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Original article

# Correlation between the rheological and technological properties of biscuits with different levels of replacement of wheat flour by rice and azuki bean flour

**Daisy Jacqueline Sousa Silva,[1] Jorge Minoru Hashimoto,[2*] Elizabeth Harumi Nabeshima,[3] Rafaela Teixeira Salgado,[3] Thaise Kessiane Teixeira Freitas[1] & Kaesel Jackson Damasceno e Silva[2]**

1 Department of Nutrition, Federal University of Piauí (UFPI), Campus Universitário Ministro Petrônio Portela, Bairro Ininga, Teresina PI, 64049-550, Brazil

2 Brazilian Agricultural Research Corporation Mid-North (Embrapa Meio-Norte), Avenida Duque de Caxias, 5650, Bairro Buenos Aires, Teresina PI, CEP 64008-780, Brazil

3 Institute of Food Technology (ITAL), Av. Brasil, 2880, Jardim Chapadão, Campinas SP, Zip Code 13.070-178, Brazil

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**Summary** A mixture design was used to evaluate the effect of replacing (0 to 100%) wheat flour (WF) with whole-grain azuki bean (WABF) and rice (RF) flours on the biscuits quality. High proportions of WABF increased diameter and decreased thickness, whereas WF affected inversely and RF intermediate. The WF biscuit specific volume was 17% greater than that of the dough, and the WABF and RF biscuits were 5 and 13% smaller, respectively. The WABF biscuit hardness was 26.25 N, close to WF (28.14 N), and higher than RF (7.6 N). The WABF increased the colour difference (ΔE) values for biscuits by up to 22.19 and the RF by 1.87. Peak viscosity, breakdown and setback of mixture flours showed positive correlations with the dough hardness and L*, a*, b* and Aw of biscuits and negatively with the radial expansion index and ΔE. The highest global desirability was for biscuits without WF, regardless of crust colour.

**Keywords** Adzuki, centroid simplex, cookie, *Vigna angularis*, whole flour.

## Introduction

Azuki beans [*Vigna angularis* (Willd.) Ohwi & H. Ohashi] have high levels of protein (21.4 to 24.5%, db), fibre (3.3 to 4.3%, db), vitamins and minerals and only 0.4 to 2.1% (db) of lipids (Yousif *et al.*, 2007). Reddish integuments are rich in polyphenols, substances with high antioxidant activity (Orsi *et al.*, 2017), associated with several health benefits, such as slower digestion and absorption of carbohydrates, hypocholesterolaemic, hypoglycaemic, antihypertensive and anticancer action (Mukai & Sato, 2011; Kim *et al.*, 2015; Orsi *et al.*, 2017; Kawahara *et al.*, 2019).

The azuki bean is one of the main legumes grown in East Asia. In China, it is the 2nd most cultivated pulse, with a production of 350 thousand t year$^{-1}$; in Japan, it is the first (70 thousand t year$^{-1}$); and in and Korea, it produces 15 000 t year$^{-1}$, being appreciated for its flavour of sweetened nutty (Shahrajabian *et al.*, 2019), but in other countries, it is little known. If the grains are transformed into flour, it will expand the possibilities of use and could become a potentially interesting option for the preparation of gluten-free baked products, similar to other legumes (Crockett *et al.*, 2011; Cappelli *et al.*, 2020b), mainly if they contain components (hydrocolloids, enzymes and emulsifiers) that can mimic the viscoelastic properties of gluten (Ferrero, 2017), avoiding negative impacts on the dough rheology and quality of the baked product (Cappelli *et al.*, 2020a). The substitution of wheat flour (WF) with whole azuki bean flour (WABF) in the production of biscuits is a way of popularizing consumption as biscuits are widely consumed bakery products (Chauhan *et al.*, 2015).

Similar to other pulses, azuki beans are limited in sulphur amino acids (Tjahjadi *et al.*, 1988). Complementation can be made with the introduction of rice flour (RF) (Oliveira *et al.*, 2017), from broken rice. Rice flour has a bland taste, is hypoallergenic, is easy to digest and is white in colour (Kadan *et al.*, 2003).

Several authors investigated the partial WF substitution with pulse derivatives in biscuit formulations

*Correspondent: E-mail: jorge.hashimoto@embrapa.br

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(McWatters *et al.*, 2003; Dhull *et al.*, 2006; Zucco *et al.*, 2011; Silky *et al.*, 2014; Chandra *et al.*, 2015; Thongram *et al.*, 2016; Benkadri *et al.*, 2018; Zhao *et al.*, 2019); however, there are no studies on the use of WABF in biscuits. Biscuits resulting from the combination of WABF and RF may be consumed by about 7 to 14% of the population with restrictions on gluten intake (Cabrera-Chávez *et al.*, 2017).

To evaluate the effect of WABF and RF in a traditional biscuit formulation based in WF, a mixture design was used, which also allows to obtain global desirability (Sahín *et al.*, 2016). For a more complete scan, the special cubic model simplex-centroide was used to evaluate the effect of the composite flours on the biscuits' physical properties.

## Materials and methods

### Raw material

WF, RF, beans and other ingredients for the formulation of the biscuits were purchased from the local grain market (Campinas, Brazil). The azuki beans were ground in a KitchenAid (Kl418ARONA) attachment adjusted to obtain the smallest particle size, coupled to the Stander mixer (KitchenAid, K5SS, Whirlpool Corporation, Springfield, Ohio, USA) drive shaft, operating at the lowest speed (144 rpm). Particle size was reduced in a cone mill [Risch-Rotkreuz, METRO International AG (MIAG), 975118, Zug, Switzerland] after subsequent milling with intercalated adjustments to reduce the particle size to 60, 40 and 20 and finally to obtain the WABF with particles diameter ≥10 μm.

The chemical composition (AOAC, 2010), water absorption and solubility indexes (Anderson *et al.*, 1969) were determined in three replicates, and the carbohydrate content was determined by difference. The total energy value was estimated using Atwater conversion factors.

### Biscuits formulations and processing

A standard biscuit formulation (Manley, 1983) which contained 59.07% WF was used. Only the WF ingredient was replaced by WABF and / or RF (Table 1). The biscuit dough of each formulation was obtained by the two-phase method according to Manley (1983). Palm fat (13.67%), honey (2.18%) and sugar (19.69%) were placed in the mixer's stainless steel container (K5ASBP) (KitchenAid K5SS, Whirlpool Corp., Springfield, Ohio, USA) and mixed with the K5AB accessory attached, at 96 rpm for 4 min. Powdered egg yolk (3.7%) and 18 mL of water were added and homogenised at 144 rpm for an additional 3 min. Subsequently, one of the 10 flour compositions was added (Table 1) with the salt (0.22%), ammonium bicarbonate (0.5%), chemical leavening agent (0.9%), water (65 mL), and mixed at

**Table 1** Simplex-centroid design for ternary mixtures with 3 additional equidistant internal points, physical and physicochemical characteristics of the biscuits

| Flour proportions | | | Pasting profile (cP) | | | Texture parameters (N) | | Biscuit characteristics | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WF ($x_1$) | WA BF ($x_2$) | RF ($x_3$) | Peak viscosity | Breakdown | Setback | Hardness | Springiness | REI | TEI | SF | VEI | SV (g cm$^{-3}$) | Hardness (N) | L* | a* | b* | ΔE | Aw |
| 1 | 0 | 0 | 3790 ± 34.04 | 2183 ± 40.10 | 2169 ± 52.84 | 7.27 ± 0.53 | 0.054 ± 0.00 | 0.99 ± 0.03 | 2.27 ± 0.10 | 3.23 ± 0.25 | 1.17 ± 0.01 | 2.04 ± 0.03 | 28.14 ± 2.45 | 36.46 ± 1.01 | 35.73 ± 1.17 | 24.68 ± 1.09 | 0.00 | 0.47 ± 0.00 |
| 0 | 1 | 0 | 467 ± 33.13 | 21 ± 3.60 | 194 ± 8.62 | 5.44 ± 0.60 | 0.055 ± 0.00 | 1.08 ± 0.00 | 1.48 ± 0.01 | 5.36 ± 0.02 | 0.95 ± 0.07 | 1.81 ± 0.15 | 26.25 ± 3.37 | 21.29 ± 0.41 | 21.12 ± 0.42 | 17.73 ± 0.38 | 22.19 | 0.33 ± 0.01 |
| 0 | 0 | 1 | 6079 ± 101.17 | 4032 ± 87.52 | 2929 ± 5.29 | 10.57 ± 0.27 | 0.042 ± 0.01 | 1.01 ± 0.01 | 1.48 ± 0.03 | 5.06 ± 0.15 | 0.87 ± 0.03 | 1.61 ± 0.00 | 7.60 ± 2.38 | 36.33 ± 1.12 | 35.51 ± 1.21 | 24.37 ± 0.85 | 1.87 | 0.54 ± 0.00 |
| 1/2 | 1/2 | 0 | 1605 ± 111.22 | 700 ± 49.37 | 1010 ± 47.63 | 4.24 ± 0.26 | 0.025 ± 0.00 | 1.10 ± 0.02 | 1.70 ± 0.02 | 4.81 ± 0.05 | 0.84 ± 0.10 | 1.53 ± 0.17 | 26.83 ± 4.06 | 22.45 ± 0.33 | 21.94 ± 0.37 | 16.93 ± 0.30 | 21.13 | 0.46 ± 0.01 |
| 1/2 | 0 | 1/2 | 4633 ± 38.31 | 2595 ± 46.36 | 2658 ± 9.16 | 7.81 ± 0.22 | 0.033 ± 0.00 | 0.98 ± 0.02 | 2.03 ± 0.04 | 3.58 ± 0.15 | 0.70 ± 0.09 | 1.32 ± 0.15 | 21.62 ± 3.33 | 33.78 ± 1.43 | 32.80 ± 1.47 | 22.67 ± 0.82 | 4.47 | 0.40 ± 0.00 |
| 0 | 1/2 | 1/2 | 1540 ± 13.32 | 328 ± 8.74 | 1415 ± 30.53 | 5.55 ± 0.13 | 0.024 ± 0.00 | 1.07 ± 0.00 | 1.67 ± 0.07 | 4.76 ± 0.21 | 0.76 ± 0.12 | 1.48 ± 0.25 | 14.14 ± 2.41 | 22.99 ± 0.68 | 22.39 ± 0.77 | 17.06 ± 0.60 | 20.44 | 0.32 ± 0.00 |
| 1/3 | 1/3 | 1/3 | 2331 ± 13.89 | 1004 ± 16.65 | 1708 ± 30.99 | 6.39 ± 0.27 | 0.027 ± 0.00 | 1.09 ± 0.00 | 1.82 ± 0.02 | 4.43 ± 0.04 | 1.00 ± 0.08 | 1.89 ± 0.14 | 22.71 ± 3.13 | 26.31 ± 0.48 | 25.74 ± 0.48 | 19.34 ± 0.15 | 15.23 | 0.38 ± 0.00 |
| 2/3 | 1/6 | 1/6 | 2941 ± 83.34 | 1458 ± 45.96 | 1824 ± 27.57 | 6.83 ± 0.27 | 0.032 ± 0.00 | 1.03 ± 0.01 | 2.02 ± 0.06 | 3.77 ± 0.14 | 1.12 ± 0.06 | 1.98 ± 0.10 | 27.27 ± 4.85 | 29.92 ± 0.39 | 29.25 ± 0.41 | 20.97 ± 0.15 | 9.94 | 0.46 ± 0.01 |
| 1/6 | 2/3 | 1/6 | 956 ± 11.24 | 73 ± 5.57 | 667 ± 7.00 | 4.50 ± 0.30 | 0.027 ± 0.01 | 1.10 ± 0.00 | 1.68 ± 0.03 | 4.85 ± 0.08 | 0.98 ± 0.14 | 1.85 ± 0.26 | 19.49 ± 3.18 | 22.48 ± 0.95 | 22.04 ± 1.06 | 17.14 ± 0.91 | 20.97 | 0.32 ± 0.01 |
| 1/6 | 1/6 | 2/3 | 3690 ± 93.02 | 1904 ± 67.44 | 2352 ± 18.68 | 7.22 ± 0.25 | 0.036 ± 0.00 | 1.06 ± 0.01 | 1.75 ± 0.02 | 4.46 ± 0.04 | 0.87 ± 0.05 | 1.65 ± 0.13 | 15.53 ± 2.53 | 29.13 ± 0.27 | 28.44 ± 0.26 | 20.23 ± 0.13 | 11.28 | 0.41 ± 0.01 |

a*, a colour value; Aw, water activity; b*, b colour value; L*, L colour value; REI, radial expansion index; RF, rice flour or $x_3$; SF, spread factor; SV, specific volume; TEI, thickness expansion index; VEI, volumetric expansion index; WABF, whole azuki bean flour or $x_2$; WF, wheat flour or $x_1$; ΔE, colour difference.

96 rpm for 1.5 min. The biscuit dough was laminated to a thickness of 5 mm, cut into a circular shape of 37 mm in diameter and baked in an electric oven (Titã, FGE 4, Titã Electrocomercial Ind. E Com. Ltda., Araraquara, Brazil) at 160 °C for 18 min, with a forced air circulation system and rotating support tray enabled. Then they were cooled to room temperature, airtight-packaged (BOPP-M 25 µm) and stored at room temperature and protected from light for 1 week.

### Rheological properties

The pasting profile (Table 1) was determined in three replicates according to the ICC 162 method (International Association for Cereal Science and Technology, 1996) using an RVA viscometer (Rapid Visco Analyzer, RVA 4500, Perten Instruments, Hägersten, Sweden). The analysis was performed according to the equipment's standard program 2, using a flour mixture according to mixture design, 3.5 g of solids (corrected to 14% moisture) suspended in 25 mL of deionised water. The texture profile (Fustier *et al.*, 2008) of the doughs of each formulation was determined in three replicates with the TAXT2i texturometer (Stable Micro Systems, London, England). The doughs were cut into cylindrical dimensions of 25 mm in diameter and 5 mm in height and subjected to compression by a P100 probe, pre- and post-test speed of 2 mm $s^{-1}$, test speed 0.8 mm $s^{-1}$, compression of 40% of the original thickness and waiting time of 5 s between strokes. Hardness (N) and springiness (N) were determined.

### Physical analysis of biscuits

Biscuits were assessed according to methods 10.50.05 [diameter, thickness and spread factor (SF)] and 10.50.01 [specific volume (SV)] of AACC (2010). From the relationships between the dimensions of the biscuits and the respective dough cut in a cylindrical shape, the expansion indices were determined: radial (REI), thickness (TEI) and volumetric (VEI). The hardness (N) was determined in five replicates in the TAXT2i (Stable Micro Systems, London, England), with the HDP/3PB and HDP0/90 accessories, using the analytical parameters: pre-test speed = 1.0 mm $s^{-1}$; test speed = 3.0 mm $s^{-1}$; post-test speed = 10.0 - mm $s^{-1}$; distance 5 mm and three point bending test probe. The water activity (Aw) was determined in three replicates on the Aqualab 4TEV (Decagon, Pullman, USA) at 25.0 ± 0.30 °C, and the colour parameters in three replicates with the CR-410 colorimeter (Konica Minolta, Japan) and the colour difference ($\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$) were calculated in relation to the standard biscuit.

### Statistical analysis

The data were submitted to multiple regression analysis using the Statistica program (StatSoft, Version 10, OK, USA). The special or reduced cubic model was used to predict the response in the ternary diagram, expressed as the general equation (Eq. 1):

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3, \quad (1)$$

where $y$ represents the response variable and the estimated coefficients represented by β1, β2 and β3 (linear); β12, β13 and β23 (binary interactions); and β123 (ternary interaction). The significant models were submitted to the analysis of global desirability (D), under equity, for 's' and 't' equal to 1.

## Results and discussion

WABF differs from WF and RF in terms of protein and ash content, with 24.61%, and 3.84%, respectively, and in terms of the higher water solubility index (Table 2).

**Table 2** Chemical and physicochemical characteristics of the flours

| | Wheat flour | Whole azuki bean flour | Rice flour |
|---|---|---|---|
| Moisture (%, w.b.) | 12.42 ± 0.13 | 9.44 ± 0.69 | 8.95 ± 0.04 |
| Protein (%, d.b.) | 11.82 ± 0.16 | 24.61 ± 0.40 | 7.92 ± 0.01 |
| Fat (%, d.b.) | 1.25 ± 0.03 | 0.21 ± 0.12 | 0.48 ± 0.02 |
| Ash (%, d.b.) | 0.31 ± 0.21 | 3.84 ± 0.40 | 0.32 ± 0.04 |
| Carbohydrates (%, d.b.) | 83.86 ± 0.30 | 45.71 ± 0.10 | 89.63 ± 1.52 |
| Total dietary fibre (%, d.b.) | 2.76 ± 1.54 | 25.63 ± 1.67 | 1.65 ± 1.06 |
| Energy (kcal $g^{-1}$, d.b.) | 393.97 | 283.17 | 394.52 |
| Mean particle size (µm) | 355.63 | 364.22 | 471.30 |
| Aw | 0.71 ± 0.01 | 0.59 ± 0.00 | 0.49 ± 0.00 |
| WAI (g $g^{-1}$) | 1.65 ± 0.05 | 2.56 ± 0.06 | 2.44 ± 0.11 |
| WSI (%) | 15.21 ± 0.55 | 45.50 ± 2.06 | 6.73 ± 3.86 |

Aw, water activity. WAI, water absorption index, and WSI, water solubility index.

## Rheological properties of mixtures

The viscosity profiles of flour mixtures can predict the performance during processing and the quality of the final products (Ragaee & Abdel-Aal, 2006; Cappa *et al.*, 2020). The maximum viscosity peak was higher in mixtures with a higher carbohydrate content (Table 1). The breakdown and setback values followed the same trend (Table 1), showing a high positive correlation ($r = 0.92$) (Table 3). There was also a high positive correlation between peak viscosity and breakdown ($r = 0.99$) and setback ($r = 0.96$) (Table 3). The highest values of the viscoamylographic parameters were obtained in the samples with the higher RF contents due to the higher contents of the amylaceous fraction. Springiness was not significantly correlated with any parameter (Table 3). On the other hand, dough hardness showed high significant correlation with the viscosity parameters, being $r = 0.93$, 0.94, and 0.86 with the viscosity peak, breakdown and setback, respectively (Table 3). Springiness values were mainly influenced by the interaction between WF and WABF, which significantly decreased the value of this parameter with increasing addition of these two flours. The highest values were in the doughs that contained 100% WF or WABF, probably due to proteins (Table 4).

According to Collar (2003), the bonding profile mainly depends from the composition. This is more evident in food systems like bakery products, where protein, starch and other ingredients like pentosans and lipids provide the consistency. For Fustier *et al.* (2007), many aspects of the processing of biscuits and end products are related to the dough rheological behaviour. The dough hardness showed a negative correlation with the REI ($r = -0.74$) and ΔE ($r = -0.85$), positive correlation with the Aw ($r = 0.66$) and three equal correlation values ($r = 0.85$) with the L*, a* and b* biscuits colour parameters (Table 3).

REI and ΔE showed a negative correlation with the three rheological parameters (peak viscosity, breakdown and setback, respectively) and dough hardness, and Aw showed correlations positive with these same parameters (Table 3). The texture profile (Table 1) of the dough followed the same trend as the viscosity parameters. The RF affected positively (10.42), and the interaction (RF x WABF) showed negative effect (−10.29) (Table 4).

## Physical properties of biscuits

Physical appearance is important for purchasing decision, and the ingredients affect this characteristic (Klunklin & Savage, 2018). According to analysis of variance, the models obtained for VEI and SV were not significative ($P > 0.05$) but significative for REI,

**Table 3** Correlation coefficients between rheological characteristics of flour mixtures, dough texture and physical and physicochemical characteristics of biscuits

| | Peak viscosity | Breakdown | Setback | Dough hardness | Springiness | REI | TEI | SF | VEI | SV | Hardness | L* | a* | b* | ΔE | Aw |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peak viscosity | 1.00 | 0.99* | 0.96* | 0.93* | 0.20$^{ns}$ | -0.78* | 0.27$^{ns}$ | -0.41$^{ns}$ | -0.12$^{ns}$ | -0.21$^{ns}$ | -0.45$^{ns}$ | 0.93* | 0.93* | 0.89* | -0.91* | 0.76* |
| Breakdown | | 1.00 | 0.92* | 0.94* | 0.28$^{ns}$ | -0.77* | 0.21$^{ns}$ | -0.34$^{ns}$ | -0.09$^{ns}$ | -0.18$^{ns}$ | -0.44$^{ns}$ | 0.92* | 0.92* | 0.90* | -0.90* | 0.80* |
| Setback | | | 1.00 | 0.86* | 0.04$^{ns}$ | -0.75* | 0.38$^{ns}$ | -0.51$^{ns}$ | -0.15$^{ns}$ | -0.24$^{ns}$ | -0.44$^{ns}$ | 0.89* | 0.89* | 0.83* | -0.88* | 0.66* |
| Dough hardness | | | | 1.00 | 0.36$^{ns}$ | -0.74* | 0.08$^{ns}$ | -0.23$^{ns}$ | -0.05$^{ns}$ | -0.10$^{ns}$ | -0.53$^{ns}$ | 0.87* | 0.87* | 0.87* | -0.85* | 0.66* |
| Springiness | | | | | 1.00 | -0.48 | 0.10$^{ns}$ | -0.10$^{ns}$ | 0.40$^{ns}$ | 0.39$^{ns}$ | 0.20$^{ns}$ | 0.39$^{ns}$ | 0.41$^{ns}$ | 0.51$^{ns}$ | -0.41$^{ns}$ | 0.23$^{ns}$ |
| REI | | | | | | 1.00 | -0.58$^{ns}$ | 0.69* | -0.05$^{ns}$ | 0.07$^{ns}$ | 0.04$^{ns}$ | -0.90* | -0.90* | -0.91* | 0.91* | -0.53$^{ns}$ |
| TEI | | | | | | | 1.00 | -0.99* | 0.43$^{ns}$ | 0.31$^{ns}$ | 0.54$^{ns}$ | 0.52$^{ns}$ | 0.52$^{ns}$ | 0.50$^{ns}$ | -0.56$^{ns}$ | 0.23$^{ns}$ |
| SF | | | | | | | | 1.00 | -0.36$^{ns}$ | -0.23$^{ns}$ | -0.42$^{ns}$ | -0.63* | -0.63* | -0.60$^{ns}$ | 0.66* | -0.31$^{ns}$ |
| VEI | | | | | | | | | 1.00$^{ns}$ | 0.98* | 0.52$^{ns}$ | 0.16$^{ns}$ | 0.18$^{ns}$ | 0.23$^{ns}$ | -0.19$^{ns}$ | 0.20$^{ns}$ |
| SV | | | | | | | | | | 1.00 | 0.46$^{ns}$ | 0.05$^{ns}$ | 0.06$^{ns}$ | 0.13$^{ns}$ | -0.08$^{ns}$ | 0.06$^{ns}$ |
| Hardness | | | | | | | | | | | 1.00 | -0.19$^{ns}$ | -0.19$^{ns}$ | -0.14$^{ns}$ | 0.15$^{ns}$ | -0.08$^{ns}$ |
| L* | | | | | | | | | | | | 1.00 | 1.00* | 0.99* | -1.00* | 0.73* |
| a* | | | | | | | | | | | | | 1.00 | 0.99* | -1.00* | 0.73* |
| b* | | | | | | | | | | | | | | 1.00 | -0.99* | 0.72* |
| ΔE | | | | | | | | | | | | | | | 1.00 | -0.71* |
| Aw | | | | | | | | | | | | | | | | 1.00 |

*Significative ($P < 0.05$).

a*, a colour value; Aw, water activity; b*, b colour value; L*, L colour value; $^{ns}$, not significative; REI, radial expansion index; SF, spread factor; SV, specific volume; hardness; TEI, thickness expansion index; VEI, volume expansion index; ΔE, colour difference.

**Table 4** Coefficients obtained by multiple regression analysis and data from analysis of variance of adjusted models for rheological parameters of flour mixtures, dough texture profiles, physical and physicochemical biscuit characteristics

| | RVA parameters (cP) | | | Texture parameters (N) | | Biscuit characteristics | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coefficients | Peak viscosity | Breakdown | Setback | Hardness | Springiness | REI | TEI | SF | VEI | SV (g cm$^{-3}$) | Hardness (N) | L* | a* | b* | ΔE | Aw |
| $x_1$ | 3768.72* | 2169.56* | 2155.28* | 7.44 | 0.05 | 0.98* | 2.27* | 3.21* | 1.19* | 2.07* | 28.54* | 36.43* | 35.71* | 24.69* | 0.07$^{ns}$ | 0.48* |
| $x_2$ | 487.63* | 32.47$^{ns}$ | 157.28$^{ns}$ | 5.34 | 0.05 | 1.07* | 1.49* | 5.34* | 0.95* | 1.82* | 25.38* | 21.60* | 21.41* | 17.83* | 21.79* | 0.32* |
| $x_3$ | 6059.81* | 4005.92* | 2953.37* | 10.42 | 0.04 | 1.02* | 1.48* | 5.07* | 0.86* | 1.60* | 7.82* | 36.05* | 35.25* | 24.19* | 2.20$^{ns}$ | 0.54* |
| $x_1x_2$ | −2095.30* | −1611.96* | −786.87$^{ns}$ | −8.30 | −0.12 | 0.27* | −0.71* | 1.99* | −0.80$^{ns}$ | −1.51* | −2.42$^{ns}$ | −25.15* | −25.42* | −16.87* | 39.51* | 0.25$^{ns}$ |
| $x_1x_3$ | −1286.94$^{ns}$ | −2129.05* | 457.32$^{ns}$ | −4.38$^{ns}$ | −0.06$^{ns}$ | −0.08$^{ns}$ | 0.59* | −2.26* | −1.26* | −1.97* | 16.20$^{ns}$ | −11.06$^{ns}$ | −11.84* | −7.76* | 14.93$^{ns}$ | −0.37$^{ns}$ |
| $x_2x_3$ | −6929.12 | −6823.23* | −610.68$^{ns}$ | −10.29 | −0.10 | 0.12$^{ns}$ | 0.77* | −1.83* | −0.60$^{ns}$ | −0.95$^{ns}$ | −12.45$^{ns}$ | −23.25* | −23.66* | −16.07* | 33.52* | −0.48* |
| $x_1x_2x_3$ | −581.29$^{ns}$ | 656.47$^{ns}$ | −567.53$^{ns}$ | 27.05$^{ns}$ | 0.21$^{ns}$ | 0.79$^{ns}$ | 0.48$^{ns}$ | 1.74$^{ns}$ | 9.40* | 17.06* | 32.27$^{ns}$ | 41.86$^{ns}$ | 46.58$^{ns}$ | 38.91$^{ns}$ | −69.29$^{ns}$ | 0.09$^{ns}$ |
| $r^2$ | 0.9992 | 0.9982 | 0.9982 | 0.9758 | 0.9554 | 0.980 | 0.998 | 0.996 | 0.933 | 0.943 | 0.971 | 0.994 | 0.994 | 0.993 | 0.996 | 0.961 |
| $r^2$adj | 0.9977 | 0.9947 | 0.9947 | 0.9275 | 0.8663 | 0.941 | 0.994 | 0.989 | 0.799 | 0.829 | 0.914 | 0.982 | 0.983 | 0.980 | 0.987 | 0.882 |
| F | 659.77* | 283.09* | 283.09* | 20.18* | 10.72* | 24.73* | 265.37* | 130.30* | 6.95$^{ns}$ | 8.25$^{ns}$ | 16.85* | 82.41* | 88.10* | 75.15* | 111.74* | 12.16* |
| p | 0.001* | 0.001* | 0.001* | 0.016* | 0.039* | 0.0119* | 0.0004* | 0.0010* | 0.0701$^{ns}$ | 0.0557$^{ns}$ | 0.0207* | 0.0020* | 0.0018* | 0.0023* | 0.0013* | 0.0327* |
| CV (%) | 3.00 | 6.50 | 3.60 | 0.08 | 0.12 | 1.07 | 1.05 | 1.67 | 0.93 | 5.65 | 9.51 | 2.82 | 2.71 | 2.10 | 7.71 | 1.67 |

*Significative ($P < 0.05$).

a*, a colour value; Aw, water activity; b*, b colour value; L*, L colour value; $^{ns}$, not significative; REI, radial expansion index; SF, spread factor; SV, specific volume; hardness; TEI, thickness expansion index; VEI, volume expansion index; ΔE, colour difference.

TEI, SF and hardness, explaining 94.1%, 99.4%, 98.9% and 91.4% of the variation, respectively (Table 4). These four characteristics were positively affected by the linear effects of WF, WABF and RF.

For REI, the linear effects of each component were more balanced because the coefficients values were very close (Table 4). There was also a synergistic interaction between WF and WABF. This interaction can be seen in Fig. 1a. Zucco *et al.* (2011) found an increase in the biscuit diameter by gradually replacing the WF by up to 100% for coarse flour (average particle size from 150 to 190 μm) of pulses (navy and pinto beans, and green lentils), but when using fine flour of these pulses (<24 μm), there was reduction in diameter. Zhao *et al.* (2019) when replacing up to 50% WF for yellow pea flour with an average particle size of 49 to 83 μm also found a decrease in diameter. Silky *et al.* (2014) when using pigeon pea flour (180 μm) to gradually replaced WF by up to 30%, they mentioned that this was because the legume starch is more hydrophilic and when gelatinised during baking, it results in increased viscosity, reducing the dough spreading. The greater dough spreading contained WABF during cooking may be related to the larger particle size of the flours (Table 1), or it may be an inherent characteristic of the WABF, different from the results of Mancebo *et al.* (2016) as the increase in protein content did not limit the dough spread.

For the TEI, the linear effect of WABF and RF was very similar (Fig. 1b) as the coefficient values were close (Table 4), but there was an antagonistic effect between WF and WABF and a synergistic effect for the other two binary interactions with RF. As the linear coefficient WF was higher, the LEI values were higher when this component was greater in proportion, gradually decreasing with the WABF substitution (Fig. 1b). Similar results were obtained by Zucco *et al.* (2011); Zhao *et al.* (2019); Silky *et al.* (2014) and Dhull *et al.* (2006) when replacing WF with pulse flours; however, Zucco *et al.* (2011) observed an increase in the biscuit thickness with the use of finer leguminous flours (<24 μm).

'For the SF, the linear effect of the WABF was greater and the estimated coefficient value was close to that of the RF and well above the WF (Table 4); therefore, high proportions of WABF resulted in higher SF values, RF affected with lower intensity. There was a synergistic effect in the interaction between WABF and WF and antagonistic on the interaction of RF with WF and WABF (Fig. 1c). Zucco *et al.* (2011) mentioned that more spread out dough during the baking are more desirable. Similar results were obtained by Silky *et al.* (2014) replacing up to 30% WF with pigeon pea flour, but Dhull *et al.* (2006) found a reduction in SF, increasing the level of substitution by legume flour by up to 30%. Thongram *et al.* (2016) found that when

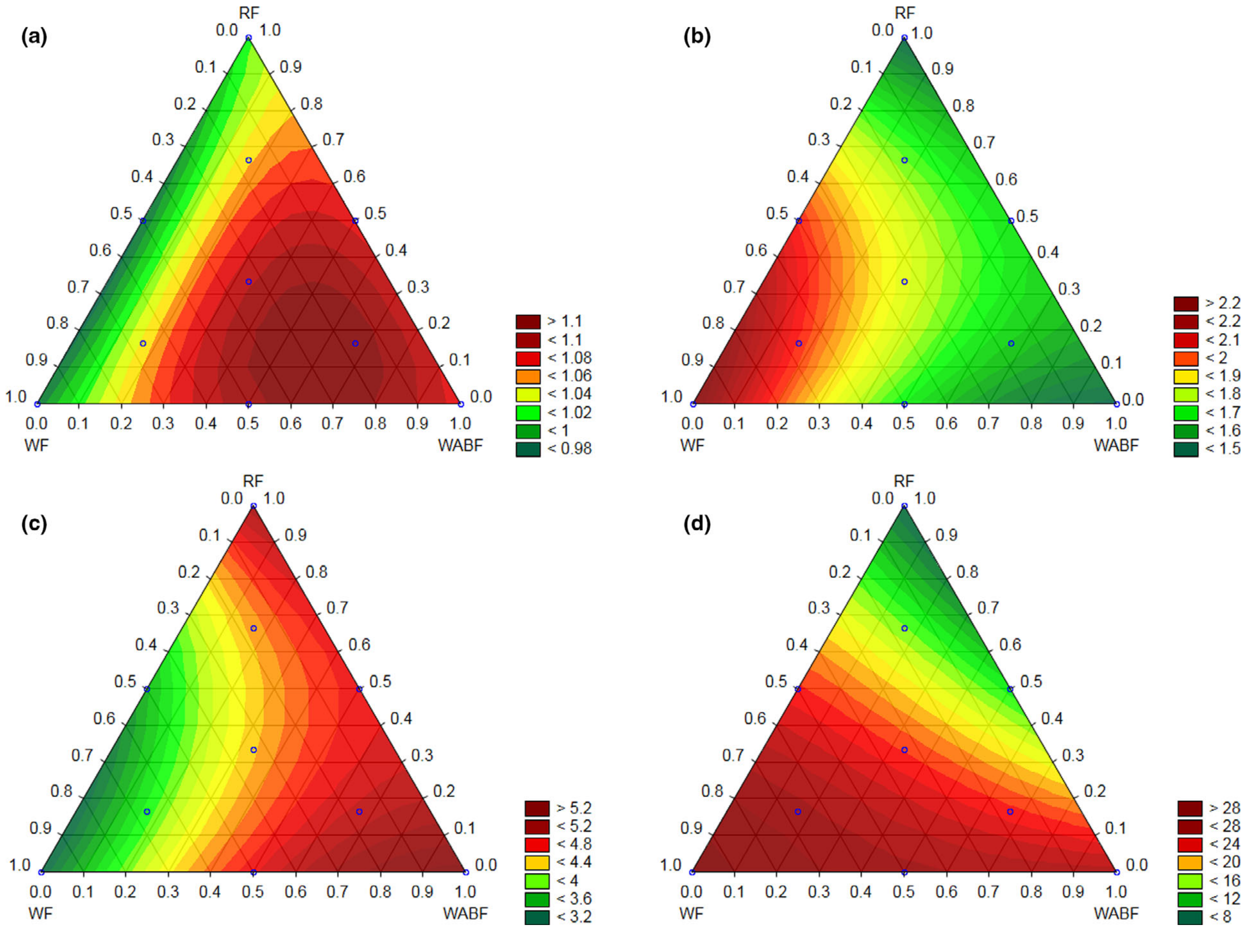


**Figure 1** Effect of wheat, rice and whole azuki bean flour content on radial expansion index (a), thickness expansion index (b), spread factor (c) and hardness (d) of biscuits.

replacing WF for 25% cowpea flour (<1 mm), there was no interference in the biscuit height, but there was an increase in diameter. SF values correlated negatively with LEI ($r = -0.98$) and positively with REI ($r = 0.70$) (Table 3).

The linear effects of WF, WABF and RF (Table 4) significantly affected the biscuit hardness values. High WF proportions resulted in harder biscuits, and increasing the WABF and RF proportions favoured the reduction in hardness. However, the effect of RF was more acute for decreasing the hardness values (Table 4 and Fig. 1d). Zucco *et al.* (2011) obtained similar results in the gradual WF replacement for coarse pulses flours up to 100%. However, Dhull *et al.* (2006) and Silky *et al.* (2014) obtained harder biscuits when replaced for fine pulse flours to 30%. The acute effect of decreasing the biscuit hardness due to the replacement of WF for RF was also reported in research carried out by Klunklin & Savage (2018) and Mancebo *et al.* (2016).

## Colour evaluation

Colour and flavour are products of non-enzymatic browning reactions (Maillard) between reducing sugars and amino acids but also due to dextrinisation and caramelisation (Zucco *et al.*, 2011). According to the analysis of variance (Table 4), the regression models for the colour parameters (L*, a*, b* and ΔE) were significant, explaining 98.2%, 98.3%, 98.0% and 98.7% of the variation, respectively.

High proportions of WF and/or RF favoured obtaining biscuits with a lighter surface, which corresponded to high values of L* (Fig. 2a) as the values of the linear coefficients of both were high and similar (Table 4). However, when the proportion of WABF was increased in the formulation, there was a greater contribution to the darker coloration (Fig. 2a). The presence of dark red pigmentation of the grain tegument resulted in low values for linear coefficients of WABF and its interaction with WF or RF (Table 4).

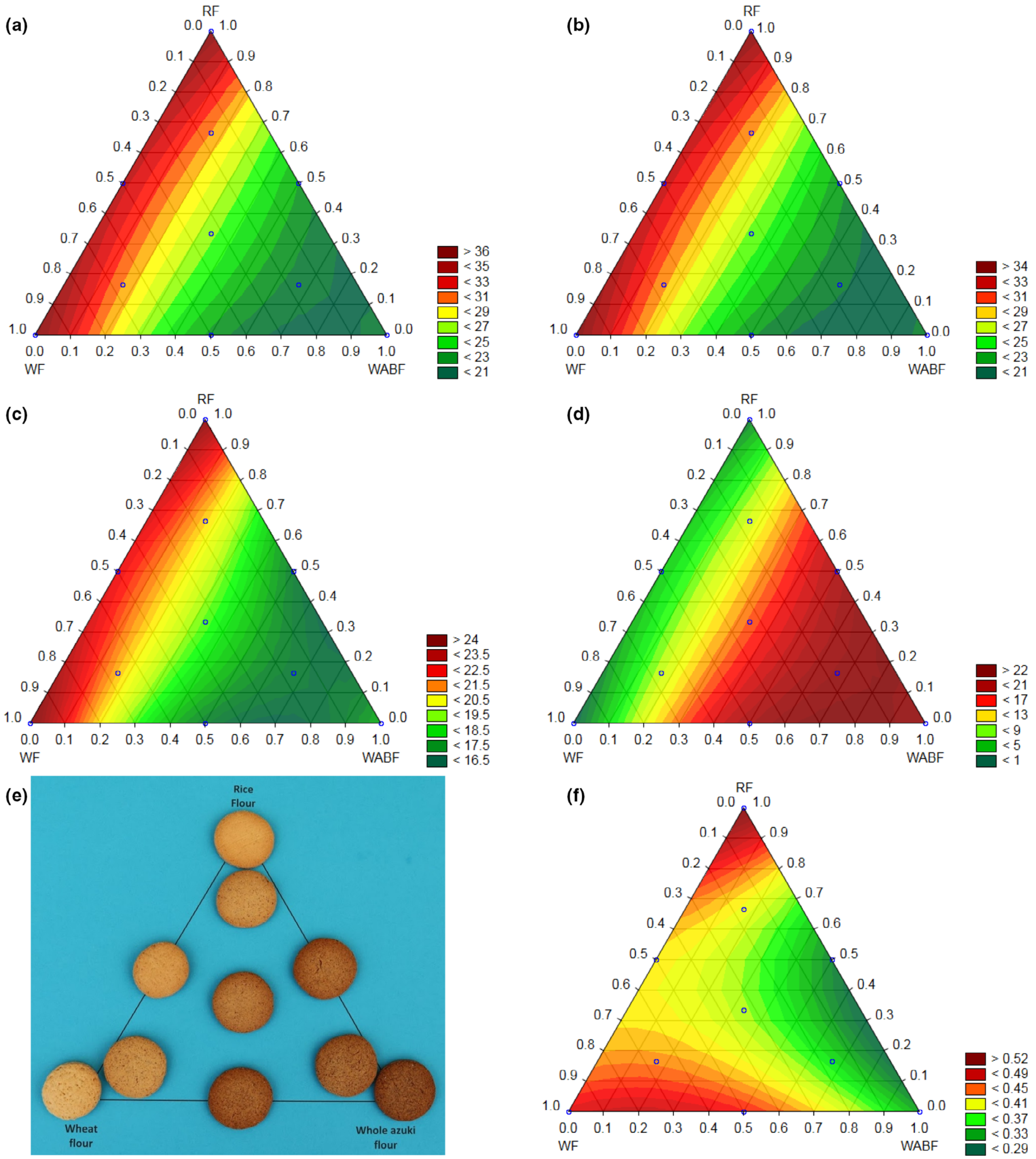


**Figure 2** Effect of wheat, rice and whole azuki bean flour content on colour value L* (a), a* (b), b* (c), colour difference (d), visual appearance (e), water activity (f) of biscuits.

The behaviour for the values of a* and b* was similar to that of L* (Fig. 2a–c), except for the WF and RF interaction for the L* value, which was not significant (Table 4).

For ΔE, the only significant linear coefficient was that of WABF, and the quadratic coefficients were those of interactions with WABF, both with positive values (Table 4), indicating that the increase in the

 

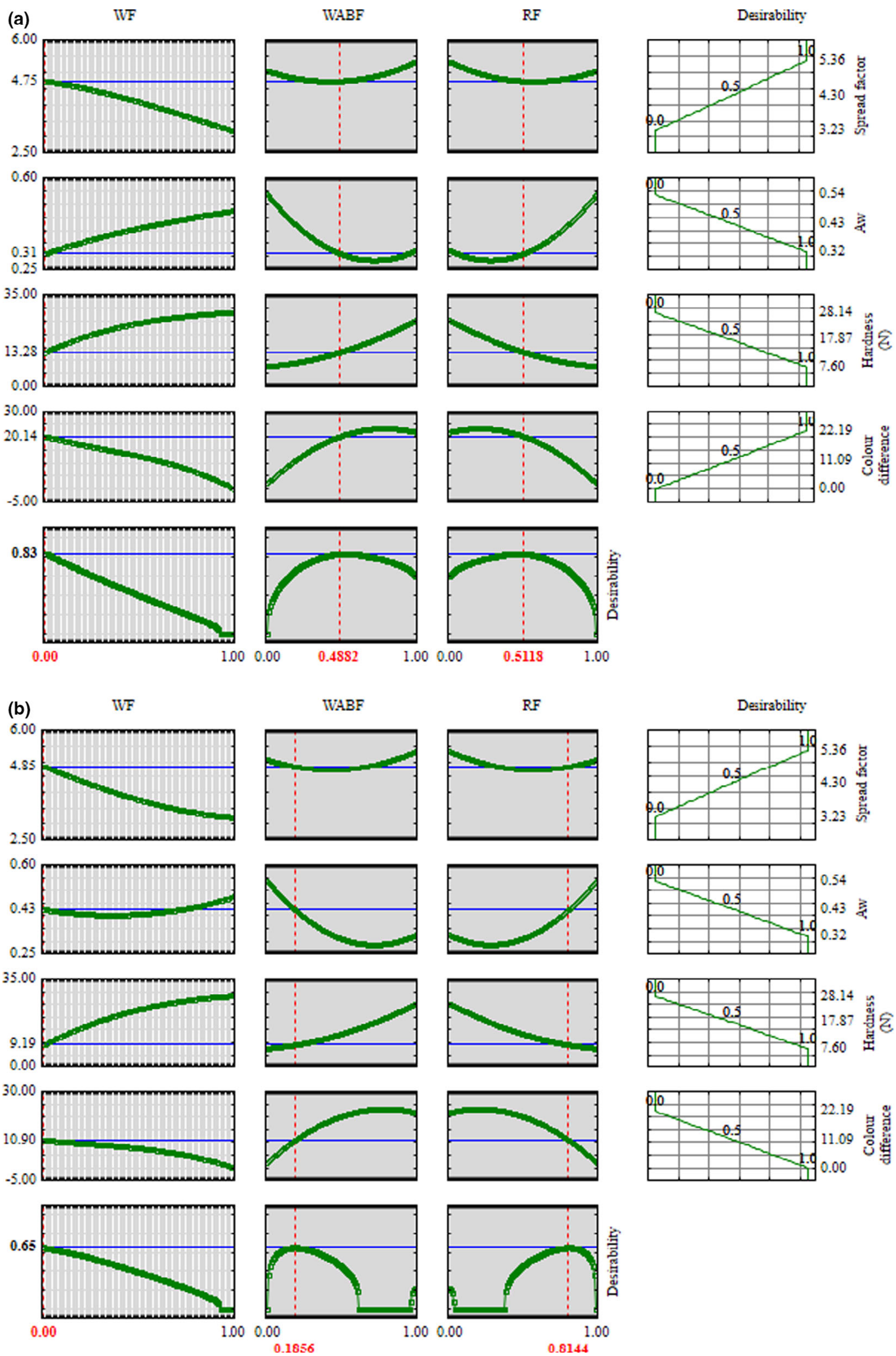


**Figure 3** Profiles for predicted values and desirability for mixture of wheat (WF), whole azuki bean (WABF), and rice (RF) flour considering the darker (a) or lighter (b) surface colour of the biscuits.

proportion of WABF increases the value of ΔE. This change in the value of ΔE is visually perceptible in the products (Fig. 2e) and represented in Fig, 2d, which presents opposite behaviour to the L*, a* and b* values (Fig. 2a–c). The ΔE values showed a positive correlation with SF ($r$ = 0.67), negative with Aw ($r$ = −0.71) and positive with REI ($r$ = 0.91) (Table 3).

The results were similar to those of Zhao *et al.* (2019). By increasing the proportion of yellow pea flour, attributed the browning to the Maillard reaction during cooking, in the case of WABF, there was also a contribution from the dark red colour of the seed coat. Zhao *et al.* (2019) also observed increases in a* and b* values and related to the reaction of reducing sugars with amino acids and pigment oxidation, respectively. Probably, the colour of the WABF masked the colour resulting from these reactions, decreasing the a* and b* values. Zucco *et al.* (2011) found that the increase in WF substitution for pulses flour, even for those with white coat, darkened the biscuit surface, which was attributed to the high protein content and the different amino acid compositions. Cady *et al.* (1987) attributed the reduction in the L* value to the increase in reducing sugars in legumes flours.

### Water activity

The Aw values ranged from 0.32 to 0.54 (Table 1). The model with $r^2$ = 0.882 presented significant linear coefficients, but the WABF × RF interaction coefficient was negative (Table 4). Figure 2f shows this antagonism. The crude WF presented the highest Aw value, followed by WABF and RF (Table 2). Biscuits with high WF contents maintained high Aw values, but biscuits with high RF contents did not present lower Aw values.

### Global desirability analysis

If the option is for darker biscuits, the highest value obtained in the simulation for the global desirability (D) was 0.83 (Fig. 3a), for the combination of 48.82% of WABF, 51.18% of RF and no WF. This combination would favour obtaining a better biscuit quality in relation to the standard biscuit made with 100% WF, such as higher SF (4.75), lower Aw (0.31), softer (13.28 N) and darker (ΔE = 20.14) (Fig. 3a). However, if the option was for lighter coloured biscuits, D value would drop to 0.65 (Fig. 3b); it would still be considered good. In this case, the best predicted mixture would contain 18.56% WABF, 81.44% RF WF (Fig. 3b) and no WF. The biscuit obtained with this mixture will not only have a lighter colour than the standard biscuit (ΔE = 0).

## Conclusions

The different proportions of WABF, WF and RF affected the characteristics evaluated in the biscuits. Higher proportions of WABF resulted in biscuits with higher radial expansion index, propagation rate and darker colour but with a hardness close to WF biscuits. The highest values of global desirability obtained were for formulations without WF, either for lighter or darker coloured biscuits, and these formulations can be destined to the population with restricted gluten intake. According to this study, WABF is an interesting alternative as an ingredient for nutritional enrichment due to its protein content, being technologically viable for the production of gluten-free biscuits.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Author contributions

**Daisy Jacqueline Sousa Silva:** Data curation (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Jorge Minoru Hashimoto:** Conceptualization (lead); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (equal); Writing-review & editing (lead). **Elizabeth Harumi Nabeshima:** Conceptualization (equal); Formal analysis (equal); Methodology (equal); Writing-review & editing (equal). **Rafaela Teixeira Salgado:** Formal analysis (supporting); Investigation (supporting); Writing-original draft (supporting). **Thaise Kessiane Teixeira Freitas:** Formal analysis (supporting); Methodology (supporting); Writing-original draft (supporting). **Kaesel Jackson Damasceno e Silva:** Investigation (equal); Project administration (equal); Supervision (equal); Writing-original draft (supporting).

## Ethical approval

Ethics approval was not required for this research.

## Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15312.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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