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Physicochemical, nutritional, and functional characterization of gluten-free ingredients and their impact on the bread texture

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ABSTRACT

Despite the commercial availability of gluten-free (GF) products, numerous nutritional, sensory, and textural limitations have been brought to the attention of the baking industries. This study aimed at the characterization of four GF flours (pregelatinized rice, pearl millet, common buckwheat, and soy protein isolate) for their nutritional and functional properties. Protein and starch were the major components in soy protein isolate and pregelatinized rice, respectively, whereas buckwheat and millet contained the highest amount of dietary fiber. Free phenolic compounds and antioxidant activity were at the highest levels in buckwheat flour followed by soy protein isolate. Likewise, all investigated ingredients varied greatly in their physicochemical properties. Based on single-ingredient baked-model, the effect on the texture and volumetric profiles of bread was reported, distinguishing GF ingredients in four different clusters with different characteristics. Accordingly, the four GF ingredients were combined to create a composite GF bread with acceptable textural properties approaching to those of a typical wheat bread. These findings might be regarded as a basis to design further innovative recipes and combinations using these raw GF ingredients.

1. Introduction

Gluten-free (GF) diet, being the only effective treatment for individuals suffering from gluten-related disorders and health trend for other people, is shifting focus towards new market for GF foods and beverages (Le Loan, Thuy, Le Tri, & Sunghoon, 2021). In fact, the market size of GF products is estimated to reach USD 7.5 billion with a compound annual growth rate (CAGR) of 7.2% from 2020 to 2027 in which the highest market share (47.5%) is attributed to Europe (*Gluten-free Products Market Size & Growth| Industry Overview by 2027, 2022*).

Simultaneously, extensive research has been conducted to overcome the absence of gluten and substitute the gluten network with other ingredients as well as texturing additives that confer the dough its unique viscoelasticity and baking quality (Bender & Schönlechner, 2020). As a result, developing GF bread with suitable cohesiveness and elasticity characteristics remains challenging. Gluten-free products commercially available are usually low in nutrition and do not always fulfill consumers' sensorial and nutritional needs (Abdelsalam, EL-Naggar, El-Soukkary, & Abdelmegiud, 2021). Many commercial GF breads have been supplemented with a wide range of additives, such as hydrocolloids, acidifiers, emulsifiers, leavening agents, preservatives, and aromas or flavorings, as well as proteins and sugars up to 81 and 87%, respectively. Although the aforementioned ingredients optimize the bread quality, they tend to further decrease the nutritional value of the GF products (Roman, Belorio, & Gomez, 2019). Conventional flours that naturally contain gluten (e.g., wheat, barley, and rye) can be deglutinated using bioprocess technologies, e.g., addition of selected sourdough is able to detoxify the immunogenic peptides by the action of enzymes secreted by lactobacilli (De Angelis et al., 2010). However, these GF formulations lack dietary fiber and bioactive compounds which justified the necessity of their fortification with processed GF food by-products' flours (e.g., seeds, peel, pips, skins, stems, and cores of the fruits and vegetables) that can add nutritive value to the product (Garkina, Kurochkin, Frolov, & Shaburova, 2021; O'Shea, Arendt, &

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Gallagher, 2014).

As an alternative, non-conventional flours are introduced in GF preparations as a relatively cheap and rich source of non-digestible carbohydrates, proteins, and bioactive compounds (Abdelsalam et al., 2021). When compared to wheat, a higher content of proteins, dietary fibers, minerals (e.g., calcium, iron, and zinc), vitamins, micronutrients, phytochemicals, and antioxidant compounds is reported for non-conventional GF cereals and legumes, but at different ratios (Martínez-Villaluenga, Peñas, & Hernández-Ledesma, 2020). Although chemical composition and nutritional features were largely investigated (Abdelsalam et al., 2021; Garkina et al., 2021), limited information is available on their physicochemical properties when applied in GF products. Indeed, the commercial value of any baked good is realized by its texture, sensory, and organoleptic features that are mainly impacted by the physicochemical properties of the ingredients. Physicochemical properties of foods reflect the interactions between the structures, molecular conformations, and the compositions existing within the food components (Chandra, Singh, & Kumari, 2015).

In our study, we aimed at providing a framework about the physicochemical, nutritional, and functional properties of potential raw GF ingredients, which included pearl millet (ML, as a source of dietary fiber), common buckwheat (BW, as a source of dietary fiber and phenolics), soy protein isolate (SPI, as a source of protein), and pregelatinized rice (PGR, as a source of starch). Such data frame can be used to optimize a GF bread formulation based on nutrient-dense GF ingredients, as an alternative to traditional low-nutrient commercial recipes.

2. Materials and methods

2.1. Materials

Raw GF ingredients were supplied by Dr. Schär, Burgstall, Italy, including millet (ML) (*Pennisetum glaucum*), buckwheat (BW) (*Fagopyrum esculentum*), soy protein isolate (SPI) and pregelatinized rice (PGR), with gross composition indicated in Supplementary Table S1. The ingredients were stored at 25 ± 3 °C and characterized within 30 days of delivery in the Micro4Food labs at NOI Techpark, Bolzano, Italy.

2.2. Nutritional and functional characterization

2.2.1. Total protein concentration

Total protein concentration (TPC) was determined in the water-soluble extracts (WSE) of all the samples using Bradford assay with slight modifications. Firstly, the WSE were prepared by suspending 2 g of sample in 8 mL (ML and BW) or 16 mL (SPI and PGR) of 50 mM Tris–HCl (pH 8.8), incubating at 4 °C for 1 h, vortexing at 15 min intervals, and recovering the supernatant after centrifugation at 20,000 × *g* for 20 min. In 96-well microplate reader (Tecan Infinite 200, Italy), the absorbance of supernatants was measured at 590 nm, and protein concentration was calculated using the calibration curve of bovine serum albumin as standard.

2.2.2. Starch content

The samples were subjected to *in vitro* starch hydrolysis by the method of Liljeberg, Åkerberg, and Björck (1996) with slight modifications. The samples were digested enzymatically with amyloglucosidase (Sigma-Aldrich, Italy), which breaks down starch into glucose units. The released glucose content was then quantified using D-Fructose/D-Glucose kit (Megazyme International, Ireland) to estimate the starch concentration of each sample with a conversion factor of 0.9.

2.2.3. Total dietary fibers

The AOAC enzymatic-gravimetric methods 993.19 for soluble dietary fiber (SDF) and 991.42 for insoluble dietary fiber (IDF) were used to determine soluble and insoluble dietary fibers, respectively. The total dietary fiber (TDF) content in the samples was calculated as the sum of IDF and SDF.

2.2.4. Anti-nutritional factors

Anti-nutritional factors (ANF), including phytic acid and raffinose, were determined in GF ingredients. The concentrations of phytic acid and raffinose were quantified using Phytic Acid (Phytate)/Total Phosphorus and Raffinose/D-Galactose kits (Megazyme International, Ireland) according to the manufacturer's instructions, respectively.

2.2.5. Antioxidant activity and total phenols

Antioxidant activity and total phenols concentration were determined in methanol/water-soluble extract (MWSE) of raw GF ingredients as described by Tlais et al. (2021). Briefly, a sample (2 g) was mixed with 8 mL of 80% methanol (prepared in water), and then the mixture was acidified with 0.1% hydrochloric acid. The mixture was then subjected to sonication (amplitude 60) using a macroprobe [Vibra-Cell sonicator (Sonic and Materials Inc., Danbury, CT)] for 1 min (2 cycles, 30 s/cycle, 5 min interval between cycles). Continuous extraction for 1 h under stirring conditions was performed followed by centrifugation at 10,000 rpm for 10 min. The supernatant was filtered and kept at −20 °C until further use. These MWSEs were then used to evaluate the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in GF ingredients. The absorbance values of an antioxidant reference [75 mg/L butylated hydroxytoluene (BHT)] were used to estimate the antioxidant activities of GF ingredients. Total phenols concentrations were determined by Folin−Ciocalteu assay and data were expressed as gallic acid equivalents (Tlais et al., 2021).

2.2.6. Free phenolic compounds

Phenolic compounds in raw GF ingredients were profiled using MWSEs. Targeted LC-MS/MS analysis of 29 free phenolic compounds was conducted as previously optimized by Tlais et al. (2021), using a UHPLC Dionex 3000 (Thermo Fisher Scientific, Germany) equipped with a Waters Acquity HSS T3 column (1.8 μm, 100 mm × 2.1 mm) (Milford, MA, USA) and coupled to a TSQ Quantum™ Access MAX Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Germany) with an electrospray source.

2.3. Physicochemical characterization

2.3.1. Moisture content

The moisture content of each sample was analyzed using MA37 Electronic Moisture Analyzer (Sartorius). Briefly, each granulated ingredient was evenly distributed onto the sample tray provided with the instrument. The preset method with standard drying at 130 °C was selected, and the sample tray was placed inside the machine at the designated area. The moisture content ended automatically depending on the flour type (range: 6–12 min) and was displayed as ratio of water in 100 g of flour.

2.3.2. Water and oil absorption capacities

The water and oil absorption capacities of the samples were determined by the method of Sosulski, Humbert, Bui, and Jones (1976) with slight modifications. One gram of ingredient was mixed with 10 mL of distilled water or soybean oil, for respective absorption capacities. The mixture was allowed to stand for 30 min at ambient temperature followed by centrifugation at 3000 rpm for 30 min. The water or oil absorption capacities was defined for each ingredient, respectively.

2.3.3. Swelling capacity

The swelling capacity of each sample was determined by the method of Okaka and Potter (1977). The sample was added to a 100 mL graduated cylinder (3 cm in diameter) up to the mark of 10 mL. This was followed by the addition of distilled water up to 50 mL, which was tightly covered with a parafilm. The suspension was mixed by inverting the cylinder twice at an interval of 2 min and then leaving it to stand for 8 min. The volume (mL) covered by the settled sample was regarded as the swelling capacity.

2.3.4. Least gelation concentration

The least gelation concentration was examined by the method of Coffman and Garcia (1977) with slight modifications. For each sample, flour-water mix was prepared using 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 30 g of flour in a glass test tube with a final volume of 100 mL of water. The mix was heated at 90 °C for 1 h in a water bath before being cooled down for 2 h. The least gelation concentration was expressed as the weight of flour per 100 mL of water at which the sample remained solidified and did not slip when the tube was inverted.

2.3.5. Emulsion activity and stability

The emulsion of 1 g GF ingredient, 10 mL distilled water, and 10 mL soybean oil was prepared to determine its activity and stability by the method of Yasumatsu et al. (1972). This emulsion was mixed thoroughly for 1 min and then centrifuged at 3000 rpm for 5 min. The emulsion activity was reported as the ratio of the height of emulsion layer to the entire height of the mix in percentage (%). For emulsion stability, the emulsion was heated at 80 °C for 30 min in a water bath, cooled for 15 min, and then centrifuged at 3000 rpm for 15 min. The ratio of the height of emulsion layer to the total height was regarded as emulsion stability expressed as percentage (%).

2.4. Baking trials with GF ingredients

Baked models were prepared with each ingredient alone at the Bakery Insperience Pilot Plant of the Micro4Food lab (Libera Universitá di Bolzano, Italy) to define the influence of physicochemical properties on the bread texture. Each GF ingredient was mixed with tap water alone to obtain a dough, where each flour particle is hydrated during the mixing process, with a dough yield (DY = [dough weight/flour weight] *100) of 190 (ML and BW), 440 (SPI), and 232 (PGR). Baked models were prepared by mixing individual GF ingredients (ML and BW = 105 g, SPI = 45 g, and PGR = 86 g), tap water (ML and BW = 95 g, SPI = 155 g, and PGR = 114 g), and 3 g of commercial baker's yeast to obtain a total dough weight of 200 g.

Moreover, composite GF and control soft wheat breads were also prepared for texture analysis with a DY of 190 and 160, respectively. The composite GF bread formula, which was tailored from a commercial GF bread with corn starch flour as the basic ingredient (30%), included ML (1.5%), BW (3%), SPI (2%), and PGR (3%), and water for the remaining part. The formulation was optimized through preliminary trials using varying percentages of the individual ingredients (data not shown). Adjustments to the formulations were made taking into account the properties of the individual ingredients revealed in this study, in particular with reference to physicochemical and textural properties. All the doughs were prepared with a continuous high-speed mixer (60 × g, dough mixing time: 5 min) and then fermented for 2.5 h at 30 °C with 1.5 g/100 g commercial baker's yeast, followed by baking at 230 °C for 45 min (Omega 2, Bongard, Italy).

2.5. Texture profile and volumetric analyses

Instrumental texture profile analysis (TPA) was performed with TVT 6700 Texture Analyzer (Perten Instruments), using a 25 mm cylindrical probe (probe P-CY25S). The test mode settings were as follows: test speed 1 mm/s, 20% compression distance; and two-compression cycle (TVT method 01–03.01). The duration between two compressions was 15 s. The samples for analysis were prepared by slicing the bread loaves up to 5 cm with a flat surface and placed on the measuring table of the instrument below the center of the probe. TPA was carried out using TexCalc 5 software, which measured firmness, gumminess, and cohesiveness of the breadcrumbs.

Instrumental volumetric analysis was performed by BVM 6600 Volumetric Analyzer (Perten Instruments), using a circular flat support plate (FSPC50). The test profile was created in the VolCalc software (Version 3.5.3.227), and the alignment of bread was verified by the 3D image to set the orientation. Specific volume (mL/g) was estimated by the software.

2.6. Statistical analysis

All analyses included two experimental replicates and two analytical replicates. Data were subjected to analysis of variance by the General Linear Model (GLM) of R statistical package (Mangiafico, 2016). Pairwise comparison of treatment means was achieved by Tukey-adjusted comparison procedure with p-value (P) < 0.05 (Mangiafico, 2016). After scaling and centering the units of variance, data were subjected to Principal Component analysis (PCA). Spearman's rank correlation matrix and p-values were generated by cor.test and visualized by corrplot package.

3. Results

3.1. Nutritional and functional properties of raw GF ingredients

3.1.1. Protein, starch, and total dietary fiber

Fig. 1 (A, B; and C) depicts the main nutritional composition of PGR, ML, BW; and SPI. As expected, the SPI showed a higher protein content (74.7 ± 4.3 g/100 g) compared to the other GF ingredients, which shared almost similar values among them. The highest starch content was found in PGR (956.0 ± 4.9 mg/g), followed by BW and ML (732.2 ± 129.6 and 728.2 ± 82.7 mg/g, respectively) and to a lesser extent in SPI (57.7 ± 35.7 mg/g). Total dietary fibers were found at the highest level in BW (11.8 ± 0.7 g/100 g) followed by ML (7.4 ± 0.7 g/100 g), PGR (6.5 ± 0.8 g/100 g), and SPI (2.5 ± 0.9 g/100 g) (Fig. 1A).

3.1.2. Anti-nutritional factors

When comparing the ANF in GF ingredients, no significant differences ($P > 0.05$) were found for raffinose which ranged from 0.26 ± 0.10 (ML) to 0.51 ± 0.15 (SPI) g/100 g. On the contrary, the amount of phytic acid varied significantly ($P < 0.05$) among the GF ingredients. The highest phytic acid content was found in SPI (1.28 ± 0.19 g/100 g) while the lowest in PGR (0.23 ± 0.1 g/100 g) (Fig. 1B).

3.1.3. Antioxidant activity and total phenols

The antioxidant activity of MWSE obtained from the raw GF was assayed as radical scavenging activity on DPPH radical. The highest ($P < 0.05$) activity towards the stable radical DPPH was found in BW (1.03 ± 0.01 mmol BHT/kg DM) followed by SPI and ML (0.78 ± 0.01 and 0.73 ± 0.00 mmol BHT/kg DM, respectively) (Fig. 1C). The total phenols content reflected the antioxidant activity. The total phenols content in PGR was scarcely detectable (Fig. 1C).

3.1.4. Phenolic compounds characterization

Analysis of phenolic profiles in MWSE was carried out through UHPLC-HESI-MS/MS. Twelve phenolic compounds were identified and quantified. As expected, BW was the ingredient with the highest number of detected phenolic compounds (Table 1). Epicatechin, catechin, epicatechin 3-gallate, isoquercetin, quercetin, luteolin, and phloridzin (in descending order by content and ranging from 32.5 ± 2.4 to 3.4 ± 0.0 μg/g DM) were only found in BW. The most abundant compound in BW was rutin (35.3 ± 1.5 μg/g DM), which was markedly higher than that found in ML (1.9 ± 0.1 μg/g DM). Vanillin was detected in all ingredients with slight but significant ($P < 0.05$) variation. The highest vanillin content was found in PGR (1.5 ± 0.1 μg/g DM), followed by SPI (1.1 ± 0.1 μg/g DM), BW (0.8 ± 0.1 μg/g DM) and ML (0.8 ± 0.0 μg/g DM). Despite the high total phenols content in SPI, only *p*-coumaric acid and kaempferol were detected at low concentrations (1.5 ± 0.0 and 0.7

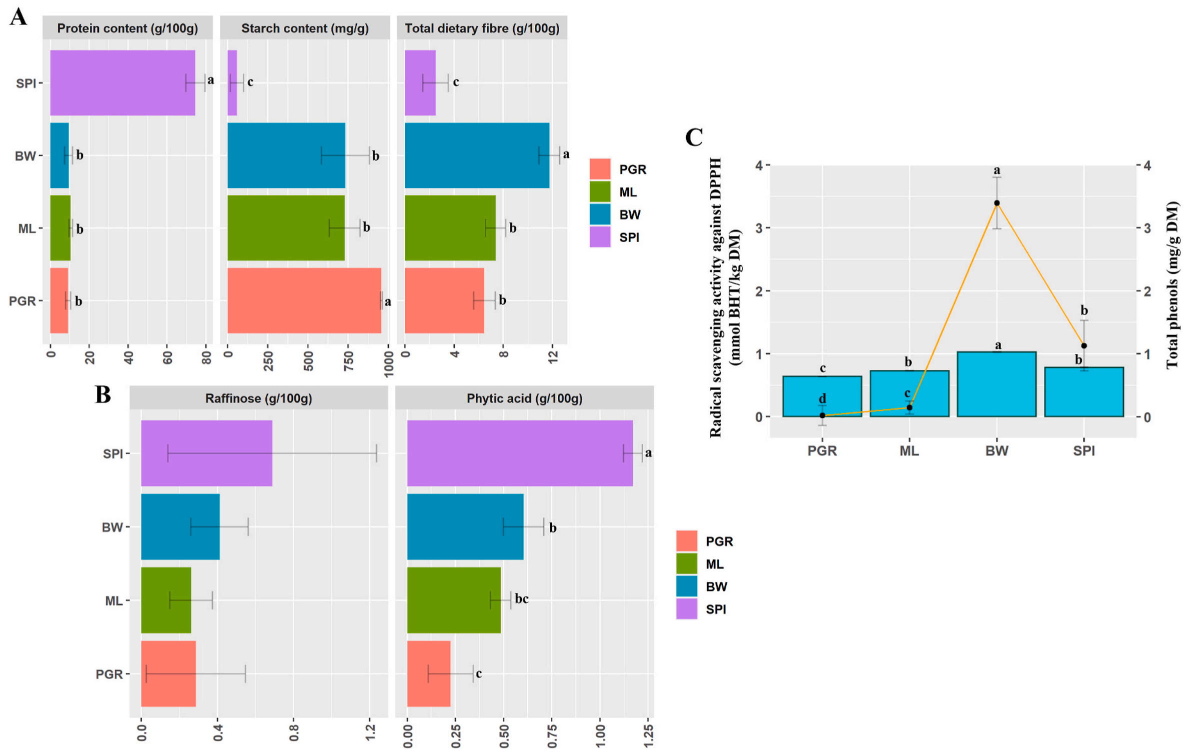

Fig. 1. Quantification of protein content (g/100 g), starch content (mg/g), total dietary fiber (g/100 g) **(panel A),** raffinose (g/100 g), and phytic acid (g/100 g) **(panel B)**, and radical scavenging activity against DPPH (blue bars), and total phenols (orange line) of methanol-water soluble extracts (MWSE) obtained from pregelatinized rice (PGR), millet (ML), buckwheat (BW), and soy protein isolate (SPI) gluten-free ingredients **(panel C).** Data are the mean of separate analyses ± standard deviations. Bars and lines with different superscript letters differ significantly (P < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Quantification of phenolic compounds (μg/g DM) by LC-ESI-MS/MS in methanol/water-soluble extracts (MWSE) obtained from pregelatinized rice (PGR), millet (ML), buckwheat (BW) and soy protein isolate (SPI) gluten-free ingredients. Data are the mean of separate analyses ± standard deviations. Means within the rows with different superscript letters differ significantly (P < 0.05).

Compounds	PGR	ML	BW	SPI
Catechin	–	–	26.7 ± 1.9	–
Epicatechin	–	–	32.5 ± 2.4	–
Epicatechin 3-gallate	–	–	14.6 ± 0.5	–
p-coumaric acid	–	–	–	1.5 ± 0.0
Vanillin	1.5 ± 0.1^{a}	0.8 ± 0.0^{c}	0.8 ± 0.1^{c}	1.1 ± 0.1^{b}
Isoquercetin	–	–	9.6 ± 1.6	–
Hyperoside	–	–	5.3 ± 0.3	–
Phloridzin	–	–	3.4 ± 0.0	–
Quercetin	–	–	7.0 ± 0.2	–
Rutin	–	1.9 ± 0.1^{b}	35.3 ± 1.5^{a}	–
Luteolin	–	–	3.6 ± 0.0	–
Kaempferol	–	–	–	0.7 ± 0.0

± 0.0 μg/g DM), along with vanillin (Table 1).

3.2. Physicochemical properties of raw GF ingredients

The physicochemical properties describing moisture content, water and oil absorption capacities, swelling capacity, least gelation concentration, and emulsion activity and stability of ingredients are shown in Fig. 2. ML showed the highest significant ($P < 0.05$) moisture content (13.1 ± 0.3 g/100 g) followed by BW and PGR (11.5 ± 0.3 and 10.9 ± 0.5 g/100 g, respectively), whereas SPI had the lowest values (6.9 ± 0.1 g/100 g). SPI, on the other hand, recorded the highest water and oil absorption capacities (285 ± 63.1 and 136.7 ± 11.1 g/100 g, respectively). The other ingredients showed lower and almost similar values of oil absorption capacity. ML and BW were lower in terms of water absorption capacity. Based on swelling capacity, SPI and PGR attained significantly higher ($P < 0.05$) values (47.2 ± 0.2 and 45.5 ± 0.4 mL, respectively) compared to ML and BW (16.9 ± 2.1 and 13.7 ± 0.8 mL, respectively). On the contrary, least gelation concentration values were the highest for ML and BW (27.5 ± 4.3 g/100 mL and 25.0 ± 8.7 g/100 mL, respectively). The highest emulsion activity and stability was

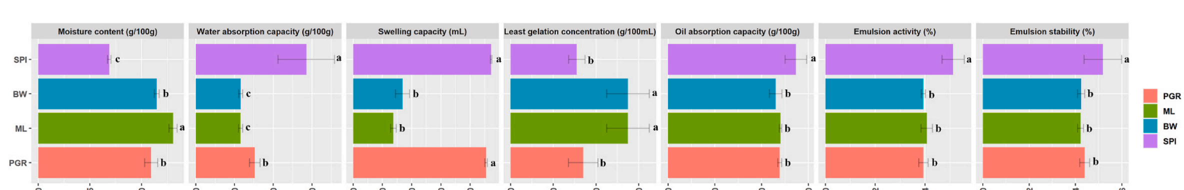

Fig. 2. Moisture content (g/100 g), water absorption capacity (g/100 g), swelling capacity (mL), least gelation concentration (g/100 g), oil absorption capacity (g/100 g), emulsion activity (%), and emulsion stability (%) of raw gluten-free ingredients: pregelatinized rice (PGR), millet (ML), buckwheat (BW), and soy protein isolate (SPI). Data are the mean of separate analyses ± standard deviations. Bars with different superscript letters differ significantly ($P < 0.05$).

observed in SPI (0.51 ± 0.04 and 0.52 ± 0.07%, respectively) when compared to the other ingredients, which had almost similar values.

3.3. Texture and volumetric analyses of single-ingredient baked-models

Aiming to investigate the influence of physicochemical properties of raw GF ingredients on the bread texture and volumetric parameters, single-GF ingredient baked-models were produced (Table 2). Different DY (190 for ML and BW, 440 for SPI, and 230 for PGR) were employed to obtain a firm dough from each GF ingredient. According to the texture profile and volumetric analyses, the highest firmness was observed for BW baked-model (6566.3 ± 131.3 N), followed by ML (5917.3 ± 608.0 N), PGR (523.5 ± 106.2 N), and SPI (75.3 ± 13.9 N) breads. Likewise, baked-model made of BW presented the highest gumminess values (5221.2 ± 122.2 N), whereas SPI baked-model had the lowest gumminess (67.9 ± 12.4 N). All the baked-models showed significantly variable ($P < 0.05$) cohesiveness and specific volume values, with SPI baked-models showing the highest values (0.9 ± 0.0 and 5.2 ± 0.4 mL/g, respectively), followed by BW, ML, and PGR (Table 2). Based on spearman correlation coefficient, a correlation matrix between physicochemical features and texture and volumetric profiles of raw GF ingredients was established (Fig. 3). Firmness and gumminess demonstrated a positive strong correlation with moisture content and least gelation concentration, but a negative strong correlation with the other properties. Cohesiveness and specific volume characteristics exhibited significant positive correlations with swelling capacity, water and oil absorption capacities, and emulsion activity and stability, whereas they were negatively correlated to moisture content (Fig. 3).

3.4. Design of GF bread formulations based on multiple GF ingredients

Principal component analysis was used to visualize the distribution of raw GF ingredients on the plot based on their nutritional, functional, physicochemical, and textural properties (Fig. 4). A considerable number of variables were reduced to a few variables, known as dimensional components (Dims), which describe the greatest variance in the data being studied. The PCA-Biplot shows an overview of the similarities and differences among the ingredients as well as the inter-relationships among the measured parameters. The first and the second Dims described 70.7% and 22% of the variance, respectively. The result of the analysis revealed distinct clustering patterns of PGR, ML, BW, and SPI, which were localized in separate quadrants (Fig. 4). The characterization activities of the individual GF ingredients allowed the most pronounced properties of each ingredient to emerge and to define a composite recipe for the production of corn starch-based GF bread supplemented with BW, ML, SPI, and PGR. Considering the outcome of the investigated parameters, 3% of BW was added (rich in dietary fiber, phenolic compounds, and antioxidants and positively correlated with firmness and gumminess), 1.5% of ML (rich in dietary fiber and moisture content), 2% of SPI (rich in protein and positively correlated with water and oil absorption capacities and emulsion activity) and 3% of PGR (rich in starch and positively correlated with the density of bread) (Fig. S1).

Table 2
Texture profile (firmness (N), gumminess (N), cohesiveness) and specific volume (mL/g) analyses of single ingredient-based breads prepared with individual raw gluten-free ingredients, including pregelatinized rice (PGR), millet (ML), buckwheat (BW), and soy protein isolate (SPI). Data are the mean of separate analyses ± standard deviations. Means within the column with different letters are significantly different (P < 0.05).

Flours	Crust Firmness	Gumminess	Cohesiveness	Specific Volume
PGR	523.5 ± 106.2^{b}	410.2 ± 86.1^{c}	0.8 ± 0.0^{b}	0.6 ± 0.1^{d}
ML	5917.3 ± 608.0^{a}	3525.6 ± 364.4^{b}	0.6 ± 0.0^{c}	1.3 ± 0.0^{c}
BW	6566.3 ± 131.3^{a}	5221.2 ± 122.2^{a}	0.8 ± 0.0^{b}	2.0 ± 0.1^{b}
SPI	75.3 ± 13.9^{b}	67.9 ± 12.4^{c}	0.9 ± 0.0^{a}	5.2 ± 0.4^{a}

This GF formulation allowed a cohesiveness value comparable to that of the control wheat bread prepared using soft wheat flour, although significant differences still persisted for other parameters (especially crumb and crust firmness) (Table 3).

4. Discussion

Despite the commercial availability of GF products, numerous limitations have been brought to the attention of the baking industries. Variable sensory and textural perceptions as well as limited variety of the GF products are some of the observations reported in a consumer behavior study by Nascimento, Fiates, dos Anjos, and Teixeira (2013). Therefore, a comprehensive analysis of the nutritional, functional, and physicochemical properties of raw GF ingredients was carried out, also making use of single-ingredient baked-models and composite GF formulations.

The protein content and quality of GF flours differed based on plant origin. In terms of nutritional characteristics, SPI obtained by extraction from the soybean was noted as the richest protein source. Soybean proteins are well-known for their high content of the essential amino acids, namely, lysine and methionine (Thrane, Paulsen, Orcutt, & Krieger, 2017). Among all the analyzed raw GF ingredients, PGR contained the highest amount of starch, which makes it the most suitable binding agent for the production of GF bread (Wani et al., 2012). BW and ML provided other important dietary nutrients, such as dietary fibers (Culetu, Susman, Duta, & Belc, 2021). A bread with high content of fiber might be associated with lower protein digestibility, which could display immunogenic effects due to undigested peptides (Wu, Taylor, Nebl, Ng, & Bennett, 2017). Nevertheless, the worldwide dietary guidelines recommend a high intake of dietary fiber for a healthy diet which can influence and shape the functional microbiome, restoring a balanced gut microbiota. For this aim, during the last years, efforts have been made to increase the level of dietary fiber content in GF products (Arslan, Rakha, Xiaobo, & Mahmood, 2019). Anti-nutritional compounds, such as raffinose and phytic acid, were also quantified in the raw GF ingredients. These compounds exert effects contradicting with optimal nutrition in which they can lower protein digestibility, nutrient absorption, and may cause intestinal discomfort (Kumar, Kumar, Lal, Jolly, & Sachdev, 2015). Most of our ingredients demonstrated lower raffinose and phytic acid content compared to other GF ingredients, such as oats (1.34 g/100 g for phytic acid), beans (2.2 g/100 g for raffinose and 2.38 g/100 g for phytic acid), and green pea (0.8 g/100 g for raffinose) (De Angelis et al., 2021; De Pasquale, Pontonio, Gobbetti, & Rizzello, 2020; Labba, Frøkiær, & Sandberg, 2021; Verni, De Mastro, De Cillis, Gobbetti, & Rizzello, 2019). Bioactive compounds, such as polyphenols, were profiled due to their relevance in enhancing antioxidant activity and characterizing GF ingredients, although in some respects they can also be considered ANF due to interference with protein digestion, and their benefits should be balanced with their antinutritional effects (Rocchetti et al., 2017; Cirkovic Velickovic & Stanic-Vucinic, 2018). Our findings were partially consistent with data available in literature, with some discrepancies attributable to the extraction process or to the variability associated with production of these GF ingredients. Based on our results, BW exhibited the highest antioxidant activity and total phenols content. Likewise, Culetu et al. (2021) reported stronger antioxidative potential in BW compared to other cereals and legumes, attributable to higher phenolics content. Rutin, catechin, epicatechin, epicatechin 3-gallate, and isoquercetin were the main compounds detected in BW. These compounds have been recognized to play a functional role due to their antimicrobial, anti-inflammatory, anticancer, and antidiabetic activities (Kawabata, Mukai, & Ishisaka, 2015). Conversely, lower antioxidant activity in SPI was accompanied with a poor profile of phenolics. The high antioxidant properties of soybean noted by de Camargo et al. (2019) were mainly associated with the presence and/or profile of isoflavones (especially genistein). When referring to our targeted metabolomics-based

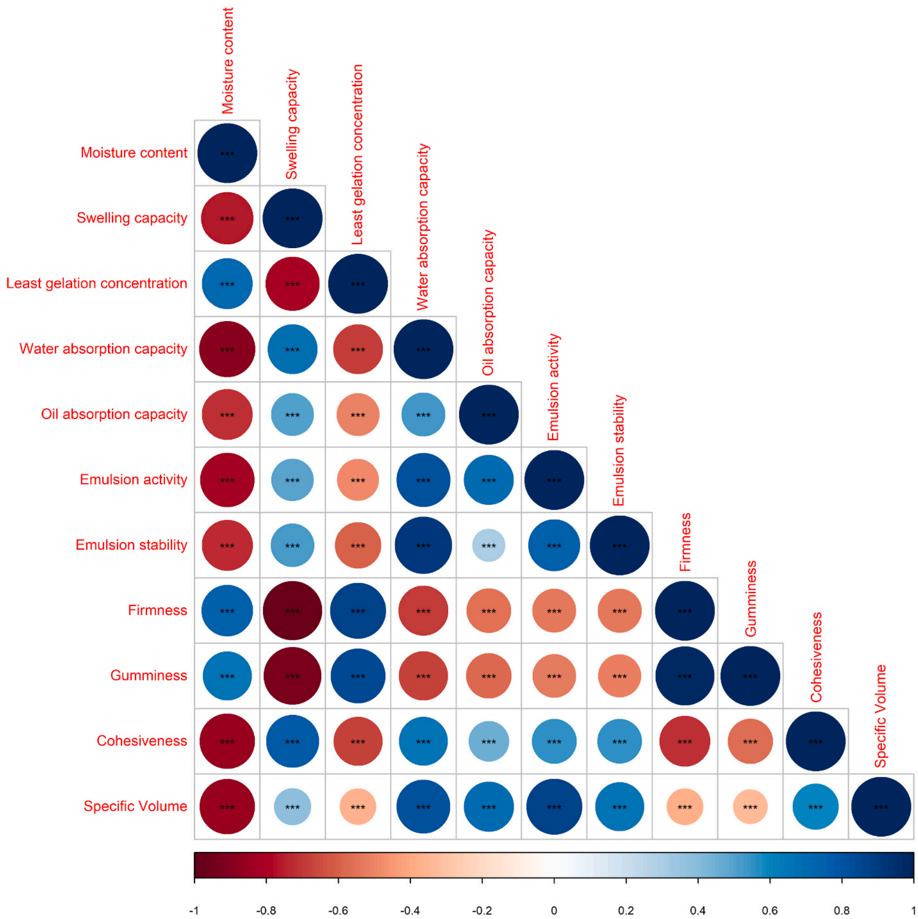

Fig. 3. Spearman's rank correlation matrix between the functional properties of pregelatinized rice, millet, buckwheat, and soy protein isolate and texture and volumetric properties of corresponding single-based gluten-free ingredient breads. Large and small circles indicate strong and weak correlations, respectively. Colors of the scale bar describe the type of correlation: 1 indicates a perfect positive correlation (dark blue) and −1 indicates a perfect negative correlation (dark red). The significance p values were not corrected by FDR and were represented by (*) < 0.05, (**) < 0.01, (***) < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

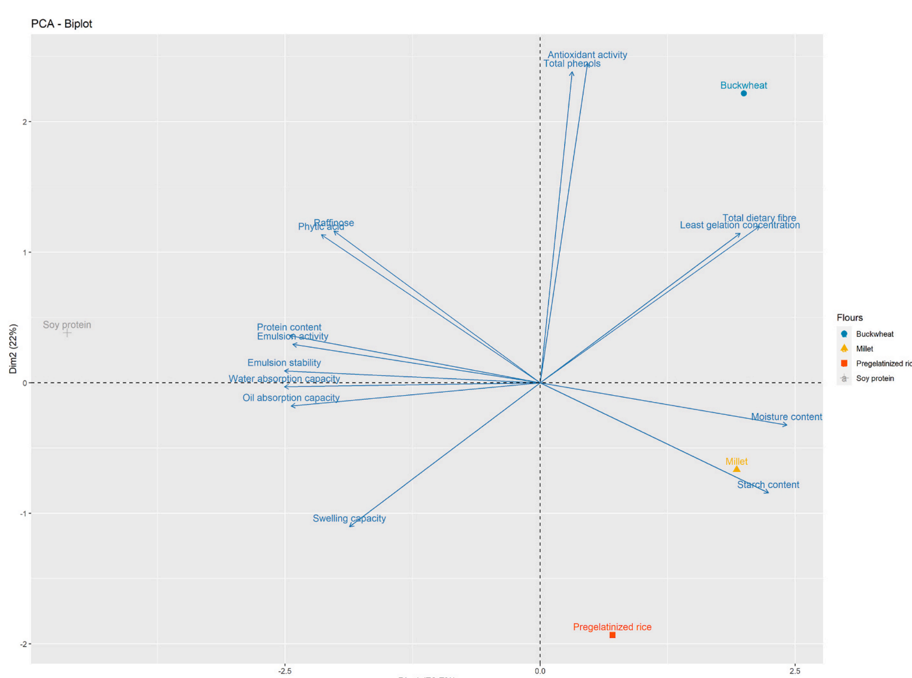

Fig. 4. Principal component analysis (PCA) of nutritional, functional, physicochemical, and textural properties of raw gluten free ingredients, including pregelatinized rice (PGR), millet (ML), buckwheat (BW), and soy protein isolate (SPI).

Table 3
Texture profile (Crumb firmness (N), crust firmness (N), cohesiveness) and volumetric [specific volume (mL/g)] analyses of composite gluten-free and soft wheat breads. Data are the mean of separate analyses ± standard deviations. Means within the column with different letters are significantly different ($P < 0.05$).

Flours	Crumb firmness	Crust firmness	Cohesiveness	Specific volume
Gluten free bread	156.0 ± 11.2[b]	328.0 ± 46.1[b]	0.9 ± 0.0	2.7 ± 0.0[b]
Wheat bread	318.0 ± 10.0[a]	1411.3 ± 53.3[a]	0.9 ± 0.0	5.7 ± 0.1[a]

approach, only *p*-coumaric acid, vanillin, and kaempferol were detected. PGR presented a low content of phenolics, represented only by vanillin, and low antioxidant activity, in accordance with Culetu et al. (2021). The same study, using different extraction approach, reported ML as a good source of phenolic compounds, which contradicts with our findings of low phenolic content.

Furthermore, we evaluated several physicochemical properties of the aforementioned raw GF ingredients that affect their utilization and textural characteristics in breadmaking. Several factors can affect the moisture content in flours, which is acceptable up to 14% at industrial level. Our results indicated moisture content slightly lower than this threshold for PGR, BW, and ML, whereas much lower for SPI. Low moisture content, as shown in several previous studies, was associated with high water absorption capacities (Roccia, Ribotta, Pérez, & León, 2009; Sapirstein, Wu, Koksel, & Graf, 2018). Higher values in flour are desired for an enhanced food texture of bread products. Our findings related to SPI and PGR confirmed that high variation in water absorption values is often attributed to high content of starch and protein (Klunklin & Savage, 2018). Likewise, the type and quantity of protein in SPI, amino acid composition, and protein polarity and hydrophobicity might explain the high oil absorption capacity (Chandra & Samsher, 2013). The oil absorption capacity can be attributed to physical trapping of the oil between flour particles, making them suitable for maintaining the flavor and enhancing the mouthfeel when used in foods (Kupirovič, Godinot, Juillerat, & Raspor, 2012). The high swelling power of PGR might be due to the high starch content, which means higher content of amylopectin (Klunklin & Savage, 2018). Additionally, it has been reported that the pre-gelatinization process (as in PGR) degrades starch molecules leading to a higher swelling power and solubility (Majzoobi et al., 2011). Whilst the low swelling capacity in ML and BW might be related to the high fiber content, which can create extensive and strongly bonded structure around the starch granules, increasing the swelling resistance (Buckman, Oduro, Plahar, & Tortoe, 2018). Least gelation concentration, defined as the lowest amount of GF ingredient required to form a firm gel network without any collapse, was higher for ML and BW. Higher least gelation concentrations indicate lower gelatinization ability. The hydrophobic interaction is a critical factor in determining the least gelation concentration that is influenced by solubility of proteins as well (Totosaus, Montejano, Salazar, & Guerrero, 2002). Focusing on protein isolates, Zhao, Shen, Wu, Zhang, and Xu (2020) stated that synergistic effect of larger amount of unhydrolyzed glutelin and very limited exposed hydrophobic polypeptides markedly reduced the gelatinization ability of rice protein. Same parameters can play a crucial role for optimal emulsifying properties. Proteins tend to stabilize the emulsions by forming a viscoelastic adsorbed layer on the oil droplets that prevents the oil from merging with the water molecules (Wilde, Mackie, Husband, Gunning, & Morris, 2004). Therefore, it is reasonable that SPI had the highest emulsifying properties due to its high protein content, as observed in previous studies (Zhao et al., 2020). It is worth noting that plant protein sources have potential as alternative natural emulsifiers from dairy proteins in food and beverage formulations (Kim, Wang, & Selomulya, 2020).

All the analyzed nutritional, functional, and physicochemical properties of these GF ingredients can influence the pasting properties of GF preparations. Thus, single-ingredient GF baked-model formulations were prepared in our study to highlight the role of each individual ingredient. The key textural properties of a typical bread are influenced by the formation of gluten network, wherein the gluten, or a protein *per se*, entraps carbon dioxide gas during fermentation to gain volume and attain acceptable crumb texture (Martínez & Gómez, 2017). According to Crockett, Ie, and Vodovotz (2011), the role of protein was relevant in SPI because it mimics the gluten action in gas entrapment, leading to an extreme increase in loaf specific volume. Based on our findings, the protein content was highly linked to several physicochemical properties, such as water and oil absorption capacity and emulsion activity and stability, all of which were negatively correlated to the firmness and gumminess of the bread and positively correlated to the cohesiveness and specific volume. In fact, high water absorption capacity moistens the bread crumb resulting in a soft texture, as evidenced by low values of firmness and gumminess (Prameswari, Manuhara, Amanto, & Atmaka, 2018). On the other side, the high starch content in PGR was responsible for increasing the density of bread by absorbing the water and leading to gelatinization (Hadnađev et al., 2013), making the bread more firm and rigid. The presence of amylose and amylopectin matrix in the starch residues can cause recrystallization and contribute to crumb firmness (Ho, Tan, Abdul Aziz, & Muhamad, 2017). Nevertheless, the role of starch is crucial when interacting with gluten to create a stable network for gas entrapment in the dough structure during fermentation preventing the collapse of bread volume (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005). In accordance with our findings, high dietary fiber content resulted in a heavier dough which is hard to grow during fermentation, thereby lowering bread volume while baking and simultaneously increasing crumb firmness and gumminess (Raczyk, Kruszewski, & Michałowska, 2021). Besides, other research studies have shown that soluble dietary fibers, such as nutriose and polydextrose, have an antagonistic action, favoring volume expansion during fermentation and decreasing crumb firmness after baking (Martinez, 2014).

Previous studies have focused on the use of stabilizers (such as corn starch, hydroxypropyl methylcellulose, hydrocolloids, etc.) to mimic gluten and improve the technological properties of resulting single-ingredient GF bread formulations (Rios et al., 2020). Such additional components may increase the calorific intake and reduce the nutritional value, as suggested by Aguiar, Santos, Krupa-Kozak, and Capriles (2021). This drawback, along with all parameters investigated, which clearly separate the flour ingredients in different clusters, drove us to modify a commercial GF bread by mixing several GF ingredients. The proportions of GF ingredients in the composite bread were proposed according to their distinctive characteristics and established correlations. The synergistic interaction between the GF flours enhanced the texture of the bread as compared to single-ingredient baked-models. Indeed, this interaction was insufficient to substitute gluten network for gas entrapment, revealing low volume of bread (Martínez & Gómez, 2017) but was able to modify other properties (low firmness or cohesiveness, etc.) required by the baking industry. Although the characteristics of composite bread differed from soft wheat bread, our set of data may represent a good starting point for further optimization of flour blends ensuring high nutritional and textural qualities (Azarbad, Mazaheri Tehrani, & Rashidi, 2019). Furthermore, baking industries are constantly inventing innovative recipes to meet the demands of the consumers, and our data will help in providing the basis for such recipes.

5. Conclusion

The characterization of individual GF ingredients (SPI, PGR, ML, and BW) allowed us to build a comprehensive and detailed picture of their peculiar nutritional, functional, physicochemical, and textural properties. This preliminary framework of data permitted to optimize a GF bread formulation approaching to wheat bread, without the aid of emulsifiers and texturing agents, which are widely employed in most commercial GF baked products and can compromise the bread's nutritional value. Our findings suggest an interaction between the properties of GF ingredients that deserves further investigation.

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CRediT authorship contribution statement

Kashika Arora: Investigation, Formal analysis, Writing – original draft. **Ali Zein Alabiden Tlais:** Investigation, Formal analysis, Writing – original draft. **Guenther Augustin:** Conceptualization. **Daniele Grano:** Conceptualization. **Pasquale Filannino:** Writing – review & editing. **Marco Gobbetti:** Funding acquisition, Writing – review & editing. **Raffaella Di Cagno:** Conceptualization, Methodology, Supervision, Writing – review & editing.

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.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114566.

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