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Exploring the Role of Baking Process on Technological, Functional, and α-Amylase Inhibition Properties of Pearl Millet Bread

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ABSTRACT: The absence of gluten is one of the greatest technological challenges in breadmaking process. This study examined how different methods (straight dough, st and sponge dough, sp) affect the properties of breads obtained exclusively with whole grain pearl millet flour. The breads were obtained by st and sp methods using different proportions of raw (RMF), extruded (EMF), and germinated millet flour (GMF). The formulation made with a mixture of raw, extruded, and germinated millet flour (REGMF) showed an increase in dietary fiber (6.3−10.8 g/100 g), phenolic compounds (3160.2−4131.8 mg GAE/100 g), and antioxidant capacity (1041.0−1923.3 μmol/100 g) compared with the st and sp processes, respectively. However, REGMFsp showed the greatest hardness (19.85 N) and amylose content (41.2%), but the combination of germinated flour and sponge dough method led to the highest value of total phenolic content (TPC = 4131.8 mg GAE/100 g) ($p < 0.05$). However, the (50:50) REMF formulation obtained by the sponge dough process showed the highest antioxidant capacity (2133.0 μmol/100 g) by the FRAP method ($p < 0.05$). Additionally, the REMF sample also presented the lowest IC_{50} value in both breadmaking processes (2.06 mg/mL, st, and 1.36 mg/mL, sp), which implies high α-amylase inhibition activity than the controls (wheat-based formulations). The results suggest that pearl millet-based breads are an excellent food resource for health promotion and can be included in the dietary management of both healthy individuals and celiacs or diabetics.

KEYWORDS: *gluten-free, breadmaking process, phenolic compounds, IC_{50}*

1. INTRODUCTION

Bread is one of the most representative foods in the world, consisting in an important food of the modern human diet.[1] Bread is commonly obtained by using wheat, which is mixed with water and salt, forming a viscoelastic matrix that provides the necessary development of the dough in order to be obtained. However, the market driver for gluten-free products is due to the wheat-related disorders in addition to the popular belief that these products are healthier.[2]

Different breadmaking processes are used to produce bread with different qualities and palatability,[3] in which straight dough has been the most used method.[4,5] On the other hand, the sponge dough method is characterized by a longer fermentation time that results in a more acidic dough, hence improving the texture and palatability of fiber-rich products besides enhancing the levels of bioactive compounds.[6]

Alternative matrices that mimic the breadmaking requirements are studied to obtain gluten-free breads since the lack of a viscoelastic network compromises product quality, especially texture.[7] Intentional addition of ingredients in order to compensate the absence of gluten leads to high carbohydrate and fat contents (especially saturated fatty acids), and low concentration of dietary fiber may increase the risk factor for the development of chronic noncommunicable diseases (NCDs).[8]

In this context, millets can be considered as an excellent source of alternative material due to their high nutritional value and hypoglycemic properties.[9] Pearl millet (*Pennisetum glaucum* (L) R. Br.) is an interesting source of proteins (9.9 g/100 g), zinc (5.5 mg/100 g), iron (8.48 mg/100 g), and dietary fiber content (9.5 g/100 g).[10,11] Furthermore, millets contain significant amounts of phytochemicals that are associated with antioxidant and hypoglycemic properties by inhibiting the activity of pancreatic α-glucosidases, and α-amylases.[12] However, the type of millet processing affects the concentration and bioavailability of nutrients in terms of phytochemicals such as polyphenols.[13] Phenolics, particularly ferulic and *p*-coumaric, increase the nutritional value of pearl millet grains, and together with dietary fiber, can contribute to improving glycemic and insulinemic control.[14]

Breads made exclusively from 100% whole pearl millet flour demonstrated appropriate physical characteristics, presenting antihypoglycemic activity.[15] However, comparison between breadmaking processes of pearl millet-based breads has not been reported in the literature. Therefore, the present study was conducted in order to evaluate the impact of straight and sponge dough processes on the physical properties, nutritional quality, bioactive compounds profile, antioxidant capacity, and α-amylase inhibition activity.

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Table 1. Bread Formulations and Particle Size Distribution of the Millet Flours[a]

	ingredients (%)									
samples	raw flour	extruded flour	germinated millet flour	fat	yeast	sugar	eggs	salt	water absorption*	D [4,3] (μm)
RMF	100			6	3	6	35	2	45.01	164.85^{c} ± 2.6
EMF		100		6	3	6	35	2	51.00	148.08^{c} ± 26.7
REMF	50	50		6	3	6	35	2	70.80	173.60^{c} ± 12.7
REGMF	47.5	47.5	5.0	6	3	6	35	2	47.74	263.65^{b} ± 28.6
RGMF	95		5	6	3	6	35	2	45.29	150.01^{c} ± 6.4
EGMF		95	5	6	3	6	35	2	54.30	272.90^{ab} ± 11.2
WWF	100			6	3	6	35	2	55.00	314.63^{a} ± 46.9
RWF	100			6	3	6	35	2	55.00	75.92^{d} ± 3.6

[a]RMF: raw millet flour; EMF: extruded millet flour; REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; RGMF: raw and germinated millet flour; EGMF: extruded and germinated millet flour; WWF: whole wheat flour, and RWF: refined wheat flour; *Water absorption was based on farinograph parameters shown in the Supporting Information (Figure S2); D [4,3]: volume or mass moment mean. Values are mean ± standard deviation. Different letters in the same columns indicate statistical differences ($p < 0.05$) among samples.

2. MATERIALS AND METHODS

2.1. Materials. Pearl millet (*P. glaucum* (L.) R. Br.) (hybrid ADR 9070) was donated by Atto Sementes (Rondonópolis, Brazil). Whole wheat flour (WWF), refined wheat flour (RWF), and other ingredients (whole eggs, sugar, palm fat, salt, and dry yeast) were acquired at a local market in Rio de Janeiro, Brazil. Acarbose (ref 56180-94-0) and α-amylase from porcine pancreas (ref A3176) were purchased from Sigma-Aldrich (Darmstadt, Alemanha).

2.2. Technological Flour Characterization. *2.2.1. Preparation of Flour.* Grains were cleaned using a Clipper Office Tester 400/B (A.T. Ferrell Co., Indiana, USA) and ground in a disc mill 3600 (Perten Instruments, Huddinge, Sweden) and a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm opening screen for obtaining fine raw millet flour (RMF).

2.2.2. Germination. Grains were soaked in water (1:3) (grain/water) for 1 h and then drained. The grains were germinated in a bread fermentation cabinet (National Mfg. Co., Lincoln, USA) at 30 °C and a relative humidity of 90%. After 24 h, the grains were dried in a fan oven model DMS-G.E (Macanuda, Joinville, Brazil) at 45 °C/24 h and ground using a disc mill and hammer mill (Perten Instruments, Huddinge, Sweden) in order to obtain germinated whole grain millet flour (GMF).

2.2.3. Extrusion Cooking. Prior to extrusion, RMF was preconditioned to reach a final moisture content of 15% (wb). The flour was extruded in a single-screw laboratory extruder 19/20 DN (Brabender, Duisburg, Germany) attached to a Plasti-Corder Lab-Station (Brabender, Duisburg, Germany). The extruder was equipped with a 3 mm circular die, a 3:1 compression screw, running at a screw speed of 200 rpm, and a temperature profile of 40, 80, and 90 °C, from the inlet to the outlet. The extrudates were dried in a fan oven at 55 °C for 10 h, followed by grinding the dried extrudates in order to obtain an extruded millet flour (EMF) using the same conditions as raw millet flour preparation. The torque and temperature profile versus time recording are presented in Figure S1 (Supporting Information).

2.2.4. Particle Size Distribution. Flour particle size distribution was measured by laser diffraction using a S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) according to method 55−40.01[16] using deionized water as a dispersant. Particle sizes were expressed in terms of a mean particle size of D [4,3] (volume or mass moment mean) calculated by the Flex Software, version 11.0.0.3 (Microtrac Inc., Montgomeryville, USA).

2.2.5. Paste Viscosity. The apparent paste viscosity was analyzed in a Rapid Viscosity Analyzer series RVA-4 (Newport Scientific Pty Ltd., Warriewood, Australia) according to the methodology reported by Carvalho et al.[17] The following apparent viscosity properties were evaluated as a function of temperature: paste temperature (T_p) (°C), cold viscosity at the beginning 25 °C (CV, cP), peak viscosity (PV, cP), trough viscosity or holding strength (TV, cP), breakdown viscosity (BDV = PV − TV, cP), final viscosity (FV, cP), and setback viscosity (SBV = FV − TV, cP).

2.3. Bread Elaboration. A straight and sponge dough were prepared according to 10-10.03 and 10-11.01 methods,[18,19] with modifications. Breads were obtained following the formulations presented in Table 1. The maximum water absorption of doughs was previously determined using a FD0234H Farinograph (Brabender, Duisburg, Germany) (Supporting Information, Figure S2) according to the 54-21.02 method[20] fitted with a 50 g mixer capacity.

Both dough preparations were performed using a 35 g Micromixer (National MFG. CO., Lincoln, USA) (Supporting Information, Figure S3 a). In the case of the straight dough (st) method, instant yeast (Fleischmann, Pederneiras, Brazil) was previously activated with deionized water (1/3 of the total formulation water) at 35 °C and placed in a fermentation chamber Crescepão ACT20 (Venâncio Metalúrgica, Venâncio Aires, Brazil) at 85% relative humidity for 10 min. Then, the activated yeast and all dry ingredients were mixed before adding the liquid ingredients and palm fat.

Regarding the sponge dough (sp) method, a light and airy "sponge" was created by mixing a portion of flour, instant yeast (previously activated), and water, followed by 4 h of fermentation and subsequent incorporation of the remaining ingredients into the "sponge". After mixing all the ingredients, the dough was portioned in 20 g, molded, and placed into a previously greased and floured steel molds of 45 mL capacity (Supporting Information, Figure S3 b), then placed in a fermentation cabinet chamber Crescepão ACT20 (Venâncio Metalúrgica, Venâncio Aires, Brazil) at 30 °C and 85% relative humidity for 45 min. The breads were baked using a Maxiconv VP convection oven (Metalúrgica Skymsen, Brusque, Brazil) at 180 °C for 12 min and then left at room temperature for 1 h for further analysis (Supporting Information, Figure S3c). Two control samples were also prepared using whole wheat flour (WWF) and refined wheat flour (RWF).

2.4. Bread Technological Characterization. *2.4.1. Color Measurements.* The colors of bread crust (ct) and crumb (cb) were measured by reflectance with a portable colorimeter CR-400 (Konica Minolta, Tokyo, Japan) using illuminant D65/2°. The results were expressed according to the International Commission on Illumination (CIE) L^* a^* b^* color space parameters. The changes in the bread crust and crumb color were estimated according to browning index (BI) and the Chroma (C^*) as follows[21]

$$\mathrm{BI} = \frac{100(x - 0.31)}{0.17} \tag{1}$$

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \tag{2}$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \tag{3}$$

2.4.2. Specific Volume Analysis. Bread-specific volume was determined using a standard seed displacement method 10-05.01 using proso millet instead of rape seeds.[22] The specific volume (SV) was calculated as bread volume was divided by bread weight (cm^3/g).

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2.4.3. Texture Analysis. The texture profile analysis (TPA) was applied to evaluate hardness (Hd, N), adhesiveness (Ad, g·s), cohesiveness (Co), springiness (Sp), chewiness (Ch, N), and resilience (R) using a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell and a 15 mm aluminum cylindrical probe. Each formulation was measured using 12 slices that were 20 mm thick. The analysis was controlled by the Exponent software version 6.1.11.0 (Stable Micro Systems, Surrey, UK) at a compression of 50% and 30 s cycle according to Comettant-Rabanal et al.[5]

2.5. Bread Nutritional Characterization. *2.5.1. Chemical Composition.* The chemical composition of the experimental breads and commercial samples was performed according to the AOAC[23] methods: 2001.11 (factor of 5.75) (protein), 945.38 (fat), 923.03 (ash), 991.43 (total dietary fiber), and 925.09 (moisture). The energy value was determined according to the Collegiate Board Resolution CBR $n°$ 360, and the carbohydrate content was calculated by difference.[24]

2.5.2. Total Starch, Amylose, and Resistant Starch Contents. Total starch (TS), amylose, and resistant starch (RS) contents of breads were determined using the Assay kits K-TSTA-100A method 76−13.01, K-AMYL, method 79-13, and K-RSTAR, method 32-40.01 (Megazyme, Wicklow, Ireland), respectively.[25]

2.6. Determination of Phenolic Content and Antioxidant Capacity. *2.6.1. Sequential Extraction of Free and Bound Phenolic Compounds.* In order to evaluate phenolic compounds and antioxidant capacity, the breads were freeze-dried using a benchtop lyophilizer L101 (Liobras, São Carlos, Brazil), resulting in a moisture content that varied from 3.16 to 4.20%. Phenolic compounds of lyophilized breads were extracted in triplicate according to the method reported by Santos et al.,[26] with modifications. Free phenolic compounds were extracted by manual maceration of 210 mg of each sample and 50 mg of Celite in the presence of 3.0 mL of ethanol (80%). The samples were shaken (200 rpm, 10 min, 25 °C) and centrifuged at 5000 g for 10 min at 25 °C (Sorvall ST8, Thermo Fisher Scientific, Massachusetts, USA). The supernatants were retained, and the extraction was repeated. The supernatants of each replicate were dried in an evaporator centrifuge Savant, SpeedVac Concentrator (Thermo Fisher Scientific, Waltham, USA). The pellets resulting from extraction of free phenolic compounds were resuspended with 4 M NaOH and then placed in an ultrasonic bath (42 kHz) for 90 min at 40 °C (Cristófoli Ultrasound Cuba, Campo Mourão, Brazil). After the alkaline hydrolysis, the pH was adjusted to below 2.0 with concentrated HCl, and the samples were centrifuged at 2000 g for 5 min. The supernatant was washed three times with ethyl acetate and centrifuged between each step (10,000 g, 5 min, 10 °C). All supernatants were collected and dried. The dried extracts were resuspended in 1.5 mL of solution A containing 2% methanol, 5% acetonitrile, and 93% ultrapure water and then filtered (13 mm, 0.22 μm), transferred to vials, and stored at −20 °C until analysis.

2.6.2. Determination of Total Phenolic Content (TPC). Total phenolic content was estimated by measuring their capacity to reduce Folin−Ciocalteu reagent in triplicate according to Singleton et al.,[27] adapted to microplates. Absorbance was determined at 750 nm on a FlexStation III microplate reader (Molecular Devices, LLC., San Jose, USA). The phenolic content was calculated using a gallic acid standard (5−130 mg/L) calibration curve. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample (dry basis).

2.6.3. DPPH Free Radical Scavenging Capacity. The DPPH radical scavenging ability of bread extracts was evaluated according to Pires et al.[28] Briefly, the extract was mixed with DPPH solution and incubated for 30 min in the dark at room temperature after shaking vigorously. Samples were analyzed at 517 nm by using a microplate reader. The antioxidant capacity was calculated using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as the standard for the calibration curve (0−200 μg/mL; R^2 = 0.9976), and the results were expressed in mg of Trolox equivalents (mg TE/g of sample, dry basis).

2.6.4. Determination of Ferric Reducing Antioxidant Power (FRAP). The reducing capacity of the bread extract was evaluated according to Urrea-Victoria et al.[29] Absorption was measured at 595 nm using a microplate reader, after 30 min incubation at 37 °C. Antioxidant capacity was calculated using a standard curve (0−6.5 μmol/mL; R^2 = 0, 9972), and the results were expressed in μmol of Trolox equivalents (μmol Trolox/100 g of sample, dry basis).

2.7. α-Amylase Inhibition Activity of Breads. The inhibition assay in vitro for the α-amylase activity was based on Aleixandre et al.,[30] with some modifications. Briefly, the substrate (6.25 mg/mL) was prepared from a solution of wheat starch in sodium phosphate buffer (0.02 M, pH 6.9, containing NaCl 6 mM), followed by starch gelatinization in a water bath at 100 °C for 20 min and kept at 37 °C until used in the reaction. 50 μL of sample extract was added to a test tube and placed in a water bath at 37 °C, followed by the addition of 50 μL of α-amylase (2.5 U/mL) and homogenization. The tubes were kept in incubation for 10 min, and then 400 μL of substrate was added for another 10 min. Afterward, 0.5 mL of DNS (3,5-dinitrosalicylic acid) was added, followed by a new incubation at 100 °C for 10 min and finally added of deionized water (9 mL). Samples were analyzed at 540 nm in a spectrophotometer model wuv-m51 (Weblabor, Milano, Italy). A reference sample was obtained using the hypoglycemic drug Acarbose, ref 56180−94−0 (Sigma-Aldrich, Darmstadt, Germany). The percentage of inhibition of the extracts was calculated according to eq 4.

$$\%\ \text{Enzyme inhibition} = \left[1 - \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{negative control}}}{\text{Abs}_{\text{positive control}} - \text{Abs}_{\text{blank control}}}\right] \times 100 \quad (4)$$

where $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (extract + substrate + enzyme), $\text{Abs}_{\text{negative control}}$ is the absorbance obtained without the enzyme, $\text{Abs}_{\text{positive control}}$ is the absorbance without extracts, and $\text{Abs}_{\text{blank}}$ is the absorbance of the substrate.

IC_{50} was calculated from a linear regression curve obtained by different concentrations (140.00−0.14 mg/mL) of free and bound extracts.

2.8. Statistical Analysis. Statistical analysis was performed with one-way ANOVA, followed by Tukey's HSD test ($p < 0.05$) using XLSTAT software version 2023.2.0 (Lumivero, Denver, USA). Principal Component Analysis (PCA), Clustering Analysis (HCA), and Radar Chart analyses were performed by XLSTAT. Pearson correlation and the significance test were generated in the R package "corrplot" by RStudio software version 1.2.5042 (RStudio, Boston, USA).

3. RESULTS AND DISCUSSION

3.1. Flour Characterization. *3.1.1. Particle Size Distribution of Flours.* The particle size (D, [4.3]) of EMF (148.0 μm) and RGMF (150.0 μm) presented the lowest values among pearl millet flours (Table 1). Particle size is an important factor related to bakery products, as it affects the rheological characteristics of the dough and the final product texture. Flours that present smaller particles increased the level of starch damage, i.e., starch is more available to enzymatic reaction. Greater accessibility to starch increases CO_2 production during fermentation in addition to promoting a high degree of starch breakdown and retrogradation favored by the formation of a three-dimensional network, hence mimicking the viscoelastic properties of gluten at some extent and contributing to improve the final structure of the gluten-free dough.[31,32] As demonstrated by Azeem et al.,[33] gluten-free breads using sweet potato flour with a smaller particle size (45 μm) exhibited better physical characteristics compared to other particle sizes (75, 106, 180, and 355 μm).

3.1.2. Pasting Profile of Flours. Pasting temperature, peak viscosity, breakdown viscosity, final viscosity, and setback of

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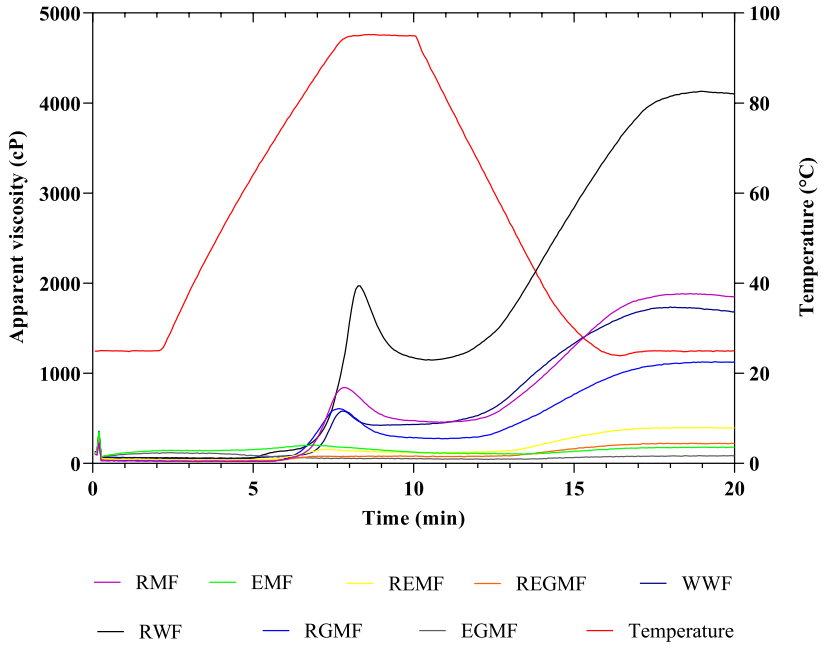

Figure 1. Paste viscosity properties of (i) raw millet flour (RMF); (ii) extruded millet flour (EMF); (iii) raw and extruded millet flour (REMF); (iv) raw, extruded, and germinated millet flour (REGMF); (v) extruded and germinated millet flour (EGMF); (vi) raw and germinated millet flour (RGMF); (vii) WWF (whole wheat flour), and (viii) RWF (refined wheat flour).

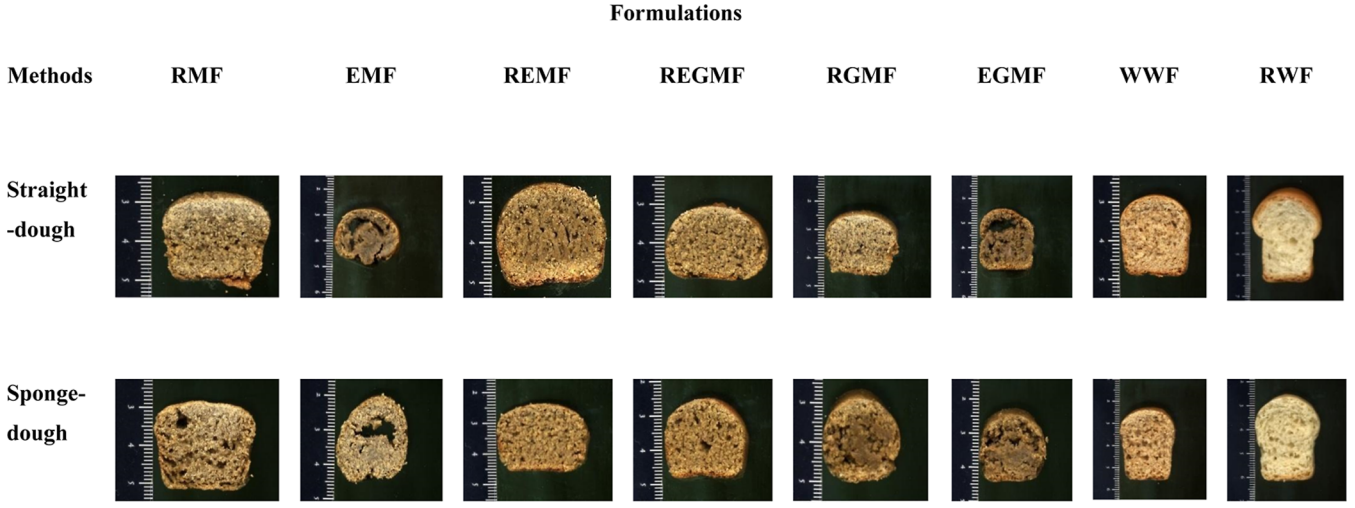

Figure 2. Photographs of slice section millet breads elaborated with (i) raw millet flour (RMF); (ii) extruded millet flour (EMF); (iii) raw and extruded millet flour (REMF); (iv) raw, extruded, and germinated millet flour (REGMF); (v) extruded and germinated millet flour (EGMF); (vi) raw and germinated millet flour (RGMF); controls (vii) WWF (whole wheat flour); and (viii) RWF (refined wheat flour) obtained by two breadmaking processes.

millet flours ranged from 28.2 to 90.8 °C, 80.5 to 844.5 cP, 3.5 to 389.0 cP, 81.5 to 1840.0 cP, and 38.5 to 1384.5 cP, respectively. The REGMF (Figure 1) showed the lowest BDV value (Supporting Information, Table S1), which may characterize advantageous effects of using this flour due to the starch resistance to heat and shear stress during cooking, besides showing lower tendency to retrograde.[34] In contrast, pasting properties of refined wheat flour showed maximum values of PV (1232.0 cP), setback (4097.5 cP), and FV (4097.5 cP), as expected due to its high total starch content.

EMF and EGMF presented values of PV, BDV, SBV, and FV significantly lower than RMF ($p < 0.05$) (Supporting Information, Table S1). These findings can be explained by the combination of heat and mechanical shear during extrusion cooking, leading to broken starch granules. As the crystalline arrangement and cold water solubility are changed by the shear heat process cause a reduction in viscosity readings by RVA.[35] This observation was in accordance with Patil et al.[36] and Kumar et al.[9] that show a significant decrease of the same pasting properties of pearl millet flour when extruded finger millet flour was added. The addition of GMF to EMF also contributed to a decrease in viscosities ranging from 203.0 cP (EMF) to 120.0 cP (EGMF). The decrease in viscosity after germination can be attributed to starch degradation caused by α-amylase activity.[37]

3.2. Quality Evaluation of Bread. *3.2.1. Instrumental Color Analysis.* The crumb and crust color parameters of breads are presented in Figure 2 and Table 2. As expected, the

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Table 2. Instrumental Color Parameters and Specific Volume of Pearl Millet-Based Breads[a]

samples		crumb color				crust color				SV (cm^3/g)
		L^*	a^*	b^*	BI	L^*	a^*	b^*	BI	
straight-dough	RMF	$50.67^{fgh} \pm 0.82$	$3.34^{cde} \pm 0.17$	$11.53^{e} \pm 0.19$	$30.22^{de} \pm 1.28$	$40.07^{g} \pm 2.83$	$9.35^{b} \pm 0.34$	$12.06^{efg} \pm 2.08$	$52.27^{d} \pm 2.56$	$1.39^{ef} \pm 0.05$
	EMF	$54.87^{d} \pm 0.85$	$3.26^{de} \pm 0.03$	$12.52^{de} \pm 0.09$	$29.80^{def} \pm 0.28$	$41.86^{defg} \pm 0.17$	$6.14^{bc} \pm 0.09$	$11.92^{efg} \pm 0.37$	$43.77^{de} \pm 1.18$	$1.63^{cdef} \pm 0.07$
	REMF	$62.59^{c} \pm 1.17$	$2.78^{e} \pm 0.15$	$15.69^{bc} \pm 0.11$	$31.56^{bcde} \pm 0.63$	$42.24^{defg} \pm 0.94$	$8.44^{bc} \pm 0.41$	$12.93^{def} \pm 0.35$	$50.62^{d} \pm 0.81$	$1.45^{def} \pm 0.12$
	REGMF	$53.34^{defg} \pm 0.17$	$3.67^{bcd} \pm 0.06$	$14.14^{cd} \pm 0.27$	$35.32^{abcd} \pm 0.64$	$40.49^{fg} \pm 1.65$	$9.30^{b} \pm 1.29$	$9.46^{fg} \pm 1.10$	$42.86^{de} \pm 0.57$	$1.43^{def} \pm 0.10$
	EGMF	$50.64^{fgh} \pm 0.04$	$3.51^{bcde} \pm 0.03$	$11.25^{e} \pm 0.17$	$29.76^{def} \pm 0.45$	$41.83^{defg} \pm 0.72$	$7.38^{bc} \pm 0.27$	$9.03^{g} \pm 0.49$	$36.80^{d} \pm 0.33$	$1.73^{ef} \pm 0.15$
	RGMF	$51.55^{defgh} \pm 1.81$	$3.14^{de} \pm 0.19$	$11.91^{de} \pm 0.45$	$30.27^{de} \pm 0.36$	$41.65^{efg} \pm 2.51$	$8.89^{bc} \pm 2.25$	$12.29^{efg} \pm 1.24$	$50.10^{e} \pm 3.10$	$1.57^{def} \pm 0.06$
	WWF	$65.24^{c} \pm 0.78$	$5.81^{a} \pm 0.26$	$17.61^{ab} \pm 0.13$	$37.49^{a} \pm 1.09$	$47.05^{cde} \pm 2.38$	$15.18^{a} \pm 0.15$	$16.63^{cd} \pm 2.33$	$66.25^{bc} \pm 3.95$	$1.96^{cd} \pm 0.23$
	RWF	$77.80^{a} \pm 0.91$	$-0.46^{f} \pm 0.02$	$17.33^{ab} \pm 0.04$	$24.15^{f} \pm 0.37$	$54.01^{b} \pm 0.0$	$15.43^{a} \pm 0.45$	$25.93^{ab} \pm 0.62$	$84.65^{a} \pm 2.64$	$3.83^{a} \pm 0.52$
sponge-dough	RMF	$54.42^{de} \pm 0.42$	$3.20^{de} \pm 0.14$	$12.30^{de} \pm 0.45$	$29.49^{def} \pm 1.50$	$46.53^{cdef} \pm 0.21$	$9.12^{b} \pm 0.49$	$18.09^{c} \pm 0.26$	$62.77^{c} \pm 1.32$	$1.69^{cde} \pm 0.13$
	EMF	$49.72^{gh} \pm 1.29$	$3.62^{bcd} \pm 0.18$	$11.49^{e} \pm 0.22$	$31.16^{cde} \pm 0.62$	$42.88^{defg} \pm 0.16$	$7.56^{bc} \pm 0.01$	$12.32^{efg} \pm 0.23$	$46.30^{de} \pm 0.56$	$1.54^{def} \pm 0.16$
	REMF	$53.50^{def} \pm 1.50$	$3.48^{bcde} \pm 0.07$	$13.44^{cde} \pm 0.40$	$33.19^{abcde} \pm 0.20$	$42.82^{defg} \pm 0.55$	$6.76^{bc} \pm 0.07$	$12.73^{efg} \pm 0.50$	$46.32^{de} \pm 0.82$	$1.14^{f} \pm 0.06$
	REGMF	$50.83^{efgh} \pm 0.37$	$4.06^{bc} \pm 0.02$	$13.48^{cde} \pm 0.16$	$36.16^{abc} \pm 0.07$	$42.96^{defg} \pm 0.45$	$8.13^{bc} \pm 0.17$	$12.87^{defg} \pm 0.60$	$48.97^{d} \pm 2.85$	$1.61^{def} \pm 0.20$
	EGMF	$49.62^{h} \pm 0.24$	$4.12^{b} \pm 0.27$	$13.09^{de} \pm 0.83$	$36.20^{abc} \pm 2.45$	$43.69^{defg} \pm 0.26$	$6.56^{bc} \pm 0.31$	$12.69^{efg} \pm 0.42$	$44.82^{de} \pm 1.57$	$1.36^{ef} \pm 0.06$
	RGMF	$53.79^{def} \pm 0.27$	$3.38^{bcde} \pm 0.01$	$13.07^{de} \pm 0.06$	$31.95^{abcde} \pm 0.35$	$47.79^{cd} \pm 1.38$	$5.45^{c} \pm 0.67$	$15.47^{cde} \pm 0.12$	$46.89^{d} \pm 3.00$	$1.41^{ef} \pm 0.09$
	WWF	$63.68^{c} \pm 0.25$	$5.65^{a} \pm 0.25$	$17.00^{ab} \pm 0.24$	$37.07^{ab} \pm 0.62$	$51.28^{bc} \pm 1.11$	$13.29^{a} \pm 0.53$	$22.53^{b} \pm 0.79$	$75.39^{ab} \pm 0.26$	$2.16^{c} \pm 0.09$
	RWF	$71.03^{b} \pm 1.14$	$-1.04^{f} \pm 0.50$	$18.09^{a} \pm 1.92$	$27.62^{ef} \pm 4.57$	$67.46^{a} \pm 2.99$	$5.95^{bc} \pm 1.94$	$29.63^{a} \pm 0.06$	$62.97^{c} \pm 5.97$	$2.75^{b} \pm 0.07$

[a]RMF: raw millet flour; EMF: extruded millet flour; GMF: germinated wheat flour; REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; EGMF: extruded and germinated millet flour; RGMF: raw and germinated millet flour; WWF: whole wheat flour; RWF: refined wheat flour; BI: browning index; and SV: specific volume. Values are mean ± standard deviation. Different letters in the same columns indicate statistical differences ($p < 0.05$) among samples.

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Table 3. Texture Profile Analysis (TPA) of Crumb Millet-Based Breads by Straight and Sponge Dough Methods[a]

	samples	hardness (N)	adhesiveness (g s)	cohesiveness (−)	springiness (−)	chewiness (N)	resilience (−)
straight-dough	REMF	$12.62^{c} \pm 1.62$	$44.49^{ab} \pm 39.56$	$0.22^{d} \pm 0.03$	$0.80^{a} \pm 0.40$	$2.35^{c} \pm 1.86$	$0.08^{d} \pm 0.01$
	REGMF	$12.59^{c} \pm 0.61$	$64.48^{ab} \pm 27.18$	$0.30^{c} \pm 0.04$	$0.71^{a} \pm 0.25$	$2.83^{abc} \pm 1.35$	$0.09^{d} \pm 0.0$
	WWF	$9.208^{d} \pm 0.82$	$11.54^{a} \pm 4.11$	$0.47^{b} \pm 0.03$	$0.94^{a} \pm 0.02$	$4.03^{ab} \pm 0.29$	$0.15^{c} \pm 0.01$
	RWF	$7.48^{de} \pm 1.11$	$2.31^{b} \pm 3.92$	$0.58^{a} \pm 0.04$	$0.97^{a} \pm 0.02$	$4.28^{a} \pm 0.61$	$0.22^{b} \pm 0.01$
sponge-dough	REMF	$15.51^{b} \pm 1.50$	$40.25^{ab} \pm 34.74$	$0.27^{cd} \pm 0.09$	$0.70^{a} \pm 0.19$	$3.03^{abc} \pm 1.72$	$0.09^{d} \pm 0.0$
	REGMF	$20.25^{a} \pm 2.78$	$20.96^{ab} \pm 28.41$	$0.20^{d} \pm 0.04$	$0.79^{a} \pm 0.18$	$3.30^{abc} \pm 1.14$	$0.06^{d} \pm 0.01$
	WWF	$5.88^{e} \pm 1.31$	$4.17^{ab} \pm 3.22$	$0.44^{b} \pm 0.02$	$0.96^{a} \pm 0.03$	$2.54^{bc} \pm 0.64$	$0.15^{c} \pm 0.01$
	RWF	$3.16^{f} \pm 0.83$	$0.51^{a} \pm 0.85$	$0.58^{a} \pm 0.05$	$1.05^{a} \pm 0.24$	$1.97^{c} \pm 0.77$	$0.26^{a} \pm 0.03$

[a]REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; WWF: whole wheat flour; RWF: refined wheat flour; values are mean ± standard deviation, $n = 12$ (TPA). Different letters in the same columns indicate statistical differences ($p < 0.05$) among samples.

Table 4. Proximate Composition (g/100 g), Dietary Fiber (g/100 g), and Energy Value (kcal/100 g) of Pearl Millet-Based Breads[a]

	samples	moisture	ash	protein	lipids	carbohydrates	dietary fiber	energy value
straight-dough	RMF	$26.94^{i} \pm 0.12$	$2.37^{ab} \pm 0.08$	$10.14^{def} \pm 0.03$	$7.55^{def} \pm 0.41$	$53.0^{cd} \pm 0.16$	$6.96^{cde} \pm 0.00$	292.62
	EMF	$34.10^{c} \pm 0.04$	$2.11^{cde} \pm 0.09$	$9.48^{g} \pm 0.16$	$4.78^{k} \pm 0.12$	$49.52^{i} \pm 0.34$	$6.37^{cde} \pm 0.11$	253.58
	REMF	$28.94^{fg} \pm 0.31$	$2.34^{abc} \pm 0.03$	$10.06^{defg} \pm 0.08$	$7.02^{efgh} \pm 0.18$	$51.64^{def} \pm 0.61$	$6.42^{cde} \pm 0.42$	284.34
	REGMF	$30.55^{e} \pm 0.48$	$2.24^{bcd} \pm 0.04$	$9.82^{efg} \pm 0.16$	$7.94^{cde} \pm 0.04$	$49.45^{i} \pm 0.55$	$6.34^{cde} \pm 0.77$	283.17
	EGMF	$28.23^{gh} \pm 0.28$	$2.25^{bcd} \pm 0.04$	$10.06^{defg} \pm 0.08$	$5.98^{ijk} \pm 0.25$	$53.49^{c} \pm 1.0$	$7.72^{bcd} \pm 1.16$	277.18
	RGMF	$26.24^{ij} \pm 0.18$	$2.33^{abc} \pm 0.13$	$10.37^{cde} \pm 0.12$	$8.26^{bcd} \pm 0.02$	$52.8^{cd} \pm 0.16$	$7.55^{bcde} \pm 0.84$	296.66
	WWF	$22.03^{m} \pm 0.00$	$2.03^{de} \pm 0.04$	$11.23^{ab} \pm 0.20$	$8.60^{bc} \pm 0.27$	$56.11^{b} \pm 0.03$	$5.44^{de} \pm 0.00$	324.99
	RWF	$20.78^{l} \pm 0.09$	$1.65^{g} \pm 0.05$	$9.83^{efg} \pm 0.08$	$6.61^{fghi} \pm 0.20$	$61.14^{a} \pm 0.43$	$4.5^{de} \pm 0.73$	325.37
sponge-dough	RMF	$25.94^{j} \pm 0.09$	$2.48^{ab} \pm 0.04$	$10.83^{bc} \pm 0.12$	$9.69^{a} \pm 0.02$	$51.06^{efgh} \pm 0.09$	$7.07^{bcde} \pm 0.56$	306.53
	EMF	$32.36^{d} \pm 0.03$	$2.25^{bcd} \pm 0.06$	$9.68^{fg} \pm 0.20$	$5.58^{jk} \pm 0.20$	$50.14^{ghi} \pm 0.43$	$4.71^{de} \pm 1.22$	270.7
	REMF	$28.92^{fg} \pm 0.22$	$2.40^{ab} \pm 0.01$	$10.60^{cd} \pm 0.03$	$7.29^{efg} \pm 0.09$	$50.79^{fgh} \pm 0.26$	$9.75^{abc} \pm 0.93$	272.12
	REGMF	$27.86^{h} \pm 0.17$	$2.50^{a} \pm 0.04$	$10.86^{bc} \pm 0.07$	$7.81^{cde} \pm 0.18$	$50.97^{fgh} \pm 0.48$	$10.83^{ab} \pm 0.98$	274.33
	EGMF	$29.15^{f} \pm 0.09$	$2.40^{ab} \pm 0.07$	$10.29^{cdef} \pm 0.0$	$5.72^{jk} \pm 0.11$	$52.45^{cde} \pm 0.28$	$8.05^{bcd} \pm 1.14$	270.28
	RGMF	$26.33^{ij} \pm 0.12$	$2.44^{ab} \pm 0.04$	$10.86^{bc} \pm 0.07$	$9.12^{ab} \pm 0.25$	$51.25^{efg} \pm 0.0$	$12.61^{a} \pm 1.97$	280.12
	WWF	$23.20^{k} \pm 0.07$	$2.26^{abcd} \pm 0.06$	$11.61^{a} \pm 0.32$	$6.13^{hij} \pm 0.14$	$56.81^{b} \pm 0.45$	$9.94^{abc} \pm 0.77$	289.13
	RWF	$21.95^{l} \pm 0.07$	$1.72^{fg} \pm 0.02$	$9.88^{efg} \pm 0.16$	$6.32^{ghij} \pm 0.66$	$60.13^{a} \pm 0.45$	$3.84^{e} \pm 1.69$	321.60
commercial	WWB	$37.05^{b} \pm 0.07$	$2.01^{de} \pm 0.03$	$10.78^{bc} \pm 0.28$	$0.47^{m} \pm 0.09$	$49.70^{hi} \pm 0.14$	$7.18^{bcde} \pm 0.37$	217.42
	GFB	$39.70^{a} \pm 0.24$	$1.94^{ef} \pm 0.05$	$3.44^{h} \pm 0.07$	$2.92^{l} \pm 0.06$	$52.0^{def} \pm 0.43$	$6.43^{cde} \pm 0.24$	222.32

[a]RMF: raw millet flour; EMF: extruded millet flour; REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; WWF: whole wheat flour; RWF: refined wheat flour; WWB: commercial whole wheat bread; and GFB: commercial gluten-free bread. Values are mean ± standard deviation, $n = 2$. Different letters in the same columns indicate statistical differences ($p < 0.05$) among samples.

crumb of refined wheat flour bread presented the highest value of L^* and the lowest BI ($p < 0.05$) in both breadmaking processes. In general, all millet breads showed lower values of L^* and BI than the controls (whole and refined wheat breads) as much as crumb or crust. These results can be attributed to the polyphenol content present in the pericarp, in the aleurone layer, and in the endosperm of the millet grain.[38] A similar decrease of L^* values of bread with finger millet flour substitution was also reported in Mudau et al.[39] However, the L^* of sponge dough crust breads was higher ($p < 0.05$) than straight dough samples. Higher values in the crust compared to the crumb are derived from the Maillard and caramelization reactions, i.e., a nonenzymatic browning that is influenced by the distribution of water and the reaction between reducing sugars and amino acids during the baking step.[40] Color is an important parameter that drives consumer preferences mainly related to purchase intentions since gluten-free breads commonly present a pale color, showing an artificial appearance as opposite to wheat-based bread expectances.[41,42]

3.2.2. Specific Volume. Visual appearance and specific volume are shown in Figure 2 and Table 2. The specific volume ranged from 1.14 to 1.73 cm^3/g in which all formulations that used millet flour showed lower significant values ($p < 0.05$) than WWF (1.96−2.16 cm^3/g) and RWF (2.75−3.83 cm^3/g), as expected and visualized in Figure 2. Chandrasekar et al.[43] also reported that although the specific volume of fermented flour finger millet breads was lower than the commercial samples, it was higher than the specific volume of unfermented millet flour breads. In the present study, specific volume showed higher values than those reported by Comettant-Rabanal et al.[5] that developed gluten-free breads using extruded whole grain flours (parboiled brown rice, corn, and sorghum) and sprout pearl millet flour with values ranging from 1.11 to 1.15 cm^3/g.

Gluten-free breads obtained by mixing proso millet and xanthan gum (XG), guar gum (GG), and hydroxypropyl methylcellulose (HPMC) at varied concentrations (0.5−2.0 g/100 g) showed higher specific volume values. The highest specific volume (2.88 cm^3/g) was observed using HPMC 2.0 g/100 g.[44] Some authors attributed that HPMC may promote the association of the gum's hydrophilic groups with the OH-groups of starch and water by hydrogen bonds. The hydrophobic component acts as a surfactant between the starch components and the air cell interphase in the food matrix, reinforcing this structure.[45]

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Table 5. Total Starch, Resistant Starch, Amylose, and Amylopectin Contents of Millet-Based Breads[a]

	samples	total starch (g/100 g)	resistant starch (g/100 g)	amylose (%)	amylopectin (%)
straight-dough	REMF	$57.51^{bc} \pm 0.21$	$0.64^{b} \pm 0.01$	$22.44^{d} \pm 1.57$	$77.55^{a} \pm 1.57$
	REGMF	$59.36^{b} \pm 0.63$	$0.50^{b} \pm 0.03$	$28.39^{cd} \pm 1.11$	$71.60^{ab} \pm 1.11$
	WWF	$53.50^{bc} \pm 0.21$	$0.77^{ab} \pm 0.01$	$35.79^{ab} \pm 2.40$	$64.20^{cd} \pm 2.40$
	RWF	$71.02^{a} \pm 1.38$	$0.97^{ab} \pm 0.08$	$32.75^{bc} \pm 1.58$	$67.24^{bc} \pm 1.58$
sponge-dough	REMF	$53.38^{bc} \pm 2.54$	$0.61^{b} \pm 0.08$	$36.87^{ab} \pm 0.03$	$63.12^{cd} \pm 0.03$
	REGMF	$52.08^{c} \pm 1.45$	$0.45^{b} \pm 0.05$	$41.18^{a} \pm 1.07$	$58.81^{d} \pm 1.07$
	WWF	$55.30^{bc} \pm 0.64$	$0.71^{b} \pm 0.07$	$29.02^{c} \pm 1.22$	$70.97^{b} \pm 1.22$
	RWF	$74.68^{a} \pm 2.17$	$1.18^{a} \pm 0.07$	$31.97^{bc} \pm 2.60$	$68.02^{bc} \pm 2.60$

[a]REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; WWF: whole wheat flour; and RWF: refined wheat flour. Values are mean ± standard deviation, $n = 2$. Different letters in the same columns indicate statistical differences ($p < 0.05$) among samples.

By comparing straight and sponge dough baking methods, RMF and REGMF samples demonstrated an increase in specific volume, ranging from 1.39 to 1.69 cm^3/g and from 1.43 to 1.61 cm^3/g, respectively. The positive correlation between specific volume and parameters in breadmaking processing (mixing dough, fermentation, and baking) was observed during elaboration of Australian sweet lupin wheat bread reported by Villarino et al.[46] These authors presented specific volumes ranging from 2.6 to 4.3 cm^3/g.

3.2.3. Texture Profile Analysis (TPA). The texture profile of the breads is shown in Table 3. As expected, RWF exhibited the lowest hardness value ($p < 0.05$) independently of the used method. In general, sponge dough millet breads showed the high values of hardness (15.51−20.25 N) in relation to straight dough (12.59−12.62 N) that could be related to the increase in dietary fiber content. High fiber content stiffens the walls surrounding the gas cells during fermentation, making the dough firmer.[15] Hardness is mainly related to the density and chewiness that represents the strength of the internal resistance of the food structure.[47] Millet breads showed high hardness in accordance with Torbica et al.[48]

The addition of germinated flour did not significantly affect the elasticity and resilience parameters. However, a strong correlation was observed between these parameters ($R = 0.98$), indicating that the addition of germinated flour leads to an increase of softness and ability to quickly recover after removing the applied stress, which was also observed by Comettant-Rabanal et al.[5] Except to WWF and RWF, the REGMF bread obtained by the straight dough method showed the highest cohesiveness (0.30) when compared to the findings reported in gluten-free breads using extruded whole grain flours and germinated pearl millet (0.07−0.12).[5] According to Onyango et al.,[49] high cohesiveness is a desirable attribute because during chewing, it forms a cake-like mouthfeel instead of disintegrated crumb.

3.3. Characterization of Nutritional Composition. *3.3.1. Proximate Composition.* The WWF sample by the sponge dough method presented the highest protein content (11.61 g/100 g), followed by REGMF which showed a significant increase ($p < 0.05$) in both methods, ranging from 9.82 to 10.86 g/100 g (Table 4). The increase in crude protein values was due to the production of some additional amino acids as a result of fermentation.[50]

The lipid content of RMF by the sponge dough method was significantly higher (9.69 g/100 g) than EMF by straight dough (4.78 g/100 g). The apparent reduction of lipids may be attributed to the formation of amylose−lipid complexes after the extrusion process that cannot be extracted by the conventional petroleum ether solvent method.[51] The protein and lipid content of the GFB (commercial) sample was significantly lower (3.44 and 2.92 g/100 g, respectively), than that reported by Torbica et al. 2019, that worked with millet-based breads without additives, although they did not identify what type of millet was used.[48]

The carbohydrate content ranged from 49.45 to 61.10 g/100 g, and RWF straight dough showed the highest value ($p < 0.05$), which was attributed to the partial removal of the pericarp after the refining process, hence causing a reduction of dietary fiber content. Dietary fiber contents are highlighted using RGMF and REGMF (12.6 and 10.8 g/100 g, respectively). Similar values were found by Horstmann et al.[52] for breads obtained using germinated brown millet (*Brachiaria ramosa*) flour. The germination process promotes less interaction between protein and carbohydrate, favoring the biosynthesis of the cell wall and consequent increase in dietary fiber.[53] Furthermore, an increase in fiber values was observed comparing straight dough and sponge dough methods, especially in the REMF and REGMF formulations, showing an increase from 6.42 to 9.75 and 6.34 to 10.83 g/100 g, respectively. It is expected that great amount of fibers thickens the walls surrounding the gas cells, thus increasing dough hardness as a result of alveoli reinforcement, in addition to promoting differences in the elastic properties of the dough.[36,54]

The ash content was higher in REGMF by sponge dough. Ingredients rich in mineral contents offer the potential improvement of the nutritional profile of products since gluten-free breads are made exclusively by refined flours and added starches.[52] The highest moisture content of the EMF sample in the straight method was observed; however, it was statistically smaller than the commercial gluten-free samples. The moisture of a product can affect processing since it interferes in terms of stability, quality, and composition. It can also impact the shelf life as it is one of the main factors that accelerate chemical and enzymatic reactions.[55]

3.3.2. Total Starch, Resistant Starch, and Amylose Contents. Concerning total starch, RWF presented the highest values in both methods. However, the REGMF sample by the sponge dough method demonstrated a significant ($p < 0.05$) reduction (12%) in starch content if compared to the straight dough sample (Table 5). A reduction in starch content after the fermentation process may be associated with the amylolytic action of microorganisms in the fermented dough.[56] In addition, the total starch values of millet-based breads are comparable to the WWF. In terms of resistant starch content, significant difference ($p < 0.05$) was not observed in millet-based breads, except for RWF using sponge dough processing (1.18 g/100 g).

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The amylose content was significant ($p < 0.05$) higher in the REGMF by the sponge dough sample (41.18%). These findings were also reported by Pessanha et al.,[15] in which REGMF obtained by the sponge dough method showed the highest hardness and chewiness values (Table 3), although did not show the higher setback and FV values among the samples (Figure 1), demonstrating the impact of processing rather than solely the rheological characteristics of the flour.

Comparing the baking processes, the increase in amylose content may be related to a secondary effect caused by the decrease in lipid content as a result of the fermentation process, pointed out as an effect reported by Chinenye et al.[57] Consequently, there is less interaction and formation of starch−lipid complexes. However, the tendency to form binary complexes is due to the helical structure of amylose, which depends on the availability of lipids in the matrix.[58]

3.3.3. Total Phenolic Content (TPC) and Antioxidant Properties of Breads. TPC values of pearl millet breads obtained by straight dough and sponge dough methods are shown in Table 6. It was found that the bound extract presented more phenolic content than the free extract, which has already been reported in other botanical species of millets such as kodo, finger, foxtail, proso, little, and pearl.[59,60]

Concerning different baking processes, we observed a decrease in the TPC content in the free extracts (except for REGMF samples) and an increase in the bound extracts, which influenced the total TPC values in contrast to the results reported by Santetti et al. (2022) that presented an increase in free phenolics and a decrease in bound phenolics. This phenomenon could be attributed to an apparent increase in free extracts as a result of a combination of fermentation process and cooking temperature that can lead to the hydrolysis of some complex phenols.[61] The action of microorganisms that metabolize soluble and fermentable fibers for their growth promotes the release of phenolic compounds "mechanically trapped" from the polymeric fiber structure.[62] On the other hand, the Maillard reaction during the baking process can also promote the incorporation of certain compounds into melanoidins, promoting an increase in bound compounds.[63] The TPC value of the REGMF sample presented a significant increase by sponge (4131.8 mg GAE/100 g) compared to the straight dough method (3160.2 mg GAE/100 g) ($p < 0.05$). It is known that cell wall-degrading enzymes in the grain become active during germination, which may contribute to the cell wall structure modification, thus leading to the release of bound phenolic compounds. In addition, this increment could be a result of increased polyphenol oxidase activity, which leads to key enzymes involved in biosynthesis of phenols.[64,65] Other studies of gluten-free formulated breads also have demonstrated positive effects on the content of phenolic compounds using germinated flour.[66,67]

Antioxidant properties of the extruded millet flours determined by DPPH and FRAP demonstrated a higher peroxide scavenging capacity than the WWF and RWF (Table 6). Sharma et al.[68] using finger millet flour in chapati bread formulation (ratio 3:1) showed a significant increase in antioxidant activity (49.51%) compared to the control sample (13.99%).

The REGMF bread prepared by the straight dough method showed stronger antioxidant activity among formulations, 3.69 mg/g (DPPH) and 1041.0 μmol/100 g (FRAP) and, in the case of sponge dough method, 3.06 mg/g (DPPH) and 1923.3

Table 6. Total Phenolic Content (TPC), Antioxidant Capacity (DPPH and FRAP), and Inhibitory Activity α-Amylase (IC_{50} Values of Extracts) of Millet-Based Breads by the Straight Dough and Sponge Dough Baking Methods[a]

		straight-dough				sponge-dough			
		REMF	REGMF	WWF	RWF	REMF	REGMF	WWF	RWF
TPC (mg GAE/100 g)	free	$1497.24^{a} \pm 105.35$	$1134.73^{b} \pm 73.27$	$655.03^{c} \pm 58.67$	$384.25^{d} \pm 25.14$	$1166.53^{b} \pm 73.27$	$1558.94^{a} \pm 50.79$	$523.34^{cd} \pm 19.79$	$197.92^{e} \pm 12.02$
	bound	$1587.80^{c} \pm 130.32$	$2025.50^{b} \pm 152.18$	$964.85^{d} \pm 47.52$	$108.18^{e} \pm 9.96$	$2044.09^{b} \pm 191.99$	$2572.93^{a} \pm 233.51$	$766.19^{d} \pm 68.88$	$89.45^{e} \pm 6.46$
	total	$3085.04^{b} \pm 130.20$	$3160.2^{b} \pm 115.70$	$1619.8^{c} \pm 93.20$	$492.4^{d} \pm 22.30$	$3210.6^{b} \pm 217.50$	$4131.8^{a} \pm 284.20$	$1289.5^{c} \pm 54.4$	$287.3^{d} \pm 5.50$
DPPH (mg/g)	free	$1.12^{b} \pm 0.03$	$1.84^{a} \pm 0.10$	$0.78^{c} \pm 0.04$	$0.49^{d} \pm 0.03$	$1.02^{b} \pm 0.05$	$1.13^{b} \pm 0.12$	$0.42^{d} \pm 0.05$	$0.42^{d} \pm 0.02$
	bound	$1.29^{c} \pm 0.08$	$1.84^{b} \pm 0.09$	$0.93^{d} \pm 0.02$	$0.26^{f} \pm 0.03$	$2.02^{a} \pm 0.02$	$1.93^{ab} \pm 0.06$	$1.00^{d} \pm 0.07$	$0.50^{e} \pm 0.04$
	total	$2.41^{c} \pm 0.10$	$3.69^{a} \pm 0.12$	$1.70^{d} \pm 0.05$	$0.75^{f} \pm 0.03$	$3.04^{b} \pm 0.06$	$3.06^{b} \pm 0.17$	$1.42^{e} \pm 0.03$	$0.92^{f} \pm 0.04$
FRAP (μmol/100 g)	free	$408.45^{b} \pm 14.42$	$410.69^{b} \pm 20.29$	$479.37^{b} \pm 46.03$	$405.68^{b} \pm 52.73$	$987.50^{a} \pm 113.07$	$998.03^{a} \pm 68.73$	$212.37^{c} \pm 1.36$	$181.59^{c} \pm 4.62$
	bound	$460.85^{d} \pm 8.65$	$630.26^{c} \pm 56.0$	$500.29^{cd} \pm 14.47$	$111.17^{e} \pm 3.58$	$1145.52^{a} \pm 134.72$	$925.24^{b} \pm 62.52$	$649.14^{c} \pm 22.19$	$162.61^{e} \pm 12.78$
	total	$869.30^{b} \pm 20.90$	$1041.0^{b} \pm 72.90$	$979.7^{b} \pm 60.20$	$516.9^{c} \pm 51.40$	$2133.0^{a} \pm 236.90$	$1923.3^{a} \pm 85.80$	$861.5^{b} \pm 23.5$	$344.2^{c} \pm 10.2$
IC_{50} (mg/mL)	free	-	-	-	-	-	-	-	$1.27^{d} \pm 0.02$
	bound	$2.06^{cd} \pm 0.03$	$1.46^{d} \pm 0.04$	$7.29^{a} \pm 0.18$	$2.50^{c} \pm 0.05$	$1.36^{d} \pm 0.20$	$1.78^{cd} \pm 0.05$	$5.40^{b} \pm 0.06$	$7.68^{a} \pm 0.02$

[a]REMF: raw and extruded millet flour; REGMF: raw, extruded, and germinated millet flour; WWF: whole wheat flour; RWF: refined wheat flour; and GAE: gallic acid equivalent. Values are mean ± standard deviation, $n = 3$. The minimum inhibitory concentration (IC_{50}) for control (acarbose) was 0.024 mg/mL. Different letters in the same rows indicate statistic differences ($p < 0.05$) among samples.

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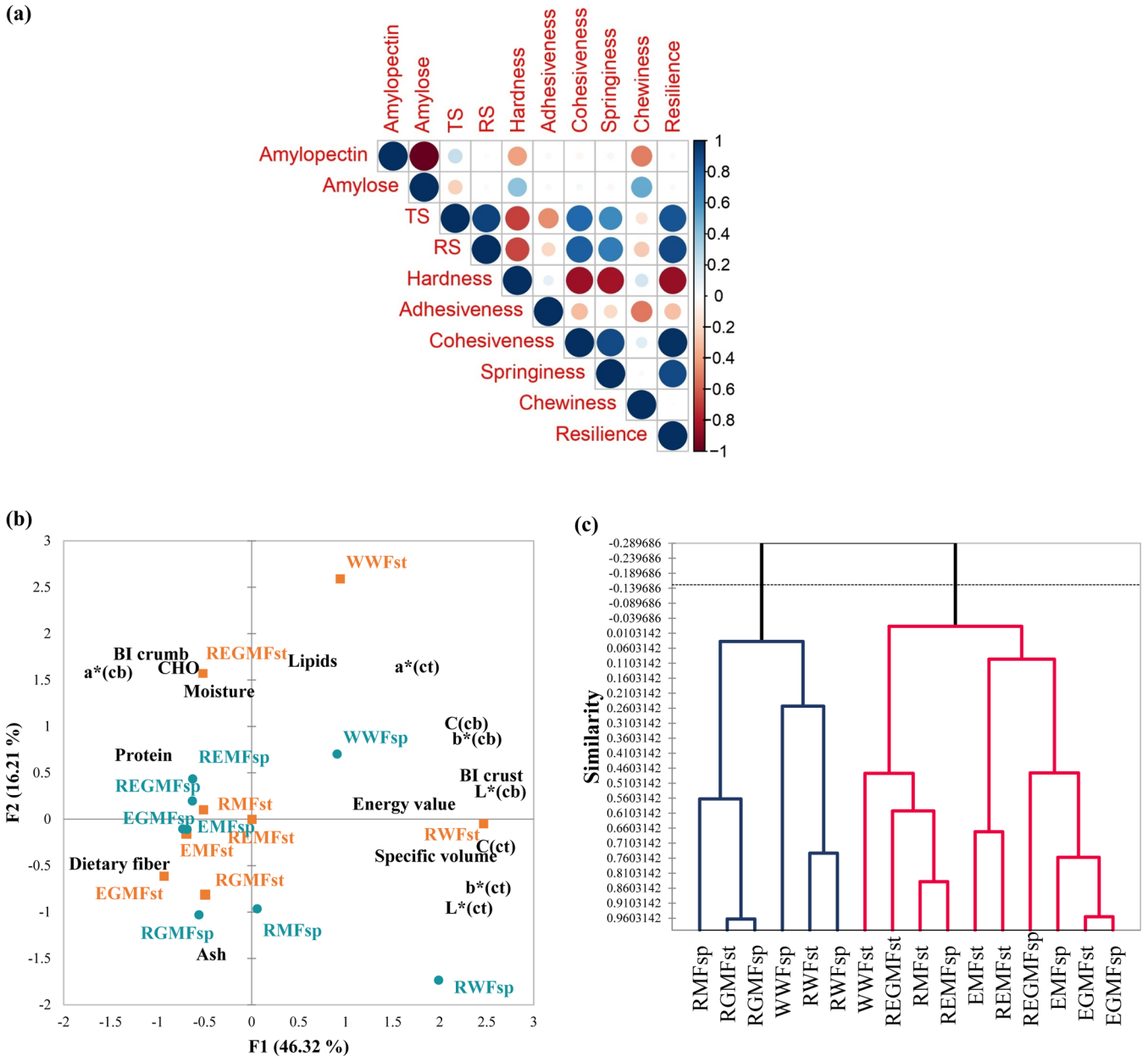

Figure 3. Correlogram for texture parameters and starch properties (TS: total starch and RS: resistant starch) of millet bread (a). Two-dimensional loading plot from principal component analysis (PCA) (C: chroma; ct: crust; cb: crumb; BI: browning index; and CHO: carbohydrate) using PCA Dimension 1 (F1) and PCA Dimension 2 (F2) (b). Loading plot based on different variables of millet bread properties elaborated with (i) raw millet flour (RMF); (ii) extruded millet flour (EMF); (iii) raw and extruded millet flour (REMF); (iv) raw, extruded, and germinated millet flour (REGMF); (v) extruded and germinated millet flour (EGMF); (vi) raw and germinated millet flour (RGMF); and controls (vii) WWF (whole wheat flour) and (viii) RWF (refined wheat flour) obtained by different methods (straight-dough, st, and sponge-dough, sp) and Hierarchical tree and individual factor map by the principal components of PCA (c).

μmol/100 g (FRAP). These findings showed high antioxidant activity, especially if compared to RWF formulation by the straight dough method that is known to be more preferable by consumers and commercially available (0.75 mg/g and 516.9 μmol/100 g).

3.3.4. α-Amylase Inhibition Activity of Breads. The results of α-amylase inhibition expressed by the minimum inhibitory concentration (IC_{50}) are presented in Table 6. Concerning the free extracts, only the RWF (sponge dough) sample showed an inhibition effect on the α-amylase (1.27 mg/mL). These findings are reported by previous studies that observed a high amount of ferulic acid and its isoforms even in refined wheat flour.[69] In vivo studies have shown that ferulic acid has the ability to neutralize free radicals present in the pancreas induced by streptozotocin that facilitates the proliferation of β-cells in order to secrete insulin, which in turn enhances the use of glucose by extra hepatic tissues and reduction of blood glucose levels.[70]

In contrast, all bound extracts presented an IC_{50} significantly higher ($p < 0.05$) than acarbose (IC_{50} = 0.024 mg/mL), as expected by considering that this drug is widely used in glycemic control. In addition, it was observed that pearl millet-bound extracts demonstrated higher α-amylase inhibition activity than wheat extracts (WWF and RWF). The lowest

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IC_{50} value for the REMF sample (2.06 mg/mL straight-dough and 1.36 mg/mL sponge-dough) means that the inhibition capacity of the extracts is dependent on the breadmaking process and is also influenced by the extrusion process. These results are in agreement with the TPC values reached in bound extracts, which showed a value of 1587.80 mg GAE/100 g by the straight-dough and 2044.09 mg GAE/100 g by the sponge-dough method, confirming that high phenolic contents were associated with higher α-amylase inhibition activities. Besides, the REMF sample revealed an increase from 1.29 mg/g (straight-dough) to 2.02 mg/g (sponge-dough) by DPPH and 460.8 μmol/100 g (straight-dough) to 1145.5 μmol/100 g (sponge-dough) by the FRAP method. Regarding the use of germinated flour, the REGMF sample showed an increase ranging from 1.84 mg/g (straight-dough) to 1.93 mg/g (sponge-dough) by DPPH and 630.2 μmol/100 g (straight-dough) to 925.2 μmol/100 g (sponge-dough) by the FRAP method, but the intensity of this increase was lower (0.47-fold) than the formulation that did not contain GMF addition (1.48 fold).

Many studies have shown that there is a positive correlation between the amounts of phenolic contents in plant extracts and their ability to inhibit digestive enzymes. However, the inhibition strength may not only depend on the phenolic concentration but also on the composition because high levels of phenolic contents in plant extracts are not always associated with strong inhibition, suggesting that the type of phenolic compounds and the interaction among them may also be important factors determining the inhibition activity.[71] The interaction between phenolic compounds and the starch digestion process has been widely reported. Two main mechanisms have been proposed and can be classified as (i) the modulation of glycolytic enzymes and (ii) the formation of supramolecular complexes between starch and phenolic compounds. These mechanisms probably play a complementary role and do not exclude the other.[72]

Hyperglycemia is a factor associated with an increased risk of developing metabolic diseases, such as type 2 diabetes.[73] Health loss has become a growing burden from NCDs and risk factors that are responsible for half of the lost years of healthy life lost worldwide. Regarding this scenario, it is important to reduce exposure to inappropriate behavioral and metabolic risks that would bring enormous health benefits; hence, there is a need of re-engineering foods in order to address low glycemic products.

4. PAIRWISE CORRELATION COEFFICIENT (*R*) AND PRINCIPAL COMPONENT ANALYSIS (PCA)

In order to explore the relations between texture profile and starch, amylose, and amylopectin content in bread samples, a correlation matrix was calculated (Figure 3 a). The correlogram indicates a positive relationship between total and resistant starch with cohesiveness (0.78 and 0.82, respectively), springiness (0.62 and 0.69, respectively), and resilience (0.85 and 0.89, respectively), but a negative correlation with hardness (−0.68 and −0.66, respectively). A positive correlation was also found between amylose content with hardness (0.40) and chewiness (0.50) that can be explained due to the reassociation and reordering of amorphous amylose, which was largely responsible for the short-term retrogradation of starch.[74]

PCA was applied to evaluate the relationship among the 17 variables related to bread characteristics (Figure 3b). PC1 and PC2 explained 65.26% of the total variance among three quality characteristics of breads (specific volume, color, and proximate composition). PC analysis evidenced the differences between the pearl millet and the control breads (WWF and RWF). Dim 1 is described by energy value, lipids, a^* (ct), b^* (ct), L^* (ct), C^* (ct), BI crust, b^* (cb), L^* (cb), C^* (cb), and specific volume. In contrast, the other variables described by Dim 2 are carbohydrates, protein, dietary fiber, and ash. The parameters of the close vectors are positive and correlated.

A hierarchical tree of the principal components of PCA was used to better explain the relationship between the variables (physicochemical composition) and factors (pearl millet samples). HCPC formed two sample groups according to their similarities (Figure 3c). Cluster 1 (EMFsp, RMFst, REMFsp, EMFst, and REGMFst) was composed by the samples with similar carbohydrate values, while cluster 2 was constituted by RGMFst, RMFsp, REGMFsp, and RGMFsp that presented analogous values of ash, and cluster 3 grouped the RWF samples showing greater correlation between a^* (ct), b^* (cb), C^* (cb), BI crust, L^*, and energy values. Cluster 4 was characterized by RWF values that are similar in terms of specific volume, C* (ct), b* (ct), and L* (ct).

Our findings indicated that different types of breadmaking had a positive impact on the nutritional characteristics of gluten-free pearl millet-based breads led to high dietary fiber products (10.83 g/100 g). Although pearl millet-based samples presented a significant lower specific volume than wheat breads (WWF and RWF), they demonstrated a higher value in terms of ash (43%) and dietary fiber (228%). Furthermore, the content of phenolic compounds and antioxidant capacity of millet-based breads was significantly higher by at least 50% compared to WWF and RWF ($p < 0.05$). Millet-based formulations demonstrated positive effects in inhibiting the activity of the α-amylase, especially in the case of the sponge dough breadmaking process. The ternary formulation of pearl millet (raw, extruded, and germinated millet flour) demonstrated excellent effects mainly in terms of the content of phenolic compounds, antioxidant capacity, and the nutritional characteristics. Therefore, it can be concluded that pearl millet has the potential to be used as a gluten-free cereal in breadmaking with adequate nutritional and technological characteristics in order to be a useful alternative for the diet management.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.4c00987.

Torque and temperature curve as a function of extrusion processing time of pearl millet flour; farinograph profile of millet flours; mixing of ingredients during breadmaking process; and pasting properties' characteristics by RVA (PDF)

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Notes

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■ ABBREVIATIONS

BDV, breakdown viscosity
BI, browning index
C, Chroma
Cb, crumb
CD, celiac disease
CIE, International Commission on Illumination
Ct, crust
CV, cold paste viscosity
DPPH, 2,2-difenil-1-picrylhydrazyl
EGMF, extruded and germinated millet flour
EMF, extruded millet flour
FRAP, ferric reducing antioxidant power
GAE, gallic acid equivalents
GFB, gluten-free bread
GG, guar gum
GMF, germinated millet flour
HCA, hierarchical clustering analysis
HPMC, hydroxypropyl methylcellulose
NCDs, chronic noncommunicable diseases
PCA, principal component analysis
PSD, particle size distribution
PV, peak viscosity
REGMF, raw, extruded and germinated millet flour
REMF, raw and extruded millet flour
RGMF, raw and germinated millet flour
RMF, raw millet flour
RS, resistant starch
RWF, refined wheat flour
Sp, sponge dough
St, straight dough
SV, specific volume
TPA, texture profile analysis
TPC, total phenolic content
T_p, paste temperature
TPTZ, 2,4,6-tris(2-pyridyl)-*s*-triazine
TS, total starch
TV, trough viscosity
WWB, whole wheat bread
WWF, whole wheat flour
XG, xanthan gum

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