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Enrichment of bread with powder from the aerial parts of purslane: effects on mineral content, phenolic compounds, antioxidant activity, sensory properties, and nutritional enhancement

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antioxidant activity; fortified bread, minerals; phenolic compounds; *Portulaca Oleracea* L.

KEY CONTRIBUTION
A nutritious bread product was developed using powdered *Portulaca oleracea* L. Purslane enrichment increased phenolics, antioxidant activity, and mineral content. Fortified breads significantly improved the daily intake of key minerals such as iron, magnesium, manganese, and copper. All fortified breads remained acceptable to consumers despite reduced sensory scores at higher inclusion levels. The 5% formulation offered the best balance between health benefits and taste.

ABSTRACT

As a widely consumed staple, bread serves as an excellent vehicle for incorporating plant-based ingredients with demonstrated health-promoting properties. This study evaluated the total phenolic content, antioxidant activity, mineral composition, sensory properties, and nutrient contribution of wheat bread enriched with 0%, 5%, 10%, and 15% purslane powder. Mineral content was determined using ICP-AES, phenolic compounds using the Folin-Ciocalteu method, and antioxidant activity through the DPPH assay. Sensory evaluation was conducted using a five-point hedonic scale. Results showed that PO-enriched breads had significantly higher levels of phenolic compounds, antioxidant activity, and mineral content compared to the control ($p < 0.05$), with the 15% PO formulation exhibiting the highest values. Fortification enhanced the daily intake of key minerals such as iron, magnesium, manganese, and copper. Sensory analysis revealed significant differences in consumer acceptance breads with 10% and 15% PO received lower scores than the control and 5% PO, although all samples remained acceptable (overall liking score >3). Correlation and principal component analysis (PCA) demonstrated strong associations between polyphenol content, antioxidant activity, and mineral levels. The 5% PO formulation emerged as the most balanced option, offering improved nutritional value while maintaining acceptable sensory qualities. This study highlights the potential of PO as a functional ingredient to enhance the nutritional and antioxidant properties of bread, with 5% enrichment offering the best compromise between health benefits and consumer preference.

Introduction

Wild edible plants (WEPs) are crucial for enhancing nutrition, promoting sustainability, and improving food security (Hassen, 2021). These plants have been part of human diets for thousands of years and are now gaining importance as a source of nutrients and medicinal benefits (Duguma, 2020). In the context of global challenges such as drought, biodiversity loss, and climate change, WEPs can serve as a viable strategy to combat malnutrition, particularly in regions with limited access to conventional agricultural products (Aboukhalaf et al., 2023). Additionally, WEPs play a significant role in alleviating poverty and providing income sources for communities in developing countries (Delang, 2006). One such plant, *Portulaca oleracea* L. (common name: purslane), is a nutritious wild edible that is receiving increased attention for its rich content of carbohydrates, proteins, omega-3 fatty acids, fibre, minerals and vitamins (Kumar et al., 2022). In addition to its impressive nutritional profile, purslane contains significant amounts of bioactive compounds, such as carotenoids, polyphenols, flavonoids, and alkaloids (Kumar et al., 2022). These constituents have been linked to various biological and pharmacological effects, including antibacterial, anti-inflammatory, antitumor, immunomodulatory, antioxidant, anti-insomnia, anticancer, gastroprotective, hepatoprotective, and wound-healing properties (Kumar et al., 2022; Li et al., 2024). Moreover, its ability to thrive in diverse environmental conditions and its minimal cultivation requirements further enhance its potential as a sustainable food source (Srivastava et al., 2023). In Morocco, the leaves and stems of this species can be eaten raw in salads, cooked as a vegetable, or used as an ingredient in bread and other dishes (Aboukhalaf et al., 2022; Naciri et al., 2022). Bread is one of the most popular and widely consumed foods globally, due to its nutritional value, cultural acceptability, and affordability (Govender and Siwela, 2020). It is an excellent medium for incorporating plant-based ingredients, including herbs, spices, and extracts (Ranasinghe et al., 2022). Adding nutrient-dense plants such as *P. oleracea* to bread formulations can enhance its nutritional profile and increase its content of beneficial phytochemicals, antioxidants, and other bioactive compounds. This innovative strategy could help address micronutrient deficiencies in populations that rely on bread as a staple food. Although several studies have explored the addition of purslane powder to bread, most have focused mainly on rheological, physicochemical, and sensory properties. Few have assessed its detailed nutritional and mineral composition or its contribution to daily mineral intake. This study addresses these gaps by evaluating the effects of adding aerial parts of *P. oleracea* (5%, 10%, and 15%) on the nutritional, biochemical, mineral, and sensory qualities of wheat bread, and uses statistical analysis to identify the optimal fortification level.

Materials and methods

Collection and pretreatment of plant samples

The aerial parts of the purslane plant were collected from the province of El Jadida, located in central Morocco, in June 2023. After harvesting, the plant material was thoroughly washed with distilled water to remove dirt and foreign matter. The cleaned samples were then oven-dried at 37 °C until completely dehydrated. Once dried, the plant material was ground into a powder using a laboratory electric grinder (Moulinex type LM 207, France).

Preparation of bread making

The mixing ratios for dough used to make white bread with varying amounts of purslane aerial parts are presented in Table 1. The dough was prepared using the direct kneading method until a cohesive mixture was achieved, followed by fermentation at 30 °C for 60 minutes. The dough was then divided into 150 g portions and baked at 195 ± 5 °C for 30 minutes. After baking, the breads were packed in polyethylene bags for further analysis. The percentages of purslane powder incorporated into the bread were 0%, 5%, 10%, and 15%, respectively. The control sample contained 0% purslane powder, while the experimental samples were labelled as PO5 (5% purslane powder), PO10 (10% purslane powder), and PO15 (15% purslane powder).

Table 1 Ingredient composition of bread with different amounts of *Portulaca oleracea* L.

Ingredients (g)	Control	PO5	PO10	PO15
Wheat flour	200	190	180	170
Purslane	0	10	20	30
Yeast	14	14	14	14
Salt	7	7	7	7
Water (mL)	120	120	120	120

Determination of total phenolic content (TPC)

The total phenolic content (TPC) in the bread samples was quantified using the colourimetric Folin–Ciocalteu method, as previously described by Aboukhalaf et al. (2023), with minor modifications. A five gram sample of bread powder was extracted by maceration in methanol. The obtained extract was filtered and concentrated using a rotary evaporator. The concentrated extract was then refrigerated at 4 °C for subsequent analysis. For TPC determination, 1 mL of the Folin–Ciocalteu reagent (in a 10:1 v/v ratio) was added to 100 µL of the sample extract., After a 5 min-incubation, 1mL of 7% sodium carbonate (Na2CO3) solution was added. This was followed by immediate dilution with 400 µL of distilled water, and the mixture was incubated in the dark for 90 min. Absorbance was measured spectrophotometrically at 760 nm, and TPC was expressed as milligrams of gallic acid equivalent per gram of dried bread samples (mg GAE/g DW).

Antioxidant activity

The antioxidant activity was evaluated using the free radical scavenging assay of the 2,2-diphenyl-2 picrylhydrazyl (DPPH●), a stable free radical commonly used to assess antioxidant capacity, following the procedure described by Aboukhalaf et al., (2023). Various concentrations of extracts from bread samples were mixed with an equal volume of a methanol/DPPH solution (0.004%). After a 30-minute incubation period in the dark, the absorbance was measured at 517 nm using a spectrophotometer. The assay was performed in triplicate, and results were expressed as an IC_{50} value (µg/mL), representing the concentration required to scavenge 50% of the initial DPPH● radicals.

Mineral content analysis

The concentrations of both macrominerals (Na, K, Ca, Mg, and P) and microminerals (Fe, Cu, Zn, and Mn) in the bread samples were analysed using an atomic absorption spectrophotometer, specifically the ICP-AES model (Jobin Yvon, Ultima 2), equipped with an axial viewing plasma, as described by Mohammed et al. (2013).

Contribution of bread consumption to daily mineral intake

The contribution to daily mineral requirements was assessed using two reference standards: the Recommended Daily Allowance (RDA) and Adequate Intake (AI). Based on the consumption of a defined quantity of bread, the mineral intake was calculated and adjusted to reflect the nutritional needs of adult men and women aged 18 years and older.

Sensorial analysis of bread

Sensory attributes of bread samples were evaluated by an untrained panel of 48 participants from El Jadida, Morocco, aged between 25 and 60 years. The evaluation focused on various sensory specifications, including taste, colour, texture, odour, and overall acceptability. A grading test method was employed, utilising a five-point scale where scores ranged from 1 (very bad) to 5 (very good) (Ghanbari and Farmani, 2013). The overall acceptability of each bread sample was determined by calculating the average score obtained from the evaluations.

Sensory panel

All participants received written information about the test and provided informed consent to take part in the study. Ethical committee approval was not required for this study.

Statistical analysis

Data are presented as means ± standard deviation (SD) from at least three independent replicates. Differences between bread samples were analysed using one-way analysis of variance (ANOVA) in SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered statistically significant. Additionally, principal component analysis (PCA) was performed using RStudio (version 2023.06.1 + 524) to explore the relationships between the nutritional composition (bioactive compounds and minerals) and the sensory characteristics of purslane-fortified breads. Correlation analysis (r) was also conducted in RStudio to examine associations among these variables.

Results and discussion

Total phenolic content

Figure 1 shows the total phenolic content (TPC) of wheat breads enriched with various concentrations of purslane powder. The TPC values range from 13.209 to 29.790 mg GAE/g DW. Breads fortified with 5% (PO5), 10% (PO10), and 15% (PO15) purslane powder showed a significant increase in TPC ($p < 0.05$) compared to the control. These results highlight the potential health benefits of incorporating purslane powder into bread formulations.

Phenolic compounds are plant molecules characterised by an aromatic hydrocarbon with one or more hydroxyl groups (Khoddami et al., 2013). These compounds act as antioxidants and may contribute to the prevention of heart disease, reduction of inflammation, and a lowered risk of cancer and diabetes rates, as well as reducing mutagenesis in human cells (Griffiths et al., 2016; Rahman et al., 2022). Purslane is notable for its high content of phenolic compounds, including flavonoids, phenolic acids, and anthocyanins (Montoya-García et al., 2023). Major phenolics commonly reported in purslane include catechin, ferulic acid, gallic acid, and quercetin (Kumar et al., 2022; Sicari et al., 2018). These components are associated with the increased phenolic content observed in bread fortified with purslane (Lee et al., 2015). Several studies have shown that the phenolic content of bread increases proportionally with the amount of dried purslane added (Lee et al., 2015; El Gindy, 2017).

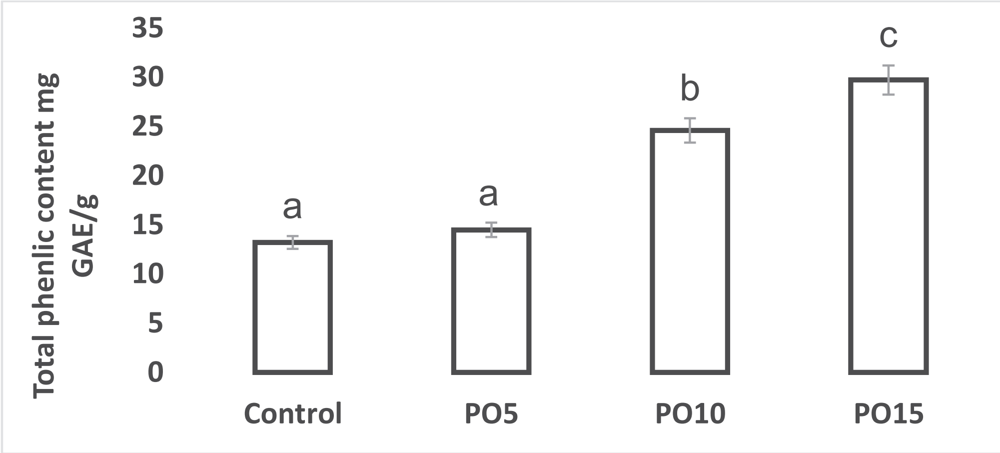

Figure 1 Total phenolic compounds of wheat breads enriched with varied amounts of purslane powder. Different letters (a-c) among bars represent significant differences among means at a 0.05 probability level

Antioxidant activity

Figure 2 shows the antioxidant activity of the bread samples. Significant differences ($p<0.05$) were observed among the samples. The bread fortified with 15% purslane showed the highest antioxidant activity (IC_{50} = 12.045 mg/mL), whereas the control bread displayed the lowest antioxidant activity (IC_{50} = 34.434 mg/mL).

Similar findings have been reported in studies investigating the antioxidant properties of breads enriched with various ingredients, such as purslane, vegetable waste, and pumpkin flour (Baiano et al., 2015; Lee et al., 2015; Wahyono et al., 2020). These studies also demonstrated that increasing the level of ingredient addition leads to a concurrent increase in the breads' antioxidant potential. Moreover, a strong correlation (R=-0.87) was observed between the antioxidant potential and phenolic content in the bread samples. Salehi et al. (2021) similarly found that higher phenolic content corresponds to a greater ability to neutralise free radicals. These results demonstrate that incorporating purslane powder into bread not only boosts its antioxidant activity but also enhances its overall bioactive properties, thereby contributing to improved potential health benefits.

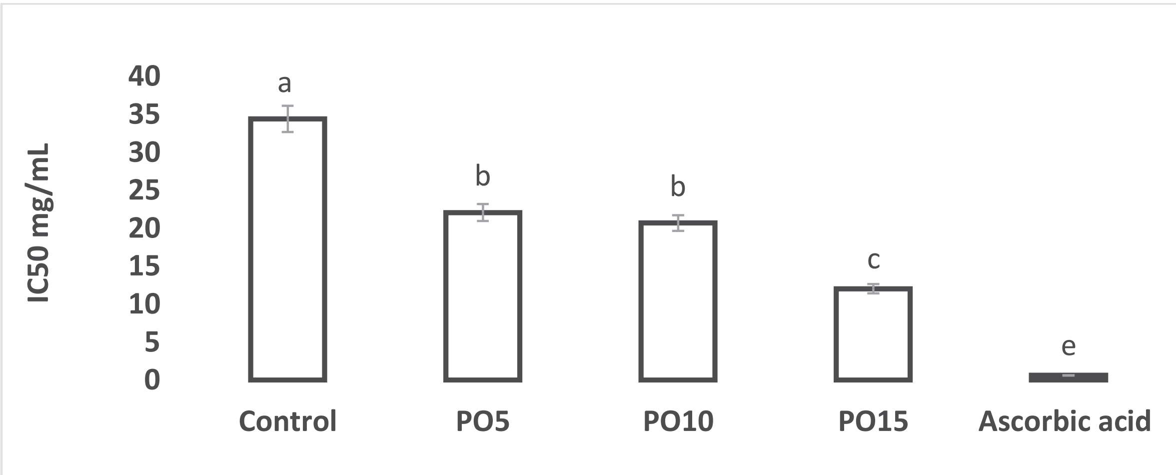

Figure 2 Antioxidant activity of wheat breads enriched with varied amounts of purslane. Different letters (a-e) among bars represent significant differences among means at a 0.05 probability level

Mineral analysis

As shown in Table 2, the macro- and micromineral contents of breads enriched with purslane varied significantly with the amount of purslane added ($p<0.05$). Sodium was the most abundant mineral across all bread samples, likely due to the addition of salt during preparation. Calcium (Ca) content increased from 58.6 mg/100 g in the control to 109.5 mg/100 g in PO15, while potassium (K) rose from 307.8 to 370.4 mg/100g. Magnesium (Mg) levels ranged from 51.7 mg/100 g (control) to 81.4 mg/100 g (PO15), and phosphorus (P) from 213.9 mg/100 g (control) to 244.9 mg/100 g (PO15). Iron (Fe) content increased from 206 3.6 to 4.7 mg/100 g, and zinc (Zn) from 1.9 to 2.9 mg/100 g with increasing levels of purslane. A study by Delvarianzadeh et al. (2020) on breads enriched with purslane powder demonstrated that higher levels of purslane powder led to increased mineral content. Hussien and Salem (2016) also reported that incorporating purslane powder into gluten-free snack formulations resulted in a significant increase in iron, zinc, and calcium content, effectively doubling their concentrations. These findings are consistent with the present study and suggest that incorporating purslane powder into bread formulations could be a promising approach for enhancing dietary mineral intake, making it an attractive option for consumers and manufacturers focused on healthier eating.

Table 2 Mineral content of wheat breads enriched with varying levels of purslane powder

	Macrominerals					Microminerals			
Samples	**Ca**	**Na**	**K**	**Mg**	**P**	**Fe**	**Mn**	**Cu**	**Zn**
Control	58.60±0.04ª	459.6±0.05ª	307.8±0.07ª	51.7±0.02ª	213.9±0.04ª	3.6±0.03ª	1.2±0.02ª	0.7±0.07ª	1.9±0.04ª
PO5	73.65±0.03ᵇ	460.8±0.04ª	337.8±0.02ᵇ	57.4±0.04 ᵇ	221.5±0.09ᵇ	3.7±0.03ᵇ	1.3±0.03ᵇ	0.7±0.04ª	2.2±0.08ᵇ
PO10	90.74±0.02ᶜ	520.8±0.01ᵇ	369.7±0.02ᶜ	77.8±0.03ᶜ	221.8±0.02ᵇ	4.68±0.03ᶜ	1.4±0.04ᶜ	0.92±0.05ᵇ	2.6±0.01ᶜ
PO15	109.5±0.03ᵈ	475.2±0.04ᶜ	370.4±0.06ᵈ	81.4±0.04ᵈ	244.9±0.01ᶜ	4.7±0.04ᶜ	1.7±0.05ᵈ	0.97±0.04ᵇ	2.9±0.02ᵈ

Means within a column followed by different letters are significantly different ($p<0.05$)

Contribution of bread consumption to daily mineral intake

The incorporation of purslane (*Portulaca oleracea*) powder into bread significantly increased the daily intake of essential minerals and trace elements, particularly iron, magnesium, manganese, and copper (Table 3). As the level of fortification increased from 0% (control bread) to 15%, a progressive enhancement in breads' mineral content was observed. This trend translated into a greater nutritional contribution per portion, with each fortification level offering additional health benefits while preserving acceptable sensory attributes for consumers. For example, iron content increased proportionally with higher inclusion rates, covering approximately 45% of the recommended daily intake (RDI) for post-menopausal women and adult men in the control bread, and up to 58.75% at 15% fortification. For women of reproductive age, the RDI coverage dose rose from 20% to 26.11%. Iron is an essential micronutrient required for haemoglobin synthesis and plays a vital role in muscle function, cellular respiration, and oxygen transport (Zoroddu et al., 2019). Iron deficiency, affecting over one billion people worldwide, remains a leading cause of anaemia (Aboukhalaf et al., 2023).

Similarly, magnesium availability improved with increasing purslane content, rising from 12.92% to 20.35% of the RDI for men aged 19-30 and from 16.67% to 26.25% for women. Magnesium is a vital structural component of ribosomes and plays a key role in protein synthesis and neuromuscular signal transmission, thereby contributing to muscle relaxation and overall neuromuscular function (Zoroddu et al., 2019). Manganese content also increased substantially, covering 52.17% to 73.91% of the RDI for men and 66.66% to 94.44% for women. Manganese supports numerous metabolic functions, including glucose and lipid metabolism, enzyme activation, protein and vitamin synthesis, as well as immune and endocrine system regulation (Li and Yang, 2018). Furthermore, the fortified breads proved to be excellent sources of copper. Copper content increased from 77.77% to 107.77% of the RDI for both sexes, highlighting the nutritional value of this fortification. Copper is essential for numerous physiological processes, including erythropoiesis, bone maintenance, and the proper functioning of the nervous system (Zoroddu et al., 2019). In summary, fortifying bread with 5% to 15% purslane powder presents a promising strategy to improve dietary mineral intake while maintaining acceptable sensory quality.

Table 3 Estimated coverage of selected mineral RDIs (%) following consumption of 100 g of control or purslane-enriched wheat bread

Mineral elements	Sex	Age (years)	RDA mg/day	Control bread	Enriched breads		
					PO5	PO10	PO15
Ca	M	19-70	1000	5.86	7.36	9.07	10.95
		>70	1200	4.88	6.13	7.55	9.125
	W	19-50	1000	5.86	7.36	9.07	10.95
		>50	1200	4.88	6.13	7.55	9.125
Na	M/W	≥19	1500	30,64	30.72	34.72	31.68
K	M	≥19	3400	9.05	9.93	10.87	10.90
	W	≥19	2600	11.84	13.00	14.21	14.25
Mg	M	19-30	400	12.92	14.35	19.45	20.35
		>31	420	12.30	13.66	18.52	19.38
	W	19-30	310	16.67	18.51	25.09	26.25
		>31	320	16.15	17.94	24.31	25.43
P	M/W	≥19	700	30.55	31.64	31.68	34.99
Fe	M	≥19	8	45.00	46.25	58.5	58,75
	W	19-50	18	20.00	20.55	26.00	26.11
		>50	8	45.00	46.25	58.5	58,75
Mn	M	≥19	2.3	52.17	56.52	60.86	73.91
	W	≥19	1.8	66.66	72.22	77.77	94.44
Cu	M/W	≥19	0.9	77.77	77.78	102.22	107.77
Zn	M	≥19	11	17.27	20.00	23.63	26.36
	W	≥19	8	23.75	27.5	32.5	36.25

Results of sensory analysis

Table 4 presents the average consumer evaluation scores, while Figure 3 provides a visual representation of the sensory study results. The sensory scores for nearly all attributes of the control bread were not significantly different (p>0.05) from those of the wheat bread fortified with 5% purslane aerial parts powder. However, significant differences (p<0.05) were observed in the sensory attributes between the control and the bread samples fortified with purslane powder at levels greater than 5%. Across all sensory parametres, the control bread was significantly preferred compared to the other experimental groups (PO5, PO10, and PO15), receiving the highest scores for overall acceptability. Furthermore, the radar graph (Figure 3) shows that the vertices corresponding to the control, PO5, and PO10 samples exhibit visible symmetry across most parametres, although a significant difference remains between the control bread and the bread fortified with 10% purslane powder. Overall, all samples consistently received scores close to grade 4 across all parametres and groups, indicating a general rating of 'good'.

In summary, while the addition of purslane powder influenced consumer evaluations, all formulations remained within an acceptable range. Supporting this, Melilli et al. (2020) reported that bread samples containing purslane powder at substitution levels of 5%, 10%, and 15% received overall acceptability scores close to 6, which is just above the threshold for consumer acceptance.

Table 4 Means of sensory attributes of bread fortified with different levels of purslane

Groups	Colour	Taste	Odour	Texture	General acceptability
Control	4.06 ± 0.04^{a}	4.06 ± 0.1^{a}	3.93 ± 0.05^{a}	4.12 ± 0.05^{a}	4.06 ± 0.01^{a}
PO5	4.02 ± 0.05^{a}	3.93 ± 0.08^{a}	3.91 ± 0.01^{a}	4.04 ± 0.08^{a}	4.02 ± 0.04^{a}
PO10	3.91 ± 0.03^{b}	3.81 ± 0.01^{b}	3.89 ± 0.08^{a}	3.91 ± 0.02^{b}	3.83 ± 0.02^{b}
PO15	3.60 ± 0.08^{c}	3.46 ± 0.03^{c}	3.68 ± 0.01^{b}	3.83 ± 0.02^{d}	3.72 ± 0.01^{c}

Means within a column followed by different letters are significantly different ($p < 0.05$)

This suggests that even with the addition of purslane, consumers found the bread to be both palatable and satisfactory. Further supporting these findings, Delvarianzadeh et al. (2020) reported that wheat breads fortified with less than 15% purslane retained acceptable sensory qualities, including taste, texture, colour, and odour. Their study highlighted that the sensory attributes of the bread remained favourable, indicating that the incorporation of purslane did not detract from the overall eating experience. Overall, these studies collectively demonstrate that the addition of purslane powder not only enhances the nutritional profile of bread but also aligns well with consumer preferences.

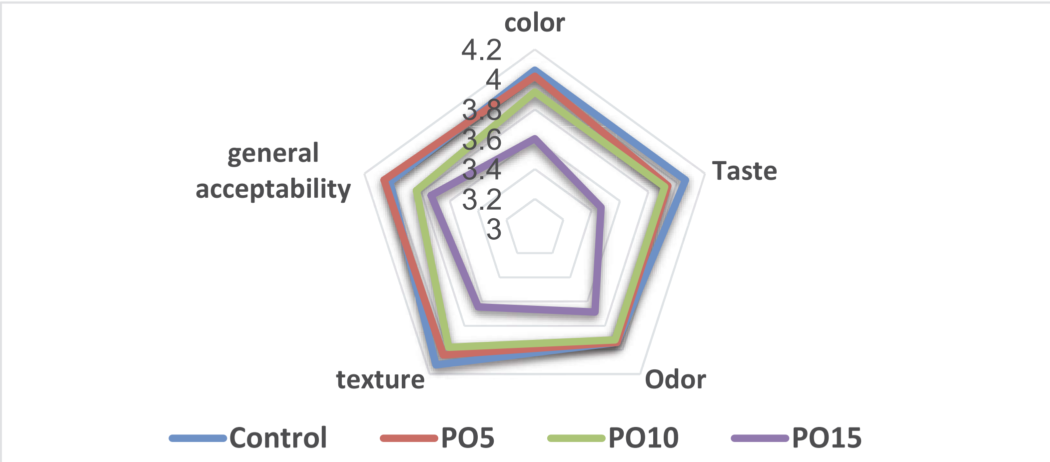

Figure 3 Effect of different replacement levels with purslane (PO0, PO5, PO10, and PO15) on sensory attributes of breads as evaluated using a 5-item hedonic scale (1, 2, 3, 4 and 5 standing for very bad, bad, medium, good and very good)

Heatmap correlation

Analysis of the correlation matrix (Figure 4) revealed significant associations among polyphenol content, antioxidant activity, mineral composition, and sensory characteristics of bread enriched with purslane powder. A strong negative correlation between polyphenol levels and IC50_AA suggests that the addition of purslane enhances the breads' antioxidant capacity, highlighting its functional potential. Moreover, positive correlations between polyphenol content and several minerals (Ca, K, Mg, P, Fe, Mn, Cu, Zn) indicate that purslane enrichment improves both bioactive and nutritional profiles. However, inverse correlations between polyphenols and minerals with key sensory attributes, including colour, taste, texture, and general acceptability, suggest that higher concentrations, particularly of polyphenols and zinc, may negatively affect organoleptic properties. These findings are consistent with sensory evaluations, which show reduced acceptability scores for breads containing 10% and 15% purslane, likely due to increased bitterness and altered texture. The correlation matrix thus facilitates the interpretation of the relationships among variables, in particular the link between the chemical composition of purslane and its impact on sensory characteristics.

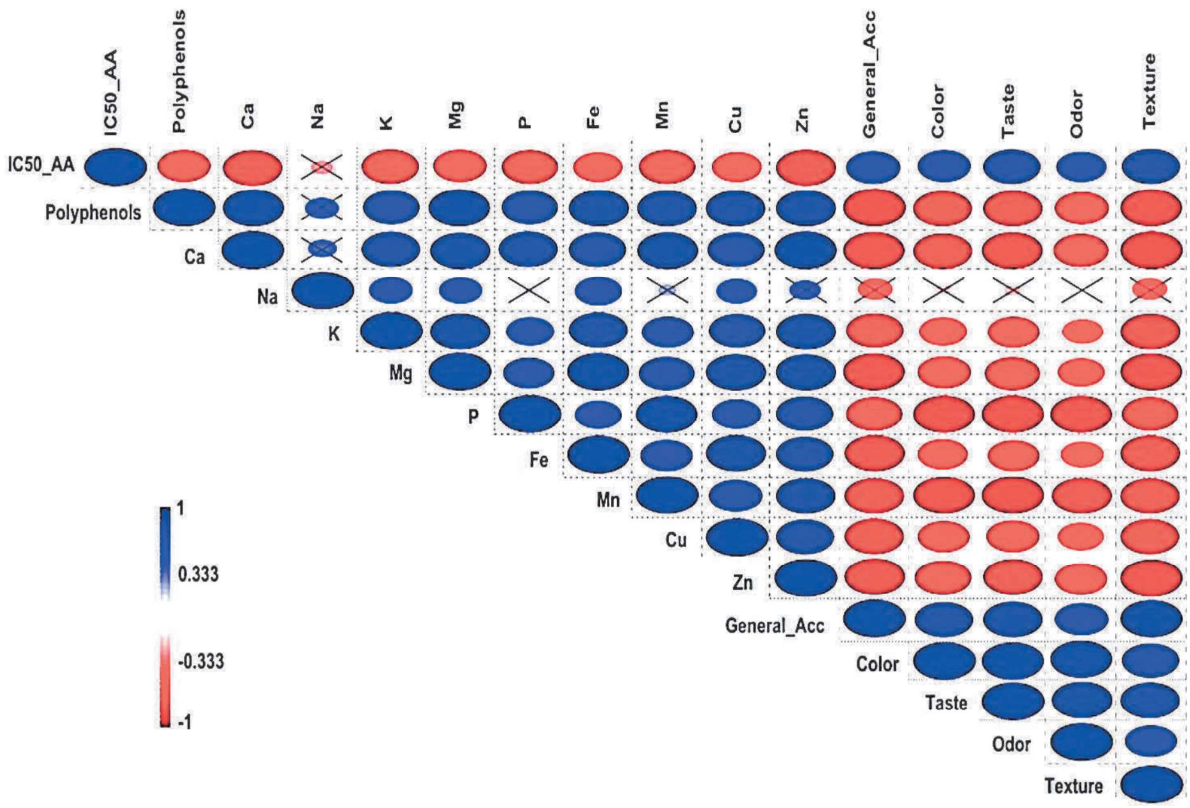

Figure 4 Correlation Heatmap of polyphenols, antioxidant activity, minerals, and sensory attributes in purslane-enriched bread

Acp analysis

The pairwise correlations (Figure 4) offer an initial insight into the relationships among the nutritional, biochemical, and sensory parametres of the enriched bread samples. To further explore the overall distribution of the samples and uncover potential multidimensional interactions, a PCA was performed. This multivariate approach not only confirms the trends revealed by the correlation matrix but also offers a deeper understanding of the data structure. The PCA results (Figure 5) demonstrate a clear separation of samples based on their level of purslane powder enrichment. The first principal component, accounting for 93.4% of the total variance, distinctly isolates the 10% and 15% PO of breads. These samples are strongly associated with higher levels of polyphenols, antioxidant activity, and various minerals (Fe, Mg, K, Zn, Cu, etc.), setting them apart from the control and 5% PO samples. This marked separation highlights purslane enrichment as the primary source of variability among the bread formulations. Moreover, sensory parametres (colour, taste, odour, texture, and general acceptability) are positioned on the opposite side of the principal axis, indicating an inverse relationship with the biochemical and nutritional variables. This pattern reflects a trade-off between nutritional enhancement and sensory appeal: as the level of functional compounds increases, the organoleptic quality tends to decrease. Notably, the 5% PO formulation occupies an intermediate position on the biplot, suggesting it offers the most balanced compromise between improved nutritional value and acceptable sensory attributes. As such, itrepresents a promising formulation for functional bread development.

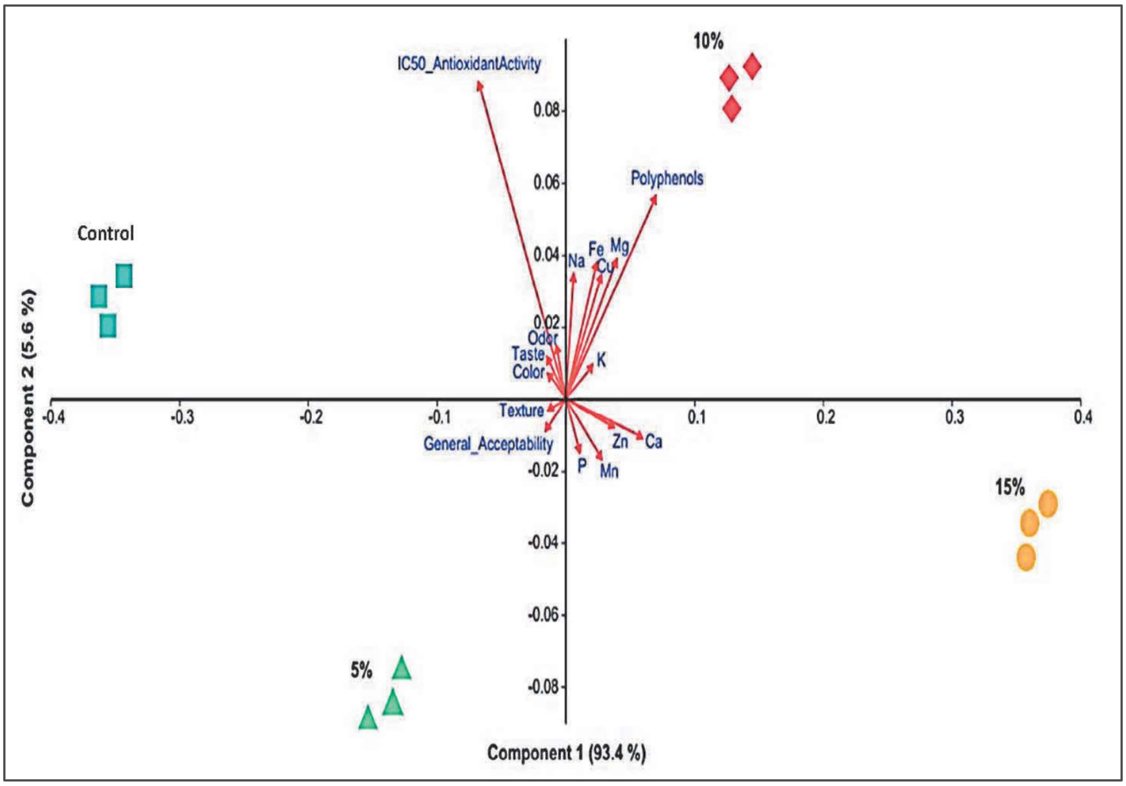

Figure 5 Principal component analysis (PCA) biplot of control and purslane-enriched bread samples

Conclusion

This study focused on the development of wheat bread enriched with powdered aerial parts of purslane (*Portulaca oleracea* L.), with an evaluation of its phenolic compounds, antioxidant activity, mineral content, and sensory attributes across various substitution levels. Our findings demonstrated that the addition of purslane powder significantly increased the total phenolic content, antioxidant activity, and mineral levels compared to the control bread. Notably, these beneficial properties were concentration-dependent, with greater enhancements observed at higher inclusion levels. Correlation analysis and principal component analysis revealed strong associations among polyphenols, antioxidant activity, and mineral composition. Furthermore, the analyses identified 5% purslane enrichment as the most favourable level for achieving an optimal balance between nutritional improvement and sensory acceptability.

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