# Impact on technological properties, sensory acceptance, bioactive compounds, and nutritional quality of blend pinto beans and red rice for novel cookies

**Camila Soares de Magalhães**
Universidade Federal do Espírito Santo
**Gleicyane de Almeida Marques**
Universidade Federal do Espírito Santo
**Ronel Joel Bazán-Colque**
Universidade Federal Rural do Rio de Janeiro
**Erica Aguiar Moraes**
Universidade Federal do Espírito Santo
**Erika Madeira Moreira da Silva** (erika.m.silva@ufes.br)
Universidade Federal do Espírito Santo
**José Luis Ramirez Ascheri**
Embrapa Food Technology

**Research Article**

**Keywords:** Food Biofortification, BRS Cometa, Bioactive Compounds, Sensory Acceptance, Gluten-free product

**Posted Date:** June 22nd, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1754480/v1

## Abstract

The objective of this work was to understand how the addition of pinto beans flour (PBF) affected the technological properties, sensory acceptance, bioactive compounds, and nutritional quality of gluten-free cookies. The factors of (PBF) addition (25–75 g/100 g) and margarine content (13.3–19.3 g/100 g dough) were varied according to a $2^2$ factorial design with center and axial points augmented. Other physicochemical analyses were carried out in the selected formulations during storage time. (PBF) addition affected the technological properties and increased the nutritional content of proteins (up to 13%), fibers (8.28 %), iron(2.13 %), zinc(1.54%), and bioactive compounds (phenolic with 139.46 mg GAE/100 g), but it negatively affected sensory acceptance. Margarine's addition improved the evaluated sensory aspects, showing above 70% acceptance for all the attributes analyzed in all the trials. Cookies with 50 g pinto beans flour, 50 g red rice, and 16.3 g margarine/100 g dough showed better technological, nutritional characteristics, and physicochemical quality up to 60 days of storage. This work contributed to the incorporation of mixtures of red rice and pinto beans for developing more nutritious cookies for celiac patients or even those who wish to consume gluten-free products.


## 1. Introduction

Beans represent 65% of the total protein and 32% of the energy consumed in Africa and Latin America. Moreover, beans are a source of fiber, vitamins, minerals, and bioactive compounds [1]. Pinto beans (BRS Cometa) are a project of biofortification through conventional breeding, originating from years of research of the BioFORT program, led by Brazilian Agricultural Research Corporation (EMBRAPA). The type of beans used in this study, besides its natural nutritional profile, presents higher levels of iron and zinc compared to commercial ones [2]. In addition to these nutritional benefits, pinto beans do not contain gluten in their structure, which represents an alternative to wheat-based products. However, foods with the direct addition of bean flour result in bean-flavored foods and other unpleasant flavors in the food, it is necessary to make mixtures with non-conventional flours to improve this characteristic.

Red rice is a type of rice grain that presents proanthocyanidins, responsible for the reddish coloration of the grain. This polyphenol has beneficial biological properties, such as strong antioxidant capacity. When compared with white rice, red rice also has higher levels of dietary fiber, minerals, and vitamins [3]. Naturally, cereals are limited in lysine and have a high amount of methionine [4]; therefore, combining rice with a legume is important to have a complete and well-balanced amino acid profile.

The development of new flours may offer the market opportunities for innovation of new products, as well as increasing the supply of gluten-free products. However, gluten is responsible for the elastic characteristic of dough and contributes to the appearance and structure of many bakery products. Gluten replacement is a major technological challenge as it is an essential building protein for the formulation of high-quality products [5]. Many commercially available gluten-free products have lower quality [6], an unwanted and residual taste, besides a high cost compared to gluten foods [7]. Cookies are an interesting alternative because of their flexibility of substitutions of many ingredients and are widely accepted and consumed by all types of consumers, as well as having a long shelf life and being convenient for consumption. Normally, this type of bakery is traditionally prepared with wheat flour, sugar, and fat [8]. Fat is fundamental for the mixing of dough ingredients and contributes to the technological, texture, and flavor quality of cookies [9]. However, consuming high amounts of fat is correlated with serious health problems. Therefore, an optimization process is suggested for the production of cookies with less fat and the use of compound flours with high amounts of bean flour, since these offer a valuable supplementation vehicle for nutritional improvement. In the light of this information, this study aimed to evaluate the effect of $PBF$ addition and margarine content, on technological properties, sensorial acceptance, nutritional characteristics, bioactive compounds, and storage of cookies of mixtures of red rice ($RRF$) and pinto beans flour ($PBF$).

## 2. Materials And Methods

The material and methods section is presented as Supplementary Material.

## 3. Results And Discussion

# 3.1 Electrophoresis of raw and processed flours

In Fig. 1, $PBF$ raw presented saturation of the main protein groups present in bean, mainly globulins (40–60 kDa) and phytohemagglutinins (27–37 kDa). However, processed $PBF$ showed weak protein bands, although the same extraction methodology was used. In contrast, $RRF$ raw and processed showed a similar protein profile, to typical rice grain protein run, such as the presence of albumin (13–110 kDa), α glutenin (30–40 kDa), β glutenin (19–23 kDa) and prolamines (~ 13 kDa). The presence of these proteins indicates their maintenance after processing. $PBF$ raw showed strong protein bands when compared to $RRF$ raw and processed results since beans have higher protein content when compared to rice. No legumin band was found, and only a weak vicillin band (~ 47.82 kDa) was observed in the $PBF$ processed (Fig. 1). These results may be related to the fact that proteins in the 60 kDa range were partially extracted from a flour insoluble complex processed during sample preparation [10]. Phytohemagglutinins or lectins are globular proteins that are present in large quantities in beans. These proteins are highly resistant to proteolytic digestion, the digestive tract is constantly exposed to biologically active lectins contained in fresh and processed [11]. When consumed in large quantities, phytohemagglutinins are toxic. A pre-treatment is necessary to reduce or destroy these proteins [12]. In this study, a weak band in the range of 33.99 kDa was observed in the $PBF$ processed (Line 3, Fig. 1), indicating that all the processes for obtaining the flour were fundamental for lectin reduction.

## 3.2 Technological properties

Measurements of cookies' technological properties are shown in Table 1. Under the conditions studied, the levels of $PBF$ ($X_1$) and margarine ($X_2$) did not have significant effects (p > 0.05) on the weight reduction ($WR$), thickness ratio ($TR$), and expansion factor ($EF$) of the cookies. However, an increase in $X_1$ was able to reduce significantly (p < 0.01) the diameter ratio ($DR$) and specific volume ($SV$) (Table S1). The effect on these variables can be attributed to the functional properties of $PBF$ and $RRF$. Flours with higher protein content increase some properties, like water retention, emulsification, and viscosity of the dough, consequently, occurring an inverse correlation between expansion and volume of dough during baking [8]. The fitted models (Eqs. 1 and 2) had a non-significant $LoF$ (Table S1, p > 0.05) and the coefficients of determination ($R^2$) were acceptable for $DR$ (0.888) and $SV$ (0.712), which could be used for predictive purposes.

Table 1
Experimental design and responses for technological properties and sensory analysis of cookies

| | Experimental design | | Technological properties | | | | | | Sensory analysis | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trial | $X_1$ | $X_2$ | $WR$ | $DR$ | $TR$ | $EF$ | $SV$ | $H$ | Taste | Color | Aroma | $App$ | Texture | $PI$ |
| | g/100 g | g/100 g | g | cm/cm | cm/cm | cm/cm | mL/g | N | | | | | | |
| 1 | 25 | 13.3 | 1.15 | 1.08 | 1.41 | 5.84 | 1.35 | 1.89 | 7.30 | 8.09 | 7.33 | 7.96 | 7.47 | 7.17 |
| 2 | 25 | 19.3 | 1.18 | 1.12 | 1.32 | 6.38 | 1.32 | 1.12 | 7.39 | 8.05 | 7.56 | 7.88 | 7.68 | 7.21 |
| 3 | 75 | 13.3 | 1.11 | 1.03 | 1.34 | 6.19 | 1.21 | 3.04 | 6.30 | 7.79 | 6.84 | 7.74 | 6.30 | 5.89 |
| 4 | 75 | 19.3 | 1.16 | 1.04 | 1.27 | 6.22 | 1.25 | 1.75 | 7.00 | 7.74 | 7.25 | 7.58 | 7.28 | 7.02 |
| 5 | 14.65 | 16.3 | 1.13 | 1.14 | 1.39 | 6.11 | 1.43 | 1.64 | 7.32 | 7.53 | 7.47 | 6.98 | 6.96 | 6.79 |
| 6 | 85.35 | 16.3 | 1.08 | 1.04 | 1.36 | 5.98 | 1.19 | 1.41 | 6.74 | 7.42 | 7.12 | 7.32 | 7.04 | 6.02 |
| 7 | 50 | 12.06 | 1.06 | 1.06 | 1.45 | 5.62 | 1.31 | 2.72 | 6.51 | 7.49 | 7.25 | 7.60 | 6.32 | 6.07 |
| 8 | 50 | 20.54 | 1.06 | 1.12 | 1.45 | 5.98 | 1.41 | 1.36 | 7.46 | 7.77 | 7.32 | 7.26 | 7.33 | 7.30 |
| 9 | 50 | 16.3 | 1.09 | 1.06 | 1.41 | 5.81 | 1.24 | 1.74 | 7.00 | 7.88 | 7.37 | 7.86 | 7.33 | 6.58 |
| 10 | 50 | 16.3 | 0.97 | 1.06 | 1.35 | 5.99 | 1.28 | 1.70 | 7.12 | 8.07 | 7.49 | 7.89 | 7.63 | 7.04 |
| 11 | 50 | 16.3 | 1.08 | 1.04 | 1.28 | 6.23 | 1.24 | 1.50 | 7.11 | 7.88 | 7.60 | 7.81 | 7.33 | 7.04 |
| 12 | 50 | 16.3 | 1.13 | 1.05 | 1.33 | 5.96 | 1.20 | 1.78 | 7.25 | 7.63 | 7.39 | 7.60 | 7.39 | 7.39 |

$X_1$: $PBF$ addition; $X_2$: margarine; $WR$: weight reduction; $DR$: diameter ratio; $TR$: thickness ratio; $EF$: expansion factor; $SV$: specific volume; $H$: hardness; $App$: appearance; $PI$: purchase intention. Trials of the 9 to 12 are the center points

$$DR = 1.053 - 0.034X_1 + 0.013{X_1}^2 + 0.017X_2 + 0.013{X_2}^2 \ldots(1)$$

$$SV = 1.258 - 0.069X_1 + 0.043{X_2}^2 \ldots(2)$$

The response surface plots for $DR$ and $SV$ were constructed using Eqs. (1) and (2) and are shown in Fig. S1 and S2. The regression coefficient of $X_1$ had a negative impact on $DR$ (− 0.034) and $SV$ (− 0.069) values. On the other hand, the $H$ values ranged from 1.12 to 3.04 N (Table 1). Based on the ANOVA (Table S1), the process variability was explained by the effects, in the following impact order $X_2$ (59.51%, p < 0.01); ${X_2}^2$ (10.53%, p < 0.05), and $X_1$ (7.92%, p < 0.05). The model tested (Eq. 3) showed a non-significant $LoF$ (p > 0.05) and an acceptable $R^2$ (0.779), which could be used to predict future values within the studied range.

$$H = 1.652 + 0.182X_1 - 0.498X_2 + 0.229{X_2}^2 \ldots(3)$$

The response surface graph for H was constructed using Eq. (3) and is shown in Fig. S3. The addition of $X_1$ (up to 85.35 g/100 g) caused an increase in $H$. Some authors observed an increase in hardness when pulses were added to cookies [13, 14]. Incorporating protein-rich flours requires more water to obtain a moldable dough. With this competition and reduction of free water in the dough, it loses consistency and becomes breakable. In addition, the higher the protein content, the higher the water absorption in the dough, consequently, less expansion will make the cookie harder [8, 13]. On the other hand, the addition of $X_2$ (up to 20.54 g/100 g) reduced $H$, making the cookies softer (Fig. S3). The addition of fats in the formulation softens the dough and gives it a plastic character. Fat globules cover proteins and starch granules and isolate them. Thus, it prevents the formation of polymers and reduces the density of the net, which reduces the hardness of the cookies [15].

## 3.3 Sensory analysis

The $PBF$ addition ($X_1$) was able to negatively interfere with the acceptance of the taste, aroma, appearance, and texture, essential criteria for the consumption of cookies (Table 1). Besides, the color, as well as the purchase intention of the cookies, were not influenced by the formulations used in this study. Despite the $PBF$ content having influenced the acceptance of the cookies, all the evaluated treatments received grades, that on the hedonic scale corresponded from “liked moderately” to “liked very much” (Table 1). On the other hand, considering a relationship between sensory texture and instrumental texture ($H$) (– 0.83, p < 0.01, Fig. 2a), it can be noted that there is a rejection by harder cookies, a result achieved by increasing $X_1$. Also, the addition of margarine ($X_2$, up to 20.54 g/100 g) had a positive impact on sensory acceptance for taste and texture. Szczygiel et al. [16] reported that 25–35% of $X_1$ added to the total cracker mass weight had a negative impact on texture and taste.

Trial 1 showed the best acceptance rate (considering all the sensory attributes) while the formulation of trial 3 showed the lowest acceptance rate. However, the rates for almost all analyzed attributes were above 70% for all treatments (Table 1). This reaffirms that, although the increase in $PBF$ causes less acceptance of the product, the inclusion of a higher content of margarine improves the sensory aspects, making the product more accepted. Baked products require margarine, vegetable shortening, or butter to promote a pleasant taste, incorporate air, increase shelf life, and soften the texture of the final product [17]. Thus, a balance between these ingredients, to serve a product with greater acceptance in the market, must be sought.

## 3.4 Selection of formulations

The selected formulas were found using multiple desirable characteristics and principal component analysis. For technological properties, optimal process conditions were obtained by overlaying contour plots of $DR$, $SV$, and $H$ over the $X_1X_2$ plane (Fig. 2b), to satisfy the technological properties of cookies based on $PBF$ and $RRF$ blends. According to the AACC Method 10–54 [18], high-quality cookie flours are usually associated with larger cookie $DR$, lower $SV$, and $H$, thus desirability ranges were: 1.05 ≤ $DR$ (cm/cm), $SV$ (mL/g) ≤ 1.32, and 1.5 ≤ $H$ (N) ≤ 2.0. Figure 2b shows the feasible operating region from the technological standpoint, in the range of 27.5 ≤ $X_1$ (g/100 g) ≤ 60.0 and 14.2 ≤ $X_2$ (g/100 g) ≤ 17.5. The cookies obtained in this region guarantee the technological characteristics and necessary attributes in the development of novelty products rich in proteins. On the other hand, the dimensionality reduction of the sensory analysis responses into two principal components ($PC$), allowed retaining above 90% of the variation present in the original responses ($PC$ 1: 64.76% and $PC$ 2: 26.07%). In the correlation plot (Fig. 2a, c), the responses grouped that presented positive strong correlations (p < 0.01) were: taste-$PI$ (+ 0.91), texture-$PI$ (+ 0.83) and taste-texture (+ 0.83). Correlations between taste-aroma, color-$App$, color-texture, aroma-texture, and aroma-$PI$ reached intermediate correlations ($p$ < 0.05). The projection of the trials on the factor map (Fig. 2d) confirms the contribution of all variables in data variability. Trials 1 and 2 are close to the $CP$ point showing similarity in the results of the technological properties and sensory analysis. It was considered that the increase of $PBF$ in cookies formulated negatively affected technological and sensory parameters, while the increase in the margarine content improved these aspects. In addition, it can be considered that the excessive increase of fats in the formulations can cause nutritional problems in the long term. Thus, it is important to seek a balance between these ingredients, not forgetting that red rice, also included in the formulation, is a component with good content in fibers and antioxidant elements. The cookies selected to analyze the nutritional composition, bioactive compounds, morphology, and storage were trials 1, 2, and $CP$. Also, a control treatment with 0 g $PBF$, 100 g $RRF$, and 16.3 g margarine/100 g dough was included.

## 3.5 Chemical composition analysis, iron, and zinc content

Cookies with a higher $PBF$ addition had higher moisture, ash, protein, iron, zinc, and fiber content as well as lower total carbohydrate content (Table 2). Moreover, trial $CP$ presented almost 2 times more protein and 3.42 times more fiber than the control formulation. Some authors observed an increase in fiber content with legume incorporation in cookies [19, 20]. Adding pinto beans to cookies not only increases the amount of protein and fiber, but also minerals like iron and zinc (Table 2). Trial $CP$ had almost 4 times more iron and 1.39 times more zinc content when compared to the control treatment. Rajiv et al. [19] also observed an increase in these minerals when there was a 40 g/100 g substitution of wheat flour for legume flour in comparison to control.

Table 2
Chemical composition (selected and control cookies) and bioactive compounds of pinto beans flour ($PBF$) and red rice flour ($RRF$) raw and processed, selected cookies and control sample

| Component | Trial (cookies) | | | Control |
|---|---|---|---|---|
| | 1 | 2 | $CP$ | |
| *Proximal* | | | | |
| Ash (%, db) | 2.43 ± 0.00$^{b}$ | 2.37 ± 0.00$^{b}$ | 2.83 ± 0.05$^{a}$ | 2.05 ± 0.07$^{c}$ |
| Lipid (%, db) | 15.43 ± 0.57$^{b}$ | 22.57 ± 0.61$^{a}$ | 19.48 ± 1.71$^{ab}$ | 16.89 ± 0.23$^{b}$ |
| Protein (%, db) | 9.19 ± 0.24$^{b}$ | 9.89 ± 0.08$^{b}$ | 13.00 ± 0.16$^{a}$ | 5.77 ± 0.08$^{c}$ |
| Carbohydrates (%, db) | 67.14 ± 0.77$^{b}$ | 59.49 ± 0.55$^{c}$ | 56.41 ± 1.68$^{c}$ | 72.90 ± 0.10$^{a}$ |
| Fiber (%, db) | 5.82 ± 0.01$^{b}$ | 5.68 ± 0.01$^{c}$ | 8.28 ± 0.01$^{a}$ | 2.39 ± 0.01$^{d}$ |
| Energetic value (kcal) | 440.44 ± 3.05$^{b}$ | 477.45 ± 3.58$^{a}$ | 445.28 ± 8.02$^{b}$ | 465.24 ± 1.33$^{a}$ |
| *Mineral* | | | | |
| Iron (mg/100 g) | 1.34 ± 0.02$^{b}$ | 1.40 ± 0.70$^{b}$ | 2.13 ± 0.06$^{a}$ | 0.58 ± 0.13$^{c}$ |
| Zinc (mg/100 g) | 1.42 ± 0.01$^{b}$ | 1.45 ± 0.01$^{b}$ | 1.54 ± 0.03$^{a}$ | 1.11 ± 0.03$^{c}$ |
| *Bioactive compounds* | | | | |
| $TPC$ (mg $GAE$/100 g) | 116.43 ± 1.26$^{b}$ | 122.81 ± 3.75$^{b}$ | 139.46 ± 1.57$^{a}$ | 87.35 ± 0.21$^{c}$ |
| $AC$ (%) | 71.56 ± 1.08$^{b}$ | 74.96 ± 1.45$^{a}$ | 77.72 ± 1.70$^{a}$ | 70.56 ± 0.26$^{b}$ |
| Tannins (mg $CE$/100 g) | 3.47 ± 0.15$^{b}$ | 3.27 ± 0.43$^{b}$ | 4.78 ± 0.10$^{a}$ | 3.28 ± 0.07$^{b}$ |
| | Flours | | | |
| Component | $RRF$ | $RRF$ | $PBF$ | $PBF$ |
| | (Raw) | (Processed) | (Raw) | (Processed) |
| $TPC$ (mg $GAE$/100 g) * | 184.78 ± 5.83$^{a}$ | 79.16 ± 0.68$^{b}$ | 204.65 ± 0.50$^{a}$ | 79.16 ± 0.68$^{b}$ |
| $AC$(%) * | 99.92 ± 0.06$^{a}$ | 74.49 ± 1.06$^{b}$ | 95.22 ± 1.57$^{a}$ | 82.16 ± 0.66$^{b}$ |
| Tannins (mg $CE$/100 g) * | 17.35 ± 1.24$^{a}$ | 10.00 ± 0.59$^{b}$ | 16.14 ± 0.43$^{a}$ | 7.86 ± 0.01$^{b}$ |

Values are expressed as the mean ± standard deviation of three replications (n = 3). Cookies made of pinto bean flour ($PBF$) and red rice flour ($RRF$). Trial 1 (25 g $PBF$, 75 g $RRF$, and 13.3 g margarine/100 g dough). Trial 2 (25 g $PBF$, 75 g $RRF$, and 19.3 g margarine/100 g dough). Trial $CP$ (center points' average) (50g $PBF$, 50 g $RRF$, and 16.3 g de margarine/100 g dough). Trial control (0 g $PBF$, 100 g $RRF$, and 16.3 g margarine/100 g dough). db: dry basis; $TPC$: Total phenolic compound; $GAE$: Gallic acid equivalent; $AC$: Antioxidant capacity; $CE$: Catechin equivalent. Values with different letters in the same row differ statistically in mean using the Tukey test ($p < 0.05$) and * t-student test ($p < 0.05$).

## 3.6 Bioactive compounds

The process to obtain flours ($PBF$ and $RRF$) retained 43% and 61.4% of phenolic compounds and 74.5% and 86.3% antioxidant capacity, respectively. Only $PBF$ showed significant retention of tannins (48.7%), losing more than 50% of this component during processing (Table 2). In cookies, it is noted that the increase in the $PBF$ addition (up to 50 g/100 g) increased the content of total phenolic, tannins, and antioxidant capacity. Some studies also reported an increase in phenolic compounds when beans were added to formulations [21, 22]. However, a reduction in the concentration of total phenols, especially in beans, is expected due to its process of soaking, as these compounds are water-soluble. Additionally, high temperatures used to cook the grains may negatively impact these compounds or even the interaction with proteins, forming complexes [23]. Even with a certain loss after processing, the antioxidant capacity of the flours and cookies was above 70%, which is considered exceptionally good. It is important to highlight that the cooking of raw grains is necessary to eliminate antinutritional substances. Moreover, it increases the digestibility of proteins and starches through the inactivation of proteinase inhibitors, protein denaturation, and starch gelatinization [24]. In addition, the increase in margarine also showed a higher antioxidant capacity (Table 2), this could be due to the addition of antioxidant additives in the margarine.

## 3.7 Scanning electron microscopy (SEM)

All cookies exhibited homogeneous characteristics, however, with variable sizes of protuberances (Fig. 3). Starch granules are surrounded by a non-gluten-forming protein film and melted and re-solidified fat [25]. The components of the cookie formulations are capable of interaction when ingredients are mixed and kneaded during dough formation, especially in the presence of an emulsifier, such as lecithin. However, it is observed that trial 1 (with less margarine content) presents a different aspect from the other treatments, with less evidence of complexes made with starch, proteins, and lipids (Fig. 3). As expected, the presence of a gluten protein network was not observed in all formulations. However, air bubbles can be observed in various parts of the cookie (Fig. 3). This is due to the formation of gases by chemical agents and the evaporation of water when baked. Higher temperatures increase gas production and water vapor; this expands the dough to go outside, producing air bubbles inside the cookies [9].

## 3.8 Storage

The water activity ($a_w$) increased after day 60 of storage, for all samples. However, these values remained around 0.3. This level of $a_w$ is important to reduce risks of biological, chemical, and physical deterioration and, consequently, increase shelf-life [26]. The use of margarine may affect reducing $a_w$ Trial 2 showed a lower $a_w$, among the other samples (Table 3). On the other hand, the hardness of the cookies was not affected by the storage period (Table 3, $p > 0.05$), possibly due to the maintenance of low levels of $a_w$ throughout this period. The cookie samples showed very close pH values, around 6. However, there is a slight increase in pH from day 30, remaining stable until 60 days. At the same time, the samples showed low acidity, especially the control sample. The acidity level did not change significantly (Table 3, $p > 0.05$). The thermal processing of beans, red rice, and the cookies may have contributed to the stability of these parameters as heat inhibits enzymes that can cause rancidity in whole flours. Regarding the color, there is a darkening of all the cookie samples after day 30, keeping up to 60 days of storage. Cookies tended to become reddish from the same period. The same was observed for the values of hue and saturation. The cookie samples showed more intense colors and within the range of red, after day 30 of storage, remaining stable within up to 60 days. This effect may be due to the additional anthocyanidins released from proanthocyanidins [27]. Also, the formation of anthocyanin-derived pigments stabilizes the flavylium red-colored form [28]. At the end of the storage, all samples, including the control sample, showed similar values for chroma a* and b*, hue, and saturation (Table 3). Changes in product color during storage indicate changes in freshness shelf life and therefore play an important role in food selection and purchase [29].

Table 3
Storage analysis of selected cookies and control sample

| Component | Day | Trial (cookies) | | | Control |
|---|---|---|---|---|---|
| | | 1 | 2 | *CP* | |
| $a_w$ | 1 | 0.15 ± 0.01$^{cB}$ | 0.15 ± 0.02$^{bB}$ | 0.22 ± 0.01$^{cA}$ | 0.17 ± 0.01$^{cAB}$ |
| | 30 | 0.22 ± 0.01$^{bAB}$ | 0.19 ± 0.01$^{bB}$ | 0.24 ± 0.01$^{bA}$ | 0.23 ± 0.01$^{bAB}$ |
| | 60 | 0.32 ± 0.01$^{aB}$ | 0.32 ± 0.01$^{aB}$ | 0.35 ± 0.01$^{aAB}$ | 0.36 ± 0.01$^{aA}$ |
| $H$ | 1 | 1.95 ± 0.49$^{aA}$ | 1.42 ± 0.47$^{aA}$ | 2.71 ± 1.65$^{aA}$ | 1.57 ± 0.87$^{aA}$ |
| | 30 | 2.31 ± 0.82$^{aAB}$ | 1.40 ± 0.58$^{aB}$ | 2.80 ± 1.49$^{aA}$ | 1.65 ± 0.55$^{aAB}$ |
| | 60 | 2.53 ± 0.75$^{aA}$ | 1.35 ± 0.45$^{aAB}$ | 1.96 ± 1.03$^{aAB}$ | 1.46 ± 0.63$^{aB}$ |
| pH | 1 | 6.52 ± 0.03$^{bA}$ | 6.28 ± 0.06$^{bB}$ | 6.44 ± 0.06$^{bAB}$ | 6.50 ± 0.06$^{bAB}$ |
| | 30 | 6.83 ± 0.01$^{aA}$ | 6.71 ± 0.01$^{aC}$ | 6.73 ± 0.01$^{aC}$ | 6.79 ± 0.01$^{aB}$ |
| | 60 | 6.71 ± 0.04$^{aA}$ | 6.66 ± 0.04$^{aA}$ | 6.72 ± 0.03$^{aA}$ | 6.77 ± 0.09$^{abA}$ |
| $TTA$ | 1 | 0.18 ± 0.01$^{aB}$ | 0.17 ± 0.01$^{bC}$ | 0.21 ± 0.01$^{aA}$ | 0.14 ± 0.01$^{aD}$ |
| | 30 | 0.19 ± 0.01$^{aAB}$ | 0.21 ± 0.01$^{aA}$ | 0.22 ± 0.01$^{aA}$ | 0.17 ± 0.02$^{aB}$ |
| | 60 | 0.16 ± 0.01$^{bB}$ | 0.19 ± 0.01$^{abA}$ | 0.21 ± 0.01$^{aA}$ | 0.15 ± 0.01$^{aB}$ |
| L* | 1 | 39.26 ± 0.62$^{bA}$ | 39.64 ± 0.52$^{bA}$ | 38.25 ± 1.74$^{bA}$ | 38.36 ± 0.84$^{bA}$ |
| | 30 | 44.76 ± 0.52$^{aA}$ | 44.63 ± 0.49$^{aA}$ | 44.11 ± 0.09$^{aA}$ | 44.75 ± 0.18$^{aA}$ |
| | 60 | 44.34 ± 0.73$^{aA}$ | 43.59 ± 1.06$^{aA}$ | 44.38 ± 0.41$^{aA}$ | 43.80 ± 0.25$^{aA}$ |
| a* | 1 | 7.31 ± 0.20$^{bA}$ | 6.58 ± 0.10$^{bA}$ | 7.36 ± 0.74$^{aA}$ | 6.70 ± 0.24$^{bA}$ |
| | 30 | 8.14 ± 0.07$^{aA}$ | 7.76 ± 0.02$^{aA}$ | 7.90 ± 0.23$^{aA}$ | 7.26 ± 0.18$^{abB}$ |
| | 60 | 8.25 ± 0.29$^{aA}$ | 7.87 ± 0.69$^{aA}$ | 8.13 ± 0.16$^{aA}$ | 7.61 ± 0.54$^{aA}$ |
| b* | 1 | 4.57 ± 0.20$^{bA}$ | 3.85 ± 0.16$^{bB}$ | 3.55 ± 0.30$^{bB}$ | 3.55 ± 0.30$^{bB}$ |
| | 30 | 8.19 ± 0.14$^{aA}$ | 8.10 ± 0.16$^{aA}$ | 7.81 ± 0.37$^{aA}$ | 6.85 ± 0.35$^{aB}$ |
| | 60 | 8.38 ± 0.31$^{aA}$ | 8.12 ± 1.38$^{aA}$ | 8.41 ± 0.27$^{aA}$ | 7.36 ± 0.84$^{aA}$ |
| Hue | 1 | 0.56 ± 0.02$^{bA}$ | 0.53 ± 0.02$^{bAB}$ | 0.45 ± 0.06$^{bB}$ | 0.49 ± 0.03$^{bAB}$ |
| | 30 | 0.79 ± 0.01$^{aA}$ | 0.81 ± 0.01$^{aA}$ | 0.78 ± 0.01$^{aAB}$ | 0.76 ± 0.01$^{aB}$ |
| | 60 | 0.79 ± 0.00$^{aA}$ | 0.80 ± 0.04$^{aA}$ | 0.80 ± 0.01$^{aA}$ | 0.77 ± 0.02$^{aA}$ |
| C* | 1 | 9.59 ± 0.27$^{bA}$ | 8.51 ± 0.03$^{bB}$ | 9.14 ± 0.69$^{bAB}$ | 8.48 ± 0.36$^{bB}$ |
| | 30 | 12.23 ± .010$^{aA}$ | 11.81 ± 0.06$^{aA}$ | 11.81 ± 0.41$^{aA}$ | 10.68 ± 0.35$^{aB}$ |
| | 60 | 12.44 ± 0.44$^{aA}$ | 11.93 ± 1.38$^{aA}$ | 12.33 ± 0.26$^{aA}$ | 11.29 ± 0.96$^{aA}$ |

Values are expressed as the mean ± standard deviation of three replications (n = 3). $a_w$: water activity; $H$: hardness; $TTA$: titratable total acidity; L*: luminosity; a*: variation from green to red; b*: variation from blue to yellow; C*: saturation. Cookies made of pinto bean flour ($PBF$) and red rice flour ($RRF$). Trial 1 (25 g $PBF$, 75 g $RRF$, and 13.3 g margarine/100 g dough). Trial 2 (25 g $PBF$, 75 g $RRF$, and 19.3 g margarine/100 g dough). Trial $CP$ (center points' average) (50g $PBF$, 50 g $RRF$, and 16.3 g de margarine/100 g dough). Trial control (0 g $PBF$, 100 g $RRF$, and 16.3 g margarine/100 g dough). Values with different lowercase letters in the same column differ statistically in mean using the Tukey test ($p < 0.05$). Values with different uppercase letters in the same row differ statistically in mean using the Tukey test ($p < 0.05$)

## 4. Conclusion

Considering the technological, nutritional, and sensory quality of the products, it was possible to formulate cookies with a mixture of $PBF$ (50 g/100 g), $RRF$ (50 g/100 g), and margarine (16.3 g/100 g of dough). In addition, cookies showed acceptable technological quality up to 60 days of storage. The increase in the proportion of pinto beans in the cookies promoted a nutritional increase in proteins (up to 13%), minerals (iron and zinc, 2.13 and 1.54%, respectively), and phenolic compounds (139.46 mg GAE/100 g) with antioxidant capacity (77.72%) compared with the control cookies. Besides, margarine content exerted a greater influence on the acceptance (above 70%) of the formulated cookies. The cookies produced in this study become an excellent alternative to the gluten-free products available on the market, because they are intended for celiac patients or even those who wish to consume products based on rice and beans as a way to increase the nutritional content of their diet.

## Declarations

**Acknowledgments** The authors would like to extend a special thanks to FAPES (Edital nº 04/2017 – PPP) and CAPES – Finance Code 001 for the financial support, to the Federal University of Espirito Santo, EMBRAPA Food Technology, and BioFort biofortification program for their facilities.

**Declarations**

**Funding** Not applicable.

**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' Contributions** de Magalhães CS, Marques GA, Bazán-Colque RJ, and Moraes EA prepared figures, acquired data, and wrote the main text of the manuscript. da Silva EMM and Ascheri JLR supervised, revised, and edited the manuscript.

**Ethics approval** This study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human subjects were approved by the Research Ethics Committee of the Federal University of Espirito Santo (CAAE nº: 55894016.5.0000.5060).

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of Interest** The authors declare no conflict of interest.

## References

1. Petry N, Boy E, Wirth JP, Hurrell RF (2015) Review: The Potential of the Common Bean (*Phaseolus vulgaris*) as a Vehicle for Iron Biofortification. Nutr 2015, Vol 7, Pages 1144–1173 7:1144–1173. https://doi.org/10.3390/NU7021144
2. Martins SM, Melo PGS, Faria LC et al (2016) Genetic parameters and breeding strategies for high levels of iron and zinc in *Phaseolus vulgaris* L. - Portal Embrapa. Genet Mol Res 15:1–14
3. Min B, McClung AM, Chen MH (2011) Phytochemicals and Antioxidant Capacities in Rice Brans of Different Color. J Food Sci 76:C117–C126. https://doi.org/10.1111/J.1750-3841.2010.01929.X
4. Shih FF (2003) An update on the processing of high-protein rice products. Food/Nahrung 47:420–424. https://doi.org/10.1002/FOOD.200390093
5. Mancebo CM, Picón J, Gómez M (2015) Effect of flour properties on the quality characteristics of gluten free sugar-snap cookies. LWT - Food Sci Technol 64:264–269. https://doi.org/10.1016/J.LWT.2015.05.057
6. de la Barca AMC, Rojas-Martínez ME, Islas-Rubio AR, Cabrera-Chávez F (2010) Gluten-Free Breads and Cookies of Raw and Popped Amaranth Flours with Attractive Technological and Nutritional Qualities. Plant Foods Hum Nutr 65:241–246. https://doi.org/10.1007/S11130-010-0187-Z/TABLES/2
7. Souza A, de Pereira M, Yokoo RA EM, et al (2013) Most consumed foods in Brazil: National Dietary Survey 2008–2009. Rev Saude Publica 47:190s-199s. https://doi.org/10.1590/S0034-89102013000700005
8. Cappa C, Kelly JD, Ng PKW (2020) Baking performance of 25 edible dry bean powders: Correlation between cookie quality and rapid test indices. Food Chem 302:125338. https://doi.org/10.1016/J.FOODCHEM.2019.125338
9. Mamat H, Hill SE (2014) Effect of fat types on the structural and textural properties of dough and semi-sweet biscuit. J Food Sci Technol 51:1998–2005. https://doi.org/10.1007/S13197-012-0708-X/FIGURES/2
10. Kohnhorst AL, Smith DM, Uebersax MA, Bennink MR (1991) Production and characterization of a protein concentrate from navy beans (*Phaseolus vulgaris*). Food Chem 41:33–42. https://doi.org/10.1016/0308-8146(91)90129-C

11. MuraMoto K (2017) Lectins as Bioactive Proteins in Foods and Feeds. Food Sci Technol Res 23:487–494. https://doi.org/10.3136/FSTR.23.487
12. Kumar S, Verma AK, Das M et al (2013) Clinical complications of kidney bean (*Phaseolus vulgaris* L.) consumption. Nutrition 29:821–827. https://doi.org/10.1016/J.NUT.2012.11.010
13. Zucco F, Borsuk Y, Arntfield SD (2011) Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. LWT - Food Sci Technol 44:2070–2076. https://doi.org/10.1016/J.LWT.2011.06.007
14. de la Rosa-Millán J, Pérez-Carrillo E, Guajardo-Flores S (2017) Effect of germinated black bean cotyledons (*Phaseolus vulgaris* L.) as an extruded flour ingredient on physicochemical characteristics, in vitro digestibility starch, and protein of nixtamalized blue maize cookies. Starch - Stärke 69:1600085. https://doi.org/10.1002/STAR.201600085
15. Maache-Rezzoug Z, Bouvier JM, Allaf K, Patras C (1998) Effect of principal ingredients on rheological behaviour of biscuit dough and on quality of biscuits. J Food Eng 35:23–42. https://doi.org/10.1016/S0260-8774(98)00017-X
16. Szczygiel EJ, Harte JB, Strasburg GM, Cho S (2017) Consumer acceptance and aroma characterization of navy bean (*Phaseolus vulgaris*) powders prepared by extrusion and conventional processing methods. J Sci Food Agric 97:4142–4150. https://doi.org/10.1002/JSFA.8284
17. Hwang HS, Singh M, Lee S (2016) Properties of Cookies Made with Natural Wax–Vegetable Oil Organogels. J Food Sci 81:C1045–C1054. https://doi.org/10.1111/1750-3841.13279
18. AACC (1995) Approved Methods of the American. Association of Cereal Chemists
19. Rajiv J, Lobo S, Jyothi Lakshmi A, Venkateswara Rao G (2012) Influence of green gram flour (*Phaseolus aureus*) on the rheology, microstructure and quality of cookies. J Texture Stud 43:350–360. https://doi.org/10.1111/J.1745-4603.2012.00346.X
20. Tulse SB, Reshma V, Rajiv J, Sakhare SD (2015) Effect of co-milled wheat, green gram and barley on the rheological and quality characteristics of cookies. Food Sci Technol Int 21:492–502. https://doi.org/10.1177/1082013214550112
21. Chávez-Santoscoy RA, Gutiérrez-Uribe JA, Serna-Saldivar SO, Perez-Carrillo E (2016) Production of maize tortillas and cookies from nixtamalized flour enriched with anthocyanins, flavonoids and saponins extracted from black bean (*Phaseolus vulgaris*) seed coats. Food Chem 192:90–97. https://doi.org/10.1016/J.FOODCHEM.2015.06.113
22. Ramírez-Cárdenas L, Leonel AJ, Costa NMB (2008) Effect of domestic processing on nutrient and antinutritional factor content in different cultivars of common beans. Food Sci Technol 28:200–213. https://doi.org/10.1590/S0101-20612008000100029
23. Aguilera Y, Estrella I, Benitez V et al (2011) Bioactive phenolic compounds and functional properties of dehydrated bean flours. Food Res Int 44:774–780. https://doi.org/10.1016/J.FOODRES.2011.01.004
24. Sumczynski D, Kotásková E, Družbíková H, Mlček J (2016) Determination of contents and antioxidant activity of free and bound phenolics compounds and in vitro digestibility of commercial black and red rice (*Oryza sativa* L.) varieties. Food Chem 211:339–346. https://doi.org/10.1016/J.FOODCHEM.2016.05.081
25. Brites LTGF, Ortolan F, da SILVA, DW et al (2018) Gluten-free cookies elaborated with buckwheat flour, millet flour and chia seeds. Food Sci Technol 39:458–466. https://doi.org/10.1590/FST.30416
26. Klein ÂL, Bresciani L, Oliveira EC (2015) Physicochemical characteristics of commercial biscuits of the Type cookies acquired in the taquari valley. Rev Destaques Acadêmicos - Cetec/Univates 7:167–173
27. Kaderides K, Mourtzinos I, Goula AM (2020) Stability of pomegranate peel polyphenols encapsulated in orange juice industry by-product and their incorporation in cookies. Food Chem 310:125849. https://doi.org/10.1016/J.FOODCHEM.2019.125849
28. Moser P, Telis VRN, de Andrade Neves N et al (2017) Storage stability of phenolic compounds in powdered BRS Violeta grape juice microencapsulated with protein and maltodextrin blends. Food Chem 214:308–318. https://doi.org/10.1016/J.FOODCHEM.2016.07.081
29. Krystyjan M, Gumul D, Ziobro R, Sikora M (2015) The Effect of Inulin as a Fat Replacement on Dough and Biscuit Properties. J Food Qual 38:305–315. https://doi.org/10.1111/JFQ.12148

## Figures

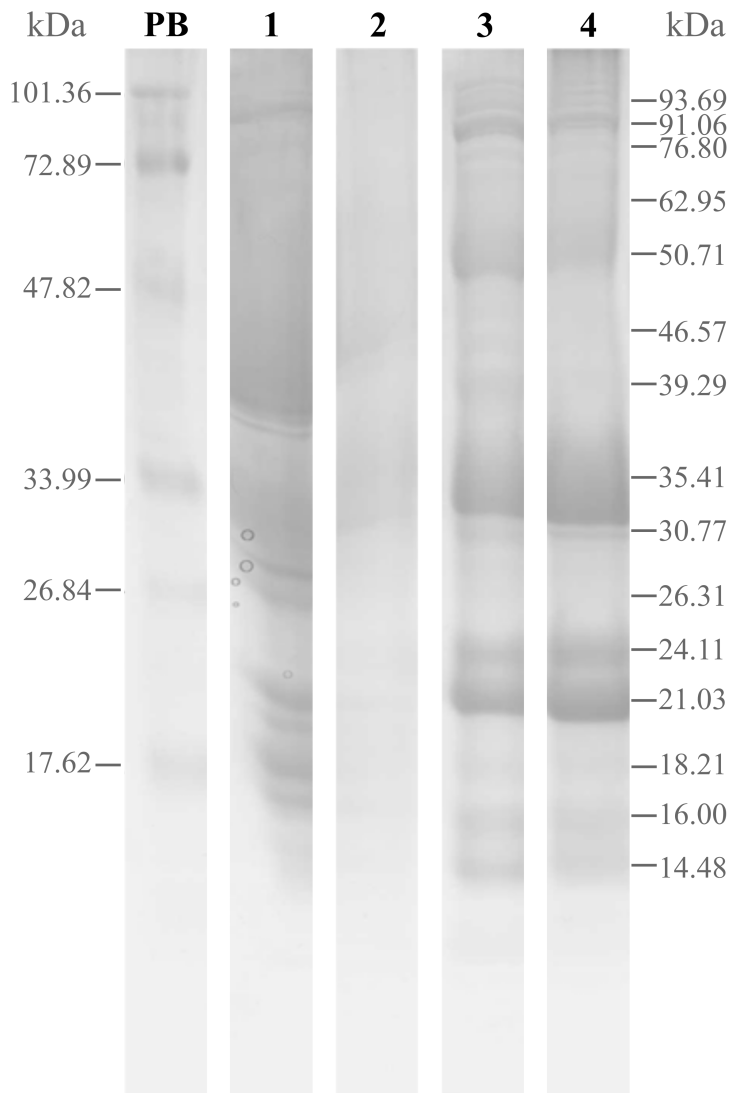
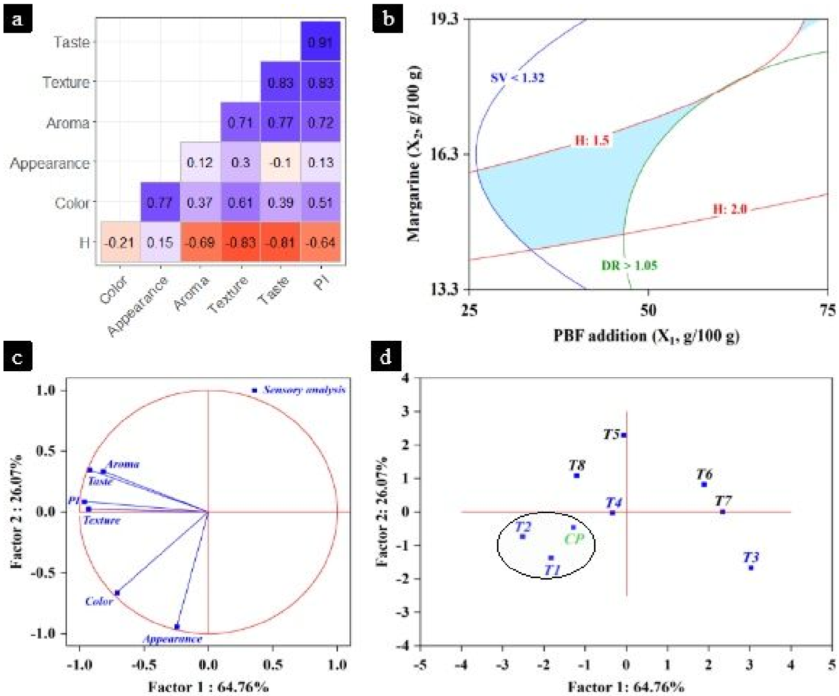



**Figure 1**

Gel electrophoresis (SDS/PAGE) of pinto bean flour (PBF) and red rice flour (RRF). PB (Marker); *PBF* raw (Line 1); *PBF* processed (Line 2); *RRF* raw (Line 3); RRF processed (Line 4)




## Figure 2

Pearson's correlation for response variables (a). Region of the optimum found by overlaying response contour (b). Principal components analysis: sensory analysis correlation plot (c) and projection of the treatments on the factor map (d). PI: purchase intention. H: hardness; DR: diameter ratio; SV: specific volume; CP: center points' average

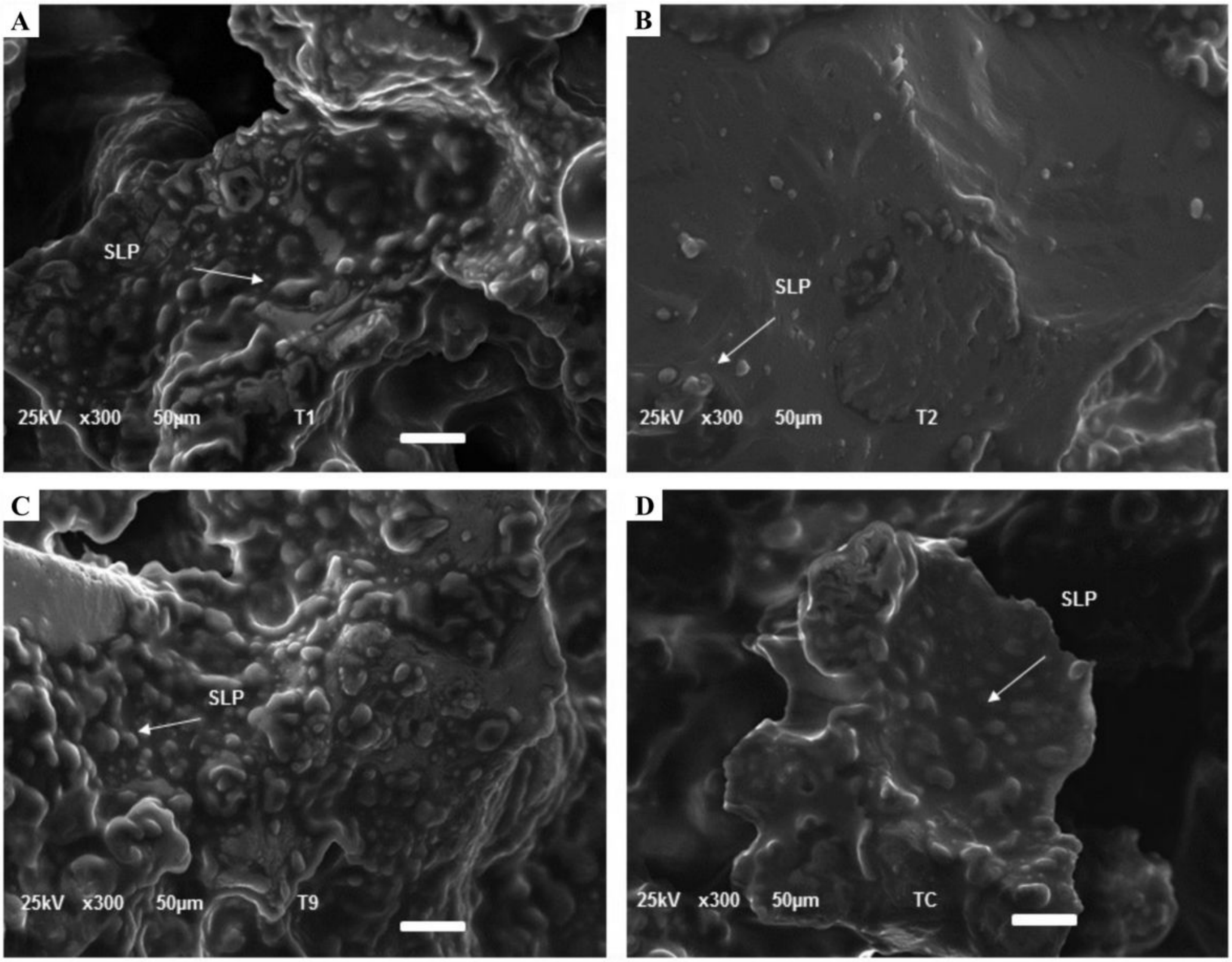



**Figure 3**

Electron micrographs at 300x magnification of cookies made of pinto bean flour (*PBF*) and red rice flour (*RRF*). **A)** Trial 1 (25 g PBF, 75 g *RRF*, and 13.3 g margarine/100 g dough). **B)** Trial 2 (25 g PBF, 75 g RRF, and 19.3 g margarine/100 g dough). **C)** Trial 9 = center points' average (CP) (50g PBF, 50 g RRF, and 16.3 g de margarine/100 g dough). **D)** TC = trial control (0 g PBF, 100 g RRF, and 16.3 g margarine/100 g dough). AB: air bubble. SLP: starch, lipid and protein complex

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata1.docx
- Supplementarydata2.docx