







## Chemical, Physical, and Sensory Properties of Bread with Popped Amaranth Flour

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This study investigated the effect of substitution of wheat flour with popped amaranth flour in bread formulation on the chemical, physical, and sensory characteristics of breads. The raw and popped amaranth grain flours of four Peruvian varieties: Oscar Blanco, Centenario, Taray, and Imperial, were characterised for chemical composition and pasting properties using Rapid Visco-Analyzer (RVA). Both types of amaranth flour had a high nutritional value, but the peak and final viscosity of popped amaranth flour were closer to the wheat flour. Breads were formulated with the popped amaranth flour, at four substitution levels of 0, 10, 20, and 30%. A significant increase in contents of protein (around 12%) and raw fiber (more than 100%), and a decrease in carbohydrate content (around 6%) in breads at the highest substitution level compared to wheat bread were observed. At this substitution level, the RVA profile parameters, specific volume, pore area, and colour coordinates ( $L^*$ ,  $a^*$ , and  $b^*$ ) differed significantly. In the sensory analysis using Flash profile technique, consumers identified that the Taray and Imperial bread varieties at 10 and 20% substitution level were similar to the wheat bread. Adding popped amaranth flour to bread improved the nutritional value, ensuring good physical and sensory properties. Popped amaranth flour can, thus, be an alternative to wheat flour in the development of healthy bakery products.

**Keywords:** *Amaranthus caudatus*, popped grains, pasting properties, bread physical properties, Flash profile

### INTRODUCTION

The genus *Amaranthus* is a pseudocereal from the Amaranthaceae family with more than 60 species. Only three species are used in the production of edible grains: *Amaranthus hypochondriacus*, *Amaranthus cruentus*, and *Amaranthus caudatus* [Kaur *et al.*, 2010]. The most important Andean species is *A. caudatus*, cultivated in the Andes of Peru, Bolivia, Ecuador, and Argentina [Repo-Carrasco-Valencia *et al.*, 2009]. Its grains have recently attracted

interest for their high protein content (12.5–16 g/100 g) [Bressani *et al.*, 1987]. Additionally, the amino acid profile of proteins is well-balanced in the context of human nutritional requirements [Drzewiecki, 2001], with the content of lysine, an amino acid with a high biological value, being two to three times higher than in other cereals, and a high content of methionine, cysteine, tryptophan, threonine, leucine and phenylalanine, which are the limiting amino acids in the protein profile of other cereals

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such as wheat [Bresani *et al.*, 1987; Cotovanu & Mironeasa, 2021; Palombini *et al.*, 2013; Paucar-Menacho *et al.*, 2018; Pavlik, 2012]. In addition, 100 g of amaranth grain contains between 7.7 to 12.8 g of lipids, which are rich in unsaturated fatty acids [Bressani *et al.*, 1987]. Moreover squalene, beneficial for human health, has a significant contribution to the lipid profile of amaranth [He *et al.*, 2002; Venskutonis & Kraujalis, 2013]. It is a natural biosynthetic precursor of cholesterol and has photoprotective properties. The amaranth grains also contain high amounts of dietary fiber and minerals such as calcium and magnesium [Bodroza-Solarov *et al.*, 2008]. They are also a rich source of nutrients; however, the presence of phytic acid, has adverse effects on the bioavailability of their minerals [Sanz-Penella *et al.*, 2013].

The amaranth grain can be consumed roasted, popped, extruded, in flour, or as an added ingredient for bread, cakes, muffins, pancakes, cookies, dumplings, crepes, and noodles [Sanz-Penella *et al.*, 2013]. Researches have demonstrated that the partial substitution of wheat flour with amaranth flour (5 to 20%) improves nutritional value and final sensory acceptance of breads [Cotovanu & Mironeasa, 2021; Kamoto *et al.*, 2018; Tömösközi *et al.*, 2011]. Others studies have pointed out that a higher addition of amaranth flour to bread dough (greater than 40% substitution) not only improves the nutritional value of the product, but also allows the physical and sensory properties to be maintained at acceptable levels [Martínez *et al.*, 2013; Miranda-Ramos *et al.*, 2019; Rosell *et al.*, 2009; Sanz-Penella *et al.*, 2013]. Both raw and popped amaranth grain flour was used as a substitute for wheat flour in the bread preparation. Bodroza-Solarov *et al.* [2008] obtained a denser crumb structure, more uniform porosity, improved crust colour and great sensory acceptance of bread with popped *A. cruentus* grain flour at substitution level of 10–20% compared to wheat bread. Calderón de la Barca *et al.* [2010] produced gluten-free bread with up to 70% popped amaranth and 40% raw amaranth. They obtained acceptable physical characteristics of bread (loaves with homogeneous crumb and high specific volume) and rheological behaviour of the doughs, with a high nutritional value even without added hydrocolloids. Thus, popped amaranth could improve rheological and digestibility properties of breads since it is mainly composed of starches and proteins [Bodroza-Solarov *et al.*, 2008]. Furthermore, the partial removal of the pericarp could reduce the content of antinutrients, such as phytic acid (IP6), since phytates are known to be concentrated in the bran of most cereals [Hama *et al.*, 2011].

Several varieties of *A. caudatus* are cultivated in Peru, including Oscar Blanco, Centenario, INIA 414 Taray, and INIA 430 Imperial. This raised the interest in evaluating popped amaranth flour in various bread formulations. However, flours from popped grains of these varieties have not yet been studied in terms of addition to bread. Therefore, the objective of the present study was to compare the chemical and rheological properties of flour from raw and popped amaranth grains of four Peruvian varieties and evaluate the effect of the addition of popped amaranth flour on the chemical, physical, and sensory properties of bread.

## MATERIALS AND METHODS

### Materials

Grains of four Peruvian varieties of *Amaranthus caudatus* were used in study. The varieties Oscar Blanco (OB), INIA 414 Taray (T), and INIA 430 Imperial (I) were provided by Instituto Nacional de Innovación Agraria (INIA) station Andenes Cusco, Peru (harvest 2019). The Centenario (C) variety was acquired at UNALM, Lima, Peru (harvest 2019). The grain sizes of the four amaranth varieties used were as follows: Oscar Blanco –  $1.1 \pm 0.18$  mm, Centenario –  $1.4 \pm 0.13$  mm, Taray –  $1.2 \pm 0.09$  mm, Imperial –  $1.3 \pm 0.12$  mm. Wheat flour from “Los gallos mill” in Hermosillo, Sonora-México was used.

### Preparation of popped grain flours

The amaranth grains were placed in a stainless-steel pot at 200°C for 15 s until complete popping was achieved [Amare *et al.*, 2015; Bodroza-Solarov *et al.*, 2008]. Subsequently, each variety of popped grains was pulverized in a disc mill, and the resulting flour was sieved through a 0.5 mm mesh, and identified as popped Oscar Blanco (POB), popped Taray (PT), popped Imperial (PI), and popped Centenario (PC). The flours were stored in polyethylene bags at 4°C until use.

### Dough preparation and baking

The formulation for the control bread (with wheat flour) and the breads with 10, 20 and 30% substitution of wheat flour with popped amaranth flour are shown in **Table 1**. The solid ingredients were mixed and kneaded with water. Then the dough was placed in stainless steel molds, fermented at 40°C for 40 min, and baked at 150°C for 40 min. The loaves were cooled to room temperature (19°C), removed from the mold, and packed in high-density polyethylene containers.

### Proximate analysis

The proximate composition of raw amaranth flours, popped amaranth flours and breads was analysed. The determinations were performed using the official methods of the AOAC International [AOAC, 2005]. The contents of proteins (method 984.13), lipids (method 2003.05), ash (method 942.05), moisture (method 950.46), and raw fiber (method 962.09) were determined. Carbohydrate content was determined by subtraction of the total content of other constituents (expressed in g/100 g) from 100 g. Moisture determination for pasting profile analysis was performed using AACC International method 44-19 [AACC, 1995].

### Determination of pasting properties

The pasting properties of the flours were analysed using the AACC International method 76-21 [AACC, 2000]. Wheat flour (WF), raw and popped amaranth flours, and blends of WF with popped amaranth flour (in proportion as in bread formulations) were analyzed on  $3.5 \pm 0.01$  g of sample adjusted to 14% moisture. The amount of water incorporated was  $25 \pm 0.01$  g, which was obtained from the flour adjustment [Shittu *et al.*, 2007]. Rapid Visco-Analyzer (RVA, Super 4, Newport Scientific, Sydney, Australia) and Thermocline software (Newport Scientific) were used to

**TABLE 1.** Formulations of breads without (control) and with the popped amaranth flour.

Ingredient	Control	Substitution level (% of total flour)		
		10	20	30
Wheat flour (g)	300	270	240	210
Flour of popped amaranth (g)	0	30	60	90
Instant yeast (g)	8	8	8	8
Brown sugar (g)	40	40	40	40
Vegetable shortening (g)	30	30	30	30
Sodium chloride (g)	2	2	2	2
Water (mL)	170	Variable	Variable	Variable

obtain pasting profiles. An initial temperature of 50°C and mixing at 960 rpm were applied, decreasing the speed to 160 rpm after 10 s. The temperature was maintained at 50°C for 1 min and then increased to 95°C at 4.42 min, remaining until 7.42 min. At 11 min the temperature dropped to 50°C and the test ended at 13 min. The parameters recorded were pasting temperature, peak and final viscosities.

#### ■ Bread specific volume determination

Volume of bread loaves (mL) was measured using laser topographic equipment (BVM-6610, Perten Instruments, Sweden) and their weight (g) with analytical balance (Entris 224-IS, Sartorius Lab Instruments GmbH & Co.). The specific volume (mL/g) was calculated by dividing volume by weight [Vidaurre-Ruiz *et al.*, 2019].

#### ■ Bread porosity determination

Bread slices were photographed in colour using a camera. The images were scanned (Canon MG3610, Tokyo, Japan) at 600 dpi resolution, converted to gray colour, and processed with ImageJ software, version 1.51j8 (National Institutes of Health, Bethesda, MD, USA). The pixel values were converted into length units using dimensions of known lengths. The images were then binarized (pores in black and crumb in white), determining the number of pores *per cm*<sup>2</sup> and the area percentage of the pores [Vidaurre-Ruiz *et al.*, 2019].

#### ■ Instrumental bread colour measurement

The bread colour coordinates were measured in the CIELab space using a CSM7 portable colourimeter (PCE instruments, Deutschland GmbH, Meschede, Germany). *L*\* (0 – black, 100 – white), *a*\* (positive value – red, negative value – green), and *b*\* (positive value – yellow, negative value – blue) were recorded. Three points were measured for each bread slice [Yamsaengsung *et al.*, 2010].

#### ■ Flash profile sensory evaluation

For the sensory evaluation of bread, Flash profile (FP) technique was used, which is a descriptive sensory technique derived

from the free-choice profile (each evaluator qualifies samples comparatively with their own words). Breads were evaluated in three sessions. In the first session, the samples were shown simultaneously and randomly, and the evaluators were asked to list sensory characteristics (attributes). The second session consisted of a consensus avoiding the repetition of two terms describing the same thing and then choosing their definitive list of attributes. In the third session, the samples were again presented simultaneously and randomly with a sensory evaluation of the chosen attributes. Finally, the evaluators were instructed to classify them in increasing order of intensities on an ordinal scale, allowing ties [Dairou & Sieffermann, 2002]. The information was analysed *via* a generalized Procrustes analysis (GPA). The evaluation was carried out with 24 evaluators (consumers) between men and women.

#### ■ Statistical analysis

Data of nutritional composition and physical characteristics were expressed as mean and standard deviation and analyzed using the InfoStat free version 2017 software (InfoStat Group, Universidad Nacional de Córdoba, Argentina). The differences between treatments were established through the analysis of variance and Tukey multiple comparisons, considering statistically significant values of  $p < 0.05$ . Flash profile sensory evaluation data were evaluated by PGA with XLSTAT 2014 trial version software (Addinsoft, New York, NY, USA).

## RESULTS AND DISCUSSION

#### ■ Raw and popped amaranth flours and bread proximate analysis

The proximate composition of the flours of raw and popped amaranth grains of four varieties is shown in **Table 2**. Significant differences ( $p < 0.05$ ) between varieties were found in contents of protein (highlighting varieties I and T), lipids (highlighting the OB and C varieties), and ash (the highest content in flour of I variety grains). No differences ( $p \geq 0.05$ ) were found in raw fiber and carbohydrate content in raw amaranth flours. The results were similar to those reported for *A. caudatus* [Alvarez-Jubete *et al.*, 2009; Amare *et al.*, 2016; Repo-Carrasco-Valencia *et al.*, 2009] and *Amaranthus* spp. [USDA, 2019]. **Table 2** also shows an increase in protein, fat, ash and a decrease in carbohydrate and raw fiber content in the four popped amaranth varieties compared to the raw samples. The protein contents in PI and PT were different ( $p < 0.05$ ) from the rest of the varieties; ash content was significantly higher ( $p < 0.05$ ) in PC than in POB and PT. No differences ( $p \geq 0.05$ ) between varieties were found in lipid, raw fiber, and carbohydrate contents. These data were consistent with findings from other studies for popped *A. cruentus* (increasing content compared to raw grains of lipids from 5.88 to 7.27% and soluble fiber from 8.61 to 9.22% [Bodroza-Solarov *et al.*, 2008] and popped *A. caudatus* var. Centenary (increasing content of total protein from 10.30 to 11.81 g/100 g dry weight and lipids from 7 to 8.17 g/100 g dry weight, decreasing content of carbohydrates from 80.3 to 70.15 g/100 g dry weight) [Pau-car-Menacho *et al.*, 2018].

**TABLE 2.** Proximate chemical composition (g/100 g dry matter) of flours of raw and popped amaranth grains of four Peruvian varieties, and breads without (control) and with popped amaranth flours

Flour/bread	Proteins (N×6.25)	Lipids	Raw fiber	Ash	Carbohydrates
<b>Raw amaranth flour</b>					
OB	13.45±0.29 <sup>b</sup>	6.90±0.21 <sup>a</sup>	3.89±0.12 <sup>a</sup>	1.97±0.09 <sup>c</sup>	73.79±0.47 <sup>a</sup>
C	13.16±0.40 <sup>b</sup>	6.64±0.00 <sup>a</sup>	3.98±0.23 <sup>a</sup>	2.35±0.04 <sup>b</sup>	73.87±0.13 <sup>a</sup>
T	14.9±0.20 <sup>a</sup>	6.42±0.16 <sup>ab</sup>	4.25±0.09 <sup>a</sup>	1.90±0.02 <sup>c</sup>	72.53±0.43 <sup>a</sup>
I	14.22±0.35 <sup>ab</sup>	5.96±0.16 <sup>b</sup>	3.76±0.19 <sup>a</sup>	2.64±0.05 <sup>a</sup>	73.42±0.74 <sup>a</sup>
<b>Popped amaranth flour</b>					
POB	14.59±0.07 <sup>b</sup>	8.03±0.04 <sup>a</sup>	3.58±0.17 <sup>a</sup>	2.10±0.12 <sup>c</sup>	71.7±0.16 <sup>a</sup>
PC	14.08±0.03 <sup>c</sup>	7.49±0.14 <sup>a</sup>	3.08±0.39 <sup>a</sup>	3.14±0.08 <sup>a</sup>	72.21±0.57 <sup>a</sup>
PT	15.25±0.15 <sup>a</sup>	7.72±0.43 <sup>a</sup>	2.87±0.10 <sup>a</sup>	2.37±0.05 <sup>bc</sup>	71.79±0.33 <sup>a</sup>
PI	15.54±0.15 <sup>a</sup>	7.23±0.09 <sup>a</sup>	3.24±0.08 <sup>a</sup>	2.80±0.28 <sup>ab</sup>	71.19±0.05 <sup>a</sup>
<b>Bread</b>					
Control	11.35±0.82 <sup>b</sup>	8.77±0.05 <sup>g</sup>	0.53±0.04 <sup>d</sup>	1.29 ±0.02 <sup>de</sup>	78.06±0.85 <sup>a</sup>
POB10	12.96±0.12 <sup>a</sup>	10.80±0.02 <sup>ab</sup>	0.73±0.14 <sup>cd</sup>	1.17 ±0.10 <sup>e</sup>	74.36±0.39 <sup>cde</sup>
POB20	12.64±0.12 <sup>a</sup>	9.07±0.07 <sup>fg</sup>	1.09±0.18 <sup>abcd</sup>	2.04±0.02 <sup>abc</sup>	75.16±0.35 <sup>bc</sup>
POB30	12.74±0.34 <sup>a</sup>	9.32±0.01 <sup>efg</sup>	1.27±0.03 <sup>abc</sup>	1.82±0.01 <sup>abcd</sup>	74.85±0.30 <sup>bcd</sup>
PC10	12.39±0.09 <sup>ab</sup>	10.15±0.36 <sup>bcd</sup>	0.78±0.02 <sup>bcd</sup>	1.48±0.25 <sup>cde</sup>	75.2±0.73 <sup>bc</sup>
PC20	12.41±0.06 <sup>ab</sup>	10.94±0.09 <sup>a</sup>	1.05±0.24 <sup>abcd</sup>	2.06±0.25 <sup>abc</sup>	73.54±0.16 <sup>de</sup>
PC30	12.54±0.00 <sup>ab</sup>	10.90±0.03 <sup>a</sup>	1.36±0.17 <sup>ab</sup>	2.04±0.13 <sup>abc</sup>	73.17±0.27 <sup>e</sup>
PT10	12.29±0.21 <sup>ab</sup>	10.60±0.04 <sup>abc</sup>	0.86±0.00 <sup>bcd</sup>	1.21±0.03 <sup>e</sup>	75.05±0.22 <sup>bcd</sup>
PT20	12.35±0.07 <sup>ab</sup>	10.32±0.09 <sup>abc</sup>	1.18±0.25 <sup>abc</sup>	1.26±0.14 <sup>de</sup>	74.98±0.54 <sup>bcd</sup>
PT30	12.36±0.06 <sup>ab</sup>	9.49±0.19 <sup>def</sup>	1.53±0.01 <sup>a</sup>	2.23±0.29 <sup>a</sup>	74.39±0.03 <sup>cde</sup>
PI10	12.26±0.10 <sup>ab</sup>	9.97±0.17 <sup>cde</sup>	0.97±0.18 <sup>abcd</sup>	2.01±0.06 <sup>abc</sup>	74.78±0.06 <sup>bcd</sup>
PI20	12.29±0.54 <sup>ab</sup>	8.69±0.08 <sup>g</sup>	1.11±0.10 <sup>abcd</sup>	1.53±0.20 <sup>bcdde</sup>	76.38±0.16 <sup>b</sup>
PI30	12.26 ±0.01 <sup>ab</sup>	8.87±0.44 <sup>fg</sup>	1.32±0.25 <sup>abc</sup>	2.11±0.05 <sup>ab</sup>	75.44±0.22 <sup>bc</sup>

Results are shown as mean ± standard deviation (n=3). Different letters in superscript in each column, separately for raw amaranth flours, popped amaranth flours and breads, represent significant differences ( $p<0.05$ ). OB, Oscar Blanco variety; C, Centenario variety; T, Taray variety; I, Imperial variety; POB, PC, PT and PI, popped OB, C, T and I, respectively; POB10, PC10, PT10 and PI10, breads with 10% substitution of wheat flour with POB, PC, PT and PI, respectively; POB20, PC20, PT20, PI20, breads with 20% substitution of wheat flour with POB, PC, PT and PI, respectively; POB30, PC30, PT30, PI30, breads with 30% substitution of wheat flour with POB, PC, PT and PI, respectively.

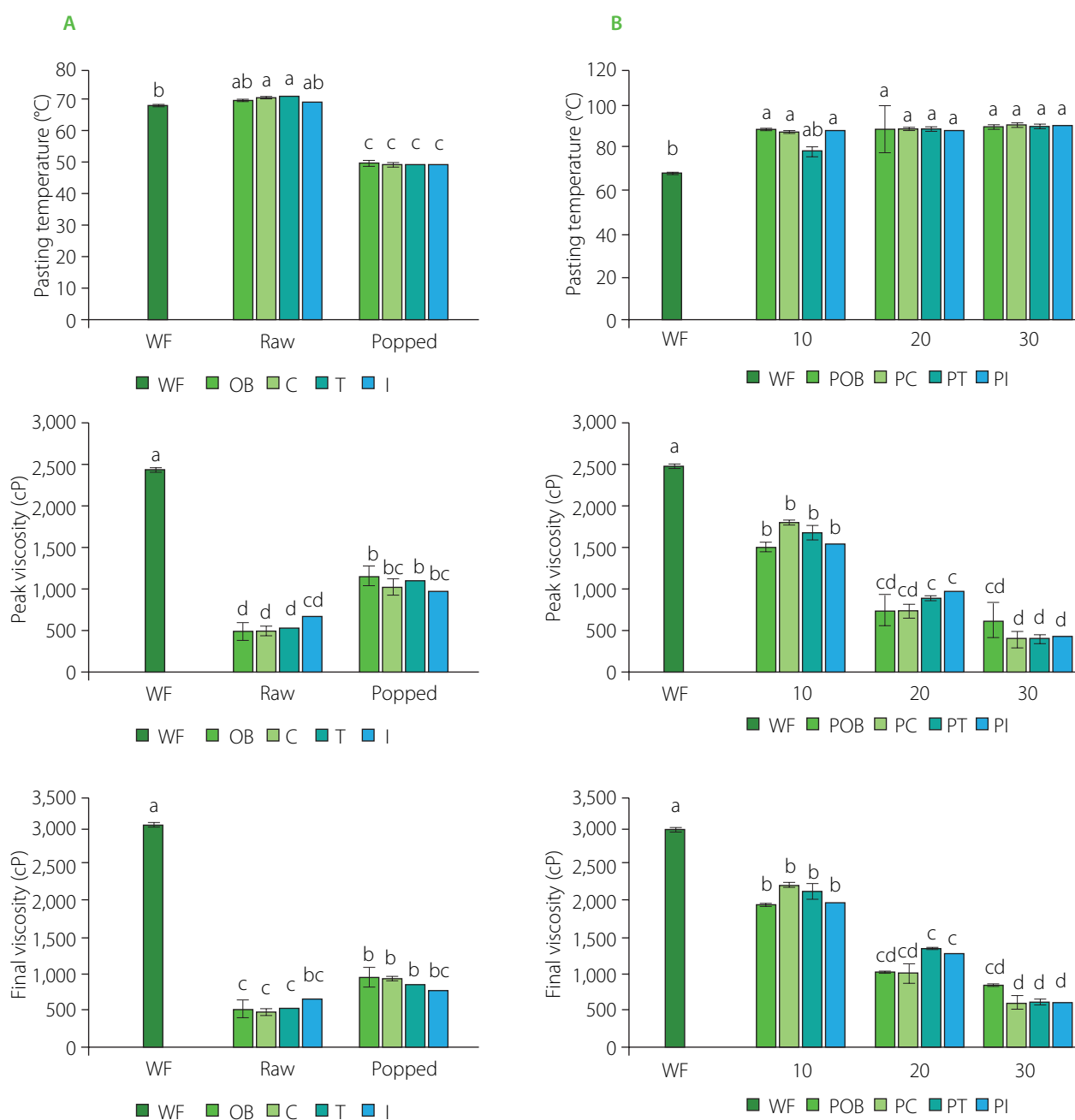
Finally, **Table 2** shows the proximate composition of breads with substitution in formula from 0 to 30% of wheat flour with amaranth popped flour of the four Peruvian varieties. As the percentage of WF substitution increased, the content of protein and raw fiber in bread showed an increasing tendency, while the content of carbohydrates – a decreasing one. The increase in the contents of protein and raw fiber and a decrease in carbohydrate content in breads with the highest substitution level compared to WF accounted for around 12%, more than 100% and around 6%, respectively. These trends were similar to those reported by Bodroza-Solarov *et al.* [2008], who made bread with popped *A. cruentus* at substitution levels of 10, 15, and 20%, resulting in higher contents of protein and crude fiber but a lower content of carbohydrates. Similar results were also reported

for breads with raw flours of *A. cruentus* [Sanz-Penella *et al.*, 2013], *A. spinosus* and *A. hypochondriacus* [Miranda-Ramos *et al.*, 2019], *A. hypochondriacus* [Kamoto *et al.*, 2018], and *Amaranthus* spp. [Cotovanu & Mironeasa, 2021] with substitution levels ranging from 5 to 50%. Moreover, a significant increase ( $p<0.05$ ) in ash and lipid contents was observed in bread with popped amaranth flours compared to the control bread (with a few exceptions) (**Table 2**). These results agree with those reported in bread made from flours of popped *A. cruentus* [Bodroza-Solarov *et al.*, 2008], raw *A. cruentus* [Sanz-Penella *et al.*, 2013], and raw *Amaranthus* spp. [Cotovanu & Mironeasa, 2021]. The improved nutritional properties of breads with popped amaranth flour can result from the favorable chemical composition of amaranth flour compared to wheat flour (higher protein and fiber content). A larger share of amaranth

in bread is beneficial not only because of its protein content, but also because of a high nutritional value of these proteins. Amaranth albumins and globulins are rich in essential amino acids, including lysine, and are easily digestible [Venskutonis & Kraujalis, 2013]. Moreover, according to the literature, heat treatment in popping of amaranth grains increases the protein efficiency ratio and the gelatinisation of starch positively affects the stability, strength and freshness of the crumb [Bodroza-Solarov *et al.*, 2008]. Furthermore, it could affect protein digestibility due to the reduction of exogenous factors such as tannins, phytates, and trypsin inhibitors that reduce protein digestibility [Amare *et al.*, 2015].

### ■ Pasting properties

Parameters of the pasting profile of raw and popped amaranth flours and WF, including pasting temperature, peak and final viscosities, determined using RVA are shown in **Figure 1A**. Pasting profiles show the flour viscosity changes during heating in excess water under constant agitation. The flour behaviours of raw and popped amaranth grain flours and WF showed significant differences ( $p < 0.05$ ). A slightly higher pasting temperature was determined for raw amaranth flours than WF. However, the viscosity parameters of raw grain flours showed a considerably lower value. These results were similar, although slightly smaller differences were observed,



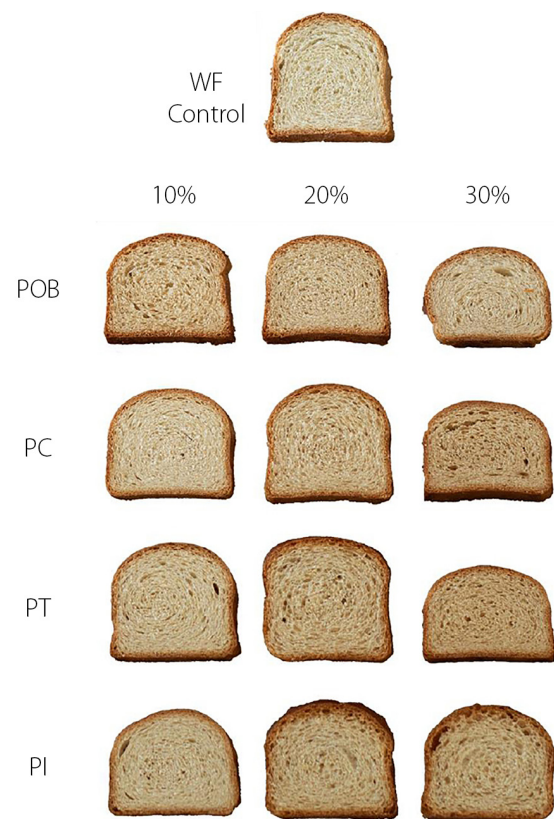
**FIGURE 1.** Pasting characteristics of wheat flour (WF) and flours of raw and popped amaranth grains of four Peruvian varieties (A), and blends of WF with 10, 20 and 30% popped amaranth flour (B). OB; Oscar Blanco; C, Centenario; T, Taray; I, Imperial; POB, PC, PT and PI, popped OB, C, T and I, respectively. Results are shown as mean and standard deviation ( $n=3$ ). Different letters above bars indicate that the treatments are significantly different ( $p < 0.05$ ).

to those reported in 11 lines of *A. caudatus* [Kaur *et al.*, 2010] and *A. hypochondriacus* [Sindhu & Khatkar, 2016]. The pasting temperature was the lowest for flours of popped amaranth grains (Figure 1A). The other RVA profile parameters were also lower for popped grain flours than WF but higher than for raw grain flours. Our analyses showed lower values of pasting profile parameters than those reported for flours of raw and popped *A. cruentus* and *A. hypochondriacus* grains by Muyonga *et al.* [2014]. Popped samples would have better viscosity properties than raw amaranth flour because starch granules disintegrate during heating, becoming more susceptible to hydration, which is associated with increased viscosity [Lai, 2001] and possibly due to the impact of extreme dehydration when raw grain bursts [Muyonga *et al.*, 2014].

Figure 1B also shows the differences in every RVA profile parameter of blends of wheat flour with popped amaranth flour of the four varieties used in bread formulation. The pasting temperature was higher ( $p < 0.05$ ) for the blends compared to WF; however, the proportion of WF and amaranth flour in the blend did not change pasting temperature significantly ( $p \geq 0.05$ ). In the rest of the viscosity characteristics, considerably lower values were found for blends than in the control flour and as the substitution level of WF with popped amaranth flour in the blend increased. These results were similar to the findings from a study of low-gluten bread made with amaranth flour, with substitution level of 0 to 40% [Duda *et al.*, 2019]. It was found that as the substitution level increased, the peak and final viscosity were considerably reduced, and a slight increase in the pasting temperature was observed. Another study described bread with raw quinoa flour addition. The peak and final viscosity of the flours decreased slightly, while the dough temperature did not show differences as the substitution level increased from 0 to 20% [Vásquez *et al.*, 2016]. In another study on pastes, in which wheat was substituted with *Amaranthus mantegazzianus* flour, the viscosity values were reduced as the substitution level increased up to 50% [Martínez *et al.*, 2013]. This is possibly due to the low starch and amylose contents of the whole meal amaranth flour with respect to bread wheat flour. The grains with a low starch content swell and release amylose, resulting in a lower viscosity [Martínez *et al.*, 2013]. Amaranth starch is characterised by a low amylose content from 4.7 to 12.5% [Kong *et al.*, 2009] and a higher amylopectin content from 20 to 25% [Cotovanu & Mironeasa, 2022] affecting its functional properties [Kong *et al.*, 2009]. Starch gelatinisation is a key factor in starch behaviour, which occurs when the dough is heated to 60°C. Low consistency values reached at the starch gelatinisation stage can be explained by the increased interactions between the low amount of amylose and the large length of amylopectin chain of amaranth starch, which generates a synergistic effect on the final viscosity and these on starch retrogradation [Corke *et al.*, 2016; Piga *et al.*, 2021]. This would indicate that the addition of amaranth flour could limit starch retrogradation and increase the shelf life of bread [Cotovanu & Mironeasa, 2022], but it would also contribute to the weakening of gluten [Šárka & Dvořáček, 2017].

## Physical characteristics

The physical characteristics of the breads with different levels of popped amaranth substitution from the four Peruvian varieties are shown in Table 3. Additionally, the appearance of bread cross-sections is shown in Figure 2. The breads POB10 and POB30 had lower weight compared to the others. The volume and specific volume decreased with the increase in the substitution of WF with popped amaranth flour in bread formulations. The WF bread presented the highest specific volume, although POB10, POB30 and PT10 did not differ significantly ( $p \geq 0.05$ ) from control in this respect. Our findings were consistent with the study by Bodroza-Solarov *et al.* [2008] who reported a reduction in the specific volume of bread by 33% when a 20% popped *A. cruentus* flour was used in formulation. Similar results were also obtained for amaranth flours of other species [Cotovanu & Mironeasa, 2021; Miranda-Ramos *et al.*, 2019; Sanz-Penella *et al.*, 2013; Tömösközi *et al.*, 2011]. The reduction in the specific volume results from high-fiber ingredients [Iglesias-Puig *et al.*, 2015]. This could also be explained by the dilution of gluten and decrease of  $\alpha$ -amylase activity by globular proteins (11S and 9P) of amaranth, which reduces maltose availability for yeast during the bread-making process [Cotovanu & Mironeasa, 2021]. Whole-grain pseudo-cereal flours are high in dietary fiber but are gluten-free. However, proteins such as albumins and glutenins from WF can interact through disulfide bonds, maintaining the viscoelastic properties of gluten under acceptable conditions [Osvald *et al.*, 2009].



**FIGURE 2.** Appearance of wheat flour (WF) bread and breads with 10, 20 and 30% substitution in formulation of WF with popped amaranth flours from four varieties including Popped Oscar Blanco (POB), Popped Centenario (PC), Popped Taray (PT), Popped Imperial (PI).

**TABLE 3.** Physical characteristics and colour coordinates of breads without (control) and with popped amaranth flours.

Bread	Weight (g)	Volume (mL)	Specific volume (mL/g)	Number of pores per cm <sup>2</sup>	Pore area (%)	Colour coordinate		
						L*	a*	b*
Control	129.2±1.1 <sup>a</sup>	487.00±16.97 <sup>a</sup>	3.76±74.96 <sup>a</sup>	60.00±2.20 <sup>b</sup>	46.95±2.26 <sup>bcd</sup>	74.96±1.23 <sup>a</sup>	3.99±0.48 <sup>a</sup>	16.46±0.88 <sup>a</sup>
POB10	120.85±0.35 <sup>b</sup>	448.50±0.7 <sup>ab</sup>	3.71±61.94 <sup>ab</sup>	23.28±0.24 <sup>d</sup>	63.85±1.66 <sup>a</sup>	71.74±2.28 <sup>ab</sup>	6.28±0.30 <sup>bc</sup>	20.25±0.44 <sup>ab</sup>
POB20	132.85±1.34 <sup>a</sup>	417.00±12.72 <sup>bc</sup>	3.14±69.89 <sup>cd</sup>	33.06±2.12 <sup>d</sup>	57.77±0.94 <sup>ab</sup>	69.89±1.08 <sup>abc</sup>	6.92±0.46 <sup>bcd</sup>	21.64±1.04 <sup>bcd</sup>
POB30	122.90±1.27 <sup>b</sup>	411.00±26.87 <sup>bc</sup>	3.34±71.74 <sup>abc</sup>	78.61±7.46 <sup>a</sup>	34.76±1.04 <sup>def</sup>	61.94±0.96 <sup>def</sup>	9.14±0.39 <sup>e</sup>	25.10±0.95 <sup>cde</sup>
PC10	132.70±0.28 <sup>a</sup>	429.50±7.77 <sup>abc</sup>	3.24±70.38 <sup>bcd</sup>	26.11±2.20 <sup>d</sup>	52.04±1.38 <sup>abc</sup>	70.38±1.47 <sup>abc</sup>	6.02±0.35 <sup>bc</sup>	20.18±0.33 <sup>ab</sup>
PC20	134.50±2.26 <sup>a</sup>	413.50±6.36 <sup>bc</sup>	3.07±66.91 <sup>cd</sup>	48.89±0.31 <sup>c</sup>	27.74±4.05 <sup>ef</sup>	66.91±4.78 <sup>bcd</sup>	6.75±1.71 <sup>bc</sup>	21.50±2.62 <sup>bcd</sup>
PC30	133.30±0.98 <sup>a</sup>	396.50±0.7 <sup>bc</sup>	2.97±61.25 <sup>cd</sup>	75.94±4.32 <sup>a</sup>	29.40±3.35 <sup>ef</sup>	61.25±0.35 <sup>ef</sup>	9.19±0.42 <sup>e</sup>	25.66±1.01 <sup>e</sup>
PT10	131.10±0.14 <sup>a</sup>	453.50±7.77 <sup>ab</sup>	3.46±69.49 <sup>abc</sup>	24.44±0.79 <sup>d</sup>	53.21±2.23 <sup>abc</sup>	69.49±1.11 <sup>bc</sup>	5.76±0.19 <sup>ab</sup>	20.20±0.44 <sup>ab</sup>
PT20	134.65±0.07 <sup>a</sup>	405.50±9.19 <sup>bc</sup>	3.01±61.91 <sup>cd</sup>	49.39±0.71 <sup>bc</sup>	44.43±0.80 <sup>bcd</sup>	61.91±0.71 <sup>def</sup>	6.93±0.96 <sup>bcd</sup>	21.11±2.30 <sup>bc</sup>
PT30	131.85±0.35 <sup>a</sup>	372.50±4.94 <sup>c</sup>	2.83±59.29 <sup>d</sup>	78.17±1.96 <sup>a</sup>	36.07±0.38 <sup>def</sup>	59.29±1.26 <sup>f</sup>	8.83±0.23 <sup>de</sup>	25.26±0.41 <sup>de</sup>
PI10	129.40±2.26 <sup>a</sup>	419.50±9.19 <sup>bc</sup>	3.24±65.97 <sup>bcd</sup>	47.33±1.26 <sup>c</sup>	27.51±2.96 <sup>f</sup>	65.97±0.78 <sup>cde</sup>	5.98±0.41 <sup>abc</sup>	20.77±1.19 <sup>b</sup>
PI20	133.10±2.69 <sup>a</sup>	418.00±12.73 <sup>bc</sup>	3.14±62.74 <sup>cd</sup>	53.61±0.39 <sup>bc</sup>	41.85±2.22 <sup>cde</sup>	62.74±0.62 <sup>def</sup>	7.53±0.85 <sup>bcdde</sup>	22.66±2.20 <sup>bcdde</sup>
PI30	132.05±1.62 <sup>a</sup>	371.50±43.13 <sup>c</sup>	2.82±58.84 <sup>d</sup>	74.78±2.36 <sup>a</sup>	39.86±10.48 <sup>cdef</sup>	58.84±0.84 <sup>f</sup>	7.85±0.40 <sup>cde</sup>	23.06±0.95 <sup>bcdde</sup>

Results are shown as mean ± standard deviation (n=3). Different letters in superscript in each column, separately for raw amaranth flours, popped amaranth flours and breads, represent significant differences ( $p < 0.05$ ). POB, Popped Oscar Blanco; PC, Popped Centenario; PT, Popped Taray; PI, Popped Imperial; POB10, PC10, PT10 and PI10, breads with 10% substitution of wheat flour with POB, PC, PT and PI, respectively; POB20, PC20, PT20 and PI20, breads with 20% substitution of wheat flour with POB, PC, PT and PI, respectively; POB30, PC30, PT30 and PI30, breads with 30% substitution of wheat flour with POB, PC, PT and PI, respectively; L\*, lightness; a\*, redness–greenness; b\*, yellowness–blueness.

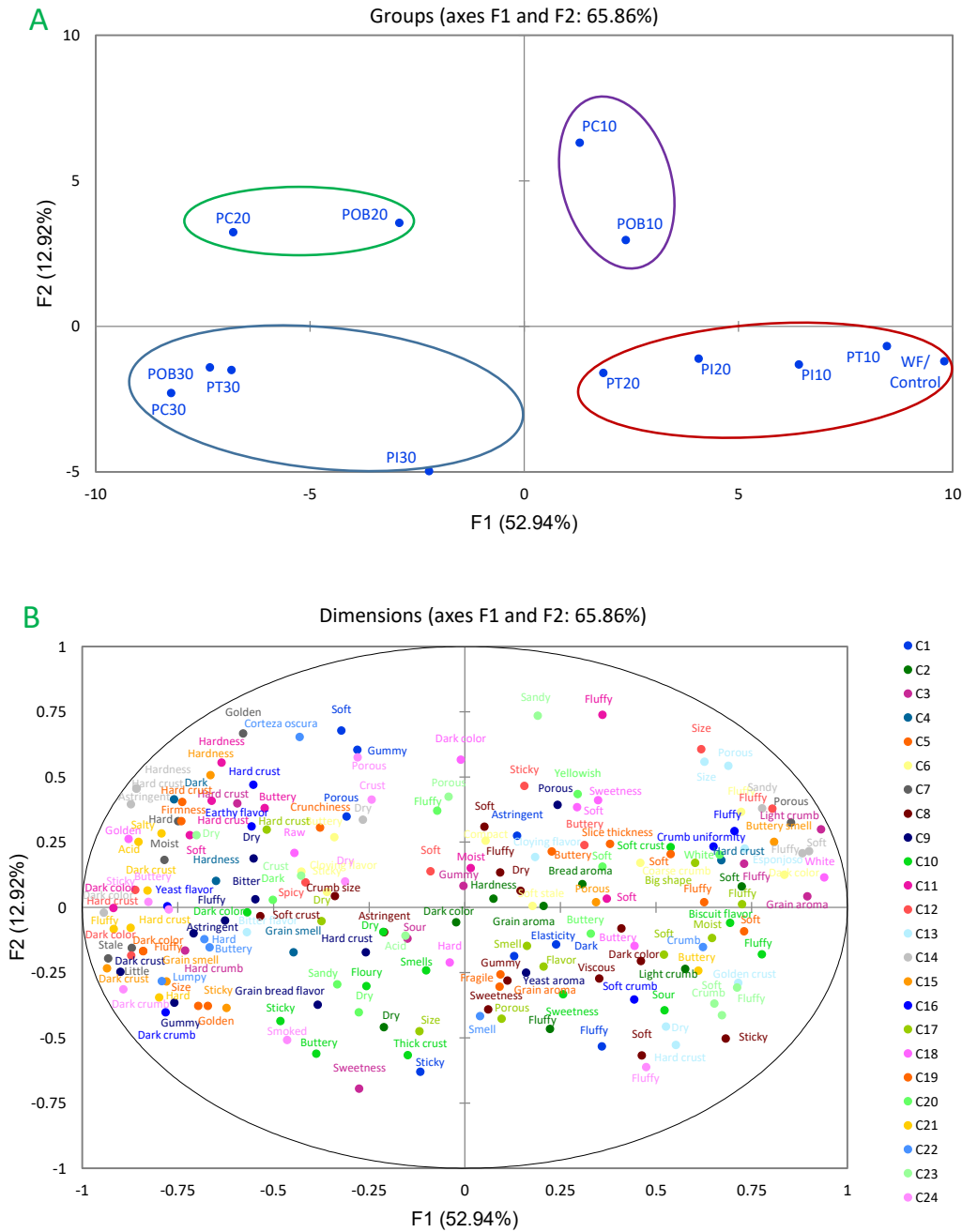
The number of pores per cm<sup>2</sup> and the pore area percentage in the bread crumbs are shown in **Table 3**. A higher substitution of WF with popped amaranth flour caused a higher number of pores/cm<sup>2</sup> and, for most amaranth varieties, a decrease in pore area (for flour from variety Imperial, an increase in pore area was determined with an increase in the share of flour in the bread formula). A bread substituted with *A. spinosus* and *A. hypochondriacus* showed a higher number of cells/cm<sup>2</sup> as the substitution percentage increased [Miranda-Ramos *et al.*, 2019]. In bread fortified with germinated *Amaranthus sp* flour, the number of pores/cm<sup>2</sup> and the pore area also increased as the substitution increased [Guardianelli *et al.*, 2021]. A bread formulation with 50% raw amaranth flour from *A. spinosus* and *A. hypochondriacus* showed a higher number of cells with a lower specific volume compared to control, but was found not significant [Miranda-Ramos *et al.*, 2019]. It could be due to a loss of dough elasticity since low gluten availability. These results can be compared to those obtained in our research. On the other hand, the technological parameters significantly decrease compared to the control because of the weakening of the gluten network, which leads to a decrease in volume, porosity, and elasticity in formulations greater than 20% [Cotovanu & Mironeasa, 2021], since, when mixed with water, wheat proteins are hydrated, allowing the development of a dough with a balanced interrelation of cohesiveness, elasticity (glutenins) and viscosity, extensibility (gliadins), forming gluten network through S–S bonds and hydrogen bonding, retaining gas during fermentation and baking [Wieser *et al.*, 2022]. The increased level of substitution results in a dilution of the protein fractions involved in gluten formation. Besides,

the amaranth 11S proteins and globulin P are not enough to have a gluten network in the mixture, resulting in a softer dough [Cotovanu & Mironeasa, 2021]. Bread shape, crumb porosity, and other characteristics depend mainly on new grain ingredients, which generally cause quantitative and qualitative changes in the protein-proteinase and carbohydrate-amylase complex of the flour, modifying the sensory properties of the product [Derkanosova *et al.*, 2020].

Finally, the results of the instrumental colour evaluation of breads are shown in **Table 3**. Lightness (L\*) had a progressive decrease ( $p < 0.05$ ) as popped amaranth substitution increased, resulting in darker bread. The values of a\* and b\* also significantly ( $p < 0.05$ ) increased. The samples changed to red as a\* increased and yellow as b\* increased. Popped amaranth caused hue changes, obtaining a darker and opaque crumb at the highest substitution level. Similar behaviour was observed in raw amaranth bread from *A. cruentus* substituted for up to 40% [Sanz-Penella *et al.*, 2013], in crude *A. caudatus* var Centenario with substitution of 0 to 100% [Rosell *et al.*, 2009], and crude *Amaranthus* spp. flour [Cotovanu & Mironeasa, 2021; Nasir *et al.*, 2020].

### ■ Sensory evaluation

The bread samples of the 12 formulations were evaluated, including the control. Each consumer described between four to ten sensory attributes of their own, generating a total of 71 terms, subdivided into: general appearance (20), texture (29), aroma (05), colour (07), and taste (10). In addition, a consumer consensus index (Rc) value of 0.61 (61%) was obtained, resulting in a positive correlation.



**FIGURE 3.** Generalized Procrustes analysis (GPA) plots of Flash profile data for wheat bread and breads with 10, 20 and 30% substitution in formulation of WF with popped amaranth flours. (A) Sensory space of samples – breads, (B) Sensory space of attributes or descriptors. POB, Popped Oscar Blanco; PC, Popped Centenario; PT, Popped Taray; PI, Popped Imperial; WF, wheat flour (control); C1–C24, individual consumers.

The results of the Flash profile analysis (sensory attributes submitted by the consumers) were subjected into the GPA, which results are shown in **Figure 3**. The first two factors of GPA explained 65.86% of data variability (F1=52.94% and F2=12.92%). This value was lower than that reported for GPA of data for breads with raw *A. hypochondriacus* (86% variability) [Kamoto *et al.*, 2018] and with germinated basul flour (72.99%) [Vilcanqui-Pérez *et al.*, 2022]. The sample location in the sensory space is shown in **Figure 3A**. Four groups were evident in dimensions F1 and F2. Consumers identified the same sensory attributes within

groups. The first group included the control and PT10, PI10, PT20 and PI20 samples, located in the positive areas of dimension 1 and negative ones of dimension 2. The second group included POB10 and PC10 samples, located in the positive areas of the two dimensions. The third group included POB20 and PC20, located in the negative zones of dimension 1 and positive of dimension 2. Finally, the fourth group comprised the treatments with the highest level of addition (30), located in the negative zones of the two dimensions. These results coincide with those reported by Kamoto *et al.* [2018] for breads with raw amaranth flour,



which were divided in GPA into three groups (breads with substitution levels 0–5%, 10–15, and 20–25) and by Vilcanqui-Pérez *et al.* [2022], showing similar attributes in three groups consisting of breads with 0%, 5–10%, and 15–20% of WF substitution with germinated basul flour.

Figures 3A and 3B show the sensory space for samples and attributes, respectively. Sensory attribute differences are observed between groups. The first group (control, PT and PI, at 10 and 20% substitution) was characterised as fluffy, sticky, moist, light, and yeasty, with a soft crumb and sweetness. Groups with PC10 and POB10 were described as buttery, sticky, astringent, soft, smooth, porous, and smelling like bread. Third group (PC20 and POB20) had attributes such as yeasty flavour, hardness, dryness, and gold. Finally, all the samples at 30% substitution were characterised as lumpy, small, not very fluffy, and with a dark crumb. Thus, PT and PI samples at 10 and 20% substitution were similar in their sensory profile to the control and showed the best attributes in taste and appearance. Descriptors found for bread with raw amaranth were crusts browning, alveolus size and regularity, earthy aroma, yeasty aroma, saltiness, sweetness, chewiness, crisp crust, elasticity, coarseness, crumb graininess, and stickiness [Kamoto *et al.*, 2018]. Attributes found in sprout basul breads were a sweet taste, sticky texture, and fluffy appearance [Vilcanqui-Pérez *et al.*, 2022].

The higher addition of popped amaranth to bread affects the structure of the crumb, elasticity, and crumb colour. Thicker cell walls and the grayish colour of the crumb are also observed, but uniform cells with thicker walls ensure higher breadcrumb stability and strength [Bodroza-Solarov *et al.*, 2008]. The lipid content of amaranth flour is six times higher than WF; hence, it can act as a surface-active agent and gas-stabilizing agent during baking, which could contribute to bread elasticity [Alvarez-Jubete *et al.*, 2010].

## CONCLUSIONS

Bread with 30% substitution of FW with popped amaranth flour had improved nutritional value with a lower carbohydrate content and higher protein and raw fiber contents compared to FW bread. The physical characteristics (peak and final viscosity, specific volume, pore percentage, and colour coordinates) showed reductions as substitution levels increased. According to the sensory evaluation, Taray and Imperial bread varieties at 10 and 20% substitution level were similar to the control with the best sensory attributes, specifically in taste and appearance. The results suggest that bread with popped amaranth flour could become a healthy alternative to wheat bread. However, further study on the phytate and mineral effect in bread is needed.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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