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Original Article

Bread composite by wheat and novel Andean purple corn: dough rheology and physical and bioactive characteristics

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Abstract

The aim of this work was to evaluate the dough rheological properties along with the textural, physical, colorimetric, structural, total phenolics, anthocyanins and antioxidant properties of breads composed of wheat and Peruvian INIA 601 purple corn flour at three levels of substitution (5%, 15%, and 25%). Paste profiles describe the gelatinization, swelling, and decomposition phenomena of starch-based systems. These profiles were strongly affected at 15% and 25% replacement levels of purple corn INIA 601, attributed to the presence of phenolic compounds, which retarded starch gelatinization by increasing energy demands to reach paste temperature. Farinographic analysis evaluates key quality properties of the dough and provides insight into its rheological behaviour. In them, 5% purple corn flour substitution had a gluten-enhancing effect that increased the dough consistency, given the reducing capacity of the polyphenols that caused the aggregation of gluten proteins. But at levels of 15% and 25% it affected dough stability and tolerance properties ($p < .05$). In the rheometry properties, the addition of purple corn increased the elastic (G′) and viscous (G″) modulus. However, the hardness as the most important textural parameter of breadcrumbs was not affected up to 15% purple corn incorporation levels ($p < .05$). Multivariate, heatmap, and correlation analyses identify patterns and relationships, offering insights into variable interactions. Correlation studies showed interesting associations ($0.90 > r > 0.99$) between the bread responses. In conclusion, the incorporation of INIA 601 purple corn can generate anthocyanin-rich pigmented breads with higher concentration of phenolic compounds, anthocyanins and antioxidant capacity, which showed physical characteristics like wheat without drastically affecting crumb cell structure and bread volume.

Keywords: anthocyanin-rich bread, crumb features, dough rheometry, INIA 601 maize, multivariate analysis

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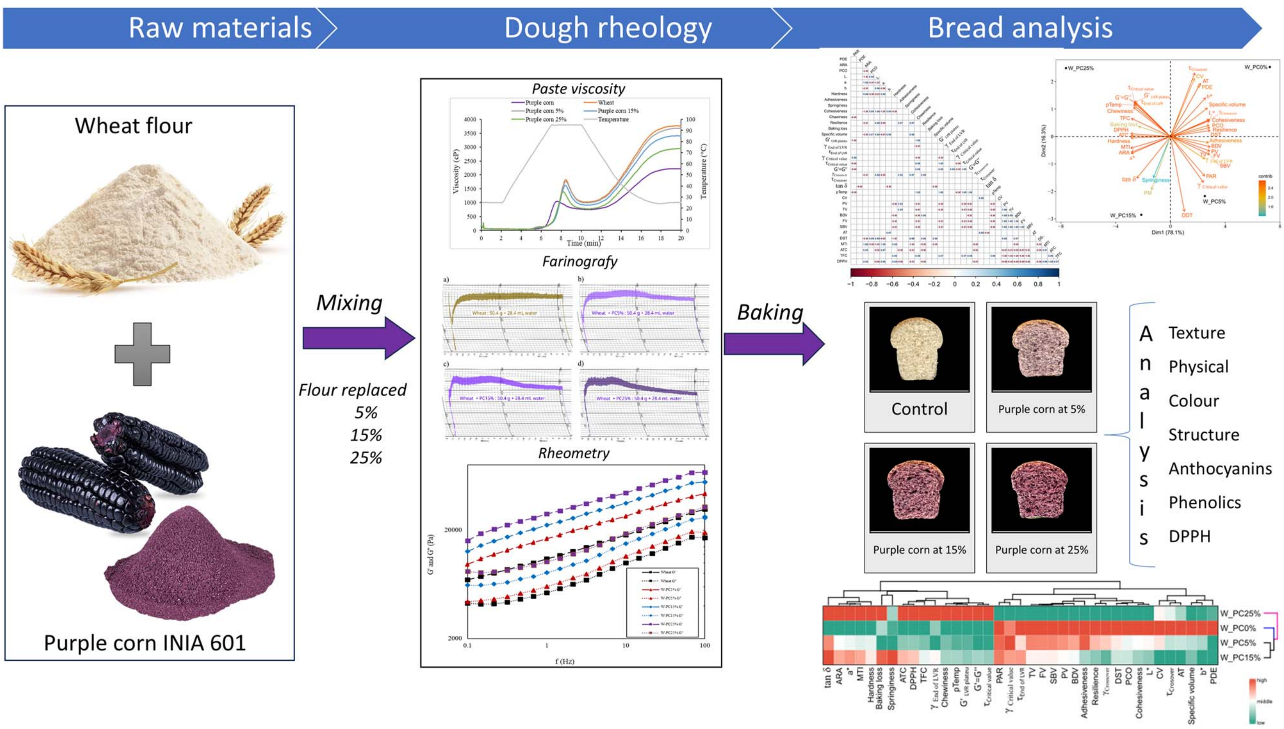
Graphical abstract



Introduction

Andean purple corn (*Zea mays* L.) is an ancient Peruvian cereal, mainly used to produce the non-fermented culinary drink named "chicha de morada" in Peru. In addition, this type of corn has been used to produce cereal-based products, such as snacks and bakery products rich in anthocyanins, in order to impart their functional properties to these products (Li et al., 2019). Purple corn is mainly composed of 85% kernel and 15% cob and its distinctive purple colour is attributed to the presence of major anthocyanins of type cyanidin-3-glucoside, cyanidin-3-malonylglucoside, peonidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-malonylglucoside, which are found both in the cob and in the pericarp of the kernel (Salinas et al., 2013; Suriano et al., 2021). These bioactive compounds possess strong antioxidant and protective capacities against reactive oxygen species, which trigger degenerative processes linked to non-communicable diseases such as obesity, diabetes, and cardiovascular diseases, often associated with oxidative damage (Rodriguez et al., 2024).

In addition, its phytochemical composition also includes phenolic acids such as p-coumaric acid, vanillic acid, protocatechuic acid, and ferulic acid (Pedreschi & Cisneros-Zevallos, 2007; Yang et al., 2009) and anthocyanin-flavanol condensed pigments, such as catechin-(4,8)-cyanidin-3,5-diglucoside, catechin-(4,8)-cyanidin-3-malonylglucoside-5-glycoside, and epicatechin-(4,8)-cyanidin-3-malonylglucoside-5-glycoside (González-Manzano et al., 2008; Peniche-Pavía & Tiessen, 2020). These nutraceutical compounds are known to inhibit colorectal carcinogenesis in male rats, possessing anti-mutagenic, anti-inflammatory, anti-diabetic, and anti-adipogenic activities and improving insulin sensitivity in insulin-resistant adipocytes and radical scavenging (Bhaswant et al., 2017; Singh et al., 2019; Zhang et al., 2019).

Currently, the most recognized Peruvian purple corn species in research is the improved variety "INIA-601" developed by the Instituto Nacional de Innovación Agraria (INIA) from Peru. It is known for its high yield and anthocyanin content in both kernels and cobs. In addition, it has pigmentation in bracts and stalks, which distinguishes it from other Peruvian purple corn species (Guzzon et al., 2021; Medina-Hoyos et al., 2020; Montenegro et al., 2022; Rabanal-Atalaya & Medina-Hoyos, 2021; Romero et al., 2022). The highest yields of this variety are obtained in the high Andean zone of Peru; with proper agronomic management of the cultivar, yields of exceeding 2.8 t ha^{-1} and average anthocyanin contents in the crowns and bracts of 9.36% can be achieved (Medina-Hoyos et al., 2020). Due to these characteristics it has gained popularity and its properties have been used in the food industry having applications as an ingredient in the production of different foods such as tea (Díaz et al., 2021), whisky, bakery products, among others.

Bread is a baked food produced mainly from wheat flour and contains storage proteins called gluten prolamins, composed of glutenin and gliadin, which give the dough viscoelasticity and play a key role in the structure and quality of the final product (Świeca et al., 2015), to which ingredients such as yeast, sugar, edible oil, and salt are often added (Zhang et al., 2023a). It is widely consumed around the world and is part of people's daily diet, especially for breakfast (Forsberg et al., 2014; Isaksson et al., 2009; Sandvik et al., 2014), as it provides energy, mainly through the starch it contains, along with dietary fibre, protein, and a variety of vitamins, minerals, and bioactive compounds essential for the human organism (Fardet, 2010; Nanditha & Prabhasankar, 2009). However, the incorporation of purple corn into refined bread could improve the phytochemical profile. In turn, it offers a naturally pigmented and healthy bread alternative with chemoprotective potential.

Therefore, some previous studies such as those by Rodríguez et al. (2013), Slavu et al. (2022), Simic et al. (2018), and Guo et al. (2022) have included purple corn in bakery products to improve their therapeutic, functional, and/or nutraceutical properties by enhancing antioxidant capacity. However, the application of INIA 601 purple corn as an ingredient in mass-market products, particularly regarding its effects on dough rheology and bread quality, has not been widely studied. Therefore, the aim of this work was to evaluate the effect of the incorporation of INIA-601 purple corn on the rheological properties of dough when replaced in different proportions into wheat flour, as well as to determine the physical, structural, and bioactive characteristics of breads enriched with this anthocyanin-rich cereal.

Materials and methods

Plant material

The purple corn INIA 601 was acquired by the Instituto Nacional de Investigación Agraria (INIA—Cajamarca), which was ground with an LM3100 hammer mill (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm sieve to obtain fine whole purple corn flour whose particle size distribution was determined on a laser diffraction analyser (S3500 series, Microtrac Inc., Montgomeryville, United States); the flour had 77.8% very fine particles (1.60–150 μm), 14.1% fine particles (150–200 μm), and 8.10% coarse particles (200–600 μm). The refined hard wheat flour (Mulino Padano, Italy) containing 12.9% protein, 1.10% fat, 2.0% dietary fibre, and 67% carbohydrates was purchased from the Rio de Janeiro. Ingredients such as sugar, yeast (Fleishmann, Brazil), vegetable fat (non-hydrogenated of palm), and salt were purchased from local markets.

Paste viscosity profile

The paste profile of the samples was measured in duplicate, following the methodology of Comettant-Rabanal et al. (2021), using an RVA series 4 rapid viscosity analyser (Newport Scientific Pty Ltd., Warriewood, Australia). Three grams of flour with an adjusted moisture content of 14% (wet basis) were combined with 25 ml of distilled water in an aluminium sample holder used in the experimental equipment. The test conditions were set as follows: mixing at 160 rpm at 25 °C for 2 min, heating to 95 °C at a constant rate of 14 °C/min, and holding for 3 min and then cooling to 25 °C in 5 min at the same rate, for a total time of 20 min. The paste properties measured were as follows:

(a) Paste temperature (PTem, cP): The temperature reached for the onset of both the paste viscosity and the gelatinization phenomenon.
(b) Cold viscosity at onset of 25 °C (CV, cP): Viscosity measured at 25 °C and indicates the starch capacity to interact with water molecules and generate paste without heating.
(c) Peak viscosity (PV, cP): The maximum viscosity reached during heating and indicates the highest degree of swelling and gelatinization of the starch.
(d) Trough viscosity or holding strength (TV, cP): The minimum viscosity after PV indicates the collapse of swollen starch granules and their stability under shear.
(e) Breakdown viscosity (BDV = PV − TV, cP): It represents the degree of granule disintegration or paste breakdown under continuous heating and shearing.
(f) Final viscosity (FV, cP): The viscosity at the end of the cooling phase, which reflects the capacity of the gelatinized starch to retrograde or form a gel upon cooling.
(g) Setback viscosity (SBV = FV − TV, cP): Represents the degree of starch fragment reorganization during cooling, important for predicting product texture after cooling.

Farinographic evaluation

The evaluation of the resistance of the dough to mixing was carried out using the Farinograph© model FD0234H from Brabender (Duisburg, Germany) following the AACC (2000b) method. Fifty grams of flour was used, and different levels of water addition (55% to 60%) were added until the dough consistency was reached with optimal hydration of wheat flour corresponding to 500 Brabender Units. From the resulting farinograms, the readings of interest were water absorption (%), farinographic consistency (Brabender Units), dough development time (DDT, min), dough stability time (DST, min), and mixing tolerance index (MTI, min), which were determined 5 min after the peak.

Oscillation rheometry

The dynamic mechanical properties of the bread doughs with purple corn addition were evaluated using a HAAKE Mars II rotational rheometer (Thermo Fisher Scientific, Karlsruhe, Germany). Prior to rheometric measurements, each sample was mixed with flour and water in the farinograph, using the same water absorption value to avoid influence of water content (28.4 ml) and the optimal DDT of each sample was used following the protocol proposed by Comettant-Rabanal et al. (2021). Then, the dough was extracted and wrapped in polyethylene film and placed in airtight container to avoid dehydration until its use in the rheometer. The measurements were carried out at a temperature of 25 °C, using a parallel plate geometry with a diameter of 35 mm. Three grams of dough were placed on the bottom plate and then the top plate was moved closer to the dough at a constant speed of 0.6 mm/min until a gap of 2 mm was reached. Excess dough was removed from the outer edge and mineral oil was applied to prevent dehydration during the measurement. For each sample, first an amplitude sweep was performed, maintaining a constant strain amplitude (γ), within the range of the linear viscoelastic region (LVR). Then, frequency sweeps were performed between 0.1 and 100 Hz. The values of elastic or storage modulus (G′), viscous or loss modulus (G″) and the loss factor tan δ (G″/G′) were obtained at a frequency of 1 Hz. All measurements were carried out in duplicate.

Texture profile analysis

Texture profile analysis was performed on 20 mm thick bread slices 24 hr after baking, applying double compression by means of a 15 mm diameter cylindrical aluminium probe placed in the centre of the breadcrumb. This procedure was performed using a TA-XT Plus Texture Analyser (Stable Micro Systems, Surrey, United Kingdom), which was equipped with a 5 kg load cell and controlled by Exponent software version 6.1.11.0 (Stable Micro Systems, Surrey, United Kingdom) following the methodology described by Comettant-Rabanal et al. (2021). The instrument was set to compress the slice by 50%, and a waiting time of 5 s was observed between the first and second compression cycle. The resulting texture measurements included crumb hardness (N), stickiness (g-s), cohesiveness (–), springiness (–), chewiness (N), and resilience (–).

Baking loss and specific volume

The specific volume was determined with the modified standard millet seed displacement method 10-05.01 AACC (2000a). The container used for the calculation was a parallelepiped with dimensions 8.5 cm × 8.4 cm × 9.2 cm (width × length × height). Bread specific volume (cm^3/g) was calculated as the volume of bread divided by the weight of the bread measured 24 hr after baking.

Image analysis of bread crumb structure

First, images of bread slices with dimensions of approximately 50 mm × 50 mm were scanned in full colour using an Epson Perfection 1240U scanner (Seiko, Nagano-Ken, Japan). Next, recording the images in TIFF format at a resolution of 400 dpi (866 mm wide × 866 mm high). Then, the images were analysed using ImageJ software (version 1.54d, Wayne Rasband, National Institute of Health, United States). After that, the centre of the image was cropped with dimensions of 20.79 mm × 20.79 mm (Supplementary Figure 1), and then 8-bit images were adjusted using the k-means threshold clustering algorithm (Encina-Zelada et al., 2019). Finally, some features were calculated to characterize the structure and properties of the bread crumb samples, such as average pore area (PAR, mm^2); average pore density (PDE, pores/mm^2); and average pore compactness (PCO, dimensionless), with compactness defined as the ratio of the pore area of a circle having the same perimeter: values of 1 indicating a perfect circle pore shape; and average pore aspect ratio (ARA, dimensionless), with aspect ratio defined as the ratio of the major axis to the minor axis of a pore; values higher than 1 indicating an ellipse shape.

Bread crumb colour analysis (CIELAB)

The colour of the breadcrumbs was determined by analysing the RGB values obtained through ImageJ software (v.1.54d, Wayne Rasband, National Institute of Health, United States). Next, those values had converted them to the CIE $L^*a^*b^*$ colour system. After that, the mathematical model to calculate the colour difference (ΔE) was as follows:

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$$

In this equation, L^*, a^*, and b^* represent the lightness, redness, and yellowness. Next, L_0, a_0, and b_0 represents the lightness, redness, and yellowness of the pattern sample (100% wheat).

Bioactive compounds and antioxidant capacity

Extraction procedure

The anthocyanins extraction was performed according Gutierrez-Quequezana et al. (2018) method, with slight modifications. Briefly 2 g sample was extracted with 15 ml of methanol acidified 70% (0.1% HCl). For total phenolics and antioxidant capacity the extraction was according to Chirinos et al. (2007), Briefly 2 g sample was extracted with 15 ml of methanol 90%. Both extracted of the samples were kept at 4 °C for 24 hr, and then centrifuged at 1500 *g* for 10 min at 4 °C. Finally, the samples were filtered and stored for the analysis.

Total anthocyanins

Total anthocyanins (ATC) in the extracts were determined according to the paper Chirinos et al. (2007) using the pH differential method. Absorbance was measured at 514 and 700 nm in pH 1.0 and 4.5 buffers. A molar extinction coefficient of 26,900 cm^{-1} mol^{-1} and a molecular weight of 449.2 g/mol were used for anthocyanin calculation. Results were expressed as mg of cyanidin 3-glucoside equivalents per 100 g in dry mass (DM).

Total phenolic compounds

Total phenolic compounds (TPC) were determined according to Campos et al. (2022), with slight modifications. The colorimetric reaction was carried out with 500 µl of extract, 250 µl of 1 N Folin–Ciolcateau reagent, and 1,250 µl of 1.2 N sodium carbonate solution. After 30 min in the dark, the absorbance at 755 nm was determined. The results were expressed in milligram of gallic acid equivalents per 100 g DM.

Antioxidant capacity by DPPH assay

The antioxidant capacity of the samples was determined according to Chirinos et al. (2013), using the DPPH method. The stock solution was prepared by dissolving 24 mg DPPH reagent with 100 ml of methanol and then stored at −20 °C until use. The working solution was obtained by mixing 10 ml of stock solution with 45 ml of methanol to obtain an absorbance of 1.10 ± 0.02 units at 515 nm. Extracts (150 µl) were mixed with 2,850 µl of $DPPH^{\bullet+}$ solution in methanol. The mixtures were incubated for 30 min in the dark at 20 °C and the absorbance at 515 nm was measured in a UV–Vis spectrophotometer (Genesis 150, Thermo Fisher Scientific, Massachusetts, United States). Methanol was used as blank. Antioxidant activity was quantified as µmol of Trolox equivalents per 100 g DM of the sample, from a standard curve created simultaneously with the sample analysis. This curve covered Trolox concentrations of 5, 60, 150, 350, 400, and 500 µmol, resulting in the equation $y = 0.0012 \times -0.0277$, which showed linearity with a value of $R^2 = 0.99$.

Statistical analysis

Data were analysed by one-way analysis of variance, followed by Tukey multiple comparison test (for parametric data) or Kruskal–Wallis (for non-parametric data) when differences were detected. The normality of the residuals was confirmed by the Shapiro–Wilk test, along with verification of the independence of the errors and the homoscedasticity of the residuals by the Durbin–Watson and Levene tests, respectively. All tests were performed with a significance level of 5%. Additionally, multivariate statistics such as principal component analysis were applied after standardizing variables to prevent bias. Heatmap was used to reveal associations between treatments and variables. The hierarchical clustering was performed by applying the Euclidean distance and Ward's grouping methods. Finally, the Pearson's correlation coefficient was calculated to evaluate possible relationships among variables. The correlation strength was classified as follows: an r value between $0.0 \leq r < 0.3$ indicates an insignificant correlation. Values between $0.3 \leq r < 0.5$ reflect a low correlation, between $0.5 \leq r < 0.7$ a moderate correlation, and between $0.7 \leq r < 0.9$ a high correlation. Finally, an r value between $0.9 \leq r \leq 1.0$ indicates a very high correlation. Correlations with r values greater than 0.7, both positive and negative, were considered relevant.

Results and discussion

Paste viscosity properties

The paste viscosity profiles of the wheat flour were slightly modified with the addition of 5% but they decreased significantly with additions of 15% to 25% of whole purple corn (Figure 1). The paste temperature (PTemp) showed no significant difference at 5% purple corn addition ($p > .05$), while 15% and 25% additions caused temperature increases ($p < .05$) from 70.55 to 74 °C (Supplementary Table 1), suggesting that the presence of phenolic compounds such as anthocyanin delays the starch gelatinization phenomenon by increasing the energy requirements in terms of temperature. The PV associated with the point of maximum gelatinization also showed significant ($p < .05$) reductions with the addition of whole purple corn flour, owing to the fibre and anthocyanins. Insoluble dietary fibre, due to its microscopic porous structure, can bind water molecules and thus hinder the swelling

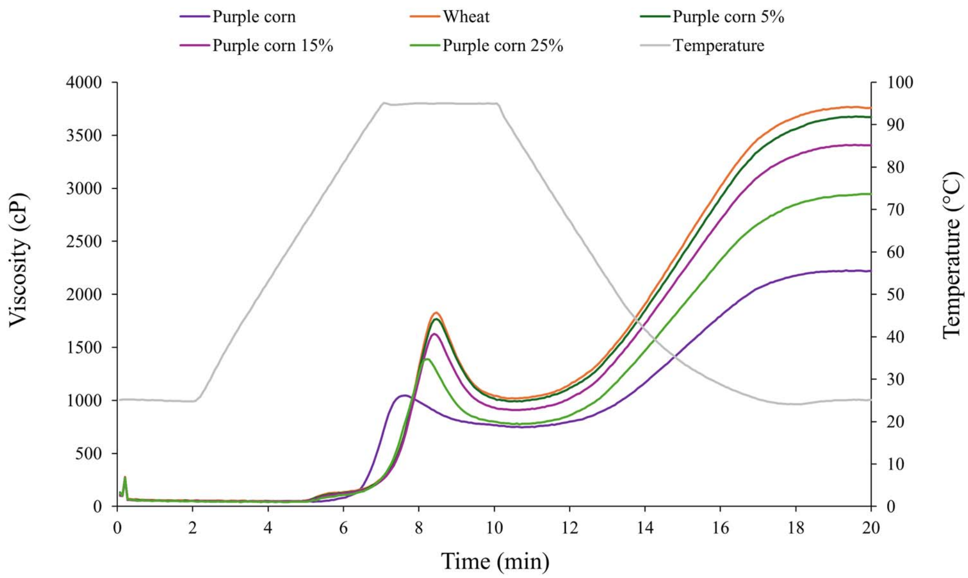


Figure 1. Pasting profile of wheat flour composite with replaced of whole purple corn flour at different percentages (5%, 15%, and 25%).

and gelatinization phenomenon of starch granules by reducing the availability of water in the medium. Anthocyanins, which contain numerous hydroxyl groups, can form hydrogen bonds with amylose, inhibiting amylose leaching, which is responsible for the swelling power during the gelatinization phenomenon (Zhang et al., 2023b).

The TV and BDV parameters, which are related to the collapse of starch after reaching its maximum absorption of water molecules, were also affected. Specifically, in the sample with 25% whole purple corn, a lower viscosity drop was observed in the profile (Figure 1), possibly due to a higher affinity of fibres and phenolic compounds for water molecules, which prevented the starch molecules from interacting with the water in the system. Likewise, SBV and FV related to the starch retrogradation phenomenon showed significant reductions at higher proportions of purple corn incorporation ($p < .05$), indicating that the effect of starch return from the amorphous to the glassy state is minimized when samples rich in fibre and phenolic compounds are added. This behaviour may indicate that the higher the addition of purple corn, the lower the rate of starch disruption and gelatinization, leading to lower starch digestibility (Aleixandre & Rosell, 2022).

Farinographic properties

The arrival time (AT) properties related to the hydration mode and dough conformation velocity showed that the addition of whole purple corn flour between 5% and 25% caused AT reductions ($p < .05$) (Supplementary Table 2), due to the lower proportion of gluten (insoluble protein) in the dough, which facilitated its conformation (Figure 2A–D). The maximum peak consistency (PM), which reflects the dough development initiation at the complete hydration time, showed that the wheat flour used had high gluten stability (Figure 2A), as no changes in dough consistency were observed during the kneading process at 20 min. In addition, slight significant increases ($p < .05$) in PM were observed with the addition of purple corn flour at 5% (Supplementary Table 2), due to the reducing effect conferred by the phenolic compounds of the corn, which possibly promoted the aggregation of the gluten proteins, thus causing modifications in the gluten network microstructure and improving the dough mixing properties (Figure 2B and C) (Wang et al., 2015).

The departure time (DT) and DST parameters explain the destructuring or collapse of the viscoelastic gluten network in the dough due to the mechanical energy applied during kneading. With the addition of higher proportions of wholemeal purple corn flour, both parameters decreased significantly (Supplementary Table 2), leading to lower dough stability. This decrease is attributed to the interference of insoluble fiber in the continuous gluten network, as evidenced by Pasqualone et al. (2018) when incorporating almond skins into wheat dough. This effect of adding whole corn flour as a replacement for very strong wheat flours may be desirable to decrease the cohesiveness of the gluten and to condition the excessive strength of this type of flour.

The DDT, which indicates the time required to develop and form the viscoelastic gluten network after reaching optimum hydration, increased significantly with the addition of 5% and 15% purple corn flour ($p < .05$); this was due to the high hygroscopicity of the purple corn fibre, which competed for water with the proteins and starch in the wheat flour, resulting in longer mixing times for the formation of the gluten network (Pasqualone et al., 2017). In contrast, with the addition of 20% purple corn the DDT was similar to the wheat control ($p > .05$). However, with regards to the MTI, which indicates the impact of mechanical energy on gluten structure and stability, it was observed that the tolerance to mechanical stress of the doughs with the addition of purple corn was significant ($p < .05$), where the higher the purple corn replacements, the lower the tolerance of the dough to mechanical stress.

Oscillatory rheometry properties

The elastic (G′) and viscous (G″) moduli of the bread doughs increased as more whole grain purple corn was added to the hard wheat flour (Figure 3A). This indicates that the polyphenols present in the purple corn produced a more compact gluten network structure, reinforcing the glutenin-rich fraction and resulting in a well-organized and ordered network for the gliadin-rich fraction (Tian et al., 2021). All the doughs with purple corn addition had increases of G′ in the plateau of the LVR (Supplementary Table 3), being the sample W-PC25% the one that presented the highest G′ (28,010 Pa) among all the samples

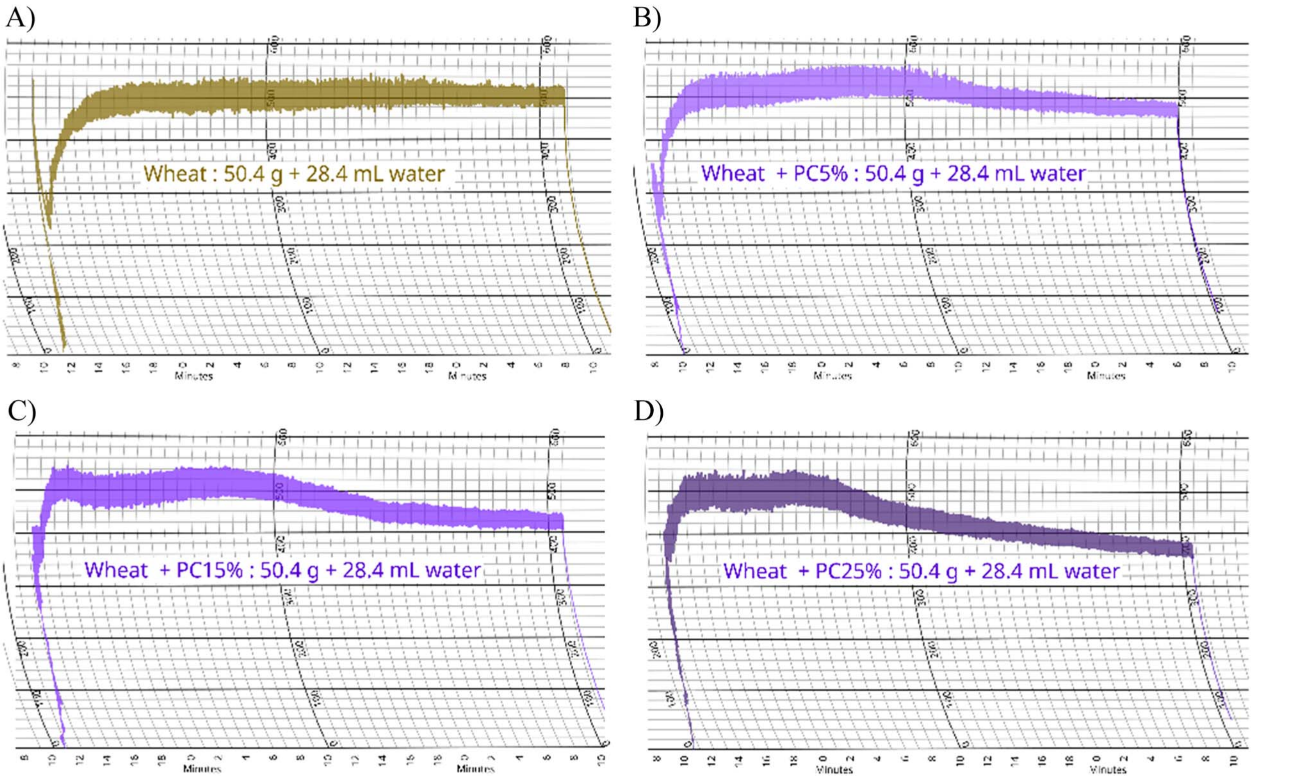


Figure 2. Farinograms of flours composed of refined hard wheat with different percentages of whole PCs (A–D). PC = purple corn addition (%).

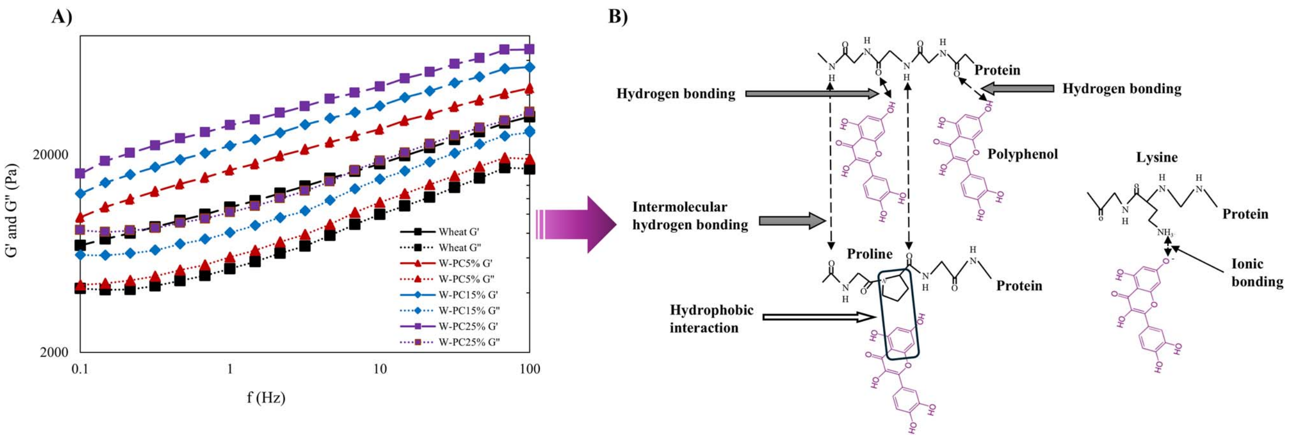


Figure 3. Oscillatory rheological properties of composite dough made from hard wheat flour containing 5%, 15%, and 25% of whole purple corn flour (A). The main mechanisms of gluten-polyphenol interactions contributing to the increase of the elastic (G′) and viscous (G″) modulus (B) adapted from Xu et al. (2019).

($p < .05$), followed by W-PC15% (18,645 Pa) and W-PC5% (17,195 Pa). These results demonstrated improvements in the rheometric properties, specifically in the elastic (G′) and viscous (G″) moduli, which were dependent on the concentration of purple whole corn flour. This effect is attributed to potential interactions between the phenolic compounds in the corn flour and the wheat gluten proteins, leading to the formation of ionic and hydrogen bonds, as well as hydrophobic forces. These interactions, in turn, promoted the aggregation of glutenin and gliadin proteins (Figure 3B) (Du et al., 2022; Ge et al., 2023). Similar behaviour was observed by Wang et al. (2015) and Girard et al. (2018) applying sorghum and grape proanthocyanidins and tannins as natural wheat dough improver additives, respectively.

Texture profile, baking loss, and volume analysis of the breads

The crumb hardness of wheat breads enriched with INIA 601 whole grain purple corn flour ranged from 1.47 to 1.60 N (Table 1), being the breads with up to 15% purple corn flour those that did not show significant differences ($p < .05$) in hardness compared with the control (1.37 N). These hardness results were much lower than those of the anthocyanin-rich pigmented wheat breads with grape pomace incorporation studied by Tolve et al. (2021), which showed hardness between 21.8 and 23.2 N. This suggests that purple corn flour can effectively substitute wheat in bread formulations without significantly compromising texture in terms of crumb hardness. In addition, substitutions of up to

Table 1. Texture profile, baking loss, and specific volume analysis of bread crumbs composed by blend of refined hard wheat at different percentage of whole-grain purple corn flour.

Treatment	Hardness (N)	Adhesiveness (g.s)	Cohesiveness	Springiness	Chewiness (N)	Resilience	Baking loss (%)	Specific volume (cm^3/g)
W-PC0%	1.37 ± 0.10^{b}	-9.98 ± 3.44^{a}	0.33 ± 0.011^{a}	0.947 ± 0.027^{a}	0.423 ± 0.033^{a}	0.096 ± 0.34^{a}	13.91 ± 0.08^{ab}	2.91 ± 0.08^{a}
W-PC5%	1.47 ± 0.08^{ab}	-10.5 ± 3.43^{a}	0.31 ± 0.018^{ab}	0.946 ± 0.021^{a}	0.426 ± 0.022^{a}	0.090 ± 0.30^{ab}	13.62 ± 0.03^{b}	2.58 ± 0.03^{b}
W-PC15%	1.50 ± 0.13^{ab}	-19.6 ± 3.99^{b}	0.30 ± 0.011^{b}	0.965 ± 0.027^{a}	0.428 ± 0.049^{a}	0.080 ± 0.57^{bc}	14.54 ± 0.09^{a}	2.50 ± 0.09^{bc}
W-PC25%	1.60 ± 0.07^{a}	-23.2 ± 6.50^{b}	0.29 ± 0.015^{b}	0.951 ± 0.028^{a}	0.442 ± 0.045^{a}	0.076 ± 0.36^{c}	14.59 ± 0.05^{a}	2.41 ± 0.05^{c}
Shapiro (Norm.Res)	0.2180	0.6903	0.8991	0.3006	0.7866	0.5133	0.7290	0.5387
Durbin–Watson (Independence.Res)	0.9097	0.8251	0.0234	0.8948	0.8190	0.3945	0.6337	0.7978
LeveneTest (Var.Homoge)	0.5851	0.4770	0.6504	0.7055	0.3876	0.0374	0.8185	0.5801

Note. Results represent the mean ± SD ($n = 8$ for texture and $n = 4$ for physical properties). W = refined wheat flour; PM = purple corn flour addition (%); W-PC0% = wheat control; W-PC5% = wheat-whole purple corn at 5%; W-PC15% = wheat-whole purple corn at 15%; W-PC25% = wheat-whole purple corn at 25%. The lowercase superscript letters indicate differences between bread samples using the Tukey test ($p < .05$) parametric test or the Kruskal–Wallis non-parametric test ($p < .05$).

15% whole purple corn INIA 601 to the strong wheat flour did not significantly affect the hardness of the bread crumb ($p < .05$).

Although breads with 25% purple corn showed significantly higher hardness values than the control ($p < .05$), these values were also lower than those obtained by Simic et al. (2018), when using 30% blue (~17.6 N) and dark (~8.8 N) corn flour to obtain anthocyanin-rich breads. Regarding cohesiveness, which is a textural parameter associated with crumb strength, it was observed that the 5% substitution of purple corn (P-WPC5%) did not affect ($p > .05$) this property, but the 15% and 25% replacements significantly affected cohesiveness ($p < .05$), coinciding with what was reported by Simic et al. (2018), while the textural parameters of springiness and chewiness associated with the degree of crumb recovery and hardening of the bread, respectively (Cauvain, 2016), were affected with replacements of whole purple corn flour INIA 601 ($p > .05$) (Table 1). This indicates that up to 25% substitutions with whole grain purple corn did not drastically affect the elastic properties and crumb staling of the bread.

Bread crumb features by image analysis

Digital images of the bread crumb showed slight differences in the alveolar structure as the proportion of purple corn flour increased (Figure 4). The bread crumb became denser, with smaller pores and more compact walls, which is characteristic of a bread with higher hydration levels. Additionally, according to Encina-Zelada et al. (2019), the bread texture is affected by pores size of bread crumb, the distribution of pore sizes having a stronger influence on texture than pore sizes; it seems that the lower PAR value could be related to the crumb with more gas pores or lower gas pore size; however, it is not a general rule, a significant positive linear correlation ($r = 0.66$) between PAR (i.e., mean pore density) and bread specific volume (Table 1–2) confirmed this result. Razavizadegan Jahromi et al. (2014) concluded that bread pore density had a negative linear correlation with a specific volume. Moreover, during proofing time, the coalescence of expanded gas pores caused a decrease in the number of pores per unit area or crumb porosity. The ARA parameter, which shows the loss of circularity and regularity of the bread crumb pores, together with PDE (number of pores) increased with the incorporation of higher proportions of purple corn flour ($p > .05$), while PCO and PAR had an opposite behaviour and their values were significantly reduced mainly at 25% purple corn flour incorporation levels ($p < .05$). These increases in shape (ARA) and pore number (PDE) parameters are related to crumb hardness and the associations between these morphological and structural crumb variables with texture are discussed in the section "Principal component analysis, heatmap, and Pearson correlation matrix for characteristics of wheat–purple corn bread composite".

Instrumental bread crumb colour (CIELAB)

An important quality parameter for consumer acceptance of bread is colour. As the proportion of purple maize flour increased, the a^* values increased concomitantly with the decrease in b* values. This indicated a shift towards red and blue tones in the breadcrumb samples (Table 2, D″ in Figure 4). Additionally, the total colour difference between bread samples can be an interesting colour measurement, since total colour differences greater than 2 ($\Delta E > 2$) indicates a colour difference noticeable for consumers (Encina-Zelada et al., 2019).

All the colorimetric measurement parameters (L^*, a^*, and b^* for crumb) were affected ($p < .001$) by higher doses of whole purple corn. When proportions of whole purple corn ($p < .001$) used

Table 2. Colorimetric values (CIE $L^*a^*b^*$) and bread porosity features of breadcrumb composed of blend of refined hard wheat at different percentage of whole-grain purple corn flour.

Treatment	L^*	a^*	b^*	ΔE	PAR (mm^2)	PDE (pores/mm^2)	ARA	PCO
W-PM0%	85.2 ± 1.35^{a}	1.32 ± 0.215^{d}	16.7 ± 1.04^{a}	–	0.834 ± 0.074^{a}	0.617 ± 0.038^{a}	1.62 ± 0.04^{a}	0.767 ± 0.007^{a}
W-PM5%	64.4 ± 3.11^{b}	9.99 ± 0.449^{c}	5.68 ± 0.29^{b}	25.0 ± 2.52^{c}	0.828 ± 0.078^{a}	0.619 ± 0.044^{a}	1.64 ± 0.03^{a}	0.762 ± 0.007^{ab}
W-PM15%	50.8 ± 1.94^{c}	16.7 ± 1.34^{b}	1.48 ± 1.22^{c}	40.6 ± 1.48^{b}	0.822 ± 0.087^{a}	0.620 ± 0.041^{a}	1.65 ± 0.06^{a}	0.761 ± 0.01^{ab}
W-PM25%	41.8 ± 4.12^{d}	20.3 ± 1.85^{a}	1.76 ± 1.61^{c}	49.7 ± 3.32^{a}	0.739 ± 0.070^{b}	0.641 ± 0.031^{a}	1.66 ± 0.03^{a}	0.757 ± 0.008^{b}
Shapiro (Norm.Res)	0.0046	0.0946	0.5455	0.088	0.00571	0.76718	0.01522	0.72261
Durbin–Watson (Independence.Res)	0.5019	0.0163	0.0034	0.241	0.0016	0.0026	4e–04	1e–04
LeveneTest (Var.Homoge)	0.3166	0.0006	0.1630	0.148	0.96782	0.66925	0.65346	0.76141

Note. Results represent the mean ± SD ($n = 3$). W = refined wheat flour; PM = purple corn flour addition (%); W-PC0% = wheat control; W-PC5% = wheat-whole purple corn at 5%; W-PC15% = wheat-whole purple corn at 15%; W-PC25% = wheat-whole purple corn at 25%. The lowercase superscript letters indicate differences between bread samples using the Tukey test ($p < .05$) parametric test or the Kruskal–Wallis non-parametric test ($p < .05$). L*: lightness scale (0 = black and 100 = white); a*: red/green coordinates (positive values indicate red and negative values indicates green); b*: yellow/blue coordinates (positive values indicate yellow and negative values indicates blue). PAR = average pore area; PDE = average pore density; ARA = average pore aspect ratio; PCO = average pore compactness.

in the bread formulations increased, the loaves crumb became darker, less yellowish (lower b^* values), and more reddish (lower a^* values). Moreover, higher bread crumb total colour differences (crumb ΔE, $p < .001$) were obtained when whole purple corn doses increased, differences that can be seen with the naked eye (Figure 4). Therefore, consumers could recognize the difference of colour between these four formulations only by appreciating the crumb.

Bioactive compounds and antioxidant capacity by DPPH of breads

Table 3 presents the content of total anthocyanins, TPC, and antioxidant capacity by DPPH scavenging of the breads after baking. The treatments with inclusion of purple corn flour (W-PC5%, W-PC15%, and W-PC25%) presented significant differences ($p < .05$) compared with wheat bread as control (W-PC0%). Although the anthocyanin content increased with higher proportions of purple corn flour, the potential degradation of the pigment during dough preparation should be considered. This degradation may be caused by the addition of other dough components, the incorporation of air (O_2) into the dough, the change in pH and acidification of the dough during fermentation, as well as the activation of enzymes such as polyphenol oxidase and peroxidase, and the high baking temperature, which is a major factor in anthocyanin degradation (Eliášová et al., 2020).

In relation to the TPC, the treatments with purple corn inclusion showed positive increases, of which W-PC25% outperformed the others, resulting in a 2.7-fold increase with respect to the control bread. This increase may be due to the release of some phenolic compounds bound to their free forms. Likewise, the baking process plays an important role in the increase of W-PC25%, due to the complex mechanism of the process, which involved starch gelatinization/pasting, protein denaturation, and a Maillard reaction (Yu & Beta, 2015). On the other hand, significant increases were also observed in antioxidant capacity by DPPH with W-PC25%, being six times higher than the control bread ($p < .05$). These results are in agreement with the findings investigated by Vieira et al. (2020), where Maillard reaction products (melanoidins) have been considered to contribute significantly to the antioxidant properties of baked cereal products. Finally, the increased antioxidant capacity by DPPH scavenging could be attributed equally to the increased polyphenol content from the purple corn incorporation. This has been well demonstrated by many previous studies on the relationships between polyphenol content and antioxidant activity of plant materials (Xu et al., 2017).

Principal component analysis, heatmap, and Pearson correlation matrix for characteristics of wheat–purple corn bread composite

PC1 and PC2 explained 94.4% of the total variance among a total of 41 variables representing rheological (paste properties, farinography, and oscillatory rheometry), textural, physical, colorimetric, structural, and bioactive characteristics of the four wheat–purple corn breads composite (W-PC5%, W-PC15%, and WPC25%) including a control (W-PC0%). Each of the samples was distributed in the four quadrants according to the properties that characterize them (Figure 5A), being the W-PC0% (control) the sample that was characterized by the highest values of the rheological properties of paste such as CV, PV, BDV, FV, and SBV; farinographic of AT and DST, rheometric of τ and γ (crossover in the non-linear region) (Supplementary Tables 1–3), as well as physical properties of the breads such as specific volume; textural

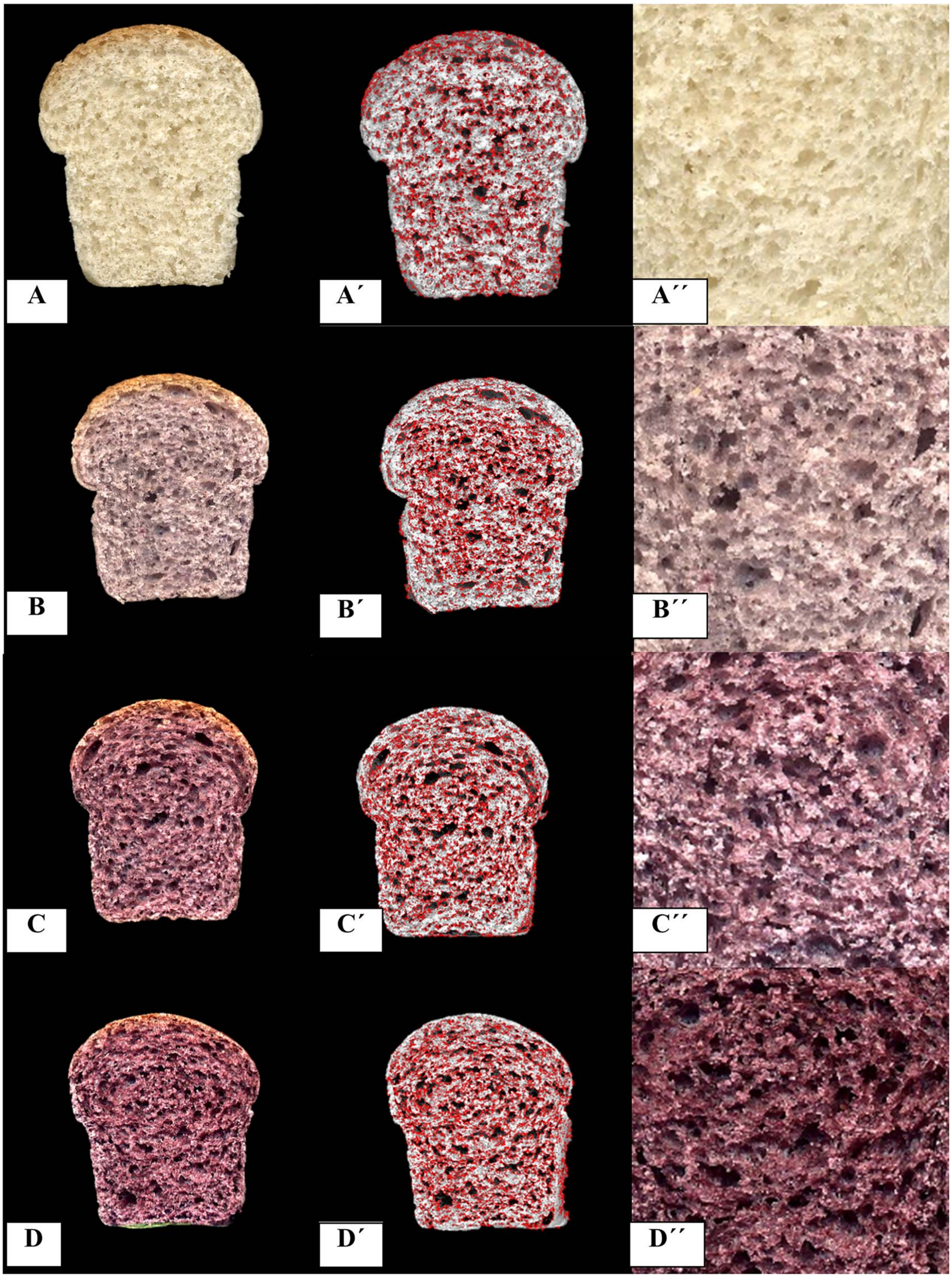


Figure 4. Digital images of bread slices showing an untreated (A, B, C, or D, left pictures) images of the crumb grain structure of breads (wheat control, A) produced by varying purple corn flour doses: 5% (B), 15% (C), and 25% (D); images of A, B, C, or D superimposed with the contours produced by binary segmentation using the k-means cluster thresholding algorithm (A′, B′, C′, or D′, middle pictures); and cropped section (A″, B″, C″, or D″, right pictures) to obtain the crumb features.

properties of cohesiveness and resilience; colorimetric properties of L^* (highest lightness values) and b^* (highest yellowness values) and structural properties of PAR, PDE, and PCO associated with the number of pores and their compactness or circularity (Table 2).

Likewise, W-PC5% had values close to the control in the same properties in which the control stood out, but it was also characterized by the highest values for the farinographic property of DDT (Supplementary Table 2), indicating that the incorporation of purple corn increased the time to develop wheat dough, due to the increase in gluten strength by the interaction between phenolics and gluten. The W-PC15% sample was characterized by the highest PM value (maximum consistency) and intermediate MTI values for farinographic properties; it also had the highest springiness value for textural properties and intermediate values

Table 3. Total anthocyanins, total phenolic compounds, and antioxidant capacity amounts of the breads after baking.

Treatment	ATC (mg CGE/100 g)	TPC (mg GAE/100 g)	DPPH (µmol TE/100 g)
W-PC0%	0.00 ± 0.00[d]	19.4 ± 0.96[d]	29.9 ± 3.24[d]
W-PC5%	39.0 ± 0.69[c]	24.7 ± 0.85[c]	60.0 ± 3.43[c]
W-PC15%	134.6 ± 5.89[b]	34.2 ± 0.87[b]	127.8 ± 4.34[b]
W-PC25%	190.7 ± 3.65[a]	52.9 ± 0.58[a]	178.1 ± 4.27[a]

Note. Results represent the mean ± *SD* ($n = 3$). W = refined wheat flour; PC = purple corn flour addition (%); W-PC0% = wheat control; W-PC5% = wheat-whole purple corn at 5%; W-PC15% = wheat-whole purple corn at 15%; W-PC25% = wheat-whole purple corn at 25%; ATC = total anthocyanins; CGE = cyanidin 3-glucoside equivalents; TPC = total phenolic compounds; GAE = gallic acid equivalents; TE = Trolox equivalent antioxidant capacity. The lowercase letters in the same column indicate differences between bread samples using the Tukey test ($p < .05$) parametric test.

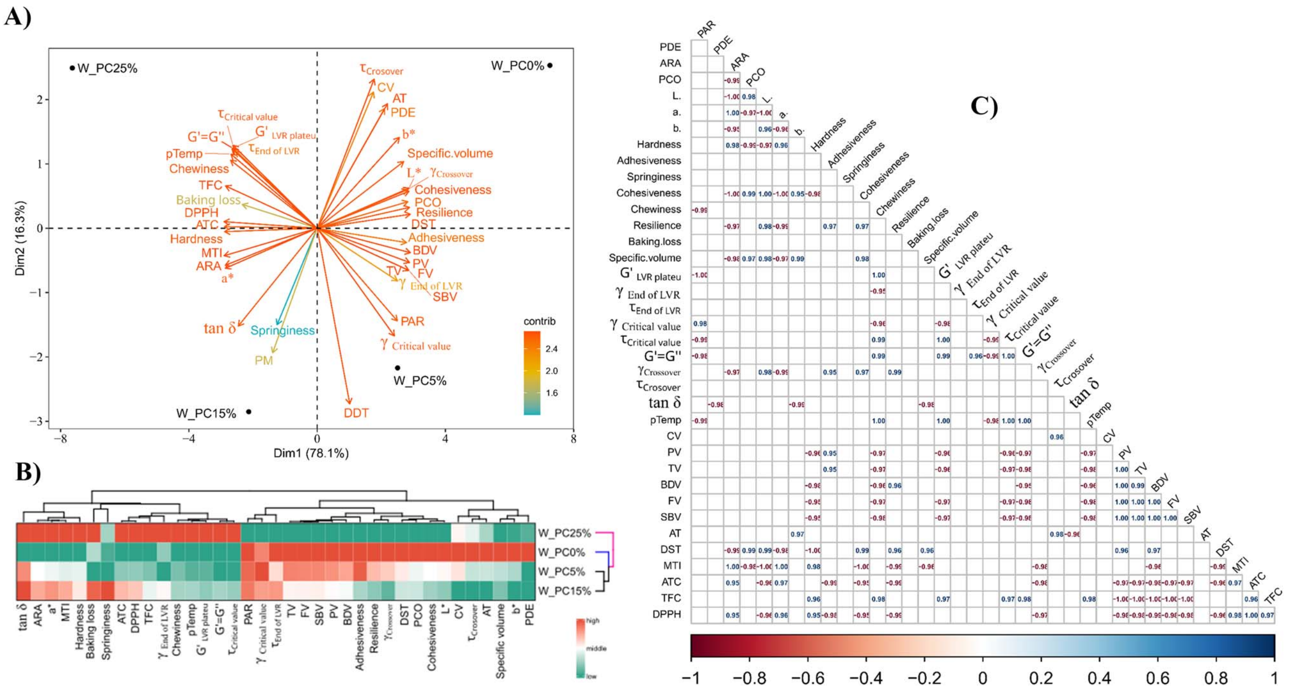


Figure 5. Principal components analysis of bread composed by wheat and whole-grain purple corn. (A) Bit-plot of score plot for samples and loading plot for response variables, (B) heat map of the samples for each response variable with hierarchical clustering, and (C) Pearson's correlation of the rheological, physical, textural, colour, and bioactive characteristics of the breads ($p < .05$).

for crumb hardness, as well as intermediate values for both colorimetric a^* and structural ARA properties, which indicate reddish colour intensity and pore aspect ratio in the breadcrumb.

While W-PC25% showed the highest values of the farinographic property of MTI (Supplementary Table 2), this property has an inverse relationship and therefore generated a lower kneading tolerance due to gluten dilution and the disruptive effect of the fibre on the viscoelastic gluten network. Furthermore, this sample was characterized by higher values in the dough properties of Ptemp, where the dough temperature increased possibly due to the influence of phenolics (anthocyanins); the same occurred in the rheometric properties of G′ (linear viscoelastic region), τ (linear and critical viscoelastic region), $G' = G''$ (non-linear viscoelastic region where polymers are destructured) (Supplementary Table 3), and tan δ (higher values indicate lower elasticity and stiffness of the bread dough). Likewise, W-PC25% excelled in the textural properties of hardness and chewiness, which indicate a higher stiffness produced by denser crumbs with lower pore count; it also showed the highest amounts of ATC, TFC, DPPH (Table 3) along with the highest a^* (intensity in red colour) and the lowest b^* (indicating higher blue colour values) (Table 2).

The heatmap illustrates the transition of each response variable (Figure 5B) by changing colour intensity (red represents highest values, white represents intermediate values, and green represents lowest values). Hierarchical clustering identified three distinct groups. The first group, represented by W-PC25%, showed the highest red intensities in rheometric properties such as pTemp, γ (end of LVR), G′ (LVR plateau), $G' = G''$, and τ (critical value), together with the highest bioactives (ATC, DPPH, and TPC), colorimetric property a^*, structural ARA, textural hardness and chewiness, and cooking loss. The second group, W-PC0%, presented the highest intensities in dough properties, where the highest intensities were observed in farinographic parameters (AT and DST), rheometric properties (τ and γ in LVR and crossover), textural parameters (adhesiveness, resilience, and cohesiveness), structural properties (PAR, PCO, and PDE), and colorimetric properties L^* and b^*. The third group, composed of W-PC5% and W-PC15%, showed statistical similarities in rheometric (pTemp, G′ LVR, and tan δ), physical (specific volume), textural (hardness, chewiness), and structural (PAR, PCO, and PDE) parameters. The heatmap corroborated these results, showing similar intensities for these properties, suggesting that the inclusion of 15% purple corn flour behaves similarly to 5%, without significantly affecting the technological properties of the dough and bread.

Very high positive correlations were observed between ARA and a^*, hardness, MTI, ATC, and DPPH ($0.95 > r \leq 0.99$) (Figure 5C). This indicates that progressive additions of whole kernel purple corn increase the pore aspect ratio, leading to greater dough destructuring during mixing, higher red color intensity and hardness, as well as increased anthocyanin content and antioxidant capacity through DPPH radical sequestration in pigmented breads. A strong positive correlation ($r = 0.98$) was found between the structural parameter ARA and crumb hardness, indicating that the higher the loss of pore circularity, the crumb hardness increases accordingly, while the PCO parameter, which denotes pore circularity, exhibited a strong negative correlation with crumb hardness ($r = -0.99$). This suggests that increasing the incorporation of purple corn flour in wheat bread reduces pore circularity, leading to a corresponding increase in hardness.

Also, very high correlations ($0.95 > r \leq 0.99$) were found between PCO and DST, L^*, cohesiveness and specific volume. These results indicate that greater pore compactness, typical of wheat-only breadcrumbs, is associated with higher dough stability during mixing (Figure 2A), as well as increased lightness, cohesiveness, and specific volume. Other very high positive correlations ($0.96 > r \leq 0.99$) were found between L^* and b^*, cohesiveness, resilience, specific volume, γ (crossover), and DST; these properties are characteristic of wheat-based breads and indicate that, as lightness increases, crumb yellowness, volume, stiffness, and resilience will increase.

Also, L^* was negatively correlated ($-0.96 > r \leq -0.99$) with the farinographic parameter of MTI, as well as with the bioactive properties of ATC and DPPH, indicating that the lower the L^* values (the darker the breadcrumb), the higher the kneading index, the amount of total anthocyanins, and the antioxidant capacity of the breadcrumb, whereas a^* correlated positively ($0.96 > r \leq 0.97$) with ATC and DPPH and negatively with DST ($r = 098$). These correlations between instrumental colour and bioactives having coefficients very close to 1 suggest that instrumental colour measurements such as L^*, a^*, and L^*/b^* correlation may be useful non-destructive and toxic solvent-free assays for predicting the amount of ATC and antioxidant capacity by DPPH radical scavenging in a wheat flour-based sample with replacement of an anthocyanin-rich substitute.

Conclusion

Substitution of wheat flour with INIA 601 whole grain purple corn at 5% produced minimal alterations in dough profiles, while 15%–20% significantly affected dough profiles, causing an increase in dough temperature (Ptemp) and a decrease in PV, TV, FV, and SBV. The 5% substitution improved dough consistency, but slightly reduced kneading tolerance. In contrast, 15%–25% substitutions significantly compromised dough stability due to dilution and interference of the fibres in the gluten viscoelastic network. Rheometric profiles were minimally affected by the 5% inclusion, but 15% and 25% substitutions increased both the elastic (G′) and viscous (G″) modulus, indicating increased dough strength. Breads produced with higher proportions of purple corn showed acceptable specific volume, higher hardness and darker, and reddish crumbs, accompanied by lower PAR and lower pore compactness. Colorimetric properties (L^*, a^*, b^*) showed high correlations (0.95–0.99) with total anthocyanins and antioxidant capacity measured by DPPH, suggesting that colorimetry could serve as a reliable indicator of the presence of these beneficial compounds in wheat flour-based products enriched with anthocyanins. In general, the incorporation of INIA 601 purple corn at levels between 5% and 25% produces anthocyanin-rich pigmented breads (190.7 mg/100 g) with 2.7 to 6 times higher phenolic content and antioxidant capacity. In particular, the 15% substitution preserved the technological dough properties and the bread quality characteristics. These results represent a breakthrough in the development of nutraceutical breads, which could improve human health through their antioxidant properties and chemoprotective effects against oxidative stress and related diseases.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

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

Author contributions

Raul Comettant-Rabanal (Conceptualization [lead], Data curation [equal], Formal analysis [lead], Methodology [lead], Visualization [equal], Writing—original draft [lead], Writing—review & editing [equal]), Roxanna T. Chavez-Llerena (Formal analysis [equal], Investigation [equal], Methodology [equal]), Davy William Hidalgo Chávez (Data curation [equal], Methodology [equal], Software [lead], Supervision [equal], Writing—review & editing [equal]), Bárbara Amorim Silva (Resources [equal], Visualization [equal]), Ronald Edson Rimari-Barzola (Writing—original draft [equal], Writing—review & editing [equal]), Christian R. Encina-Zelada (Data curation [equal], Methodology [equal], Writing—review & editing [equal]), Victor Delgado-Soriano (Methodology [equal], Writing—review & editing [equal]), and Carlos W. Carvalho (Resources [equal], Supervision [equal], Visualization [equal], Writing—review & editing [equal]).

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Conflicts of interest

The authors declare no conflict of interest for this work. All authors have participated in the design, analysis, and interpretation of the data and approved the final version. This manuscript has not been submitted to another journal. The authors are not affiliated with any organization with direct or indirect financial or personal nature interests related to the subject of the manuscript.

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