure 1d).

**Weight loss and Spread ratio.** The mathematical model obtained for weight loss (Table 2) shows a significant influence of bean flour and avocado puree on weight loss (p<0.1). Treatment 4 presented the highest weight loss because it contained 85.4% of avocado puree and 14.6% of butter (Table 1). On the other hand, bean flour replacement significantly affects weight loss (Figure 1e), has a quadratic effect on the model, and has a more drastic impact than avocado puree replacement. Avocado puree replacement negatively influences the spread ratio. The mathematical model (Table 2) and the response surface (Figure 1f) do not show an influence of bean flour on the spread ratio, nor both factor interaction (p>0.1).
Table 1. Results obtained in the Central Composite Design (CCD).

<table>
<thead>
<tr>
<th>T</th>
<th>BF* (%)</th>
<th>AP* (%)</th>
<th>Protein content (g/100g)</th>
<th>Fat content (g/100g)</th>
<th>Moisture content (g/100g)</th>
<th>Spread ratio</th>
<th>Weight loss (g/100g)</th>
<th>Aw</th>
<th>Ash content (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8</td>
<td>14.6</td>
<td>6.69±0.01</td>
<td>20.8±1.06</td>
<td>3.2±0.03</td>
<td>4.96±0.38</td>
<td>21.3±1.18</td>
<td>0.366±0.002</td>
<td>0.9±0.01</td>
</tr>
<tr>
<td>2</td>
<td>8.8</td>
<td>85.4</td>
<td>7.60±0.16</td>
<td>17.40±0.17</td>
<td>3.5±0.10</td>
<td>4.37±0.42</td>
<td>22.3±1.65</td>
<td>0.352±0.005</td>
<td>1.2±0.01</td>
</tr>
<tr>
<td>3</td>
<td>51.2</td>
<td>14.6</td>
<td>9.2±0.04</td>
<td>21.1±0.13</td>
<td>4.1±0.18</td>
<td>5.28±0.38</td>
<td>21.5±0.80</td>
<td>0.38±0.007</td>
<td>1.5±0.07</td>
</tr>
<tr>
<td>4</td>
<td>51.2</td>
<td>85.7</td>
<td>9.84±0.05</td>
<td>17.4±0.21</td>
<td>4.6±0.06</td>
<td>4.08±0.51</td>
<td>24.7±0.69</td>
<td>0.35±0.003</td>
<td>1.7±0.01</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>50</td>
<td>6.58±0.25</td>
<td>18.2±0.47</td>
<td>3.4±0.09</td>
<td>4.31±0.37</td>
<td>22.5±1.18</td>
<td>0.34±0.001</td>
<td>0.7±0.02</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>50</td>
<td>10.5±0.23</td>
<td>19.3±0.15</td>
<td>5.9±0.05</td>
<td>4.72±0.35</td>
<td>22.7±1.76</td>
<td>0.423±0.002</td>
<td>1.6±0.08</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0</td>
<td>7.62±0.28</td>
<td>22.6±0.12</td>
<td>3.5±0.10</td>
<td>4.42±0.28</td>
<td>20.9±1.13</td>
<td>0.367±0.002</td>
<td>1.2±0.04</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>100</td>
<td>8.51±0.12</td>
<td>17.5±0.18</td>
<td>4.3±0.03</td>
<td>3.92±0.26</td>
<td>21.7±1.63</td>
<td>0.431±0.011</td>
<td>1.2±0.06</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>50</td>
<td>8.59±0.24</td>
<td>19.8±0.13</td>
<td>3.6±0.08</td>
<td>4.97±0.43</td>
<td>21.1±0.58</td>
<td>0.324±0.002</td>
<td>1.2±0.02</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>50</td>
<td>8.34±0.20</td>
<td>19.3±0.28</td>
<td>3.5±0.09</td>
<td>4.63±0.19</td>
<td>21.8±1.27</td>
<td>0.35±0.001</td>
<td>1.2±0.03</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>50</td>
<td>8.58±0.24</td>
<td>19.7±0.13</td>
<td>3.4±0.01</td>
<td>4.84±0.35</td>
<td>20.9±1.15</td>
<td>0.351±0.003</td>
<td>1.2±0.02</td>
</tr>
</tbody>
</table>

*BF (Bean Flour) being the percentage of substitution considering 100% of the total flour; AP (Avocado puree) being the percentage of substitution considering 100% of the total butter.

Table 2. Mathematical models obtained with the CCD.

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Mathematical model</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (g/100g)</td>
<td>= 3.61 +0.696x1 + 0.416x1² +0.261x2 (1)</td>
<td>0.86</td>
</tr>
<tr>
<td>Protein content (g/100g)</td>
<td>= 8.52 + 1.29x1 + 0.339 x2 – 0.209x2² (2)</td>
<td>0.99</td>
</tr>
<tr>
<td>Fat content (g/100g)</td>
<td>= 19.8-0.539 x1²-1.80x2 (3)</td>
<td>0.97</td>
</tr>
<tr>
<td>Ash content (g/100g)</td>
<td>= 1.24 + 0.3297x1 + 0.0636 x2-0.04371 x2 (4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Weight loss (g/100g)</td>
<td>= 21.4 + 0.754x1²+ 0.68 x2 (5)</td>
<td>0.60</td>
</tr>
<tr>
<td>Spread ratio</td>
<td>= 4.75-0.311 x2-0.219 x2² (6)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

x1: Bean Flour; x2: Avocado Puree.
DISCUSSION

Proximal analysis

Scientific evidence shows that muffins with avocado puree instead of butter presented higher moisture due to avocado puree’s water content, which can be as high as 77%\textsuperscript{22} while butter can be as high as 16.2%\textsuperscript{23}. The bean flour replacement affects moisture content more drastically than avocado puree replacement. It can be explained due to the difference in moisture content in raw materials. Rice flour moisture content is 11.6%\textsuperscript{24} in average and 14% in red beans\textsuperscript{13}.

Regarding the protein content, the results obtained correspond with Frota et al.\textsuperscript{25}. They also observed higher protein content with increased legume flour (Vigna unguiculata L. Walp flour) in a dough of chocolate chip cookies and sweet rolled pastries. Differences in protein content between treatments explain the influence of beans addition over protein content\textsuperscript{26}. Bean flour has a more drastic effect than avocado puree (Table 2); also, the substitution of both factors created a quadratic effect, therefore the model’s curvature. Moreover, avocado puree addition showed a significant positive effect on protein content, as observed by Othman et al.\textsuperscript{27}. Avocado puree addition increased protein content in the cookies because protein content in avocado puree is 2%\textsuperscript{28} while butter protein content tends to 0%\textsuperscript{23}.

According to Dreher et al.\textsuperscript{16}, fat content in avocado is 15.4%\textsuperscript{22}, while in butter can be as high as 81.1%\textsuperscript{23}. Therefore, the mentioned fat content results correspond with Othman et al.\textsuperscript{27}, where avocado puree, used as a fat replacer, also showed a negative effect indicating a decrease in the fat content of baked products.

Ash content corresponds to the amount of minerals and trace elements found in measured samples\textsuperscript{29}. The observed influence (p<0.1) is caused by different mineral content in ingredients. Small red beans, Phaseolus vulgaris, L., contain 1.9% mineral content\textsuperscript{30}; meanwhile, Oryza sativa has around 0.4% mineral content\textsuperscript{31}. Therefore, bean flour replacement affected ash content positively (Table 2). avocado puree substitution positively influences ash content due to the different mineral content compared to butter, which is approximately 1.7%\textsuperscript{16}, while in butter is 0.1%\textsuperscript{23}.

Weight loss and Spread ratio

As already stated, avocado puree has a higher moisture content (77%)\textsuperscript{22} than butter (16.2%)\textsuperscript{23}. Baking is an industrial cooking process where moisture loss occurs\textsuperscript{32}. The observed difference in raw materials determines water loss during the cooking process and explains the positive effect of avocado puree on weight loss (Table 2). As already mentioned, beans have a higher moisture content than rice flour. Therefore, the addition of bean flour positively affects the final product’s moisture content.
Regarding the mathematical model obtained for spread ratio (Table 2), treatments with higher butter content increased diameter and decreased height, resulting in a higher spread ratio. In contrast, treatments with avocado puree presented a lower expansion and a lower spread ratio. These results are related to the melting process of high fat content batter providing a larger diameter than a low-fat one. Scientific literature affirms this result in oatmeal cookies, where treatments with butter content increased the diameter in a higher proportion than treatments with avocado in their formulations. The spread ratio enhances while the butter amount increases as air incorporate during the battering process in pastries with high-fat content. Due to the fat difference between avocado puree and butter, the spread ratio decreases because of the reduced amount of air bubbles.

Optimization

Using the simultaneous optimization technique by the desirability function, two statistical criteria were established: 1) maximize protein and mineral content and minimize moisture content, 2) maximize protein and mineral content and maintain the moisture content on average. A third criterion (no statistical) was chosen: 100% of avocado puree and 40% of bean flour to assure vegetarian products and complement amino acids between legumes and cereals (Table 3).

The first criterion was defined as gluten-free cookies with high protein content suitable for celiac consumption (Table 3). A German Dietary Survey from 2012 declared that celiac patients ingested more protein than their non–celiac pairs. As already discussed, rice has less protein than red beans; therefore, by adding bean flour, these cookies become a source of protein, making these cookies with a better nutritional profile. The blend of rice flour and bean flour reduces protein malnutrition for people who usually consume pastry made of wheat flour as the main ingredient. This blend can create a product with a complete amino acid profile.

Mineral content was maximized for creating a product with a high quantity of micronutrients like Ca, Zn, Fe, and K present in beans and rice. Moisture content was minimized to avoid lipid oxidation. Water solubilizes ions and minerals present in the product generating this detrimental effect. The desirability function increased to the first criterion (Table 3). Moisture content increased compared to the first criterion (Table 3). Proximal analysis (Table 4) shows differences among moisture content between criteria 1 and 2. Results in table 4 also indicate that criterion 2 has more risk to present lipid oxidation due to higher water content, higher Aw, and mineral content.

The third criterion is an approach for vegan consumers (Table 4, Treatment 3). The amount of bean flour chosen assured a complementary amino acid profile between cereals/legumes. The 100% of avocado puree as butter replacer is an option for the growing population looking for functional foods, which can improve personal health and increase the consumption of plant-based diets having the same characteristics as the traditional ones.

Sensory Evaluation

Results from affective tests are shown in table 5. According to the judges, treatments did not present statistical differences (p>0.1) among them and were evaluated between “Mildly like it” and “Moderately like it” (Table 5). Previous studies have shown that choco-chip cookies with 30% to 50% of rice flour substitution by bean flour did not present statistical differences for flavor among university students. Sensory evaluation test for reduced-fat muffins with 25%, 50%, 75%, and 100% avocado puree as a butter replacer demonstrated that muffins had high acceptability at up to 50% substitution. Fat substitution at higher than 50% leads to undesirable flavor and aftertaste. Therefore, the results indicate avocado puree’s feasibility for preparing fat-reduced muffins with an optimal 50% avocado purée substitution level. Othman et al. recommend using flavorants like peanut butter, chocolate, and fruits for masking colors, appearance, and flavor given by avocado puree.

Treatments 1 and 2 (Table 5) showed a significant difference (p<0.05) for hardness, appearance, and global acceptance. Nevertheless, Treatment 3 showed no difference with 1 and 2 (Table 5) for the same attributes.

Avocado puree replacement can affect the texture and the crumb size in low-fat muffins development. Ripening of climacteric fruits like avocado is fundamental for changes in taste and texture. Avocado’s creaminess is given by fat content developed during maturation.

Appearance varied from “Like slightly” and “Neither like nor dislike” (Table 5). During the sensory test, different judges commented about the unusual brown color of the cookies that affected their appearance due to the presence of avocado puree and bean flour, being different from traditional Danish cookies. Othman et al. mention that low-fat muffins with total avocado puree replacement for butter received comments about undesirable colors. General acceptance matches with Frota et al., who discussed that the higher substitution of rice flour by bean flour decreased the overall acceptance of treatments during sensory evaluation.

CONCLUSIONS

The mathematical models and equations show that bean flour and avocado puree replacement influence protein, fat, moisture, weight loss, and ash content. Avocado puree is the only factor that significantly influences overspread ratio. The protein and mineral content were maximized with the optimal formulations, obtaining cookies with a better nutritional profile. Sensory evaluation results indicated that formulas with 46% bean flour and 86% avocado puree presented the highest global acceptance. Results from this study showcase the possibility of producing gluten-free cookies with good protein content.
Table 3. Results obtained by the desirability function.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criterion 1</th>
<th>Criterion 2</th>
<th>Solution</th>
<th>Goal</th>
<th>Importance</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>Maximize</td>
<td>5</td>
<td>46.33</td>
<td>Maximize</td>
<td>5</td>
<td>59.06</td>
</tr>
<tr>
<td>AP</td>
<td>Maximize</td>
<td>5</td>
<td>84.06</td>
<td>Maximize</td>
<td>5</td>
<td>82.17</td>
</tr>
<tr>
<td>Protein content</td>
<td>Maximize</td>
<td>5</td>
<td>9.58</td>
<td>Maximize</td>
<td>5</td>
<td>10.34</td>
</tr>
<tr>
<td>Fat content</td>
<td>In range</td>
<td>3</td>
<td>17.74</td>
<td>In range</td>
<td>3</td>
<td>17.37</td>
</tr>
<tr>
<td>Moisture content</td>
<td>Minimize</td>
<td>5</td>
<td>4.72</td>
<td>In range</td>
<td>3</td>
<td>5.68</td>
</tr>
<tr>
<td>Spread ratio</td>
<td>In range</td>
<td>3</td>
<td>4.59</td>
<td>In range</td>
<td>3</td>
<td>4.59</td>
</tr>
<tr>
<td>Aw</td>
<td>In range</td>
<td>3</td>
<td>0.37</td>
<td>In range</td>
<td>3</td>
<td>0.367</td>
</tr>
<tr>
<td>Ash content</td>
<td>Maximize</td>
<td>5</td>
<td>1.53</td>
<td>Maximize</td>
<td>5</td>
<td>1.70</td>
</tr>
<tr>
<td>Weight loss</td>
<td>In range</td>
<td>3</td>
<td>23.28</td>
<td>In range</td>
<td>3</td>
<td>24.67</td>
</tr>
<tr>
<td>Desirability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
</tbody>
</table>

*BF (Bean flour), AP (Avocado puree); Criterion 1: maximize protein and mineral content and minimize moisture content; Criterion 2: maximize protein and mineral content and moisture content “in range”.

Table 4. Results of response variable for optimized treatments.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Treatment 1*</th>
<th>Treatment 2*</th>
<th>Treatment 3**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(46.3%-BF)</td>
<td>(59.1%-BF)</td>
<td>(40%-BF)</td>
</tr>
<tr>
<td></td>
<td>(84.1%-AP)</td>
<td>(82.2%-AP)</td>
<td>(100%-AP)</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>8.7±0.02</td>
<td>9.8±0.07</td>
<td>8.4±0.03</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>19 ±0.03</td>
<td>23.8±0.05</td>
<td>22.5±0.06</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>3.2±0.03</td>
<td>3.4±0.05</td>
<td>3.9±0.03</td>
</tr>
<tr>
<td>Spread ratio</td>
<td>4.11±0.31</td>
<td>4.18±0.53</td>
<td>4.26±0.18</td>
</tr>
<tr>
<td>Aw</td>
<td>0.35±0.003</td>
<td>0.39±0.006</td>
<td>0.36±0.006</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>1.7±0.05</td>
<td>1.7±0.06</td>
<td>1.6±0.01</td>
</tr>
</tbody>
</table>

BF (Bean Flour); AP (Avocado puree).
*Treatment 1 and Treatment 2: Obtained by optimization (Table 3).
**Vegan option (not statistical).
Table 5. Results for sensory evaluation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flavor*</th>
<th>Hardness*</th>
<th>Appearance*</th>
<th>Global Acceptance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (46.3%-BF) (84.1%-AP)</td>
<td>5.48±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.14±1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.56±0.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (59.1%-BF) (82.2%-AP)</td>
<td>5.13±1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.28±1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.58±1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.05±1.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 (40%-BF) (100%-AP)</td>
<td>5.36±1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.66±1.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.86±1.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.20±1.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Arithmetical means ± standard deviation (n=64); means followed by the same letter do not differ between them at 5% of probability by Tukey’s test. BF (Bean Flour); AP (Avocado puree).

Scale used: 1- dislike a lot - 4: neither like nor dislike - 7: like a lot.

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18. AACC International Methods 46-12.01, 30-25.01, 44-17.01, and 08-01.01. AACC International Approved Methods of
High protein, gluten-free cookies made with rice, beans and avocado


