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Process Standardization for Bread Preparation using Composite Blend of Wheat and Pearl Millet: Nutritional, Antioxidant and Sensory Approach

MANJU NEHRA, ANIL KUMAR SIROHA*
SNEH PUNIA and SUNIL KUMAR

Department of Food Science and Technology, Chaudhary Devi Lal University, Sirsa, India.

Abstract
In present study, the effect of incorporation of pearl millet (PM) flour (10, 20, 30, 40%) on quality and sensory characteristics of bread were studied. Ash, fat, fiber and carbohydrate content were increased with the incorporation of PM flour. For wheat flour (WF) the values of water absorption capacity (WAC) and oil absorption capacity (OAC) were observed 1.90 g/g and 1.54 g/g, while flour blends varied from 1.78-1.87g/g and 1.48-1.52g/g, respectively. After the incorporation of PM flour peak (PV), trough TV, setback (SV) and final viscosity (FV) were decreased as compared to WF. Antioxidant properties of WF and PM flour were observed 20.3% and 15.1%, and varied from 18.10% to 19.23%, respectively for flour blends. Antioxidant characteristics of breads increased as compare to their flours. Physical parameter i.e. loaf weight increases after addition of PM flour while reverse was observed for loaf volume. Bread prepared up to 30% addition of PM flour into WF showed a satisfactory sensorial score for bread further addition of PM flour, breads were not acceptable quality. Results of present study provide a better understanding of functional properties of WF, PM flour and their blends for their possible applications in preparation of gluten free products.

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Introduction

Pearl millet (*Pennisetum glaucum*) belongs to family *Poaceae* and is widely grown around the world for feed and fodder. It is native to Africa, cultivated in the dry and semidry zones of Asia and Africa. India is leading producer (1,02,80,000 tonnes) followed by Niger (38,86,079 tonnes) of millets[1] and its cultivation is next to rice and wheat. Improved essential nutrient content and the gluten free nature of PM make it a better replacement to commercial flours.

CONTACT Anil Kumar Siroha siroha01@gmail.com Department of Food Science and Technology, Chaudhary Devi Lal University, Sirsa, India.

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Protein, fat, ash, fibre and carbohydrate content was observed 9.7-14.0, 5.1-7.2, 2.0-3.8 and 69.6-76.3%, respectively for PM flours[2,3] and it contains high amount of protein, energy level, and more balanced amino acids as compared to sorghum and maize.[4] PM has many health benefits due to its higher mineral and fibre content, such as possibly helping to prevent certain cancers, lowering blood pressure and cholesterol, delaying gastric emptying, lowering the risk of cardiac diseases, celiac diseases, constipation, diabetes obesity, and ulcers.[5,6] Annor *et al*.[7] reported that PM had low values for glycemic index which help to reduce type-2 diabetes. It is also had higher amount of antioxidants such as hydroxycinnamic acid (e.g. ferulic and cinnamic acid), hydroxybenzoic acids and flavonoids.[6]

Bread is a bakery product whose main ingredients are water, flour, salt, yeast, sugar, and fat which are mixed and fermented to form viscoelastic dough before being baked.[8] Bread is an important breakfast item that is rich in health-benefiting nutrients. There is always a need for range of gluten free products to have easy lifelong sustainability for gluten intolerants. WF is avoided by gluten intolerant patients as it contains high gluten, low fiber and high glycemic level; contributes to many disorders and diseases like diabetes, obesity, and atherosclerosis.[9] Gluten free alternates are the key demand of time and prompted food scientists to design potentially nutritive and having some functional characteristics that are in easy access to all.[10,11] Many researchers prepare the bread using different flours to improve the nutritional characteristics such as barley,[12] chickpea[13] and rice flour,[14,15] yam flour,[16] banana flour,[17] apple seed cakes,[18] finger millet,[19] Okra seed and seedless pod.[20] PM is underutilized and gluten free crop; its industrial application may be increased by utilizing it in various food products. So, present study was conducted to evaluate the nutritional, antioxidant and sensory characteristics of bread prepared by addition of WF with debranned PM flour.

Materials and Methods

Materials

The PM and wheat grains were procured from the local market Sirsa, Haryana (India). The millet grains were cleaned to remove extraneous matter and debranned. After that grains were milled and then flour was prepared. Wheat grains were also cleaned, sorted and then milled. All chemicals were used analytical grade.

Proximate Composition

AOAC[21] method was opted for evaluating proximate composition (moisture, ash, fat,protein (% N×6.25) and fiber content) of flours. Total carbohydrates content was determined using the following formula:

Total carbohydrates =100−(moisture +ash + fat + protein+ fiber).

Water and oil Absorption Capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) of the flours were evaluated using the method of Sathe *et al*.[22] with slight modifications.

Foaming and Emulsion Capacity

Method described by Lin *et al*.[23] was opted for studying the foaming capacity (FC) while emulsion capacity was analysed by using method Naczk *et al*.[24]

Antioxidant Activity using DPPH

Antioxidant activity were determined using the method described by Brand-Williams *et al*.[25]

Pasting Properties

Pasting properties of flours were evaluated using a starch cell of Modular Compact Rheometer (Model-52, Anton Paar, Austria). Flour sample 8% was used for evaluation and mixed properly before analysis. Flour samples were heldat 50°C for 1 min and then sample was heated to 95°C at a heating rate of 6°C/ min, after holding 5 min at 95°C, cooled to 50°C at the same rate and again held at 50°C for 2 min. All samples were evaluated in triplicate and the pasting temperature (PT), peak viscosity (PV), trough (TV), breakdown (BD), setback (SV), and final viscosity (FV) were determined.

Bread Preparation

The breads were prepared from WF and PM flour mix in the ratios of 90:10, 80:20, 70:30, 60:40, 100%WF and refined flour, respectively. Flour was mixed with salt in desired amount of sugar, yeast, calcium and bread improver for better dough preparation in a separate laboratory mixer. Water is added to all ingredients to make dough. Then dough was kneading by hand for 20 min and put into the greased pan. Then the dough was proofing for 1.30-2.0h at 25-30°C. For baking the breads 195°C was used for 30 min. Breads were cooled to room temperature and packaged in polythene bags.

Physical Properties of Bread
Loaf Weight and Loaf Volume
After baking process, loaves were cooled to room temperature and weight of each bread samples was calculated. After holding for five hours loaf volume was calculated using rapeseed displacement method. Loaf volume (LV) was divided by loaf weight (LW) to calculate the specific loaf volumes (ml/g).

Sensory Evaluation of Breads
Method of Rajiv *et al.*[26] with slight modifications was opted to analysed the sensory properties.

Table 1a: Proximate composition flours

Sample	Ash (%)	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Carbohydrate (%)
WF	1.80±0.2^{a}	7.68±0.3^{d}	11.51±0.4^{f}	2.46±0.4^{a}	1.91±0.2^{a}	74.64±0.3^{b}
PF	2.11±0.1^{c}	7.44±0.4^{a}	9.81±0.5^{d}	5.53±0.6^{f}	3.41±0.1^{e}	71.70±0.4^{a}
WF+PF-10%	1.82±0.1^{a}	7.60±0.5^{c}	10.80±0.4^{e}	2.85±0.3^{b}	2.10±0.2^{b}	74.83±0.4^{b}
WF+PF-20%	1.83±0.2^{a}	7.55±0.3^{b}	9.65±0.4^{c}	3.10±0.4^{c}	2.29±0.1^{c}	75.58±0.3^{c}
WF+PF-30%	1.85±0.1^{b}	7.52±0.3^{b}	9.10±0.3^{b}	3.48±0.4d	2.45±0.3^{d}	75.60±0.3^{c}
WF+PF-40%	1.86±0.3^{b}	7.44±0.4^{a}	8.46±0.5^{a}	3.95±0.6^{e}	2.51±0.3^{d}	75.78±0.4^{d}

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–f) are significantly different ($p \leq .05$).

WF: Wheat flour; PF: Pearl millet flour; WF+PF-10%: Wheat flour incorporated with 10% pearl millet flour; WF+PF-20%: Wheat flour incorporated with 20% pearl millet flour; WF+PF-30%: Wheat flour incorporated with 30% pearl millet flour; WF+PF-40%: Wheat flour incorporated with 40% pearl millet flour.

Statistical Analysis
The data reported in the tables were carried out in triplicate and they were subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Software version 15 (Minitab, Inc., State College, USA).

Results and Discussions
Proximate Composition
The proximate composition of PM flour, WF and composite flours were determined and the results are tabulated in (Table 1a). Ash, crude fat, crude protein, crude fibre, moisture and carbohydrate content was observed 1.8, 2.46, 11.51, 1.91, 7.68 and 74.64%, respectively for WF while PM flour had ash (2.11%), crude fat (5.53%), crude protein (9.81%), crude fibre (3.41%), moisture (7.44%) and carbohydrate content (71.70%), respectively. Siroha *et al.*[3] reported protein, fat, fibre and carbohydrate content 9.7 to 11.3, 5.1 to 7.2, 2.9 to 3.8 and 69.6 to 72.5%, respectively for PM flour from different cultivars. Punia *et al.*[27] evaluated ash, fat and fiber contents 1.52 to 1.76, 2.62 to 3.48, and 0.79 to 0.93%, respectively for different wheat cultivars. After the substitution of PM flour in WF proximate composition of flour blends were changed as compared to control flour. Ash content, crude fat, crude protein and carbohydrate content of flour blends varied from 1.82-1.86, 2.85-3.95, 8.46-10.80, and 74.83-75.78%, respectively. Carbohydrate content was varied from 74.83 to 75.78%, the highest value was observed for flour blends with 40% PM flour. Carbohydrate content was slightly increased with increase PM flour in the WF.

Values of proximate composition of bread are presented in (Table 1b). The crude fat and crude protein content of composite breads were varied from 4.18 to 4.54% and 8.10 to 8.89%, respectively, whereas for control bread had 5.5% and 10.15%. The ash content of breads was ranged from 3.23 to 3.51%, the highest and the lowest values observed for 10% PM flour bread and control bread. The moisture content of breads ranged from 18.90 to 19.41%, the highest value for control bread and the lowest value for 40% composite bread was observed. The carbohydrate content of composite breads varied from 61.78 to 64.80%, the highest value was observed for bread containing 20% PM flour. Chauhan *et al.*[34] reported moisture content, ash, fat, protein, fibre and carbohydrate content of WF bread 25.87, 0.72, 4.38, 9.45, 1.63 and 57.95%, respectively.

Table 1b: proximate composition of breads

Sample	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)	Carbohydrate (%)
WF	23.67±0.5^{e}	3.17±0.4^{a}	5.50±0.6^{e}	10.15±0.7^{e}	1.81±0.1^{b}	55.70^{a}
RF	19.53±0.4^{d}	3.42±0.3^{c}	3.21±0.4^{a}	10.24±0.5^{d}	1.63±0.3^{a}	61.67^{b}
WF+PF-10%	19.41±0.6^{c}	3.51±0.4^{c}	4.54±0.3^{d}	8.89±0.6^{c}	1.87±0.3^{c}	61.78^{b}
WF+PF-20%	19.32±0.5^{c}	3.45±0.4^{c}	4.38±0.3^{c}	8.56±0.5^{b}	1.93±0.4^{c}	64.80^{e}
WF+PF-30%	19.10±0.6^{b}	3.32±0.3^{b}	4.21±0.4^{b}	8.41±0.3^{b}	2.15±0.2^{d}	62..81^{c}
WF+PF-40%	18.90±0.4^{a}	3.23±0.2^{a}	4.18±0.2^{b}	8.10±0.6^{a}	2.21±0.1^{d}	63.38^{d}

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–e) are significantly different (p ≤ .05).

WF: Bread formulated from wheat flour; RF: Bread formulated from refined wheat flour;
WF+PF-10%: Bread formulated from wheat flour incorporated with 10% pearl millet flour;
WF+PF-20%: Bread formulated from wheat flour incorporated with 20% pearl millet flour;
WF+PF-30%: Bread formulated from wheat flour incorporated with 30% pearl millet flour;
WF+PF-40%: Bread formulated from wheat flour incorporated with 40% pearl millet flour

Table 2: Functional properties of flours

Sample	WAC (g/g)	OAC (g/g)	FC (%)	EC (%)
WF	1.90±0.3^{d}	1.54±0.5^{d}	8.32±0.6^{a}	16.51±0.8^{d}
PF	1.71±0.5^{a}	1.43±0.4^{a}	11.38±0.9^{a}	15.46±0.6^{a}
WF+PF-10%	1.87±0.4^{c}	1.52±0.6^{c}	8.48±0.7^{a}	16.41±0.5^{c}
WF+PF-20%	1.85±0.3^{c}	1.51±0.5^{c}	9.36±0.6^{a}	16.39±0.7^{c}
WF+PF-30%	1.81±0.4^{b}	1.49±0.4^{b}	10.28±0.8^{a}	16.25±0.8^{b}
WF+PF-40%	1.78±0.2^{b}	1.48±0.6^{b}	10.39±0.5^{a}	16.13±0.9^{b}

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–d) are significantly different (p ≤ .05).

WF: Wheat flour; PF: Pearl millet flour; WF+PF-10%: Wheat flour incorporated with 10% pearl millet flour; WF+PF-20%: Wheat flour incorporated with 20% pearl millet flour; WF+PF-30%: Wheat flour incorporated with 30% pearl millet flour; WF+PF-40%: Wheat flour incorporated with 40% pearl millet flour.

WAC: Water absorption capacity; OAC: Oil absorption capacity; FC: Foaming capacity; EC: Emulsion capacity

Functional Properties

Functional properties of flours were analysed and tabulated in Table 2. WAC shows the capacity of a product to associate with water under conditions where water is a limiting factor[28] and OAC of flour helps mouth feel and flavour retention. For WF the values of WAC and OAC were observed 1.90 g/g and 1.54 g/g while PM flour had 1.71g/g and 1.43g/g values while flour blends varied from 1.78-1.87g/g and 1.48-1.52g/g, respectively. Bhat *et al.*[29] reported that the WAC and OAC of WF were 0.83-0.84 g/g and 0.90-0.98 g/g, respectively. Siroha *et al.*[3] observed WAC and OAC 153-177% and 104-124% for the flours from different PM cultivars.

Table: 3 Pasting properties of flours

Sample	PV (mPa.s)	TV (mPa.s)	BV (mPa.s)	SV (mPa.s)	FV (mPa.s)	PT (°C)
WF	490±8^{e}	322±7^{d}	168±6^{e}	516±11^{d}	838±12^{e}	79.5±0.2^{a}
RF	690±11^{f}	520±8^{e}	170±5^{e}	550±10^{e}	1070±13^{f}	79.5±0.2^{a}
WF+PF-10%	389±6^{d}	235±5^{d}	154±7^{d}	521±8^{d}	756±9^{d}	87.4±0.1^{c}
WF+PF-20%	350±7^{c}	210±6^{c}	140±6^{c}	499±9^{c}	709±7^{c}	87.4±0.1^{c}
WF+PF-30%	270±5^{b}	189±8^{b}	81±5^{a}	401±9^{b}	590±8^{b}	87.4±0.1^{c}
WF+PF-40%	198±7^{a}	90±5^{a}	108±8^{b}	225±7^{a}	315±6^{a}	86.1±0.2^{b}

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–f) are significantly different ($p \le .05$).

WF: Wheat flour; RF: Refined wheat flour; WF+PF-10%: Wheat flour incorporated with 10% pearl millet flour; WF+PF-20%: Wheat flour incorporated with 20% pearl millet flour; WF+PF-30%: Wheat flour incorporated with 30% pearl millet flour; WF+PF-40%: Wheat flour incorporated with 40% pearl millet flour

PV: Peak viscosity; TV: Trough viscosity; BV: Breakdown viscosity; SV: Setback viscosity; FV: Final viscosity; PT: Pasting temperature

Emulsion capacity (E C), which shows the capacity of flour to emulsify oil varied significantly ($p<0.05$) for different flours. Emulsions are a class of disperse systems consisting of two immiscible liquids. EC for WF and PM flour were observed 16.51% and 15.46%. It was reduced with increase in the concentration of PM flour into WF and varied from 16.13 to 16.41%, respectively. Interfacial film formed by proteins affects the foaming capacity (FC) and stability, this films help the air bubbles in suspension and slows down the rate of fusion.[30] FC for WF and PM flour blends was observed 8.48 to 10.39%, respectively, the largest value was observed for 10% PM flour sample. After the addition of PM flour into the WF FC was increased.

Pasting Properties of Flours

Pasting properties give important information regarding the cooking characteristics of flours during heating and cooling cycles (Table 3). PV, TV, BV, FV and PT were observed 490, 322, 168, 838 mPa.s and 79.5ºC, respectively for WF. Refined WF had the highest value for PV, TV, SV and FV. After the incorporation of PM flour PV, TV, SV and FV were decreased as compared to WF sample. PT of flour blends varied from 86.1 to 87.4°C, the lowest value was observed for sample containing 40% PM flour. SV ranged from 225 to 550mPa.s, the highest was observed for refined WF. The lesser tendencies to retrograde in case of PM flour are an advantage in food products which undergoes decrease the viscosity and precipitation as a result of retrogradation.[31] PV, BV, TV and FV were observed 1184-1362, 183-649, 1000-1226 and 1938-1948cP, respectively for PM flour samples.[32,33] Punia *et al.*[27] reported PV, BV, FV and PT 171-526, 8-106, 452-721cP, and 49.9-65.7°C, respectively for different wheat cultivars.

Antioxidant Properties Flour and Breads

There are numerous methods to evaluate the antioxidant activity of foods, generally methods such as metal chelating activity, reducing power; DPPH assay and total antioxidant activity are used for the analysis of antioxidant activities.[35] DPPH is a stable free-radical compound which is widely used for calculation of free-radical scavenging ability of various samples. Antioxidant properties of flours and bread are reported in Figure 1. Antioxidant properties of WF and PM flour were observed 20.3 and 15.1%. The antioxidant activity for flour blends varied from 18.10 to 19.23%, respectively, the largest value was observed for sample containing 10% PM flour. Antioxidant properties of bread were observed from 19.75 to 21.93%, respectively. Antioxidant properties of bread increased as compared to their flours sample. Several authors claim that higher antioxidant properties of thermally processed foods could be due to the formation of Maillard products such as HMF (5-hydroxymethyl-2-furaldehyde), which render high antioxidant activity.[36, 37]

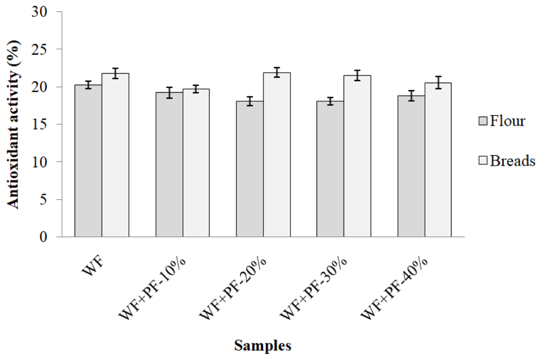

Fig. 1: Antioxidant properties of breads

Table 4: Physical characteristics of breads

Sample	LW (g)	LV (ml)	Specific loaf volume (ml/g)
WF	161±2[a]	525±6[d]	3.26±0.6[e]
WF+PF-10%	169±3[b]	512±4[c]	3.02±0.5[d]
WF+PF-20%	171±2[b]	471±5[b]	2.75±0.3[c]
WF+PF-30%	180±4[c]	467±5[b]	2.59±0.5[b]
WF+PF-40%	189±5[d]	440±6[a]	2.32±0.4[a]

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–e) are significantly different ($p \le .05$).

WF: Bread formulated from wheat flour; WF+PF-10%: Bread formulated from wheat flour incorporated with 10% pearl millet flour; WF+PF-20%: Bread formulated from wheat flour incorporated with 20% pearl millet flour; WF+PF-30%: Bread formulated from wheat flour incorporated with 30% pearl millet flour; WF+PF-40%: Bread formulated from wheat flour incorporated with 40% pearl millet flour

LW: Loaf weight; LV: Loaf volume

Physical Characteristics

Different physical properties of breads such as LV, LW and specific loaf volume were analysed and shown in Table 4. Different samples when weighed considering LW and results ranges from 161 to 189g, the highest value were observed for sample containing 40% PM flour sample. A significant ($p<0.05$) increase in LW was found with addition of PM flour resulted in extra water holding in bread during and after process of bread making process. Less retention of gas in composite dough provides denser bread texture which may be responsible for increase in LW. LV of breads prepared from WF and PM flour (10, 20, 30, & 40%) was observed 440 to 525ml, WF sample bread had the largest value. After the addition of PM flour LV was decreased, the highest decrease was observed for bread prepared from 40% PM flour. In 2012, Collar[38] explained that the reduction in LV in bread prepared from WF and PM flour is basically due to less retention of carbon dioxide gas which is caused by dilution effects on gluten and addition of non-gluten flour and also specific loaf volume is decreased when compared with the control.

Table 5: Sensory properties breads

Sample	Shape	Color	Texture	Flavor	Taste	Overall acceptability
WF	8.20±0.11^{e}	8.1±0.21^{d}	7.8±0.10^{e}	7.1±0.14^{c}	8.2±0.15^{d}	7.9±0.12^{c}
WF+PF-10%	7.9±0.14^{d}	7.1±0.18^{c}	7.3±0.12^{d}	6.8±0.12^{c}	7.9±0.11^{d}	7.1±0.14^{b}
WF+PF-20%	7.0±0.12^{c}	6.0±0.11^{b}	6.8±0.14^{c}	6.2±0.11^{b}	7.3±0.13^{c}	6.8±0.11^{b}
WF+PF-30%	6.9±0.15^{b}	6.4±0.16^{b}	6.1±0.12^{b}	5.9±0.14^{b}	6.3±0.15^{b}	5.5±0.12^{a}
WF+PF-40%	6.4±0.11^{a}	5.0±0.17^{a}	5.7±0.11^{a}	5.2±0.10^{a}	5.2±0.13^{a}	5.1±0.13^{a}

Values expressed are average of n = 3 (±standard deviation).
Averages in a column with different superscripts (a–e) are significantly different ($p \leq .05$).

WF: Bread formulated from wheat flour; WF+PF-10%: Bread formulated from wheat flour incorporated with 10% pearl millet flour; WF+PF-20%: Bread formulated from wheat flour incorporated with 20% pearl millet flour; WF+PF-30%: Bread formulated from wheat flour incorporated with 30% pearl millet flour; WF+PF-40%: Bread formulated from wheat flour incorporated with 40% pearl millet flour

Sensory Evaluation

The sensory properties and consumer acceptance are the major aspect for new products development. The sensorial scores for color, flavour, texture and overall acceptability reported by the test panel are tabulated in Table 5. Increasing levels of PM flour in bread formulation, sensory score and bread quality were affected significantly. PM flour addition show negatively effect on taste, flavour and overall acceptability of breads. A satisfactory sensory score was found when PM flour was added up to 30%into WF for bread. Addition of PM flour to WF at levels of 40% and more reduced the sensory properties due to PM properties such as flavor, bitter taste and darker color. Bread prepared from WF had the higher overall sensorial score. Although the liking by panellists decreased slightly with increasing in the level of PM flour into WF, but based on overall acceptability and general improvements in palatability scores, bread with 20% PM flour would be appropriate formulations to use in bakery goods.

Conclusion

Present study was carried out to evaluate the potential use of PM flour to enhance the nutritional and antioxidant properties of breads. Bread has high antioxidant capacity than their flours hence perform functionality to elevate the health profile of consumers. Bread prepared from PM flour had lower LV, higher LW and more bitter taste as compare to control sample. More than 30% increase in PM flour for preparation of bread showed darker and slightly bitter taste. Based on overall acceptability and general improvements in palatability scores, bread prepared with 20% PM flour would be appropriate formulations to use in bakery goods. Hence it is indeed a useful and profitable thing to consume foods in which PM flour was incorporated into WF and also improved nutritional composition. Therefore, it is possible to increase the amount of PM flour in the formulation of breads without affecting consumer acceptance.

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Conflict of interest
The authors have declared no conflict of interest.

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