LWT - Food Science and Technology 150 (2021) 112042

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Extruded whole grain flours and sprout millet as functional ingredients for gluten-free bread

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ARTICLE INFO

Keywords:
Non-gluten flour
Rheology
Thermomechanical process
Viscoelastic properties
Fiber-rich bread

ABSTRACT

This work aimed to use thermoplastic extrusion technology as a pretreatment for whole grain flours (corn, parboiled brown rice, and sorghum) and the incorporation of germinated millet at 5% for the production of gluten-free bread. The study characterized the flour (chemical composition and particle size distribution), evaluated the dough (pasting, empirical and fundamental rheological properties) and analyzed the bread quality characteristics (physical, structural, and textural measurements). Thermoplastic extrusion enabled the development of consistency, improved water absorption (105–153%) and viscoelastic properties of the doughs. This process caused an increase of the specific volume (66, 33 and 82%, respectively for corn, rice and sorghum made bread), and formation of better internal air cell distribution in the three different breads produced, especially in the sorghum bread. In addition, parboiled brown rice showed atypical pasting and rheological properties of the dough, which also affected the quality characteristics of the bread. The incorporation of 5% germinated millet enhanced breadcrumb softness in all samples, particularly for extruded rice flour added of germinated millet flour sample, which presented similar hardness values (7.3 N) and springiness (0.97) to whole wheat flour.

1. Introduction

Gluten-free (GF) products are indispensable for individuals affected by gluten ingestion (Stamnaes & Sollid, 2015). Prolamins are gluten fractions directly related to gluten-related disorders such as celiac disease, wheat allergy, and non-celiac gluten sensitivity (Scherf, Koehler, & Wieser, 2016). Furthermore, the high consumption of refined wheat-based products is attributed to low cost, wide availability, and singular properties that favor the production of bulky loaves. These modern habits result in the loss of many micronutrients and phytochemicals in the human diet, since often are separated during milling and are directly related to health benefits (Kikuchi et al., 2018).

That is why today the intake of whole grains (WG) and/or their derivatives that contain all their original components in the same proportion is being valued, since they constitute an important source of carbohydrates, proteins, fiber, bioactive phytonutrients, vitamins of the group. B and minerals (Oldways Whole Grains Council, Undated). Among the gluten-free whole grains that are grown mainly in Brazil are corn, rice and sorghum (CONAB, 2019). Whole corn has various bioactive constituents, such as carotenoids, anthocyanins (pigment depending) and phenolic compounds that have many health-promoting and disease-preventing properties (Singh, Singh, & Shevkani, 2019). Parboiled brown rice is a hypoallergenic grain with bioactive components including dietary fibers, γ-oryzanols, and phytosterols (Cho & Lim, 2016). Sorghum is a gluten-free ancient grain that stands out for having high levels of dietary fiber and phytochemicals including phenolic acids (especially 3-deoxyanthocyanidins), condensed tannins, polyflavanols (procyanidins), anthocyanins, phytosterols and policosanols, presented in the pericarp and are of considerable interest due to their possible health benefits (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003; Dykes, 2019).

In order to produce gluten-free breads some pretreatments have been tried. Among them, extrusion cooking is considered an integrated process that combines various operations (conveying, mixing, shearing, and cooking) into one only system which enables transform native biopolymers present in cereals into new functional biopolymers (Ganjyal, 2020). This process manages to modify the molecular structure of starch, which leads to an increase in its functional properties such as water

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https://doi.org/10.1016/j.lwt.2021.112042
Received 2 March 2021; Received in revised form 7 June 2021; Accepted 27 June 2021

absorption and the development of dough consistency (Espinosa-Ramírez et al., 2021). Likewise, recent studies have shown that extrusion promotes structural changes in corn proteins (zein) and also favors synergic interactions with starch that enhance dough viscoelasticity (Federici, Jones, Selling, Tagliasco, & Campanella, 2020). In addition, there is ample evidence of other favorable effects of extrusion on the conversion of insoluble to soluble fibers (Aktas-Akyildiz, Masatcioglu, & Köksel, 2020), reduction of antinutrients, increase of minerals bioavailability, flour stabilization by inactivation of lipolytic enzymes and increased antioxidant activity through the release of phenolic compounds bound in insoluble fibers (Pessanha et al., 2021). Few previous works have used extrusion under conventional temperature conditions to develop viscoelasticity and try to mimic the effect of wheat gluten to produce GF products (Clerici, Airoldi, & El-Dash, 2009; Martínez, Marcos, & Gómez, 2013; Torbica, Belovic, & Tomic, 2019).

On the other hand, one problem of GF bread is crumb hardness, but this can be reduced with the addition of germinated grains. This natural ingredient additionally contributes to CO_2 gasification during the fermentation and the retardation of bread staling (Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017). Germinated millet (*Pennisetum glaucum* (L.) R. Br.) would allow technological benefits due its high degree of germination ratio and considerable enzymatic activity compared to other grains (Horstmann et al., 2019).

In the aforementioned works related to effect of extrusion on changing starch functionality, they used high process temperatures that ranged from 140 to 220 °C to produce extruded flours that were employed at range from 10 to 70% in the GF bread formulations, whereas the present work only whole grain extruded flours were used in GF bread. Therefore, unconventional extrusion temperatures (<110 °C) can induce slight modifications in the biopolymers contained in cereal-based flours to generate potential nutritious functional ingredients that can be used 100% in GF bread formulations. The objective of this work was to evaluate the effect of thermoplastic extrusion on the pasting properties modification and development rheological properties of WG flours to improve the quality characteristics of GF bread with and without the incorporation of 5% germinated pearl millet.

2. Material and methods

2.1. Whole grain flour characterization

2.1.1. Flour preparation

Corn grains were donated by Indústrias de Alimentos Granfino (Nova Iguaçú, Brazil). Sorghum grains (red pericarp, low tannin) were donated by Embrapa Milho e Sorgo (Sete Lagoas, Brazil). Parboiled brown rice and whole wheat flour (WWF) were acquired at a market in Rio de Janeiro. WGs were cleaned and ground using a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm opening screen for obtaining fine whole corn flour (WCF), parboiled brown rice flour (PBRF), and whole sorghum flour (WSF).

2.1.2. Germination of pearl millet

The pearl millet cultivar ADR9070 was supplied by Atto Sementes (Rondonópolis, Brazil). Grains with a 99% germination index were soaked in water (1:3 grain to water) for 4 h, the water was replaced hourly and then drained following the methodology of Theodoro, Martinez, Grancieri, Toledo, Martins, Dias et al. (2021). The grains were allowed to germinate in a fermentation cabinet (National Mfg. Co., Lincoln, USA) at a controlled temperature of 30 °C and relative humidity of 90%. After 24 h, the grains were dried in a fan oven at 30 °C/24 h, until reaching a final moisture content lower than 12%, then they were ground using the same procedure as mentioned above for WG flour.

2.1.3. Chemical composition analysis

The chemical composition of raw and extruded flours was performed according to the AOAC (2000) official analytical methods: moisture (method 925.09), fat (method 945.38), total protein (method 2001.11, factor of 5.75), ash (method 923.03), total dietary fiber (soluble and insoluble) (method 991.43), and the carbohydrate was determined by the difference. The quantification of macro and micro elements was determined following the method 990.08, item 9.2.39 of AOAC (2000).

2.1.4. Particle size distribution (PZD)

The PZD of the raw flours was determined in duplicates, using a S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) according to the modified method 55–40.01 (AACC, 1999) with deionized water, using three size ranges: <0.1 mm, from 0.1 to 0.5 mm and from >0.5 to 1.7 mm.

2.2. Extrusion conditions

In this work, an Evolum HT25 co-rotating, intermeshing twin-screw extruder (Clextral Inc., Firminy, France) was used. The screw diameter was 25 mm, with a diameter ratio of 40:1, ten heating zones (25, 40, 60, 80, 100, 110, 110, 90, 80 and 70 °C), and the screw speed was set at 200 rpm. WG flours were fed through a twin-screw gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany) at a constant rate of 10 kg/h, and the process was monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Deionized water was injected between the first and second modular zones through a port with a 5.25 mm internal diameter using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China) set to compensate moisture differences in the samples and provide a final 25% moisture content. The collected extrudates were dried in a forced air oven at 55 °C/10 h. Then, they were ground under the same conditions as of the flour preparation to obtain fine extruded whole grain flours of corn (EWCF), parboiled brown rice (EPBRF), and sorghum (EWSF).

2.3. Rheological evaluation

2.3.1. Pasting properties

A Rapid Visco Analyzer series 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia) was used to measure the paste viscosity of the raw and extruded flours according to the methodology reported by Ragaee and Abdel-Aal (2006). Three grams of the gluten-free whole grain flour adjusted to 14% of moisture (wet basis) were placed along with 25 mL of distilled water in the sample holder (aluminum cup) of the equipment. The test conditions were: mixing at 160 rpm at 25 °C for 2 min, heating up to 95 °C at a constant rate of 14 °C/min and kept it for 3 min and then cooled to 25 °C in 5 min at the same rate, with a total time of 20 min. The pasting property readings measured were pasting temperature (PTem, cP), 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). Measurements were performed in duplicates.

2.3.2. Farinograph measurement

Dough resistance to mixing was performed using the Farinograph© model FD0234H (Brabender, Duisburg, Germany). The readings were obtained according to the method 54–21.01 AACC (2000b) with the following modifications: For 30 g of flour, four levels of water addition were tested (between 88 to 106.4, 100.8 to 122.2 and 93.8 to 102.9% for flours of corn, rice and sorghum, respectively) until obtaining the highest dough consistency with optimal hydration. The consistencies obtained from each cereal superpased the 500 Brabender Units (BU) wheat standard. From the farinograms the following readings were considered: water absorption (WA, %), farinographic consistence (BU), dough development time (DDT, min), dough stability time (DST, min) and mixing tolerance index (MTI, min), determined at 5 min after peak. Measurements were performed in triplicates.

2.3.3. Dynamic mechanical properties

The dynamic mechanical properties of the raw and extruded flours were performed using a rotational rheometer HAAKE Mars II (Thermo Fisher Scientific, Karlsruhe, Germany). Prior to the rheological readings, the dough of each cereal was mixed with water in the farinograph using the water absorption (WA) and dough development time (DDT) obtained from farinograms. All analyzes were performed according to Korus, Witczak, Ziobro, and Juszczak (2009) at 25 °C using a 35 mm diameter parallel plate geometry. Three grams of dough were loaded onto the bottom plate and the top plate approached onto the dough at speed of 0.6 mm/min to a gap of 2 mm. The excess dough was trimmed by removing from the outer edge and then coated with mineral oil to prevent drying during measurement. For each type of dough, a dynamic oscillatory frequency sweep was conducted at constant strain amplitude (γ) within the linear viscoelastic regime (LVR) and frequencies ranged from 0.1 to 100 Hz. Values of elastic or storage modulus (G'), viscous or loss modulus (G'') and tan δ (G''/G') were obtained at 1 Hz. Readings were performed in duplicates.

2.4. Bread making and quality evaluation

2.4.1. Formulation and bread making procedure

Breads were made following the proportions shown in Table 1. Dough preparation was performed with a 35 g micromixer (National MFG. CO., Lincoln, U.S.A.). Instant yeast (Fleischmann, Pederneiras, Brazil) was previous activated with deionized water (1/3 of the total formulation water) at 35 °C and placed in a fermentation chamber at 85% relative humidity for 15 min for activation. All dry ingredients were homogenized for 2 min, prior to adding the palm fat and liquid ingredients. Mixing times that were obtained from the farinograms for each WG flour varied: sorghum: 1.5 min, rice: 3.0 min, and corn: 2 min. Portions of 20 g were cut, formed, and placed into previously greased and floured steel molds of 45 mL capacity; after which they were placed in a fermentation cabinet at 30 °C and 85% RH for 60 min. Finally, they were put into a convection oven FVT5D (Venâncio, Venâncio Aires, Brazil) at 200 °C/14 min, then allowed to cool at room temperature. Bread analyzes were performed after 24 h, using two controls for comparison: commercial whole wheat flour (Control 1) and the mixture of non-extruded and extruded rice flour in the proportion of 50:50% (Control 2).

2.4.2. Specific volume analysis

Bread volume was determined using a modified standard seed displacement method 10–05.01 (AACC, 2000), using millet seeds. The recipient used to do the calculation was a parallelepiped with dimensions of 8.5 cm × 8.4 cm x 9.2 cm (width × length × height). Bread specific volume (cm^3/g) was calculated as bread volume divided by bread weight measured at 24 h after baking.

2.4.3. Bread crumb structure

Images of bread slices with dimensions of approximately 33 × 35 mm were captured using an Epson Perfection 1240U scanner (Seiko, Nagano-Ken, Japan), recording images at 300 dpi resolution (170 mm wide x 60 mm high). Subsequently, the images were analyzed using the ImageJ software (v.1.51j8, Wayne Rasband, National Institute of Health, USA) following the method of Crowley, Grau, and Arendt (2000). Firstly, the region of interest was cropped, then, 8-bit images with dimensions (30.0 × 33.4 mm) were generated and adjusted to threshold. Finally, total area of bread slice (TBA, mm^2), total cell area (TCA, mm^2), solid area (SA = TBA – TCA, mm^2), porosity (P = TCA/TBA, %), and height (H, mm) were determined.

2.4.4. Texture measurement

The texture profile analysis (TPA) was carried out using the center of the bread crumb slices with a thickness of 20 mm using a Texture Analyser TA-XT Plus (Stable Micro Systems, Surrey, U K) equipped with a 5 kg load cell and a 15 mm aluminum cylindrical probe. 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 Schober, Messerschmidt, Bean, Park, and Arendt (2005). TPA was performed in order to measured hardness (Hd, N), Adhesiveness (Ad, g·s), cohesiveness (Co), springiness (Sp), chewiness (Ch, N), and resilience (R) were measured.

2.5. Statistical analysis

Analysis of variance (one-way ANOVA) and LSD Fisher multiple range tests were used to determine the differences among samples. A paired T-test was used to evaluate the extrusion process effect of each cereal and for the incorporation of the germinated millet in the GF bread. The Shapiro-test and Bartlett-test were used to confer normality distribution and homoscedasticity, respectively. The Box-Cox transformation was performed only for those variables that lacked a normal distribution or homoscedasticity by the use of the lambda (λ) to achieve the normal distribution (Box & Cox, 1964). A significant level of 5% was used for all statistical tests.

Multivariate analysis was applied on the paste, farinographic and dynamic mechanical properties of the doughs (1) and on the physical, structural and textural, properties of the breads (2). Principal Component Analysis (PCA) was used to evaluate the relationship between analyzed samples. Pearson's coefficient (r) was calculated to evaluate the correlations among variables, the strongest correlation was evaluated according to the scale reported by Teles et al. (2019). Finally, the Hierarchical Clustering on Principal Components (HCPC) was performed by applying the Euclidean distance and Ward's grouping methods. All analyses were performed using the software R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. WG flour characterization

3.1.1. Chemical composition analysis

The WSF protein content was the highest ($p < 0.05$) among the three

Table 1
Gluten-free bread formulations.

Samples	Raw flour (%)	Extruded flour (%)	Fat (%)	Yeast (%)	Sugar (%)	Salt (%)	Water (%)	Germinated millet (%)
Control 1	100	–	6	3	3	1.5	70.0	–
Control 2	50	50	–	–	–	–	127.5	–
EWCF	100	–	6	3	3	1.5	100.5	–
EPBRF	100	–	6	3	3	1.5	100.8	–
EWSF	100	–	6	3	3	1.5	96.9	–
EWCF+5% GM	95	–	6	3	3	1.5	100.5	5
EPBRF+5% GM	95	–	6	3	3	1.5	100.8	5
EWSF+5% GM	95	–	6	3	3	1.5	96.9	5

Control 1-commercial whole wheat flour, control 2-mixture of raw and extruded rice flour in the proportion of 50:50%, EWSF-extruded whole sorghum flour, EPBRF-extruded parboiled brown rice flour, and EWCF-extruded whole corn flour, and 5% GM-incorporation of 5% of germinated millet, weight flour basis.

cereals followed by WCF (7.53 g/100 g) and PBRF (6.73 g/100 g), respectively (Table 2). WCF showed the highest lipid content ($p < 0.05$) of 4.19 g/100 g against WSF (3.46 g/100 g) and WCF (2.42 g/100 g). The carbohydrate content ranged from 61.7 to 71.6 g/100 g, and PBRF showed the highest value ($p < 0.05$), which was attributed to the partial removal of the pericarp after the parboiling process, causing a reduction of dietary fiber content. Dietary fiber stood out for WCF and WSF, with values of 13.2 and 10.8 g/100 g, respectively. The soluble fiber fraction in both the WCF and WSF samples were the same (1.80 g/100 g), while PBRF showed a little less at 1.14 g/100 g, whereas for insoluble fiber, WCF and WSF showed higher values 11.4 and 9.0 g/100 g than PBRF, 4.52 g/100 g. Similar values of protein, lipids, and dietary fiber content were found by Toledo, Carvalho, Vargas-Solórzano, Ascheri, and Comettant-Rabanal (2020). The high proportion of insoluble fiber present in WCF and WSF gave rise to high levels of total phenolic compounds, ferric acid content, and in the case of WCF it additionally showed high antioxidant potential, thus consolidated whole grains as a functional ingredient (Guo & Beta, 2013).

All cereals presented high amounts of macro elements such as potassium, magnesium and phosphorus and micro elements such as zinc (Table 2). WSF presented the highest ($p < 0.05$) amounts of iron, manganese, copper and zinc. PBRF showed high ($p < 0.05$) amounts of manganese and copper, and corn had high ($p < 0.05$) amounts of zinc. According to Marriott, Birt, Stallings, and Yates (2020) these important concentrations of macro and micro elements mentioned above, contribute to between 20 and 50% of the iron, 36–100% of the magnesium, 15–61% of the zinc, 24–73% of the copper and 100% of the manganese needs for the daily intake requirements of both children and adults.

3.1.2. *Particle size distribution*

WCF and WSF presented the highest percentage of particle size within the range of 0.1–0.5 mm. On the other hand, PBRF showed a bimodal behavior between the ranges of 0.1–0.5 mm and 0.5–1.7 mm, the latter range being the predominant one due to compaction of the endosperm and the sealing of the caryopsis (Nambi, Manickavasagan, & Shahir, 2017). Only 7–12% of the particles in all whole grain flours were found in the range of <0.1.

Table 2
Chemical composition of gluten-free whole grain flours of corn (WCF), parboiled brown rice (PBRF), and sorghum (WSF).

Components	Whole grain flours		
	Corn	Parboiled brown rice	Sorghum
Moisture (g/100 g)	$12.29^{b} \pm 0.04$	$12.36^{b} \pm 0.08$	$11.73^{a} \pm 0.02$
Ash (g/100 g)	$1.07^{a} \pm 0.04$	$1.26^{b} \pm 0.02$	$1.40^{c} \pm 0.02$
Protein (g/100 g)	$7.53^{b} \pm 0.13$	$6.73^{a} \pm 0.04$	$10.47^{c} \pm 0.04$
Lipids (g/100 g)	$4.19^{c} \pm 0.00$	$2.42^{a} \pm 0.01$	$3.46^{b} \pm 0.04$
Carbohydrates (g/100 g)	$61.74^{a} \pm 0.13$	$71.58^{b} \pm 0.07$	$62.14^{a} \pm 0.08$
Dietary fiber (g/100 g)	13.19	5.66	10.81
Soluble fiber (g/100 g)	1.81	1.14	1.80
Insoluble Fiber (g/100 g)	11.38	4.52	9.01
Total calories (kcal/100 g)	$314.67^{a} \pm 0.03$	$335.22^{c} \pm 0.39$	$321.64^{b} \pm 0.21$
Minerals			
Na (mg/100 g)	$6.37^{a} \pm 0.06$	$6.56^{a} \pm 2.85$	$6.39^{a} \pm 0.08$
K (mg/100 g)	$349.25^{b} \pm 2.64$	$244.77^{a} \pm 2.66$	$346.07^{b} \pm 5.30$
Mg (mg/100 g)	$84.56^{a} \pm 1.25$	$127.40^{b} \pm 2.85$	$148.82^{c} \pm 1.15$
Ca (mg/100 g)	$2.43^{a} \pm 0.18$	$7.58^{c} \pm 0.14$	$5.51^{b} \pm 0.19$
P (mg/100 g)	$243.04^{a} \pm 4.58$	$316.66^{b} \pm 0.88$	$351.36^{c} \pm 0.33$
Mn (mg/100 g)	$0.39^{a} \pm 0.00$	$3.21^{c} \pm 0.00$	$1.01^{b} \pm 0.02$
Fe (mg/100 g)	$1.61^{b} \pm 0.01$	$0.83^{a} \pm 0.01$	$3.59^{c} \pm 0.24$
Zn (mg/100 g)	$1.99^{b} \pm 0.03$	$1.68^{a} \pm 0.03$	$1.97^{b} \pm 0.00$
Cu (mg/100 g)	$0.18^{a} \pm 0.00$	$0.22^{b} \pm 0.01$	$0.22^{b} \pm 0.01$
Particle size (%)			
<0.1 (mm)	7.82^{a}	6.83^{a}	12.20^{b}
0.1–0.5 (mm)	71.63^{b}	40.40^{a}	79.02^{c}
0.5–1.7 (mm)	20.60^{b}	52.81^{c}	8.86^{a}

Values represent the mean ± SD (n = 2). Different letters in the same row indicate statistic differences ($p < 0.05$) among samples.

3.2. *Rheological evaluation of the doughs*

3.2.1. *Pasting properties*

The raw flours, particularly WSF and WCF presented similar pasting properties such as Ptemp, CV, TV, BDV, but differed in SBV, and FV (Fig. 1). On the other hand, WCF and WSF showed a PV of 626 and 696 cP, respectively. Whereas, the PBRF sample presented a peculiar significant increase ($p < 0.05$) of PV, FV, SBV values after the extrusion process. This atypical behavior was also found by Cheng et al. (2020), in buckwheat flour processed by thermoplastic extrusion at 100 °C and high moisture (58 and 70%). The authors attributed this behavior to an increase in granular rigidity resulting from an increase in the crystalline order and the interactions of the starch chain within the amorphous regions, which together caused the increase in peak viscosity due to heat resistance and shear of the modified starch granules (Wang, Wang, Wang, & Wang, 2017).

The thermal stability of all samples, during the holding at 95 °C (TV), decreased ($p < 0.05$) after the extrusion process, while the BDV increased ($p < 0.05$). The EPBRF sample showed the highest values in all pasting properties in comparison with EWCF and EWSF. On the other hand, FV and SBV showed a significant typical decrease ($p < 0.05$) for the WSF and WCF samples. This behavior is associated with the disruption of the molecular order of the starch granules during the extrusion process causing a loss of their integrity and crystallinity (Linko, Linko, & Olkku, 1983).

3.2.2. *Farinographic properties*

The water absorption levels ranged from 88.0 to 122.0% (Fig. 2a), on a flour weight basis, and within this range it was possible to detect a good dough formation and handling. The extruded flours showed shorter DDT (Table 3), due to the partial modification of their components as was evidenced in the pasting properties (Fig. 1). Namely, rapid water absorption was observed by the extruded flour, which can reduce bread processing time and energy costs. Samples EWCF and EWSF presented a significant increase ($p < 0.05$) in both DST and MTI in comparison with EPBRF (Table 3), and they showed an increased resistance to mechanical work but without affecting the structure of the dough throughout the bread-making process. The increment in both properties may be associated to the reduction of the particle size by the insoluble fiber, which consequently causes an increase in the dough viscosity (Liu, Ma, Li, & Wang, 2019). The EPBRF sample presented opposite results, possibly due to its degree of starch conversion prior to the extrusion process caused by the parboiling process, which allowed high farinographic consistency, but reduced stability when compared to PBRF.

As previously mentioned, the extrusion process had a positive impact on the increase of the WA and farinographic consistency (Fig. 2a and b); a similar behavior was observed by Bourekoua, Benatallah, Zidoune, and Rosell (2016) for rice and corn flours when they underwent hydrothermal treatment. Furthermore, the water absorption capacity of all WG flours after the extrusion process were very similar, but their corresponding levels of consistency were very variable.

All flour samples demonstrated that water levels below the optimum caused a loss of consistency of the dough due to a lack of hydration, while, doughs with water levels above the optimum caused rapid absorption leading to low consistency development and water exudation. In both cases, the absence of consistency may be associated with the higher affinity of the fiber for water, which hinders both the interaction between water and the biopolymers (mainly starch) and the consequent formation of the dough consistency. Furthermore, since EPBRF had a parboiling pretreatment it exhibited higher levels of consistency

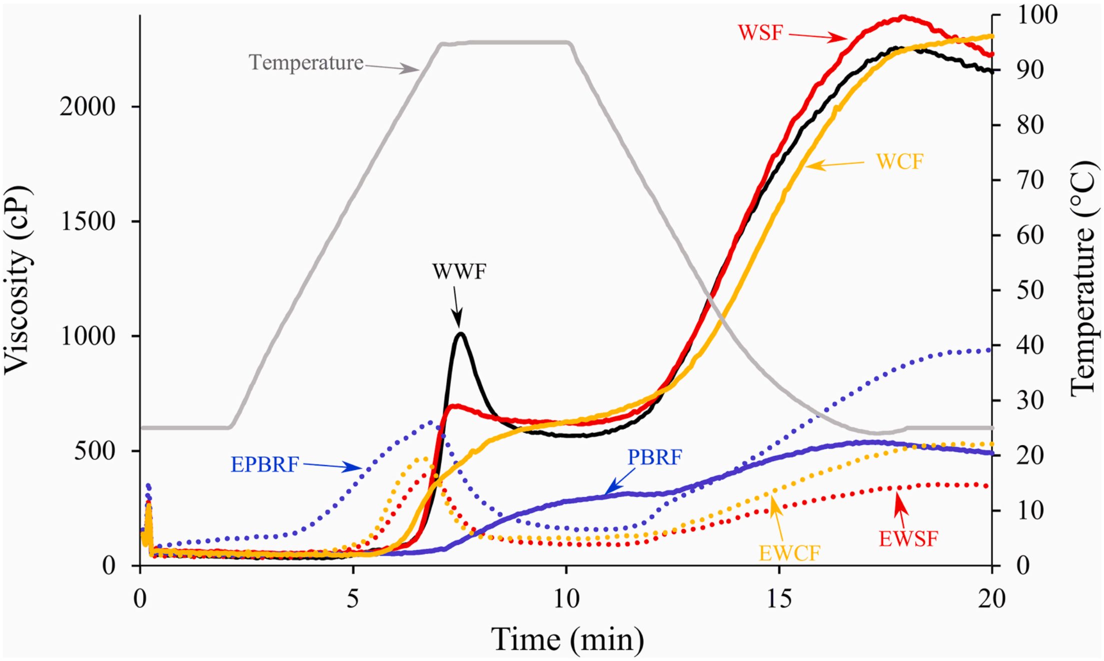

Fig. 1. Paste viscosity properties of WWF (commercial whole wheat flour), raw flours (WCF-whole corn flour, PBRF-parboiled brown rice flour, and WSF-whole sorghum flour) and extruded flours (EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, and EWSF-extruded whole sorghum flour). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

compared to EWCF, EWSF, and WWF (Fig. 2b).

3.2.3. *Dynamic mechanical properties*

Raw flours showed a rapid collapse of the biopolymeric structures when subjected to oscillatory shears between the ranges of 0.1–1 Hz (Fig. 2c). Whereas PBRF exhibited type III behavior, which is a characteristic of some molten polymers (Hyun et al., 2011; Wan et al., 2005) where G' decreases and G'' increases followed by a decrease. The extrusion process promoted a significant reduction ($p < 0.05$) of τ for EWCF and EWSF (Table 3), indicating an early disintegration of the new biopolymeric structures in comparison with the native structures. Also, it was found that the extruded flours tolerated a higher γ. Regarding the raw flours, the γ values were 0.010, 0.021, and 0.010 for WCF, PBRF, and WSF respectively, while the extruded samples presented 0.017, 0.142 and 0.020 for EWCF, EPBRF, and EWSF, respectively (Table 3). This increase in γ caused a positive effect of the extrusion process on the transformation of raw materials forming a novel biopolymeric structures with differentiated functional properties.

At frequencies (or angular velocities) greater than 1 Hz, a fall and crossover of the modulus G' and G'' was observed in the WCF and WSF, such behavior is typical in non-crosslinked polymers (Fig. 2c). The opposite effect was shown by those flours subjected to the extrusion process (Fig. 2d). The crosslinking phenomenon is typical in some extruded biopolymers (i.e. cereal melts) which display an ascending linear behavior of the elastic (G') and viscous (G'') modules (Brent, Mulvaney, Cohen, & Bartsch, 1997).

All extruded flours (Fig. 2d) presented a predominant elastic component ($G' > G''$). The tan δ of EWSF and EWCF decreased (Table 3), indicating an increase in the elasticity, which can be attributed to the plasticizing effect promoted by extrusion on the formation of binding zones among carbohydrate polymers (Brent et al., 1997). Another possible effect could be due to the starch complexation with proteins and lipids (Wu, Li, Wang, Özkan, & Mao, 2010), because those samples presented higher values (Table 2). On the contrary, tan δ of PBRF increased after the extrusion process, probably due to the previous parboiling process that caused the increase and resistance of granular stiffness of rice starch.

3.2.4. *Principal components analysis for flours*

The two first principal components explained 70.3% of the total variance among a total of 13 variables that represent three groups of results (pasting viscosity, empirical, and fundamental rheology). PC analysis evidenced the differences between the GF cereals and wheat (Fig. 3a). The main variables that characterized these three raw flours were the pasting properties of FV, SBV, and tan δ (Fig. 3b), while WWF presented high BDV (Fig. 1).

Furthermore, considering the high values of the rheological properties of WA, τ, γ, and CV and the low values of PTemp and PV, it was possible to identify and differentiate a cereal with previous heat treatment such as EPBRF and raw cereals. On the other hand, the variables with the least contribution in the PC1 and PC2 were BDV, DST, and DDT. Finally, the PCs on the raw and extruded flours were used to apply HCPC (Fig. 3c), resulting in 4 groups depending on the type of heat treatment to which the flours were subjected, as follows: raw flour samples (WWF, WSF, and WCF), parboiled brown rice flour (PBRF), the extruded brown parboiled rice flour (EPBRF) and the extruded flours (EWSF and EWCF).

Very high positive correlations ($0.90 > r \leq 0.99$) were detected between FV-TV, SBV-tan δ, SBV-TV, SBV-tan δ, DST-DDT and τ-γ (Fig. 3d). Also there were high positive correlations ($0.70 > r \leq 0.90$) between the variables PV-FV, PV-SBV, PV-tan δ, CV- τ, CV- γ, and TV-tan δ. High negative correlations between BDV-PTemp and WA-PV, SBV, and tan δ were seen as well as negative correlations between BDV-PTemp and WA-PV, SBV, and tan δ. These correlations could be explained by the effect of the extrusion process on the pasting properties, the WA and on the dynamic mechanical properties. These effects caused a disruption of the biopolymeric structures, as evidenced by an increasing τ and γ (Table 3) produced by the formation of hydrophilic bonds and protein crosslinking (Brent et al., 1997).

3.3. *Quality evaluation of GF bread*

3.3.1. *Specific volume*

The specific volume of the bread from the extruded flours were 1.04, 1.30 and 1.42 cm^3/g for EPBRF, EWCF and EWSF, respectively. In the case of sorghum, the results coincide with Torbica et al. (2019) where

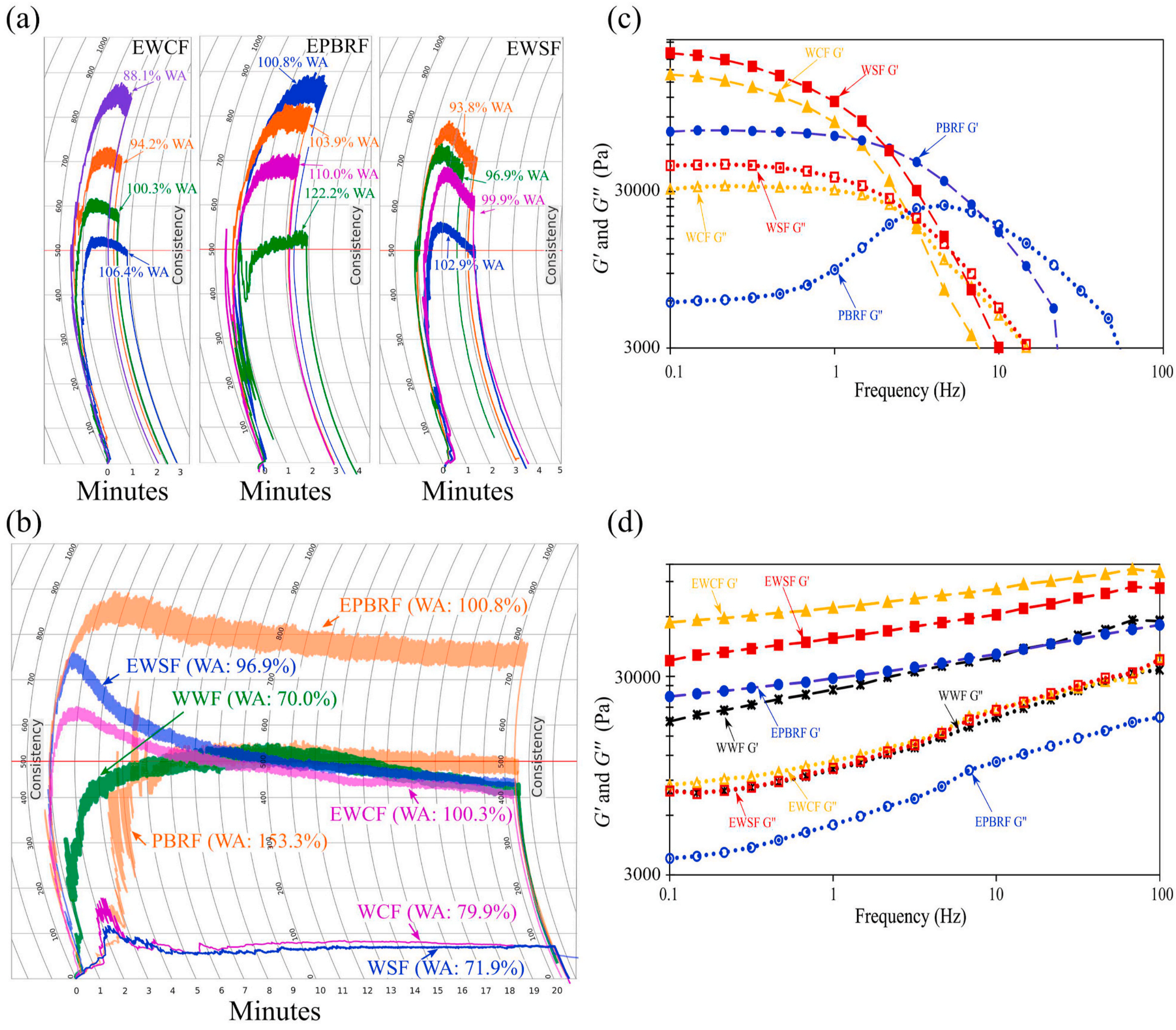

Fig. 2. Empirical and fundamental properties of raw and extruded flours. (a) optimal water absorption of extruded whole grain flours, (b) farinograms of whole grain flour of corn (WCF) parboiled brown rice (PBRF) and sorghum (WSF); extruded whole grain flours (EWCF, EPBRF, and EWSF); and whole wheat flour (WWF), (c) behavior of gluten-free whole grain flours and (d) development of elastic (G′) and viscous modulus (G″) in extruded flours. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Empirical and fundamental dynamic mechanical properties of raw and extruded whole grain flours.

Samples	Farinographic properties				Dynamic mechanical properties		
	WA (%)	DDT (min)	DST (min)	MTI(BU)	Critical stress (τ, Pa)	Critical Strain(γ)	tan δ(G''/G', at 1 Hz)
WWF	70.0	$9.8^{d} \pm 0.4$	$9.0^{d} \pm 1.4$	$45.0^{c} \pm 7.1$	$60.8^{a} \pm 0.9$	$0.002^{a} \pm 0.0000$	$0.402^{f} \pm 0.003$
WCF	80.0	$3.0^{c,\alpha} \pm 0.7$	$0.6^{a,\alpha} \pm 0.0$	$121.5^{f,\alpha} \pm 4.9$	$971.6^{b,\alpha} \pm 45.8$	$0.010^{b,\alpha} \pm 0.0001$	$0.370^{f,\alpha} \pm 0.007$
EWCF	100.5	$1.9^{ab,\beta} \pm 0.1$	$1.3^{b,\beta} \pm 0.0$	$101.5^{e,\beta} \pm 3.5$	$888.7^{b,\beta} \pm 1.1$	$0.017^{c,\beta} \pm 0.0002$	$0.170^{b,\beta} \pm 0.001$
PBRF	153.3	$11.5^{e,\alpha} \pm 0.7$	$10.0^{d,\alpha} \pm 2.1$	$21.0^{b,\alpha} \pm 1.4$	$1456.0^{d,\alpha} \pm 55.2$	$0.021^{d,\alpha} \pm 0.0014$	$0.129^{a,\alpha} \pm 0.001$
EPBRF	100.8	$2.6^{bc,\beta} \pm 0.1$	$3.8^{c,\beta} \pm 0.2$	$82.5^{d,\beta} \pm 3.5$	$3501.5^{e,\beta} \pm 265.2$	$0.142^{e,\beta} \pm 0.0143$	$0.183^{c,\beta} \pm 0.005$
WSF	71.9	$1.8^{ab,\alpha} \pm 0.4$	$0.7^{ab,\alpha} \pm 0.2$	$6.5^{a,\alpha} \pm 0.7$	$1287.0^{cd,\ \alpha} \pm 69.3$	$0.010^{b,\alpha} \pm 0.0000$	$0.330^{e,\alpha} \pm 0.008$
EWSF	96.9	$1.5^{a,\beta} \pm 0.1$	$1.0^{ab,\beta} \pm 0.1$	$20.5^{b,\beta} \pm 2.1$	$1033.2^{bc,\beta} \pm 57.8$	$0.020^{c,\beta} \pm 0.0003$	$0.222^{d,\beta} \pm 0.007$
Parametric assumptions							
Shapiro test	–	0.61	0.04	0.99	0.06	0.00	0.51
Bartlett test	–	0.43	0.00	0.69	0.01	0.00	0.56
Box-Cox							
λ	–	–	0.06	–	−0.42	−0.75	–

WA-water absorption, unique value without variation. DDT-dough development time (min), DST- dough stability time (min), BU-Brabender units, and MTI- mixing tolerance index - 5 min after peak (BU).

WWF- commercial whole wheat flour, WCF-whole corn flour, PBRF-parboiled brown rice flour, WSF-whole sorghum flour, EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, and EWSF-extruded whole sorghum flour.

Values represent the mean ± SD ($n = 3$). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t-tests for each type of cereal before and after the extrusion process. Box-Cox transformation factor (λ) for non-parametric data.

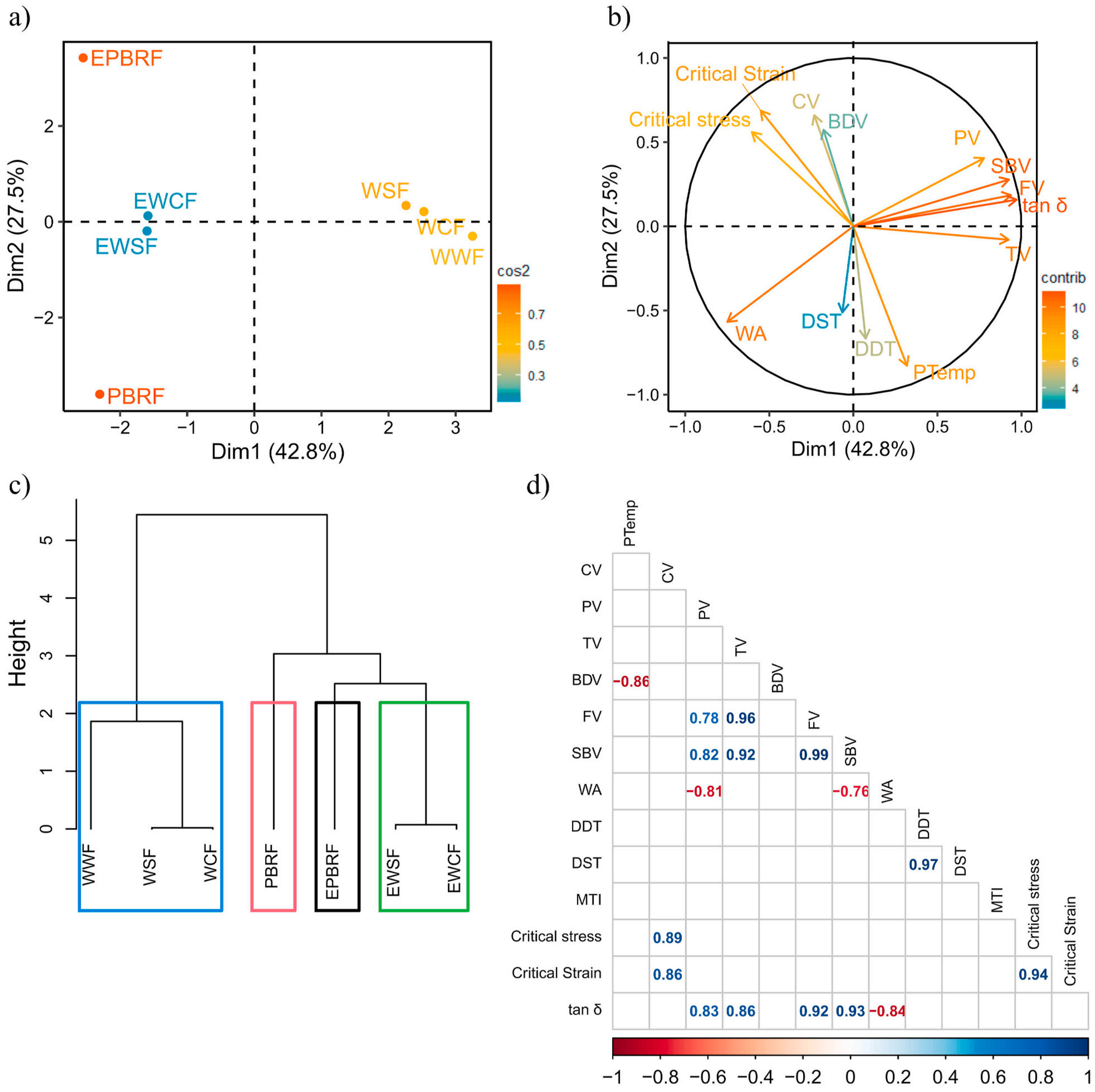

Fig. 3. Principal components analysis (PCA) of whole grain flours. a) score plot for flour samples, b) PCA loading plot for response variables, c) hierarchical clustering on principal components of raw and extruded flours and d) Pearson's correlation coefficients among pasting viscosity, empirical and fundamental rheology properties.

the sorghum bread was obtained by a combined thermal process. GF breads made from the extruded whole flours had a significant increase ($p < 0.05$) in their specific volume, which were 66% for EWCF, 33% for EPBRF and 82% for EWSF compared to Control 2 (Table 4). This could indicate the favorable effect of extrusion, corroborating the studies of Martínez et al. (2013) and Clerici et al. (2009). In addition, this increase in specific volume is related to the improvement in the dough consistency (Fig. 2b), which in turn may be attributed to the conversion of biopolymeric structures leading to an increase of water absorption, favoring the crosslinking phenomenon through the formation of molecular bonds (Barbiroli et al., 2013). However, GF breads made from extruded flours presented lower specific volume values ($p < 0.05$) than Control 1.

The incorporation of 5% GM had a negative effect ($p < 0.05$) on the specific volume of EWCF and EWSF (Table 5), due to the natural hydrolysis of the germinated millet starch, which causes a dilution effect and weakening of the dough. On the other hand, the 5% GM showed a positive effect ($p < 0.05$) on EPBRF, which may be due to its weakening biopolymeric structures with high cohesiveness (Table 3), characteristics of parboiled rice samples (Barbiroli et al., 2013).

3.3.2. *Bread crumb structure*

The EPBRF exhibited a very significant collapse of the internal air cells (Fig. 4). On the other hand, EWSF and EWCF presented a better distribution and less collapse. The extruded samples presented significant increases in TBA, TCA, P, and H than Control 2 (Table 4), and this shows that extruded flours allow the development of structural properties associated with the gas retention capacity. On the other hand, the SA in EWCF and EWSF showed significant increases ($p < 0.05$) compared to Control 2. The EPBRF presented a similar ($p > 0.05$) value, due to the significant collapse of its internal air cells, which hindered the formation of a porous structure. This may be associated with a low value of the G'' in the dough (Fig. 2c) and by the possible high air permeability

Table 4

Specific volume and structure properties of gluten-free bread.

Samples	Specific volume, SV (cm^3/g)	Total area of bread slice, TBA (mm^2)	Total cell area, TCA (mm^2)	Solid area, SA (mm^2)	Porosity, P (%)	Height, H (mm)
Control 1	$2.23^{f} \pm 0.10$	$825.48^{e} \pm 78.63$	$118.745^{e} \pm 23.04$	$735.44^{e} \pm 38.03$	$13.49^{b} \pm 2.69$	$32.7^{d} \pm 2.0$
Control 2	$0.78^{a} \pm 0.05$	$339.22^{a} \pm 1.90$	$7.47^{a} \pm 3.82$	$331.70^{a} \pm 5.63$	$1.96^{a} \pm 0.82$	$19.4^{a} \pm 0.4$
EWCF	$1.30^{d,\alpha} \pm 0.04$	$504.32^{d,\alpha} \pm 2.32$	$88.62^{bc,\alpha} \pm 21.32$	$422.93^{d,\alpha} \pm 33.88$	$16.29^{bc,\alpha} \pm 6.50$	$24.1^{c,\alpha} \pm 0.1$
EWCF+5% GM	$1.15^{c,\beta} \pm 0.01$	$373.31^{ab,\beta} \pm 36.45$	$26.44^{a,\beta} \pm 2.47$	$362.87^{abc,\beta} \pm 0.66$	$6.87^{a,\beta} \pm 0.28$	$19.5^{a,\alpha} \pm 0.9$
EPBRF	$1.04^{b,\alpha} \pm 0.06$	$410.61^{bc,\alpha} \pm 14.37$	$77.87^{bc,\alpha} \pm 4.02$	$331.41^{a,\alpha} \pm 1.99$	$18.93^{bc,\alpha} \pm 0.05$	$21.7^{b,\alpha} \pm 0.2$
EPBRF+5% GM	$1.11^{bc,\beta} \pm 0.04$	$434.73^{bcd,\beta} \pm 6.62$	$71.92^{b,\ \alpha} \pm 3.73$	$343.81^{ab,\ \beta} \pm 4.50$	$16.26^{bc,\alpha} \pm 1.01$	$22.7^{bc,\beta} \pm 0.1$
EWSF	$1.42^{e,\alpha} \pm 0.03$	$478.41^{cd,\alpha} \pm 22.85$	$101.96^{cd,\alpha} \pm 3.19$	$383.04^{bcd,\alpha} \pm 35.36$	$20.10^{c,\alpha} \pm 3.48$	$23.2^{bc,\alpha} \pm 0.4$
EWSF+5% GM	$1.12^{bc,\beta} \pm 0.06$	$497.31^{cd,\beta} \pm 0.97$	$92.06^{bcd,\beta} \pm 6.99$	$405.25^{cd,\beta} \pm 6.02$	$18.50^{bc,\alpha} \pm 1.36$	$23.6^{bc,\beta} \pm 0.5$
Parametric assumptions						
Shapiro test	0.84	0.03	0.12	0.06	0.81	0.28
Bartlett test	0.18	0.01	0.26	0.08	0.09	0.18
Box-Cox						
λ	–	−0.54	–	–	–	–

Control 1-bread of whole wheat flour, Control 2-mixture of non-extruded and extruded rice flour in the proportion of 50:50%, EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, EWSF-extruded whole sorghum flour, and 5% GM-incorporation of 5% of germinated millet.

Values represent the mean ± SD ($n = 6$). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t tests for each type of cereal with incorporation of 5% germinated millet. Box-Cox transformation (λ) for non-parametric data.

Table 5

Texture profile analysis (TPA) of gluten-free breads of corn, parboiled brown rice and sorghum.

Samples	Hardness (N)	Adhesiveness (g.s)	Cohesiveness (−)	Springiness (−)	Chewiness (N)	Resilience (−)
Control 1	$7.77^{b} \pm 0.38$	$-14.41^{bc} \pm 1.47$	$0.41^{d} \pm 0.01$	$0.95^{d} \pm 0.03$	$2.74^{c} \pm 0.45$	$0.12^{b} \pm 0.00$
Control 2	$12.66^{c} \pm 0.62$	$-1.25^{de} \pm 0.50$	$0.22^{b} \pm 0.04$	$0.73^{b} \pm 0.02$	$2.02^{c} \pm 0.44$	$0.12^{b} \pm 0.03$
EWCF	$18.83^{e,\alpha} \pm 1.05$	$-33.97^{ab,\alpha} \pm 12.22$	$0.30^{c,\alpha} \pm 0.03$	$0.94^{d,\alpha} \pm 0.03$	$5.21^{d,\alpha} \pm 1.31$	$0.10^{ab,\alpha} \pm 0.00$
EWCF+5% GM	$5.42^{a,\beta} \pm 0.22$	$-0.21^{e,\beta} \pm 0.18$	$0.08^{a,\beta} \pm 0.01$	$0.47^{a,\beta} \pm 0.01$	$0.21^{a,\beta} \pm 0.02$	$0.04^{a,\beta} \pm 0.00$
EPBRF	$15.99^{d,\alpha} \pm 1.32$	$-5.79^{cd,\alpha} \pm 1.84$	$1.00^{e,\alpha} \pm 0.05$	$1.00^{d,\alpha} \pm 0.03$	$8.97^{e,\alpha} \pm 1.32$	$0.27^{ab,\alpha} \pm 0.08$
EPBRF+5% GM	$7.31^{b,\beta} \pm 1.31$	$-4.60^{cd,\alpha} \pm 1.62$	$0.12^{a,\beta} \pm 0.01$	$0.97^{d,\alpha} \pm 0.09$	$0.77^{b,\beta} \pm 0.03$	$0.06^{ab,\beta} \pm 0.01$
EWSF	$16.45^{d,\alpha} \pm 0.11$	$-54.93^{a,\alpha} \pm 5.10$	$0.19^{b,\alpha} \pm 0.02$	$0.81^{bc,\alpha} \pm 0.06$	$2.44^{c,\alpha} \pm 0.37$	$0.08^{ab,\alpha} \pm 0.01$
EWSF+5% GM	$5.16^{a,\beta} \pm 0.66$	$-2.37^{d,\beta} \pm 1.30$	$0.07^{a,\beta} \pm 0.02$	$0.90^{cd,\alpha} \pm 0.14$	$0.35^{ab,\beta} \pm 0.18$	$0.03^{ab,\beta} \pm 0.01$
Parametric assumptions						
Shapiro test	0.59	0.03	0.56	0.80	0.80	0.00
Bartlett test	0.31	0.04	0.61	0.38	0.06	0.01
Box-Cox						
λ	–	0.26	–	–	–	0.22

Control 1-bread of whole wheat flour, Control 2-mixture of non-extruded and extruded rice flour in the proportion of 50:50%, EWCF: extruded whole corn flour, EPBRF: extruded parboiled brown rice flour, EWSF: extruded whole sorghum flour, and 5% GM-incorporation of 5% of germinated millet.

Values represent the mean ± SD ($n = 8$). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t tests for each type of cereal with incorporation at 5% of germinated millet. Box-Cox transformation (λ) for non-parametric data.

generated by the larger particle size in the parboiled rice sample (Table 2). EWSF and EWCF showed lower structural collapse and better air cell distribution, which indicates that the extrusion process greatly favored the formation of internal air cells and porous structure in GF whole grain breads. However, this was not enough to obtain GF bread with similar crumb structure to Control l.

The incorporation of 5% GM improved the appearance of the bread crumbs for all GF breads (Fig. 4c), with EWCF+5% GM being the most affected ($p < 0.05$) by the incorporation of 5% GM causing a reduction ($p < 0.05$) in TBA, TCA, and SA (Table 4), followed by EWSF+5% GM that had a significant decrease ($p < 0.05$) in TCA. However, EPBRF+5% GM was the bread sample that presented significant increases in TBA, SA, and H.

3.3.3. Texture profile analysis (TPA)

Hardness values were 15.9, 16.4 and 18.8 N for EPBRF, EWSF and EWCF, respectively (Table 5). These values could be highly related to the dietary fiber content (Table 2), indicating that within the dietary fiber, in particular insoluble fiber contributed to the hardness of the GF bread crumb (Phimolsiripol, Mukprasirt, & Schoenlechner, 2012).

The hardness values, found in EWSF breads, were lower than the values obtained by Torbica et al. (2019), who used thermal pretreatments and additives to improve their gluten-free sorghum breads. The crumb hardness values of EPBRF breads were lower than those reported by Sciarini, Ribotta, León, and Pérez (2008) in gluten-free rice breads. Breads made with EWCF exhibited lower hardness values than those found by Kotancılar, Güdük, and Seyyedcheraghı (2018), who used corn flour with the addition of eggs and yogurt as natural additives.

The incorporation of 5% GM had a positive effect, mainly on the significant reduction ($p < 0.05$) of the hardness and cohesiveness of the bread crumbs (Table 5), which could be attributed to the enzymatic system that hydrolyzed the starch present in the samples, weakening the microstructure and therefore reducing crumb rigidity. These findings differ from those found by Horstmann et al. (2019) when adding 5% of sprouted brown millet in gluten-free bread. On the other hand, in the same investigation, similar results to ours were reported, when sprouts of amaranth, quinoa, and corn were incorporated. The springiness was similar ($p > 0.05$) in breads made from EPBRF and EWSF, indicating that the addition of 5% GM did not significantly affect this parameter. In contrast, the springiness decreased ($p < 0.05$) for EWCF breads; this is probably associated with its low protein content, which reduces the degree of crosslinking, and the high content of insoluble fiber that causes network weakening.

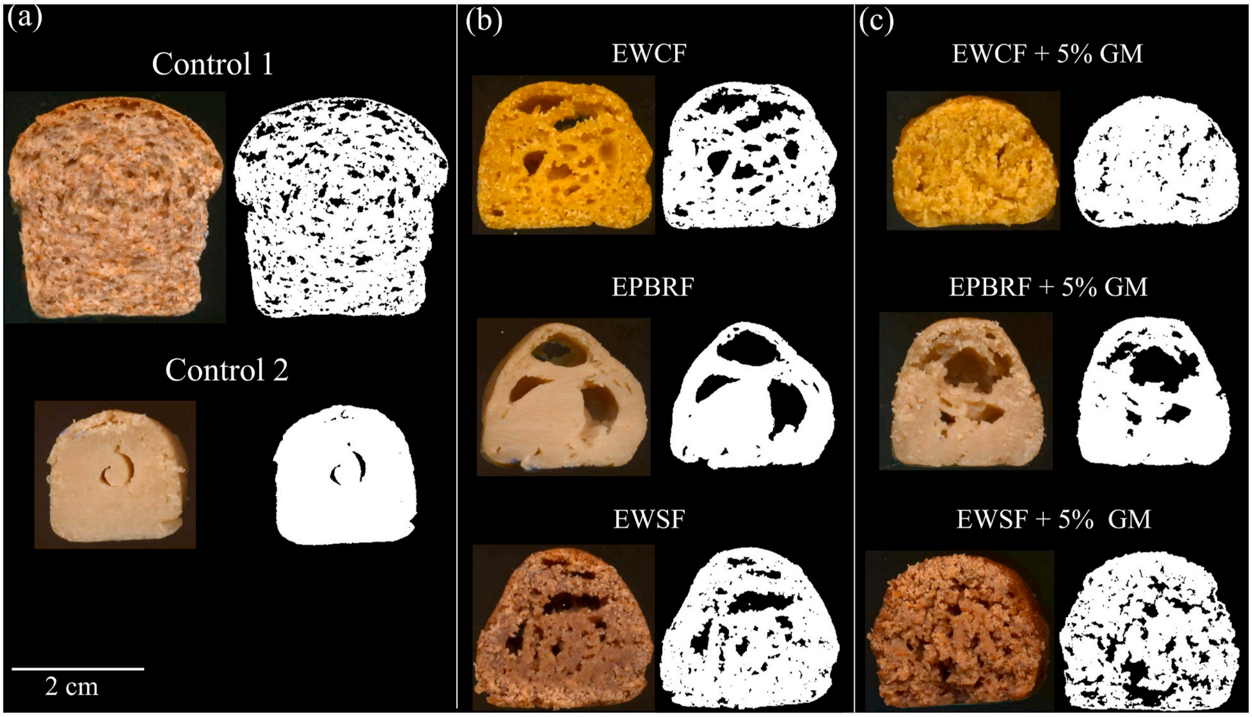

Fig. 4. Structure of the bread crumb. (a) bread controls-control 1 (commercial whole wheat flour, WWF) and control 2 (mixture of non-extruded and extruded rice flour in the proportion of 1:1), (b) GF-bread made from extruded whole flours of corn (EWCF), parboiled brown rice flour (EPBRF) and sorghum (EWSF) and (c) GF-bread with incorporation of 5% germinated millet, (5% GM). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3.4. *Principal component analysis for GF bread*

PC1 and PC2 explained 74.1% of the total variance among a total of 12 variables that represent three kinds of bread quality characteristics (physical, structural and textural properties). The EWCF and EWSF breads (Fig. 5a) were characterized by high values of the textural variables (Hd and Ad) in Fig. 5b. In addition, the EWSF bread was also represented by variables in the structure (P). Likewise, the EWCF+5% GM and EWSF+5% GM bread samples had the lowest values of the textural variables (Hd and R) in comparison to EWCF and EWSF.

The EPBRF bread presented the maximum values for the textural properties of Co, Ch and R due to the parboiling followed by extrusion. This indicates that the combined effect of the processes caused functional changes at the molecular level of the rice starch. On the other hand, the lower textural variables (Co, Ch and R) indicate the high impact that the incorporation of 5% GM had on the quality properties of corn bread (EWCF+5% GM). There was a very high correlation ($0.90 > r \leq 0.99$) between Co-Ch and R, Ch-R, SV-TBA and SA, TBA-SA and H, SA-H. Also, high significant ($p < 0.05$) positive correlations ($0.70 > r \leq 0.90$) were observed between Hd-Ch and TCA-SV, TBA, P, and H (Fig. 5c). Finally, the controls were characterized by the physical (SV), structural (TBA, TCA, SA and H) and textural (Sp) variables, with the highest and lowest values for Control 1 and Control 2, respectively.

HCPC formed five sample groups (Fig. 5d). The first group was composed of EWCF+5% GM and Control 2 samples, and was characterized by lower values of physical (SV) and structural (TBA, SA, and H) properties for Control 2 and only textural (Ch, Co, and R) for EWCF + GM 5%. The second group was formed only by Control 1, which showed the maximum values in the physical (SV), structural (TBA, TCA, SA, and H) and textural (Sp) variables among all the samples (Table 4). The third group represented by EPBRF showed the maximum values in the texture (Co, Ch, and R), indicating that the parboiled brown rice sample subjected to extrusion developed high cohesion and resistance forces in comparison to EWCF and EWSF. The fourth group was composed of breads with the inclusion of 5% GM (EWSF+5% GM and EPBRF+5% GM), with the lower values for the texture (Hd and R). Furthermore, such patterns were found for EWCF+5% GM, but the grouping technique did not consider it because it had a strong decline in the other structural and textural properties (Tables 4 and 5), leaving this category without effect.

4. Conclusions

Whole sorghum was the cereal that presented the highest amounts of iron, manganese, and copper. Parboiled brown rice showed high amounts of manganese and copper, and corn had high amounts of zinc. The modification of the paste viscosity profile in whole grain flours caused by the extrusion process was evidenced by the decrease in all the pasting properties of raw flours without pretreatment. On the other hand, the parboiled brown rice sample presented an unexpected increase in the peak viscosity, final viscosity, and seatback viscosity. Extruded flours showed high increases of water absorption and consistency as well as reduced dough development times (DDT), which would represent a lower cost for the baking processes. GF breads presented good increase in volume: 66%, 33% and 82% for EWCF, EPBRF and EWSF samples compared to the control 2, being the EWSF sample the one that approximated the bread made from wheat flour but could not reach a desired analogous volume. Addition of 5% germinated millet enhanced breadcrumb softness in all samples, being the EPBRF +5% GM sample the one that presented hardness and springiness similar to WWF. Also improved air cell distribution preventing their integration.

CRediT authorship contribution statement

Raúl Comettant-Rabanal: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Carlos Wanderlei Piler Carvalho:** Resources, Conceptualization, Methodology, Visualization, Writing – review & editing, Manuscript final version approval. **José Luis Ramírez Ascheri:** Writing –

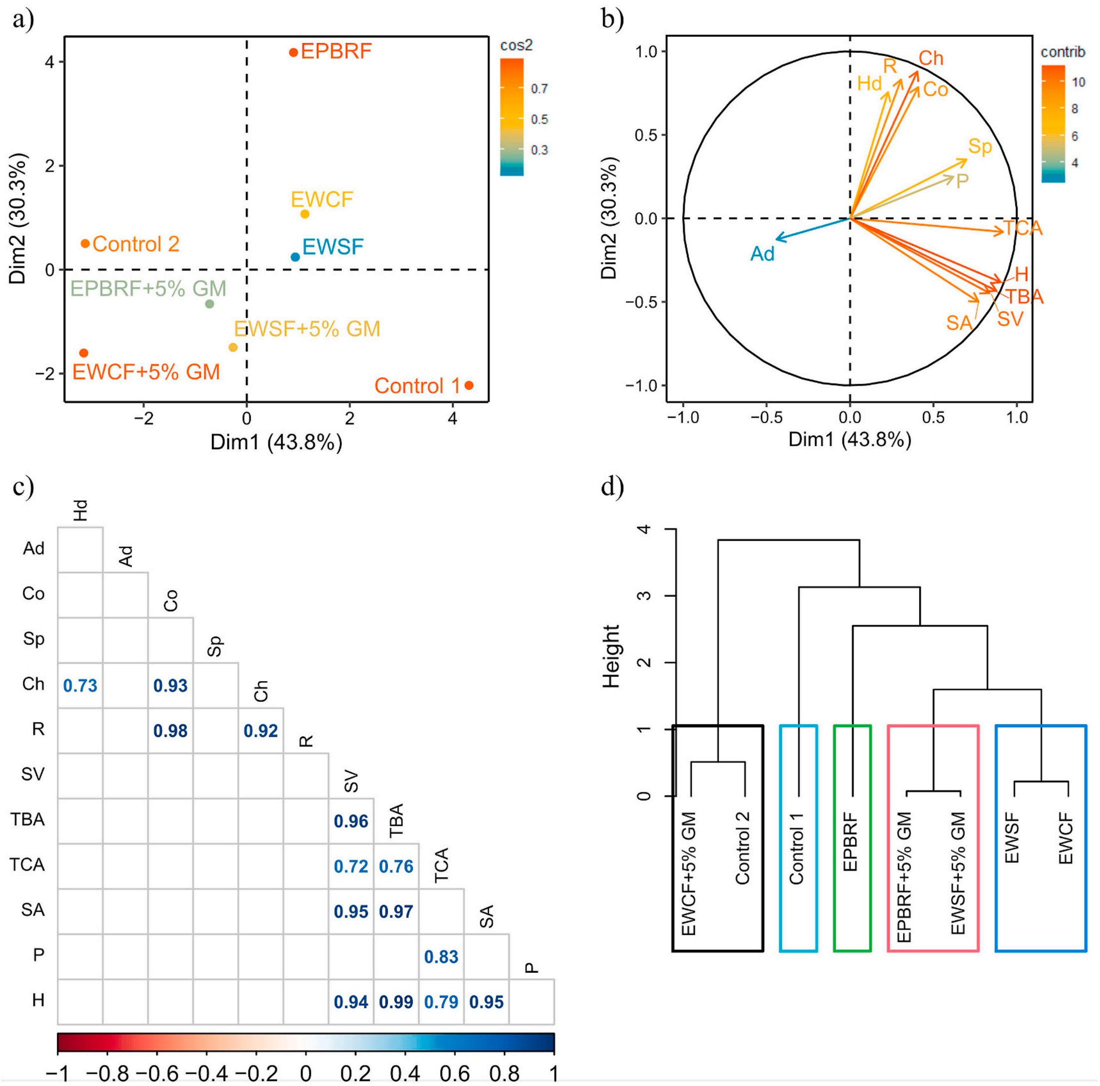

Fig. 5. Principal components analysis (PCA) of gluten-free bread. a) score plot for samples, b) PCA loading plot for response variables, c) Pearson's correlation at $p < 0.05$ and d) hierarchical clustering on principal components.

review & editing, Formal analysis, Resources, Visualization, Investigation. **Davy William Hidalgo Chávez:** Methodology, Software, Statistic process data, Writing – review & editing, Data curation. **Rogerio Germani:** Methodology, Writing – review & editing.

Declaration of competing interest

The authors declared no conflict of interest for this work.

All authors have participated in the design, analysis and interpretation of the data and approved the final version.

This manuscript has not been submitted to another journal.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject discussed in the manuscript.

Acknowledgments

The authors thank FAPERJ for the financial support, CAPES for the scholarships granted to Raúl Comettant-Rabanal and Davy W.H. Chávez and CNPq for the scholarship granted to Carlos W. P. Carvalho.

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