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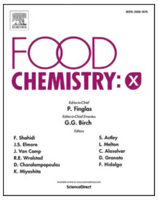

Technological, sensory, nutritional and bioactive potential of pan breads produced with refined and whole grain buckwheat flours

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ABSTRACT

The nutritional quality and bioactive potential of breads made with partial replacement of refined wheat flour (RWF) with 30% or 45% refined buckwheat flour (RBF) or whole buckwheat flour (WGBF) was assessed through mineral bioaccessibility, starch digestibility, dietary fiber content and bioactive potential by determining rutin and quercetin levels during processing. Moreover, technological quality and sensory acceptance were also evaluated. Breads made with 30% or 45% WGBF showed higher mineral and fiber contents compared to the control, while the formulations with RBF showed higher bioaccessibility. No changes were observed in the rutin levels of the dough before and after fermentation, but after baking, rutin and quercetin levels increased. The highest starch hydrolysis was found in the formulation containing 45% RBF. The formulations made with 30% RBF or 30% WGBF were well accepted by consumers. Our study shows interesting results, as few studies report the effect of processing on bioactive compounds.

1. Introduction

Buckwheat is originated from mountainous provinces of southern China and is currently cultivated in Asia, Europe and the Americas. It is an ancient pseudocereal crop under the *Polygonaceae* family and *Fagopyrum* genus, abundant in beneficial phytochemicals that provide positive effects on health (Huda et al., 2021).

The intake of foods rich in phenolic compounds is related to several health benefits, due to anti-inflammatory, anti-diabetic, anti-viral, and anti-cancer properties, from their antioxidant and free radical scavenging capacity (Costantini et al., 2014; Dziadek et al., 2016; Martín-García et al., 2021). Rutin and quercetin are the main phenolic compounds found in the buckwheat grain, with the highest concentrations detected in bran (Huda et al., 2021; Sakač et al., 2015).

Recently, phenolic compounds have received considerable attention because their dietary intake is related to lower incidence of chronic degenerative diseases, such as cancer, diabetes, Alzheimer's disease and cardiovascular diseases. Cereals, fruits, and vegetables are rich sources of phenolic compounds. In fact, the health benefits of their dietary intake have been related, at least in part, to their phenolic compounds content.

In addition to the phenolic compounds, buckwheat contains higher amounts of essential minerals when compared to the wheat grain (Huda et al., 2021; Sakač et al., 2015). The essential minerals, such as iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg), play an essential role in the human body, and are responsible for the immune system, growth and maintenance of bones and teeth (Cozzolino, 2012; Gupta & Gupta, 2014; Quintaes & Diez-Garcia, 2015). In contrast, deficiency of these minerals can lead to growth retardation, hypogonadism, decreased appetite and cognitive functions, bone loss (osteopenia/osteoporosis), among others (Gupta & Gupta, 2014; WHO, 2006).

However, the presence of a nutrient in a food does not mean its availability. Bioaccessibility is considered the fraction of a compound that is released from food in the gastrointestinal tract and becomes available for absorption. Bioaccessibility includes the entire sequence of events that occurs during gastrointestinal digestion of food and indicates the fraction of the nutrient that can be assimilated by the body (Cardoso et al., 2015a; Thakur et al., 2020). The *in vitro* digestion assays allow simulating gastrointestinal digestion, followed by determining the amount of the minerals of interest that pass through a semipermeable membrane, simulating passage through the intestinal wall (Cardoso et al., 2015b; Miller et al., 1981; Thakur et al., 2020).

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Some authors have investigated the incorporation of buckwheat flour into special foods (Bączek et al., 2020; Choy et al., 2013; Lin et al., 2009; Wolter et al., 2013). Studies have shown a good contribution of this pseudocereal in improving the nutritional and technological quality of gluten-containing and gluten-free baked products (Bączek et al., 2020; Ballabio et al., 2011; Coronel et al., 2021; Lin et al., 2009; Torbica et al., 2010; Wolter et al., 2013), due to the presence of proteins, lipids, dietary fiber, and minerals, as well as bioactive compounds. Many of these components have beneficial effects on health, such as reduction of plasma cholesterol levels, and neuroprotective, anti-carcinogenic, anti-inflammatory or anti-diabetic effects (Bączek et al., 2020; Wolter et al., 2013). Thus, the incorporation of buckwheat in the preparation of healthier foods seems to have an attractive appeal, since its components can positively affect the health of consumers.

Bread is considered a staple food worldwide and is a good source of energy for the human body. However, bread made with refined wheat flour is a nutrient-poor food and the incorporation of buckwheat in its preparation can produce healthier breads, rich in bioactive compounds, fibers and minerals (Dziki et al., 2014). Nevertheless, no studies were found in the literature on the incorporation of >15% refined and whole grain buckwheat flour in flour-based bread, aiming at investigating the technological and nutritional profile, the bioactive compounds and the sensory evaluation during processing and storage.

In this context, the objective of this study was to evaluate the use of refined buckwheat flour (RBF) and whole grain buckwheat flour (WGBF) to replace 30 and 45% refined wheat flour (RWF) in conventional bread formulations. In addition, the technological parameters, nutritional characterization, and sensory evaluation were investigated, as well as the determination of rutin and quercetin levels during bread processing, baking, and storage.

2. Material and methods

2.1. Chemicals and reagents

For starch digestibility: sodium maleate, analytical grade ethyl alcohol 99.5%, potassium hydroxide, sodium acetate and enzymatic kit (K-RSTAR, Megazyme International Ireland Ltd., Bray, Ireland) were used.

For mineral content and bioaccessibility: iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg) standard solutions were purchased from NIST. Lanthanum dioxide solution was obtained from Sigma-Aldrich (USA). Analytical grade nitric acid and hydrogen peroxide were obtained from Merck. Ultra-pure water was obtained from the Milli-Q system (Millipore Corporation, France). Enzymes: pepsin (P-7000), pancreatin (P-7545), bile salts (B-8631) and dialysis membrane (cut-off 12,000 to 16,000 and porosity 25 Å) were obtained from Sigma-Aldrich (USA).

For bioactive compounds: rutin, quercetin, and ascorbic acid standards were purchased from Sigma-Aldrich (USA). HPLC grade methanol was obtained from J.T. Backer (USA); hydrochloric acid was purchased from Êxodo Científica (Brazil) and analytical grade methanol from Synth (Brazil). Ultra-pure water was obtained from the Milli-Q system (Millipore Corporation, France). All solutions and samples were filtered through 0.22 μm pore size PVDF membranes (Millipore Corporation, France). Dihydrated quercetin and hydrated rutin standards were prepared in methanol:water (50:50) with 0.04% ascorbic acid, and kept in an ultra-freezer (−80 °C) until analysis (48 h).

2.1.1. Materials

The refined wheat flour (RWF) used was kindly donated by Anaconda Mill (São Paulo, Brazil). The buckwheat grains were obtained from Grupo Pozza (Lagoa dos Três Cantos, Brazil), and the grains were processed to obtain the flours.

Buckwheat grains presented moisture content of 12.2 ± 0.02%. Milling trials were first performed in an experimental Brabender Quadrumat Senior mill (Duisburg, Germany), with break and reduction passages, according to method 26–31.01 (AACCI, 2010), with modifications. The refined buckwheat flour (RBF) consisted of the break and reduction fractions, while the whole grain buckwheat flour (WGBF) was composed of all fractions (flour, bran, and shorts/middlings). To reduce WGBF particle size, the flour was subjected to a second milling stage in a knife mill, model 74064G, Treu SA (Rio de Janeiro, Brazil). The flours were then vacuum-packaged (1 kg) and kept in a freezer at −20 °C until physicochemical characterization and pan bread preparation.

Refined wheat flour (RWF) presented 13.19 ± 0.07% moisture; 10.29 ± 0.04% proteins; 1.13 ± 0.20% lipids; 0.53 ± 0.01% ash; and 2.07 ± 0.15% total dietary fiber. Refined buckwheat flour (RBF) presented 13.19 ± 0.37% moisture; 4.56 ± 0.15% proteins; 0.70 ± 0.02% lipids; 0.62 ± 0.02% ash; and 2.29 ± 0.20% total dietary fiber. Whole grain buckwheat flour (WGBF) presented 11.30 ± 0.05% moisture; 10.21 ± 0.90% proteins; 2.12 ± 0.07% lipids; 1.97 ± 0.03% ash; and 21.67 ± 0.91% total dietary fiber. Furthermore, RWF presented the following specifications: Falling Number: 493.50 ± 12.26 s; Wet gluten content: 29.33 ± 0.40%; Dry gluten content: 10.04 ± 0.26%; Gluten Index: 93.43 ± 4.89. Farinographic parameters: Water absorption: 58.30 ± 0.57%; Dough development time: 11.80 ± 0.35 min; Stability 16.17 ± 0.87 min. Alveographic parameters: P/L ratio: 1.64 ± 0.14; Deformation energy (W): 271.07 ± 24.95 10^{-4} J.

Bread formulation: wheat flour, sucrose, sodium chloride, instant dry yeast, whole milk powder, low-sat low-trans vegetable shortening (Triângulo Alimentos Ltda., Brazil), calcium propionate and fungal α-amylase (140,000 SKB/g) (Spring Alpha 140,000, Granotec, Curitiba, Brazil).

2.2. Methods

2.2.1. Bread preparation

Five different bread formulations were prepared: Control (made with 100% refined wheat flour); F1 (30% refined buckwheat flour and 70% refined wheat flour); F2 (30% whole grain buckwheat flour and 70% refined wheat flour); F3 (45% refined buckwheat flour and 55% refined wheat flour); F4 (45% whole grain buckwheat flour and 55% refined wheat flour). All formulations were prepared in duplicate and the formulations were calculated on a flour basis. Each batch provided 5 loaves.

The ingredients (flour basis) used in the bread making process were: flour (100%), sugar (4%), milk powder (4%), fat (4%), salt (1.8%), instant dry yeast (1.3%), calcium propionate (0.2%), α-amylase (0.0025%) and water (control: 58.3%; F1: 53.6%; F2: 56.9%; F3: 52.0%; F4: 57.0%, according to the farinographic analysis). All ingredients were mixed in an HAE10 dough mixer (Hyppolito, Ferraz de Vasconcelos, SP, Brazil) which was initially adjusted at low speed (90 rpm) for 300 s, followed by high speed (210 rpm) for 210 ± 20 s.

The dough was then divided into 200 ± 1 g portions, modeled in a 0.5 Hp HM2 molder (Hyppolito, Ferraz de Vasconcelos, SP, Brazil), placed in open molds (14 cm × 7 cm × 4 cm) and proofed in a CCKU586820-1 proofing chamber (Super Freezer, Poços de Caldas, MG, Brazil) at 38 °C and 95% relative humidity for 120 ± 8 min. The proofed dough pieces were baked in an Ipanema IP 4/80 hearth oven (Haas, Curitiba, PR, Brazil) regulated to maintain a hearth temperature of 180 °C and ceiling temperature of 195 °C, for 20 min. After baking, bread was removed from the molds, cooled to room temperature (during 2 h), packed in polyethylene bags, and stored in a controlled temperature environment (25 °C) until the time of analysis.

2.2.2. Technological characterization of pan breads

The specific volume was determined according to AACCI method 10–05.01 (2010) and expressed in mL/g. Analyses were carried out in triplicate.

Crumb color was evaluated through the parameters lightness L* (ranging from 0 = black to 100 = white), a* (−a* = green and + a* =

red), and b* (−b* = blue and + b* = yellow), using a MiniScan spectrophotometer (Hunterlab, Reston, USA), according to the CIELab system (Minolta, 1993). Analyses were carried out in triplicate.

The moisture content of the crumb and crust of the samples was determined on days 1, 5, 9, and 13 of storage, using AACCI (2010) method 44–15.02, in triplicate. The crust was considered the 1 cm portion from the bread surface and the crumb was the remaining portion.

Water activity was evaluated on days 1, 5, 9, and 13 of storage, in triplicate (AquaLab 4TEV apparatus, Decagon, Pullman, USA).

Bread crumb firmness was evaluated according to AACCI (2010) method 74–10.02, using a TA-XT2 texture analyzer (Stable Micro Systems, Surrey, England) with a load of 25 kg; P/35 aluminum probe, with a caliber of 30 mm; pre-test speed = 1.7 mm/s; test speed = 1.7 mm/s; post-test speed = 10.0 mm/s; force = 10 g; distance = 40%; compression force mode, on days 1, 5, 9, and 13 of storage. The analyses were performed on six replicates by compressing the probe on two central slices, superposed and arranged horizontally to the platform. The breads were sliced at the time of analysis into 1.25 cm thick slices, using an electric slicer.

2.2.3. *Nutritional characterization and bioactive potential of pan breads*

To determine starch digestibility, the method described by Gularte & Rosell (2011) was used. To evaluate the digestible starch of the bread samples, starch hydrolysis was performed at different periods, resulting in three distinct fractions. In the first 30 min of reaction, the rapidly digestible starch fraction was obtained, while the slowly digestible starch was obtained from 30 to 120 min. Finally, the resistant starch fraction remaining unhydrolyzed after 16 h of incubation was obtained for all samples. White bread (100% RWF) was used as control. All analyses were carried out in triplicate.

To determine hydrolysis index, the method described by to Goñi et al. (1997) was used. For the construction of the hydrolysis curve, aliquots were taken at 30, 60, 90, 120 and 180 min, and the area below the hydrolysis curve was calculated. The hydrolysis index (HI) was calculated as the ratio between the area below the hydrolysis curve of each sample and the area of the control bread. The results were expressed as a percentage. Starch hydrolysis allowed obtaining estimated glycemic index (eGI) and was calculated according to Goñi et al. (1997), in triplicate.

The *in vitro* digestion assay for the estimation of Fe, Zn Ca and Mg bioaccessibility was performed using the solubility and dialysis method, as described by Rebellato et al. (2017), on four replicates. The mineral contents, and the soluble and dialyzable fractions were quantified by Flame Atomic Absorption Spectrometry (FAAS), according to Rebellato et al. (2015), in triplicate.

The total dietary fiber of bread was calculated from the values found for the flours (RWF, RBF and WGBF, using AACCI (2010), method 32–05.01), and considering the moisture loss during the baking process.

The bioactive potential was evaluated based on rutin and quercetin levels of breads. Extraction was performed as described by Hirose et al. (2010). Approximately 0.1 g of freeze-dried sample (dough before and after fermentation and breads) was weighed in Eppendorf conical tubes and 1 mL of methanol:water solution (62.5:37.5) containing 0.04% ascorbic acid was added. Then, the tubes were placed in a water bath at 30 °C with agitation at 210 rpm for 3 h. The extractions were performed in triplicate and the extracts were filtered through PVDF membranes (0.22 µm porosity) and stored at −80 °C until the time of analysis.

For the quantification of rutin and quercetin, a high performance liquid chromatography system, Agilent 1260 (Agilent Technologies, Germany) with a quaternary pump, automatic injector, and photodiode array detector (DAD) was used. A C18 column (Ace HPLC Columns, USA), 150 mm long, 3 mm internal diameter and 5 µm particle size was used, with column oven controlled at 25 °C. The mobile phase was composed of two solvents: A (water acidified with formic acid at 0.3%) and B (methanol). The initial mobile phase was composed of 20% B, with a linear gradient increase up to 70% at 4 min and 20% at 4.1 min, remaining until the end of the analysis (7.2 min). The flow rate was 1 mL/min and the injection volume was 50 µL. The identification was performed by comparing the absorption spectra of the samples and the spectra of rutin and quercetin standards. The quantification was performed by external calibration, with detection at 370 nm, in triplicate.

Table 1
Specific volume and instrumental color parameters of breads with different RBF and WGBF incorporations.

Formulations	SV (mL/g)	L*	a*	b*
Control (100% RWF)	3.98 ± 0.05a	82.78 ± 0.37a	1.54 ± 0.07e	20.32 ± 0.49a
F1 (30% RBF)	3.62 ± 0.05b	72.45 ± 0.46b	3.14 ± 0.07d	20.89 ± 0.28a
F2 (30% WGBF)	3.05 ± 0.06c	52.68 ± 0.70d	4.45 ± 0.14b	14.77 ± 0.20b
F3 (45% RBF)	2.43 ± 0.02d	68.33 ± 0.40c	4.10 ± 0.04c	20.19 ± 0.35a
F4 (45% WGBF)	2.21 ± 0.04e	43.98 ± 0.22e	4.80 ± 0.19a	13.00 ± 0.06c

RWF: refined wheat flour; RBF: refined buckwheat flour; WGBF: whole grain buckwheat flour; SV: specific volume. Means followed by the same letter in the columns do not differ significantly by the Tukey test ($p < 0.05$).

2.2.4. *Sensory evaluation*

Sensory evaluation was carried out 24 h after bread preparation. The acceptance and purchase intention tests were applied to 116 consumers, aged between 17 and 59 years, recruited through posters and e-mails.

Prior to the sensory evaluation, the panelists read and signed the Informed Consent Form (ICF), indicating agreement to participate in the tests, according to the protocol of the Research Ethics Committee of the University of Campinas (UNICAMP) (CAAE 53020816.5.0000.5404). The consumers received half a slice of bread at room temperature in individual white-light booths. Samples were served on coded paper napkins with random three-digit numbers in complete balanced blocks along with the response form. The sensory acceptance test used an unstructured 9-cm hedonic scale (from “disliked very much” to “liked very much”) to evaluate crumb appearance, crumb color, odor, flavor, texture and overall impression. The purchase intention test, which expresses the willingness to buy a particular sample, used a 5-point structured scale (ranging from 1 = “would certainly not buy” to 5 = “would certainly buy”) (Stone et al., 2012).

2.3. *Statistical analysis*

Results were evaluated through ANOVA and the Tukey test ($p \leq 0.05$), using Statistica 7.0 software (Statsoft, Tulsa, USA).

3. Results and discussion

3.1. *Bread quality/physical properties*

The results of specific volume and instrumental color of the breads are presented in Table 1.

Specific volume values ranged from 2.21 to 3.98 mL/g, with significant differences ($p \leq 0.05$) between the formulations. Percentual reductions in specific volume were of 9% for F1 (30% RBF + 70% RWF), 23% for F2 (30% WGBF + 70% RWF), 39% for F3 (45% RBF + 55% RWF) and 44% for F4 (45% WGBF + 55% RWF), showing how compounds present in WGBF had a greater detrimental effect on volume.

As expected, it was observed that the higher the RBF and WGBF percentages incorporated into refined wheat flour (RWF), the lower the specific volume values. A decrease in the specific volume of bread with the addition of buckwheat flour was expected due to the dilution of gluten-forming proteins (gliadin and glutenin) present only in RWF.

Table 2
Crust and crumb moisture contents and firmness values of breads with different RBF and WGBF incorporations during storage.

Formulations	Day 1	Day 5	Day 9	Day 13
	Crust moisture content (%)			
Control (100% RWF)	24.79 ± 0.25aC	29.08 ± 0.27aB	29.59 ± 0.10bA	29.98 ± 0.30bA
F1 (30% RBF)	24.82 ± 0.20aC	29.33 ± 0.17aB	30.12 ± 0.16aAB	30.63 ± 0.17aA
F2 (30% WGBF)	25.24 ± 0.10aC	27.43 ± 0.23bB	28.50 ± 0.23cA	28.91 ± 0.20cA
F3 (45% RBF)	23.75 ± 0.18bB	27.11 ± 0.10bA	27.30 ± 0.12cA	27.37 ± 0.10dA
F4 (45% WGBF)	23.79 ± 0.10bC	26.92 ± 0.20cB	28.16 ± 0.20cA	28.55 ± 0.21cA
	Crumb moisture content (%)			
Control (100% RWF)	32.01 ± 0.20bB	32.02 ± 0.18bB	32.72 ± 0.10bA	31.12 ± 0.30bC
F1 (30% RBF)	33.42 ± 0.10aA	33.21 ± 0.13aA	33.20 ± 0.14aA	32.29 ± 0.14aB
F2 (30% WGBF)	32.08 ± 0.10bA	31.73 ± 0.10bB	31.19 ± 0.10cB	30.82 ± 0.10bC
F3 (45% RBF)	32.11 ± 0.20bA	30.46 ± 0.16 dB	29.85 ± 0.15dC	29.42 ± 0.10dC
F4 (45% WGBF)	33.14 ± 0.20aA	31.03 ± 0.10cB	30.82 ± 0.10cC	30.19 ± 0.25cC
	Firmness (N)			
Control (100% RWF)	5.83 ± 0.37dD	12.48 ± 1.06dC	15.11 ± 1.32eB	17.58 ± 1.02dA
F1 (30% RBF)	14.35 ± 1.98cD	26.48 ± 2.72cC	32.93 ± 3.80dA	31.48 ± 2.50cA
F2 (30% WGBF)	16.09 ± 1.47cD	30.41 ± 3.32cC	37.56 ± 3.50cA	35.85 ± 3.76cA
F3 (45% RBF)	48.72 ± 2.63aD	78.11 ± 2.35aC	86.3 ± 5.92aB	99.71 ± 6.98aA
F4 (45% WGBF)	42.53 ± 1.53bD	71.32 ± 2.81bC	77.05 ± 2.67bB	87.07 ± 3.64bA

Means followed by the same lowercase letter in the columns (for the same parameter) and by the same uppercase letter in the rows do not differ significantly by the Tukey test ($p < 0.05$). RWF: refined wheat flour; RBF: refined buckwheat flour; WGBF: whole grain buckwheat flour. Water absorption (farinographic analysis): Control: 58.3%; F1: 53.6%; F2: 56.9%; F3: 52.0%; F4: 57.0%.

These proteins are responsible for gas retention in the dough during the fermentation process, with a consequent development of bread volume (Houben et al., 2012). However, Noort et al. (2010) reported that gluten dilution has only a secondary physical effect on the reduction of specific volume, once the interaction between gluten proteins and ferulic acid monomers, glutathione, and phytate present in the bran layers have a relevant chemical effect. This fact can be verified comparing the formulations containing WGBF (that have fibers and other compounds from buckwheat outer layers) with those containing RBF.

Regarding the instrumental color, there were significant differences ($p \leq 0.05$) between the different formulations for the parameters L* and a*. The control sample had the highest lightness value (L*), which decreased with the addition of RBF and WGBF. Also, an increase in a* value (red color) was observed, once the buckwheat bran constituents (fibers and phenolic compounds) contributed to a reddish and darker flour. A similar effect was also observed by Costantini et al. (2014).

Table 2 shows the crust and crumb moisture contents (%) and the bread firmness values (N) during storage.

A significant difference ($p \leq 0.05$) was observed in the moisture contents of the different formulations, probably due to the different amounts of water used in bread making (based on farinographic water absorption). The bread made with 30% RBF (F1) presented the highest moisture content in the crust and crumb during storage. This is a positive result because, according to Cauvain & Young (2009), bread moisture has a positive effect on the quality perception, once bread with higher crumb moisture content tends to be considered fresh by consumers.

Table 3
Total and resistant starch, hydrolysis index (HI), estimated glycemic index (eGI) and estimated dietary fiber content of breads with different RBF and WGBF incorporations.

Formulations	Total starch (%)	Resistant starch (%)	HI	eGI	Estimated dietary fiber (%)
Control (100% RWF)	71.07 ± 6.06b	0.11 ± 0.02bc	100b	94.61b	–
F1 (30% RBF)	72.11 ± 3.22b	0.18 ± 0.05a	101.24 ± 1.95b	95.29 ± 0.50b	1.44
F2 (30% WGBF)	63.01 ± 5.51c	0.15 ± 0.04b	90.85 ± 4.06c	89.60 ± 0.87c	5.28
F3 (45% RBF)	74.09 ± 2.52a	0.21 ± 0.05a	115.15 ± 6.48a	102.93 ± 1.20a	1.50
F4 (45% WGBF)	59.61 ± 2.49d	0.14 ± 0.04b	82.84 ± 6.55d	85.19 ± 0.94d	7.29

Means followed by the same letter in the columns do not differ significantly by the Tukey test ($p \leq 0.05$). RWF: refined wheat flour; RBF: refined buckwheat flour; WGBF: whole grain buckwheat flour. GI values obtained by the equation: $GI = 39.71 + 0.549$ (Goñi et al., 1997).

With respect to bread moisture content (crumb and crust) over time, an increase in crust moisture and a reduction in crumb moisture were observed during storage for all formulations, due to the migration of moisture from the moister crumb to the drier crust. A similar effect of water migration from crumb to crust was observed in the water activity (Aw) results (Fig. 1 – Supplementary Material).

As shown in Table 2, the control formulation presented the lowest firmness values when compared to the other formulations, and this parameter increased with the addition of RBF and WGBF. Regarding firmness during storage, the control and formulations F3 (45% RBF) and F4 (45% WGBF) presented similar behavior, with a progressive increase in firmness. Formulations F1 (30% RBF) and F2 (30% WGBF) presented a significant difference in firmness only on day 9, making F2 promising due to the incorporation of 30% WGBF, with a greater nutritional contribution through fibers and phenolic compounds (Bonafaccia et al., 2003).

Formulation F3 (45% RBF) presented the highest firmness values on all days evaluated. This may be due to the greater content of starch in RBF, causing the dilution of gluten proteins, besides contributing to greater starch (amylose and amylopectin) retrogradation after cooling and storage (GAO et al., 2016). In contrast, the lower firmness observed in formulation F4 (45% WGBF) when compared to F3 may be due to the fiber from WGBF, which, despite diluting gluten proteins, may retard starch retrogradation during storage (Schmiele et al., 2012).

Another factor that should be considered related to the increase in firmness of breads in relation to the control is the presence of phenolic compound from buckwheat, as they may compete with starch for water. Also, they can form non-covalent bonds with starch, altering the pH of the system, impacting water absorption of starch granules, starch gelatinization and pasting properties. Apart from affecting starch gelatinization, the incorporation of phenolic compounds in wheat flour doughs can influence starch retrogradation (Xu et al., 2019; Zhu, 2015; Zhu et al., 2016). Furthermore, phenolic compounds can interact with gluten thiol groups present in wheat flour doughs, weakening the gluten network, which can affect bread volume and firmness (Koh & Ng, 2009; Nicks et al., 2013).

3.2. Nutritional characterization and bioactive potential of pan breads

Approximately 35% of the starch in the formulations was hydrolyzed within 30 min of hydrolysis, characterizing the rapidly digestible starch fraction. Regarding the slowly digestible starch fraction, 60% of the starch was hydrolyzed in the control, F1 (30% RBF), and F3 (45% RBF). From 120 to 150 min, about 70% of the total starch present in the

Table 4
Iron (Fe), zinc (Zn), calcium (Ca) and magnesium (Mg) bioaccessibility of breads with different RBF and WGBF incorporations.

Minerals	Formulations				
Iron	Control	F1	F2	F3	F4
Fe total (mg/100 g)	1.14 ± 0.05d	2.04 ± 0.07c	2.03 ± 0.05c	2.29 ± 0.11b	2.59 ± 0.07a
Fe soluble (mg/100 g)	0.53 ± 0.05c	0.91 ± 0.02a	0.75 ± 0.01b	0.74 ± 0.05b	0.86 ± 0.02a
Solubility (%)	46.83	44.83	36.65	32.35	33.05
Fe dialyzable (mg/100 g)	0.27 ± 0.03ab	0.19 ± 0.0b	0.28 ± 0.03ab	0.33 ± 0.05a	0.29 ± 0.03ab
Dialyzable (%)	23.4	9.54	13.94	14.24	11.21
Zinc					
Zn total (mg/100 g)	0.92 ± 0.01b	0.80 ± 0.03c	1.08 ± 0.03a	0.88 ± 0.02b	1.15 ± 0.02a
Zn soluble (mg/100 g)	0.35 ± 0.03d	0.54 ± 0.01b	0.45 ± 0.02c	0.61 ± 0.01a	0.43 ± 0.03c
Solubility (%)	37.90	67.94	41.71	69.54	37.67
Zn dialyzable (mg/100 g)	0.23 ± 0.02c	0.37 ± 0.03a	0.30 ± 0.02b	0.36 ± 0.02a	0.28 ± 0.03bc
Dialyzable (%)	25.17	45.76	27.57	40.77	24.01
Calcium					
Ca total (mg/100 g)	55.80 ± 0.92a	52.64 ± 0.56b	54.85 ± 0.15ab	46.46 ± 0.19c	58.15 ± 0.87a
Ca soluble (mg/100 g)	37.15 ± 1.69a	28.99 ± 0.85b	34.57 ± 2.50a	35.83 ± 1.73a	17.99 ± 0.86c
Solubility (%)	66.63	55.08	63.03	77.12	30.96
Ca dialyzable (mg/100 g)	41.75 ± 1.68a	39.14 ± 2.06a	24.48 ± 0.95b	18.81 ± 0.39c	27.87 ± 1.29b
Dialyzable (%)	74.82	74.36	44.63	40.5	47.94
Magnesium					
Mg total (mg/100 g)	28.88 ± 0.26d	30.87 ± 0.49 cd	54.16 ± 1.54b	34.83 ± 0.31c	70.49 ± 0.76a
Mg soluble (mg/100 g)	22.39 ± 1.37c	21.55 ± 1.18c	45.16 ± 1.66b	25.11 ± 0.24c	50.24 ± 3.94a
Solubility (%)	77.53	69.82	83.46	72.08	71.31
Mg dialyzable (mg/100 g)	13.68 ± 0.40d	15.28 ± 0.46 cd	24.36 ± 1.82b	17.01 ± 0.56c	33.71 ± 0.54a
Dialyzable (%)	47.37	49.51	44.98	48.84	47.82

Means followed by the same letter in the rows do not differ significantly by the Tukey test ($p \leq 0.05$).
Control: Bread elaborated with 100% refined wheat flour (RWF); F1: Bread elaborated with 30% refined buckwheat flour (RBF); F2: Bread elaborated with 30% whole grain buckwheat flour (WGBF); F3: Bread elaborated with 45% refined buckwheat flour (RBF); F4: Bread elaborated with 45% whole grain buckwheat flour (WGBF).

different formulations was hydrolyzed, and after 150 min, the percentage of hydrolysis remained constant. The starch fraction that was not digested after 16 h characterized resistant starch (0.11 to 0.21%) (Fig. 2 – Supplementary Material).

With respect to the formulations made with 30 and 45% WGBF, the slowly digestible starch fraction corresponded to 50 and 53%, respectively, and 70% of total starch was hydrolyzed within 180 min for both samples, with a reduction of 7–10% of the hydrolysis activity in relation to the other formulations (control and RBF), indicating a slower digestion of the starch present in the formulations made with WGBF.

Table 3 shows the results of total starch, resistant starch, hydrolysis index (HI), estimated glycemic index (eGI) and estimated total dietary fiber content of the different bread formulations.

Formulation F3 presented the highest total starch content, followed by the control and F1, which presented no significant differences between them ($p > 0.05$). Regarding the resistant starch, no significant differences ($p > 0.05$) were observed between the control and formulations F2 and F4 (with WGBF), with the highest resistant starch contents being observed for formulations F1 and F3 (with RBF). Although the resistant starch levels (hydrolysis time > 16 h) of the present study were considered low, they are consistent with the type of product (bread), as reported by Birt et al. (2013).

Formulation F3 presented the highest hydrolysis and eGI, followed by the control and F1, which presented no significant differences between them ($p > 0.05$), while formulation F4 presented the lowest HI and eGI values. Similar results were observed by Skrabanja et al. (2001), who evaluated the starch digestibility of bread made with whole grain buckwheat flour (30 to 70%). The authors found that whole grain buckwheat flour concentrations above 30% led to lower starch hydrolysis and glycemic index when compared to the control (wheat flour).

There is a growing interest in developing foods with increased resistant starch contents, because of the health benefits related to foods with increased resistant starch and decreased glycemic index (Birt et al., 2013).

Bread with the incorporation of WGBF can be considered promising for having lower estimated glycemix index (eGI) values, increasing satiety and reducing the possibility of a blood insulin peak after consumption, due to the lower quantity of starch and higher quantity of fibers present (Wolter et al., 2013). Studies also show that bioactive compounds such as flavonoids can modulate starch digestibility by inhibiting amylolytic enzymes or by forming complexes with starch (Giuberti et al., 2020; Rocchetti et al., 2020).

The estimated dietary fiber contents of formulations F1, F2, F3, and F4 were 1.44, 5.28, 1.50, and 7.29%, respectively.

Considering a portion of bread equivalent to 50 g, formulation F2 can be considered a “source of fiber” (>2.5 g/portion), while formulation F4 can be classified as “fiber-rich” (>5.0 g/portion), according to the Brazilian legislation (Anvisa, 2012). Fiber content of F1 and F3 did not differ, however F1 specific volume was higher than F3, due to the different amounts of RBF incorporated. Between the formulations with WGBF, F2 fiber content was lower than F4 and F2 specific volume was higher than F4.

Table 4 shows the results of the *in vitro* digestion assay for Fe, Zn, Ca, and Mg bioaccessibility of the different formulations.

Regarding the mineral levels, formulation F4 presented the highest Fe, Zn, and Mg contents, when compared to the control bread (100% RWF). This result is probably due to the greater incorporation of WGBF (45%), confirming the higher amount of minerals in the outer layer of the grain. For the total Ca levels, significant differences ($p \leq 0.05$) were observed between the control (55.80 mg/100 g) and formulations F1 and F3, which presented 52.64 and 46.46 mg/100 g, respectively, indicating a lower contribution of this mineral in the formulations made with the addition of RBF when compared to the formulation containing RWF.

In relation to the percentage of soluble minerals, the control bread (100% RWF) presented the highest Fe level (46.83%), followed by formulations F1 (44.83%), F2 (36.65%), F4 (33.05%) and F3 (32.35%). Regarding the percentage of dialyzable iron, the control formulation presented the highest levels followed by formulation F3. It is worth noting that the wheat flour used in this study is commercial grade, therefore, it is enriched with iron and folic acid, according to Resolution 150/2017 (Anvisa, 2017). The low iron solubility and dialysis in buckwheat flour may be due to the presence of organic acids, fibers and phenolic compounds, such as phytates and rutin, which can negatively affect these parameters, as they can form insoluble compounds in the presence of this mineral (Pongrac et al., 2016).

The highest percentage of soluble Zn was found in formulation F3 (45% RBF), followed by F1, F2, the control, and F4. Similar behavior was observed for the dialyzable Zn, with higher percentages for formulations F1 and F3. This result demonstrates that most of the soluble Zn is present in the refined fraction, as also pointed out by Steadman et al. (2001), who studied the mineral content in different buckwheat fractions.

With respect to Ca levels, formulation F3 (45% RBF) presented a higher solubility, followed by the control, F3, F1, and F4. However, the percentage of dialyzable Ca was higher in the control and F1, followed by formulations F4, F2, and F3, demonstrating that the formulation with higher soluble Ca did not present greater dialysis, probably due to the mineral particle size, which may present different behavior during the

Table 5
Rutin and quercetin contents in doughs before and after fermentation and in breads with different RBF and WGBF incorporations.

Formulations	Rutin contents (mg/100 g)			Quercetin contents (mg/100 g)		
	Dough before fermentation	Dough after fermentation	Bread	Dough before fermentation	Dough after fermentation	Bread
Control (100% RWF)	nd	nd	nd	nd	nd	nd
F1 (30% RBF)	0.53 ± 0.04b	0.58 ± 0.04b	0.82 ± 0.02a	0.012 ± 0.002b	0.024 ± 0.002a	0.028 ± 0.001a
F2 (30% WGBF)	2.66 ± 0.10b	2.34 ± 0.13b	3.41 ± 0.08a	0.041 ± 0.002c	0.072 ± 0.003b	0.114 ± 0.003a
F3 (45% RBF)	1.31 ± 0.07a	1.26 ± 0.03a	1.38 ± 0.09a	0.016 ± 0.002b	0.046 ± 0.002a	0.049 ± 0.003a
F4 (45% WGBF)	3.51 ± 0.2b	3.85 ± 0.15b	4.76 ± 0.06a	0.057 ± 0.004c	0.107 ± 0.006b	0.191 ± 0.002a

Means followed by the same letter in the rows (for rutin and quercetin contents separately) do not differ significantly by the Tukey test ($p < 0.05$). RWF: refined wheat flour; RBF: refined buckwheat flour; WGBF: whole grain buckwheat flour. nd: not detected.

Table 6
Acceptance scores for attributes of breads with different RBF and WGBF incorporations evaluated by the panelists.

Formulations	Attributes					
	Crumb appearance	Crumb color	Aroma	Flavor	Texture	Overall impression
Control (100% RWF)	7.55 ± 1.23a	7.46 ± 1.34a	7.14 ± 1.50a	6.97 ± 1.74a	6.88 ± 1.81a	7.25 ± 1.38a
F1 (30% RBF)	6.83 ± 1.56b	6.66 ± 1.53b	6.60 ± 1.48ab	6.35 ± 1.67ab	6.36 ± 1.87a	6.51 ± 1.52b
F2 (30% WGBF)	6.15 ± 2.05c	5.77 ± 2.22c	5.98 ± 1.98bc	5.79 ± 2.12bc	6.47 ± 1.71a	5.90 ± 1.92b
F3 (45% RBF)	5.44 ± 2.02d	5.72 ± 1.90c	6.21 ± 1.74b	5.41 ± 2.17 cd	4.00 ± 2.23b	4.89 ± 1.89c
F4 (45% WGBF)	4.30 ± 2.37e	4.99 ± 2.30d	5.40 ± 2.35c	4.87 ± 2.49d	4.10 ± 2.21b	4.40 ± 2.24c

Means followed by the same letter in the columns do not differ significantly by the Tukey test ($p \leq 0.05$). RWF: refined wheat flour; RBF: refined buckwheat flour; WGBF: whole grain buckwheat flour.

absorption simulation in the organism, as reported by Cámara et al. (2005).

Regarding soluble Mg, formulation F2 exhibited the highest content, followed by the control, F3, F4, and F1. However, the formulations made with the addition of RBF (F1 and F3) presented the highest percentages of dialyzable Mg, as also observed by Steadman et al. (2001).

Concerning the bioaccessibility of minerals determined by the dialysis assays, the formulations made with the addition of RBF presented better bioaccessibility, and formulation F1 was the most promising. This result is probably due to the higher concentration of phytates and fibers in the formulations with WGBF (F2 and F4), which have the ability to bind to minerals (Fe^{2+}, Ca^{2+}, Zn^{2+}) and consequently decrease the absorption in the human body (Bohn et al., 2004).

Steadman et al. (2001) evaluated the contents of minerals, phytic acid, tannins, and rutin in different milling fractions of buckwheat grains. The authors found that the amounts of phytic acid and tannins present in whole grain buckwheat flour were higher than those found in refined flour, which may compromise the accessibility of minerals present in the grain. Pongrac et al. (2013) studied the mineral composition of buckwheat grains, and found that the highest concentrations of Fe, Zn, Ca, and Mg are present in the outer layer of grain (bran), as well as phytates and tannins. This fact justifies the higher dialysis percentages observed in bread made with the addition of refined buckwheat flour (RBF).

Table 5 shows the rutin and quercetin levels of the dough before and after fermentation and in bread formulations.

There was no significant difference ($p > 0.05$) for the rutin contents of the dough before and after fermentation for all formulations, which indicates no phenolic degradation during fermentation. However, with the exception of the control, the rutin content after baking increased significantly in all formulations, and formulation F4 exhibited the highest rutin content in both dough and bread. Rutin is the major phenolic compound present in buckwheat, found mainly in the outer layers of the grain, both in free and bound forms. Probably, the rutin in the bound form was released during baking, thus positively affecting the formulations made with WGBF (Lee et al., 2016).

Regarding the quercetin levels, the control bread did not contain this compound in its composition, and dough fermentation had a positive effect on the release of quercetin in the other formulations. Only the formulations made with WGBF (F2 and F4) showed an increase in quercetin levels after baking. Vogrinčič et al. (2010) reported similar results during bread processing, emphasizing that the progressive increase of quercetin during bread making is due both to baking temperature and time causing transformation of rutin into quercetin, and to the addition of water and yeast, which changes the environment, releasing quercetin that may be linked to other molecules.

3.3. *Sensory evaluation*

Table 6 presents the results of the sensory evaluation of the different bread formulations. The control bread obtained the highest scores for all attributes evaluated, followed by formulations F1, F2, F3, and F4. However, no significant differences were observed between formulation F1 and the control for the acceptance of the attributes aroma, flavor, and texture.

According to Torbica et al. (2010), buckwheat flour contributes to bread aroma, and refined flour is better accepted by consumers due to the lower intensity of buckwheat aroma when compared to whole grain buckwheat flour.

For the attribute texture, no significant differences were observed between the control and formulations F1 and F2, which were the most accepted by the consumers. Formulations F3 and F4 received lower scores, in addition to negative comments about mouthfeel, dryness, and hardness. Lin et al. (2009) found similar results when evaluating bread made with 15% buckwheat flour, with and without the addition of bran. However, it is noteworthy that in the present study, breads made with 30% RBF and 30% WGBF were well accepted by the consumers.

Considering that only formulations with 70% approval have a positive acceptance (average score above 6.3) and considering the overall impression, we can state that both the control and formulation F1 were well accepted by consumers (Lazaridou et al., 2007; Torbica et al., 2010). However, formulation F2 (30% WGBF) did not differ significantly from formulation F1, with positive results for the acceptance of the attributes aroma, flavor, texture, and overall impression.

Regarding the purchase intention, the control formulation presented the best results, followed by formulations F1 and F2, which presented results of 44% and 41% corresponding to “would probably buy”, respectively.

4. Conclusion

The addition of 30% RBF or 30% WGBF to bread formulations showed minor interference with respect to technological quality. Regarding the nutritional characteristics, breads made with 30 and 45% WGBF presented higher mineral, fiber, rutin, and quercetin levels, and lower starch hydrolysis and glycemic index when compared to the control, while the formulations made with RBF presented higher mineral bioaccessibility.

The formulations made with higher percentages of buckwheat flour (45% RBF or 45% WGBF) presented a good nutritional potential, although they did not demonstrate good performance with respect to the technological and sensory properties of breads. Thus, further studies should be conducted in order to improve these properties of buckwheat breads to better please consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100243.

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