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White bean and hazelnuts flours: Application in gluten-free bread

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ABSTRACT

This study investigated the effects of white bean and hazelnut flour addition (15–30% alone or in combination) to a rice flour-corn starch mixture in gluten-free (GF) breads formulated according to a mixture design. The chemical composition of flours and pasting properties of their mixtures were investigated, as well as the spectroscopic characteristics and leavening performance of doughs. Physical properties of fresh and stored (up to 48 h) bread samples were analyzed. Bean and hazelnut flours had higher protein and fiber contents, and lower carbohydrates content than rice flour and corn starch. Although the reference bread made of rice flour-corn starch mixture (STD) resulted in the highest specific volume (7.0 mL/g) and the lowest hardness (0.43 N), the sample enriched with 15% hazelnut flour (H15) approached these characteristics the most (3.8 mL/g and 1.59 N, respectively). After 48 h of storage, H15 also showed lower hardness than STD. This study paves the way for new applications of white bean and hazelnut flours and showed as a simple reformulation can help to develop healthier bread: the European legal constraint for "fiber source" claim was achieved for breads with 15 or 30% hazelnut flour, and 30% bean-hazelnut mixture, with a fiber content of 3.34, 4.48, and 3.27 g/100g, respectively.

1. Introduction

Bread is a staple food consumed all over the world, thus it represents an ideal system for enrichment with functional ingredients to meet the growing consumers' demand for products with enhanced nutritional properties. Conventional yeast-leavened white bread made of refined wheat flours has a high glycemic index and its consumption is associated with a high rate of postprandial glucose release and lower satiety sensations (Bo et al., 2017). These factors, which are strongly related to the development of type 2 diabetes mellitus (Livesey et al., 2019), are even more evident in gluten-free (GF) bread, where wheat flours containing the gluten-forming proteins are commonly replaced by starchy ingredients (e.g., rice, corn, sorghum, buckwheat, amaranth, and quinoa flours or starches) and other techno-functional ingredients and additives (e.g., proteins, gums, hydrocolloids, and emulsifiers) (Gao et al., 2018). The addition of techno-functional ingredients and/or additives is necessary because the bottleneck in GF breadmaking is usually the poor quality of the final product compared to conventional bread, due to low volume, dry and friable crumb, pale color, a rapid staling, and lack of flavor and mouthfeel (Melini, Melini, Luziatelli, & Ruzzi, 2017). Thus, conventional and mostly GF bread can benefit from reformulation with plant-based ingredients to increase nutrient density, slow post-prandial glucose release, and provide phytochemicals that may regulate metabolic functions and have beneficial effects on health (Amoah et al., 2022). Plant-based ingredients can also provide technological functionalities useful for GF product development (i.e., water retention capacity, fat binding, foaming and gelation properties) (Melini et al., 2017).

In this context, the replacement of common GF ingredients with legumes and nuts can be a valuable strategy to produce healthier bread. Indeed, legumes and nuts are rich in bioactive compounds and dietary fiber (Hernández-López et al., 2022) and their consumption is encouraged because associated with an improved glycemic and lipid profile status (Amoah et al., 2022). Besides, legume proteins were proved to improve sensory characteristics and acceptance of GF bread, while extending the shelf-life (Melini et al., 2017).

Over the last decades, legumes have been more and more used to replace wheat flour in conventional bread formulations, despite challenges in dough technological properties and bread sensory characteristics especially when replacement levels exceed 10–30% (Melini et al., 2017). On the contrary, few studies evaluated the use of nut flours or proteins in bread (Azeez et al., 2022; Pycia & Ivanišová, 2020) and no one reported the effect of legume-nut composite flours in GF bread. Thus, the aim of this work was to investigate the role of white bean and

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hazelnut flour addition in GF bread made of rice flour and corn starch. The replacement of rice flour-corn starch mixture with white bean and hazelnut flour (15-30% alone or in combination) was studied- according to a three-component extreme vertices mixture design, to simultaneously evaluate main and interaction effects and identify an optimal formulation.

2. Materials and methods

2.1. Materials

Raw materials used to prepare GF yeast-leavened bread samples were as follows: corn starch (C; Maizena ® Unilever, Roma, Italy), rice flour (R; Il Molino F.lli Chiavazza S.p.A, Casalgrasso, Italy), white bean flour (B; Naturelka, Aydin, Turkey), hazelnut flour (H; Ingro, Karaman, Turkey), hydroxypropyl methyl cellulose (HPMC, Benecel F4M, Ashland, Wilmington, DE, USA), compressed yeast (GS S.p.A., Milano, Italy), sugar (GS S.p.A., Milano, Italy), salt (GS S.p.A., Milano, Italy), and extra virgin olive oil (Farchioni Olii S.p.A., Gualdo Cattaneo, PG, Italy).

Chemicals for analyses (i.e., sulfuric acid, ethyl ether, hydrochloric acid, sodium hydroxide, sodium carbonate, methanol, hexane, and Folin Ciocalteu reagent) were obtained from Merck KGaA (Darmstadt, Germany).

2.2. Bread formulations and experimental design

Bread formulations were defined according to a three-component extreme vertices mixture design (Minitab, demo version, State College, PA, USA; Fig. 1). A D-optimal point exchange design was chosen, with the following constraints for the flour mixture: H and B ranges between 0 and 30%; H + B range between 0 and 30%; RC (equal amounts of rice flour and corn starch) range between 70 and 100%; point candidate: 7.5% H, 7.5% B, 85% RC. The constraints on H and B were adjusted based on preliminary experiments. Thus, seven formulations were generated, including a reference sample (STD). The proportions of the three components H, B, and RC in the flour mixtures are given in Table 1. Other than STD, samples were coded as the RC replacement percentage in the flour mixtures by B, H, or their equally mixed composite (BH).

The STD formulation was defined according to Cappa, Barbosa-Cánovas, Lucisano, and Mariotti (2016) but avoiding pea protein and psyllium because in the present study B and H were used as source of proteins and fibers. Thus, STD sample contained 83.5 g/100g RC, 1.5 g/100g HPMC, 6 g/100g extra virgin olive oil, 4 g/100g sugar, 2 g/100g salt, and 3 g/100g compressed yeast. The ingredient amounts are expressed on the total recipe weight basis, water excluded.

2.3. Flour and mixtures characterization

R, C, B, and H samples were characterized in terms of moisture (AACC 44-15A, 2000; n = 3), total nitrogen content (Improved Kjeldahl Method according to AACC 46-10.0, 2000, with conversion factors of 6.25 for C, B, H and 5.95 for R; n = 3), fat content (Soxhlet method with ethyl ether, AOAC 945.38F, 920.39C, 1990; n = 3), ash content (AOAC 923.03, 1990; n = 3), crude fiber content (AOAC 14.020, AOAC 7.065, 1990; n = 3), and water retention capacity (AACC 56-11, 2000; n = 3). The total carbohydrate content of each flour was obtained by difference, subtracting moisture, protein, fat, and ash content (Choe, Osorno, Ohm, Chen, & Rao, 2022). Phenolic compounds of the flours were extracted according to Byanju, Hojilla-Evangelista and Lamsal (2021), and total phenolic contents (TPC; n = 3) were determined as explained by Rufino et al. (2010), with slight modifications (0.2 mL sample instead of 1 mL).

A scanning electron microscope (SEM; Quanta 250 FEG, FEI, Oregon, USA) equipped with Everhart-Thornley Detector (ETD) for B and H, and large-field detector (LFD) for R and C was used to examine the microscopic structures of starch and flour samples in the Center for Materials Research (CMR) at Izmir Institute of Technology (IZTECH). Dried flours were mounted with double-sided adhesive tape on aluminum stubs and sputter-coated with gold before observation.

Pasting properties (n = 2) of R, C, their mixture (RC, equally mixed), and RC with B and H flours (as given in Table 1) were evaluated by using a Brabender® Micro-Visco-Amylograph (MVA; Brabender OHG, Duisburg, Germany). The analysis was conducted according to Cappa, Lucisano, and Mariotti (2013) but sample slurry was prepared by dispersing each sample (13.5 g) in distilled water (90 mL), scaling sample and water weight on 14 g/100g sample moisture basis. The measured indices were: pasting temperature (PT, °C; temperature at which an initial increase in viscosity occurs); peak viscosity (PV, Brabender Units, BU; maximum paste viscosity achieved during heating), breakdown (BD, BU; viscosity decrease index while kept the suspension at 95 °C, calculated as difference between peak viscosity and viscosity at the end of the period at 95 °C); setback (SB, BU; index of the viscosity increase during cooling), and final viscosity (FV, BU; paste viscosity at the end of the cooling).

2.4. Dough production and characterization

Dough samples were prepared using a Brabender® farinograph (Brabender OHG, Duisburg, Germany) equipped with a 300 g bowl and set at 30 °C. The dry ingredients were pre-mixed for 5 min before adding yeast (previously suspended in a part of water), oil, and water up to the desired dough consistency of 200 ± 20 BU, considered suitable for GF formulations (Cappa et al., 2013; Cappa et al., 2016; Tufaro, Bassoli, & Cappa, 2022). The doughs were kneaded for 15 min and consistency was

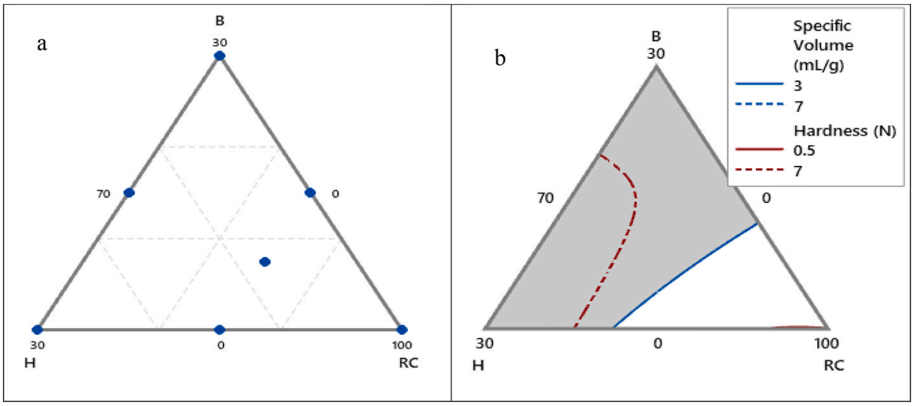

Fig. 1. (a) Experimental design space with the seven formulations studied (component amounts in percentage) (b) Overlay contour plot for specific volume (mL/g) and crumb hardness (N): the white area represents the optimal experimental space.

Table 1
Gluten-free bread sample codes, flour mixture composition according to the mixture design, total bread recipe and water added.

Sample Code	Flour mixture composition (g/100g)			Total recipe (g/100g), water excluded						Water added (g/100g total recipe)
	RC	B	H	Flour mixture	Oil	Sugar	Yeast	Salt	HPMC	
STD	100	0	0	83.5	6	4	3	2	1.5	60.1
B15	85	15	0	83.5	6	4	3	2	1.5	70.2
H15	85	0	15	83.5	6	4	3	2	1.5	51.1
B30	70	30	0	83.5	6	4	3	2	1.5	62.5
H30	70	0	30	83.5	6	4	3	2	1.5	43.9
BH15	85	7.5	7.5	83.5	6	4	3	2	1.5	60.2
BH30	70	15	15	83.5	6	4	3	2	1.5	62.5

RC: Rice flour (R) and corn starch (C) equally mixed; B: White bean flour; H: Hazelnut flour; HPMC: hydroxypropylmethylcellulose. In sample codes: STD, reference gluten-free bread formulation containing only RC as flour mixture; 15 and 30, percentages of B, H, or their equally mixed amounts in flour composite.

continuously recorded. For each formulation, water absorption (g/100g of total ingredients water excluded) was determined.

FT-IR spectra of dough samples (n = 2) were collected immediately after preparation by using a Vertex 70 spectrometer (Bruker Optics, Milan, Italy) equipped with a Germanium multiple reflection ATR cell. Dough was spread on the cell surface, paying attention to avoid empty spaces. Spectra were acquired on two dough aliquots, in duplicate for each aliquot, in the range 4000–800 cm^{-1} at room temperature, with a 4 cm^{-1} resolution and 32 scans for both background and samples. Instrument control and data acquisition were managed by Opus software (v.6; Bruker Optics, Ettlingen, Germany).

Leavening properties of dough samples (n = 3) were measured through the image analysis method by Cappa et al. (2013) with some modifications. Aliquots of each dough (10 g each) recovered from the farinograph at the end of mixing were molded in a spherical shape by using a spoon, put into a Petri dish, and leavened in an incubator (Memmert UFE500, Schwabach, Germany) at 35 °C for 1 h. At the beginning of the leavening phase and every 15 min the images of the Petri dishes were scanned full scale in 256 grey levels at 300 dpi with a flatbed scanner (Epson Perfection V850pro scanner, Seiko Epson Corporation, Suwa, Japan). The images were saved in TIFF format and processed using a dedicated software (Image Pro-Plus v. 7.0, Media Cybernetics Inc., Rockville, MD, USA). The dough area increase (%) during leavening was calculated.

2.5. Breadmaking procedure

According to Tufaro et al. (2022), the dough produced in the farinograph was collected, divided into six portions (60 g each), and placed into oiled metal molds (100 × 60 × 45 mm). According to the leavening properties evaluation, the dough was leavened at 35 °C for 40 min by using the leavening function of a multifunction oven (mod. AMW698/IXL, Whirlpool, EMEA S.p.A., Biandronno, VA, Italy) and baked in an electric static oven (mod. G2551MF816A, Whirlpool, EMEA S.p.A., Biandronno, VA, Italy) for 30 min at 175 °C. At the end of baking, the loaves were cooled for 30 min at room temperature, before being removed from the molds and characterized.

2.6. Bread characterization

The six loaves of each formulation were used immediately after cooling (t0, fresh bread) to analyze specific volume, and baking loss. The other analyses (i.e., moisture, water activity, crumb porosity, texture, and color) were performed at t0 and after 24 h (t24) and 48 h (t48) of storage, using two loaves for each time. Storage was carried out under controlled conditions (25 °C, 60% relative humidity; climatic chamber HC0020, Haereus Vötsch, Frommern, Germany) in unsealed hand-folded paper bags simulating a domestic shelf-life (Mariotti et al., 2017).

Each bread formulation was characterized in terms of baking loss (%; computed as the difference between the weight of the dough before leavening and the weight of the fresh bread, with respect to the dough weight; n = 6), slice and crumb moisture (g/100g; AACC 44-15A, 2000; n = 4), crumb water activity (AquaLab Series CX-3, Decagon Devices Inc. Pullman, WA, USA; n = 2), and specific volume (mL/g; AACC 10–05.01, 2000, replacing rapeseeds with sesame seeds; n = 6).

Crumb porosity was determined using an image analysis method: a central crumb crop (approximately 70% of the crumb) was selected from each bread slice previously scanned in 256 grey scale levels and 600 dpi resolution using an Epson Perfection V850pro scanner (Seiko Epson Corporation, Suwa, Japan). Images were processed by using the Image Pro-Plus software (v. 7.0; Media Cybernetics Inc., Rockville, MD, USA). According to previous studies (Cappa et al., 2016; Kahraman, Harsa, Casiraghi, Lucisano, & Cappa, 2022; Tufaro et al., 2022), holes within the range of 0.1–10 mm^2 were identified and measured to calculate the total area of the holes. Porosity was then expressed in percentage, considering the total hole area in the crop with respect to the total crop area (n = 4).

Bread crumb hardness was measured through a penetration test by using a dynamometer (mod. 3365, Instron Division of ITW Test and Measurement Italia S.r.l., Pianezza, TO, Italy), equipped with a 100N load cell. The BlueHill software (v. 2.9, Instron Corporation, USA) was used to control the instrument and collect data. Breads were sliced (20 mm thick) using an electric knife and each slice was penetrated up to 40% deformation at a compression speed of 1 mm/s, using a 13 mm diameter cylindrical probe. The trigger force was set at 0.098 N. Crumb resistance to 30% penetration was measured as indication of crumb hardness (N; $n \geq 4$)).

Crust and crumb color (n = 4) was evaluated by using a tristimulus colorimeter Chroma Meter II (Minolta, Osaka, Japan), with a diffused illumination integrating sphere system (d/0), the standard illuminant C, and the CIE 2° standard observer. The head of the colorimeter (8 mm aperture diameter) was laid directly on the sample surface, after calibration using the standard-white reflector plate. Results were expressed in the L*a*b* space as L* (lightness; from black (0) to white (100)), a* (from green (−60) to red (+60)), and b* (from blue (−60) to yellow (+60)). Color differences between the standard (STD) and the rest of the formulations (B15, B30, H15, H30, BH15, BH30) were determined using CIE-76 equation (Kasim & Kasim, 2015):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

During storage (24 and 48 h), GF bread samples were also characterized in terms of weight loss (%; computed as the difference between fresh and stored bread weight with respect to the fresh weight; $n \geq 2$).

2.7. Statistical analyses

The results are given as mean and standard deviation values. Statistical evaluation of the mixture design and the data was performed by using Minitab software (demo version, State College, PA, USA). All data were tested by one-way analysis of variance (ANOVA) followed by Tukey's test to check significant differences ($p \leq 0.05$).

3. Results and discussion

3.1. Flour characteristics

Proximate compositions and total phenolic content of flour samples are presented in Table 2. R and C had the significantly higher moisture, as well as significantly lower amount of proteins, fat, ash, and fiber compared to B and H. R, C, and B had carbohydrate contents higher than 68 g/100g, whereas H had a lower amount. On the other hand, H contained the significantly highest contents of fat, fiber, and TPC. B and H significantly came forward in protein content. All these properties make B and H potential ingredients for healthier GF products. In fact, Collar and Angioloni (2017) showed that high-legume wheat bread had significantly lower digestible starch linked to higher soluble and insoluble dietary fiber contents.

As a technological property, water retention capacity (WRC) for R, C, and B was determined as 131.7, 82.7, and 262%, respectively. B had the significantly highest WRC. The results are in agreement with other studies (Cappa, Kelly, & Ng, 2018; Choe et al., 2022). The WRC of H could not be determined due to the high fat content. In another study, where raw and roasted hazelnut flours were investigated, similar findings were reported (Turan, Capanoglu, & Altay, 2015).

3.2. Pasting properties of flours

The MVA is a consolidated tool for assessing the gelatinization and retrogradation properties of starchy materials in water during controlled cycles of heating and cooling. Indeed, when starch gelatinizes there is an increase of the slurry viscosity (thus pasting temperature and peak viscosity values can be measured by MVA); subsequently, during cooling, a further viscosity increase related to the starch retrogradation occurs. As expected, C had the highest viscosity profile followed by R (Table 3). However, when the pasting properties of whole flours or high-protein flour are explored, the phenomena that take place during the heating and cooling phases are more complex, since macromolecular interactions occur. In particular, the presence of proteins and fiber determines strong competitions for water during the initial hydration phase and can interfere with starch granule reorganization during the cooling phase (Cappa et al., 2013; Cappa et al., 2018). In fact, the pasting properties of flour mixtures including H at the highest levels (i.e., H30 and BH30) had the lowest pasting values (peak and final viscosity) due to low starch and high fiber and fat contents (Table 2). Furthermore, pasting temperature increased with increasing amounts of H in the mixtures (from 68 °C of R up to 72.9 °C of H30). The pasting temperatures and viscosity values were similar to previously published data showing lower gelatinization temperatures and higher viscosities for rice flour and starch, exactly opposite for legume flours (Al-Attar, Ahmed, & Thomas, 2022; Di Cairano et al., 2020). In other studies, gelatinization temperature increased with increasing content of bran (Sabaris, Lebesi, & Tzia, 2009) and with the content of amylose in the starch granules, which is high in legume flours (Aguiar et al., 2022), thus accounting for the higher values of pasting temperature registered for B15 and B30 flour mixtures. This property of legume flours could be also related to a lower accessibility of starch granules by α-amylase, possibly reflected in a low glycemic index (Zhu, Liu, Wilson, Gu, & Shi, 2011), as also reported by Gularte, Gómez, and Rosell (2012) for legume-enriched gluten-free cakes. Another index evaluated by MVA is the setback value (Table 3), which indicates the tendency of starch to retrograde, and can be used as an indicator of the bread staling rate. C showed the highest setback value (1046 BU); actually, according to Cappa et al. (2013), during cooling corn starch is able to form a strong gel, but it is highly sensitive to retrogradation, suggesting a high staling rate of the obtained GF bread. On the opposite, the presence of H at the highest amount (i.e., H30 and BH30) resulted in the lowest setback values (431 and 527 BU, respectively), suggesting that H addition potentially helps to slow down the starch retrogradation phenomenon. B mixtures, having intermediate carbohydrate content (Table 2) and reasonably intermediate starch content, showed intermediate peak viscosity and setback values due to lower starch content of bean flour as previously reported by Cappa, Kelly, and Ng (2020).

Table 3
Pasting properties of rice flour, corn starch, their mixture, and their mixtures with white bean and hazelnut flour.

Sample	Pasting temperature (°C)	Peak viscosity (BU)	Breakdown (BU)	Setback (BU)	Final viscosity (BU)
R	68.2 ± 0.2^{a}	1089 ± 28^{d}	684 ± 15^{d}	545 ± 1^{b}	950 ± 13^{cd}
C	70.7 ± 0.1^{b}	1440 ± 59^{f}	963 ± 52^{f}	1046 ± 22^{e}	1523 ± 15^{f}
RC	71.5 ± 0.1^{c}	1276 ± 2^{e}	835 ± 3^{e}	687 ± 4^{d}	1145 ± 2^{e}
B15	71.3 ± 0.1^{bc}	937 ± 19^{c}	572 ± 22^{c}	630 ± 17^{c}	995 ± 14^{d}
B30	71.6 ± 0.1^{c}	726 ± 29^{b}	404 ± 13^{b}	613 ± 1^{c}	934 ± 17^{c}
H15	71.9 ± 0.1^{cd}	948 ± 17^{c}	611 ± 11^{cd}	607 ± 15^{c}	944 ± 9^{c}
H30	72.9 ± 0.4^{e}	536 ± 40^{a}	301 ± 33^{a}	431 ± 8^{a}	666 ± 14^{a}
BH15	71.7 ± 0.1^{c}	937 ± 26^{c}	586 ± 21^{c}	625 ± 8^{c}	976 ± 13^{cd}
BH30	72.5 ± 0.2^{de}	641 ± 24^{ab}	378 ± 16^{ab}	527 ± 1^{b}	790 ± 9^{b}

R: Rice flour; C: Corn starch; RC, Rice flour and corn starch equally mixed; B: White bean flour; H: Hazelnut flour; B15 and B30, mixture containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, mixture containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 mixture containing 15 and 30% respectively of B and H in equal amounts in flour mixture; BU, Brabender Unit.$^{a\text{-}e}$, mean values ($n = 2$) in the same column with different superscript letters are significantly different ($p \leq 0.05$).

3.3. Dough characteristics

Bread doughs were produced adding different water amount (Table 1) based on the farinographic water absorption values needed to reach the desired dough consistency of 200 BU. The different water amounts were related to flour WRC. Indeed, the maximum amount of water was added to formulations with 15 or 30% replacement by B, which had the significantly highest WRC. A higher hydration level of GF bread dough with legume proteins with respect to a reference formulation containing corn starch was already reported by Sahagún and Gómez (2018). Dough prepared with 15 or 30% H required the lowest water amount due to the high amount of fat with respect to B and other

Table 2
Chemical composition of rice flour, corn starch, white bean, and hazelnut flour.

Flour	Moisture (g/100g)	Proteins (g/100g)	Fat (g/100g)	Carbohydrates* (g/100g)	Total ash (g/100g)	Crude fiber (g/100g)	TPC (mgGAE/g)
R	12.54 ± 0.03^{d}	6.03 ± 0.06^{b}	1.09 ± 0.12^{a}	79.73	0.61 ± 0.03^{b}	1.51 ± 0.02^{a}	0.22 ± 0.02^{a}
C	11.04 ± 0.05^{c}	0.52 ± 0.01^{a}	0.54 ± 0.03^{a}	87.74	0.16 ± 0.02^{a}	0.98 ± 0.03^{a}	0.15 ± 0.02^{a}
B	7.89 ± 0.05^{b}	18.77 ± 0.02^{d}	2.08 ± 0.33^{a}	68.15	3.11 ± 0.02^{d}	3.71 ± 0.19^{a}	0.38 ± 0.08^{a}
H	1.84 ± 0.02^{a}	15.60 ± 0.03^{c}	66.38 ± 1.60^{b}	14.19	1.99 ± 0.04^{c}	13.43 ± 0.06^{c}	2.05 ± 0.18^{b}

R: Rice flour; C: Corn starch; B: White bean flour; H: Hazelnut flour; TPC: Total Phenolic Content; GAE: Gallic Acid Equivalent.* Carbohydrates were estimated by difference; $^{a\text{-}d}$, mean values ($n = 3$) in the same column with different superscript letters are significantly different ($p \leq 0.05$).

fiber sources like fruit pulps, cereal bran, or even hazelnut skin (Föste, Verheyen, Jekle, & Becker, 2020). In an earlier study, higher water requirement was observed in bread dough samples with hazelnut skin, which had much lower fat and higher fiber contents compared to hazelnut flour (Anil, 2007). In the study by Sabanis, Lebesi, and Tzia (2009), the amount of added water increased with the proportion of cereal brans in the mixture, whereas in this study the amount of added water progressively decreased as the H content increased, due to the fat effect prevailing the fiber influence.

FT-IR spectra of GF dough samples are shown in Fig. 2. The major peak that dominated the spectra is attributed to O–H stretching (3700-3000 cm^{-1}), which indicates the presence of water and is representative of the starch structure (Xu, Wu, Shang, Wei, & Gao, 2023). Minor peaks between 3000 and 2850 cm^{-1} indicated the presence of carbonyl groups with C–H stretching of the methyl and methylene groups of the side chains. The peak around 1750 cm^{-1} showed a C═O stretching which is attributed to the fat triglyceride ester linkage. The peaks between 1600 and 1500 cm^{-1} are associated with the presence of Amide I (C═O stretching), and Amide II (C–N stretching and N–H bending). Characteristic peaks for polysaccharides were observed between 1200 and 1000 cm^{-1} (Sinelli, Casiraghi, & Downey et al., 2008; Skendi, Papageorgiou, & Papastergiadis, 2021). The most different spectrum was obtained for the H30 dough, which had the lowest amount of added water. Between 3000 and 2800 cm^{-1} and at around 1750 cm^{-1}, H30 dough stood out due to its high fat content. The same trend continued in the 1650 - 1000 cm^{-1} interval, and it can be attributed to the high protein and fiber content of H. The formulations containing mixtures of H and B (BH30 and BH15) followed the pattern of H30.

Dough area increase during leavening (Fig. 3) was the highest in STD sample and the lowest in the H30 formulation suggesting that H weakened the dough network; this can be attributed to the higher particle size of the flour, which created discontinuity in the network. Indeed, the effect of powder particle size on food structure is well known; previous studies reported that coarse powders resulted in less developed GF products, such as thinner cookies (Cappa et al., 2020) and bread with low volume (Qin et al., 2021). These observations are supported by SEM images, which showed different characteristics in the microscopic structures of starch and flour samples (Fig. 4). H had relatively larger compact particles compared to the other flours. C had particles homogenous in both shape and dimension. B and R flour samples had non-homogenous distribution of particle size, with some large clusters harboring round starch particles.

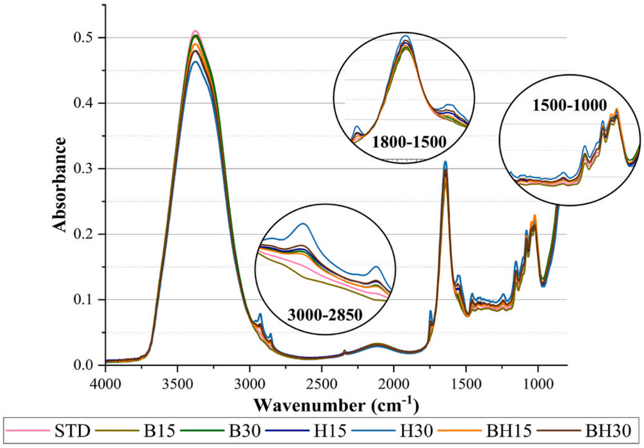

Fig. 2. FT-IR spectra of gluten-free dough samples (n = 2).
STD, reference gluten-free dough formulation containing only rice flour and corn starch as flour mixture; B15 and B30, dough containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, dough containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 dough containing 15 and 30% respectively of B and H in equal amounts in flour mixture.

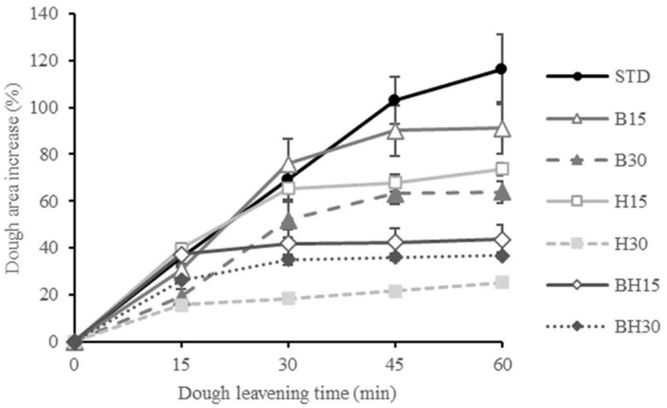

Fig. 3. Leavening profiles of gluten-free dough samples (n = 3). STD, reference gluten-free dough formulation containing only rice flour and corn starch as flour mixture; B15 and B30, dough containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, dough containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 dough containing 15 and 30% respectively of B and H in equal amounts in flour mixture.

Dough samples with 30% B, H, or combination of them showed low dough area increases (Fig. 3), whereas when the amount of H and B decreased, the area increase approached the STD formulation. This behavior can be also related to a different elasticity of the doughs depending on the specific properties of the replacement flours in terms of proteins and fiber (Bojňanská, Musilová, & Vollmannová, 2021). In a published study where hazelnut skin was used at 5–10% substitution level in wheat bread, the resistance to extensibility increased with the skin amount increase, indicating that fibrous material addition directly affected the dough strength and thus the leavening phase (Anil, 2017). Similarly, the same effect was observed with higher addition of B and H. Addition of legume flours to wheat flour also resulted in reduced extensibility and limited dough expansion capacity in other studies (Bojňanská et al., 2021; Kotsiou, Sacharidis, Matsakidou, Biliaderis, & Lazaridou, 2022).

3.4. *Fresh gluten-free bread characteristics*

GF bread characteristics are given in Table 4. Baking loss, which is basically a partial removal of water, was at a minimum level in bread samples with H in the formulation (13–19%) and at the highest in STD bread (23.7%). Consequently, the final weights were found significantly higher in H breads (data not shown). Low baking loss in samples H30 and BH30 (13.2 and 17.5%, respectively) might be linked to the high fat and fiber content of hazelnut flour. Baking loss behavior was explained by significant linear regression model ($p < 0.05$, adjusted $R^2 = 0.90$) with interaction term BxRC, and normally distributed residuals. Similarly, both slice and crumb moisture levels were explained by a significant linear regression model ($p < 0.01$, adjusted $R^2 = 0.98$, normally distributed residuals). The replacement with B caused higher moisture in both slices and crumb, whatever the content was. This is associated with the high WRC of legume flours, and the consequently higher amount of water addition in dough preparation, as resulted from farinographic results. The H-containing breads (H15 and H30) had the same moisture as STD bread or lower. Water activity, on the other hand, was significantly higher in STD and B-containing breads compared to H breads. A significant model for water activity ($p < 0.01$, adjusted $R^2 = 0.98$, normally distributed residuals) showed that lower water activity values were related to the H-containing formulations, for which the water content of dough was lower.

Volume of loaves and porosity of crumbs are appealing physical characteristics to define a preferred bread. Specific volume and porosity

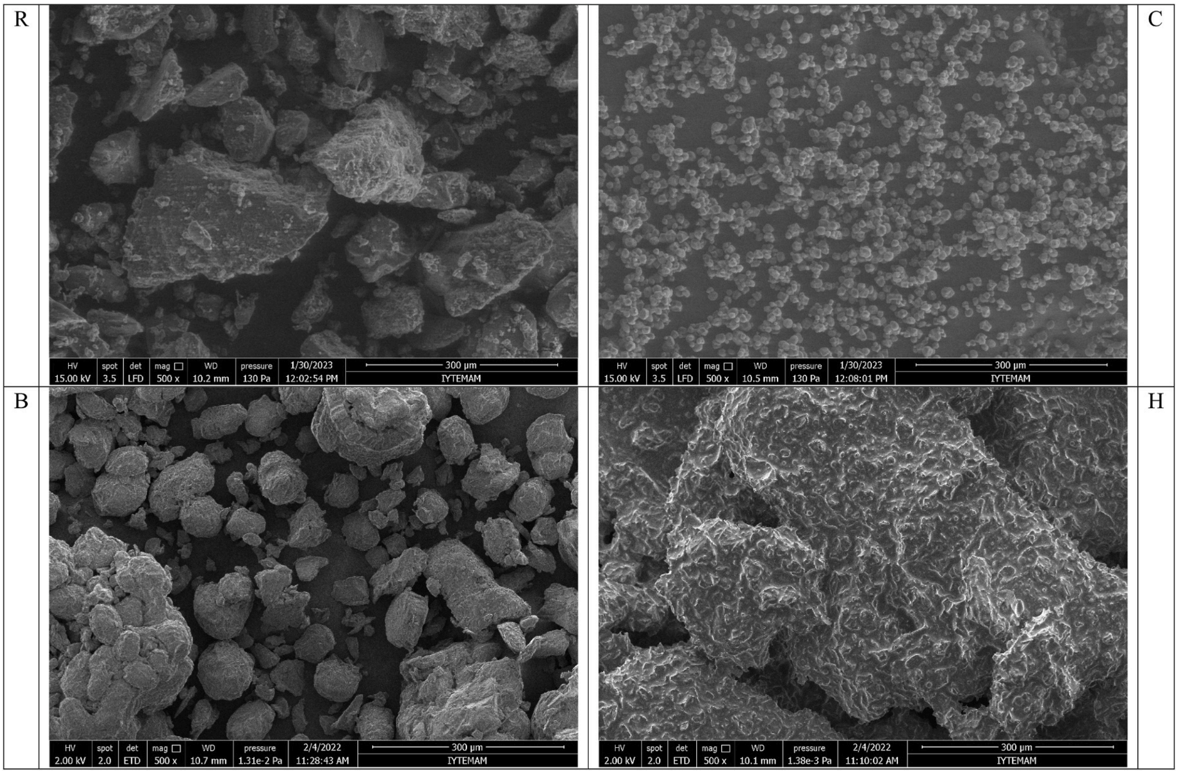

Fig. 4. SEM images of flour and starch samples. R: Rice flour; C: Corn starch; B: White bean flour; H: Hazelnut flour.

Table 4
Properties of the fresh gluten-free bread samples.

Properties	STD	B15	B30	H15	H30	BH15	BH30
Crumb moisture (g/100g)	41.1 ± 0.7^{c}	46.4 ± 0.2^{e}	50.4 ± 0.2^{f}	39.4 ± 0.3^{b}	36.3 ± 0.4^{a}	43.3 ± 0.2^{d}	44.3 ± 0.2^{d}
Slice moisture (g/100g)	27.8 ± 0.6^{a}	34.5 ± 0.4^{c}	39.4 ± 0.3^{d}	27.5 ± 0.1^{a}	27.4 ± 0.4^{a}	32.2 ± 0.6^{b}	32.7 ± 0.1^{b}
Crumb water activity	0.985 ± 0.001^{d}	0.985 ± 0.006^{d}	0.979 ± 0.001^{cd}	0.965 ± 0.003^{b}	0.951 ± 0.002^{a}	0.977 ± 0.001^{cd}	0.971 ± 0.003^{bc}
Specific volume (mL/g)	7.0 ± 0.2^{e}	2.7 ± 0.1^{c}	1.9 ± 0.1^{ab}	3.8 ± 0.1^{d}	1.7 ± 0.1^{a}	2.7 ± 0.1^{c}	2.4 ± 0.1^{bc}
Crumb porosity (%)	27.9 ± 6.1^{b}	18.0 ± 1.0^{a}	19.9 ± 1.7^{a}	17.8 ± 1.5^{a}	17.5 ± 1.7^{a}	18.8 ± 1.3^{a}	20.9 ± 3.2^{a}
Crumb hardness (N)	0.43 ± 0.04^{a}	5.27 ± 0.37^{bc}	4.92 ± 0.33^{b}	1.59 ± 0.21^{a}	14.18 ± 1.08^{e}	7.97 ± 0.64^{d}	7.15 ± 0.48^{cd}
Crust color - L*	77.6 ± 0.7^{c}	68.1 ± 0.8^{ab}	72.6 ± 3.3^{bc}	64.9 ± 0.8^{a}	67.6 ± 0.3^{ab}	69.0 ± 0.5^{ab}	66.1 ± 0.2^{a}
Crust color - a*	-0.5 ± 0.3^{a}	-0.4 ± 0.0^{a}	-0.6 ± 0.0^{a}	5.0 ± 0.1^{c}	0.9 ± 0.0^{b}	-0.6 ± 0.4^{a}	0.2 ± 0.2^{ab}
Crust color - b*	29.1 ± 0.3^{a}	30.3 ± 0.5^{a}	28.7 ± 4.5^{a}	33.1 ± 1.0^{a}	28.2 ± 0.6^{a}	28.8 ± 0.7^{a}	28.7 ± 0.5^{a}
ΔE_{Crust}	–	9.5 ± 0.0^{ab}	6.1 ± 2.3^{a}	14.4 ± 1.0^{c}	10.1 ± 0.4^{abc}	8.6 ± 0.5^{ab}	11.6 ± 0.2^{bc}
Crumb color - L*	84.6 ± 0.2^{e}	75.6 ± 0.0^{c}	75.9 ± 0.3^{c}	79.6 ± 0.8^{d}	67.6 ± 1.2^{a}	71.8 ± 0.6^{b}	72.5 ± 0.1^{b}
Crumb color - a*	-2.6 ± 0.1^{abc}	-3.0 ± 0.1^{a}	-2.8 ± 0.1^{ab}	-1.9 ± 0.1^{cd}	-0.9 ± 0.4^{e}	-2.2 ± 0.1^{bcd}	-1.8 ± 0.2^{d}
Crumb color - b*	5.2 ± 0.4^{a}	10.0 ± 0.5^{b}	12.4 ± 0.0^{d}	10.6 ± 0.0^{bc}	17.3 ± 0.7^{f}	12.2 ± 0.2^{cd}	14.4 ± 0.5^{e}
ΔE_{Crumb}	–	10.2 ± 0.3^{b}	11.3 ± 0.2^{b}	7.4 ± 0.5^{a}	21.0 ± 0.6^{d}	14.4 ± 0.5^{c}	15.2 ± 0.2^{c}

STD, reference gluten-free bread formulation containing only rice flour and corn starch as flour mixture; B15 and B30, bread containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, bread containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 bread containing 15 and 30% respectively of B and H in equal amounts in flour mixture.$^{a\text{-}f}$, mean values ($n \geq 4$) in the same row with different superscript letters are significantly different ($p \leq 0.05$).

of bread samples including H and B were significantly lower than STD bread, according to the lower baking loss (Sahagún & Gómez, 2018). The specific volume of STD bread (7 mL/g) was higher than expected; in fact, Tufaro et al. (2022), who used a similar formulation with addition of pea protein and psyllium and the same breadmaking conditions, reported a specific volume of 4 mL/g, which is closer to that of H15 sample. Furthermore, in literature a huge range of specific volume values (1.3–4 mL/g) can be found according to the ingredients used and the type of GF bread (Cappa et al., 2013; Hager & Arendt, 2013; Mariotti, Pagani, & Lucisano, 2013; Tufaro et al., 2022). The addition of H and B at levels greater than 15% caused low specific volumes as seen in B30 and H30 formulations. Similar results were observed by Sahagún and Gómez (2018) in GF bread enriched with pea proteins and by Azeez et al. (2022) in conventional bread with addition of cashew nut proteins. On the contrary, Kahraman et al. (2022) evaluated the effect of chickpea flour (25%) - differently treated (i.e., raw, roasted and dehulled) on GF bread and reported specific volume values higher than 2.5 mL/g, thus suggesting that both the amount of added flour and its composition (i.e., protein and fiber content) and physical properties (i.e., flour particle size, foaming capacity) affect bread development. The regression model for specific volume was significant ($p < 0.10$, adjusted $R^2 = 0.94$) with the interaction terms BxRC and HxRC, and had normally distributed residuals. Similarly, crumb porosity (Table 4) was the highest in STD bread as expected based on dough leavening results and specific volume data. Furthermore, as noticeable in Fig. 5, STD-crumb was the finest, while the experimental breads showed similar porosity values and large holes except for H15 and H30 samples characterized by a denser structure. The porosity model ($p < 0.01$, adjusted $R^2 = 0.99$) had

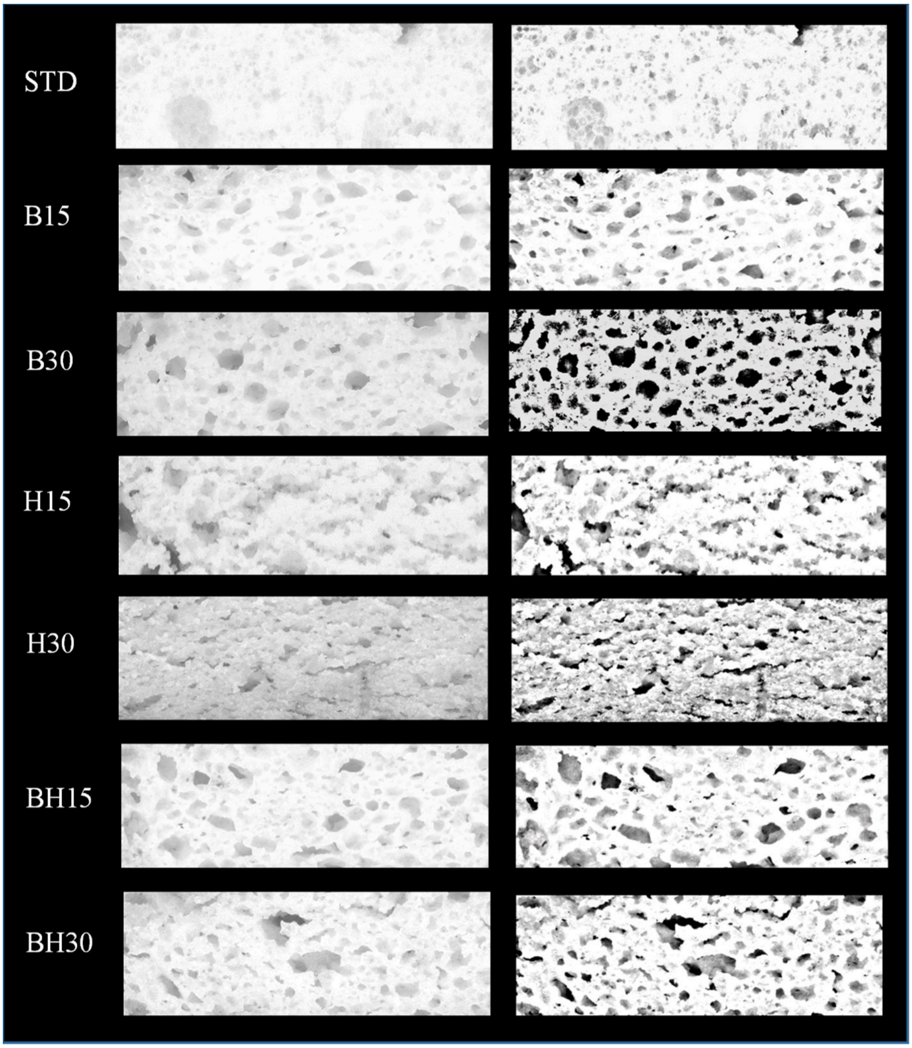

Fig. 5. Crumb crops (n = 4) of bread samples (left) and image elaboration to enhance crumb hole identification (right). STD, reference gluten-free bread formulation containing only rice flour and corn starch as flour mixture; B15 and B30, bread containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, bread containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 bread containing 15 and 30% respectively of B and H in equal amounts in flour mixture.

significant interactions of RC with both B and H.

In terms of textural characteristics, STD bread was significantly softer than the other samples, due to the highest porosity and specific volume (Table 4). The negative effect of replacement with B and H on bread porosity and specific volume also affected hardness of bread samples, which increased in presence of legume and nut flours. This is in agreement with a previous study where sourdough wheat bread formulations with mixed legume flours showed hardness increased with the amount of legume replacement (Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014). The same was found by Pycia and Ivanišová (2020) increasing the amount of walnut and hazelnut flour in wheat bread. Also Collar and Angioloni (2017) found high hardness and low specific volume in high-legume wheat-based bread. For the H- and B-enriched bread a lower dough consistency could have potentially resulted in softer bread, as suggested by data reported by Kahraman et al. (2022) for chickpea enriched bread, in which a dough consistency of 125 BU was used. Hardness produced a not significant model ($p > 0.10$, adjusted $R^2 = 0.55$), however, BxRC and HxRC interactions were found relatively more effective than linear terms.

Color of bread samples was measured on both crust and crumb. Lightness values (L*) of STD bread were significantly higher, which might be the result of the lowest protein content (Tables 1 and 2), giving limited Maillard reactions. L* values of all breads containing H were the lowest due to the brown color of hazelnut flour. Crumb b* values were higher in B and H breads (regression model with $p < 0.05$, adjusted $R^2 = 0.72$), unlike crust b* values that were significantly similar. STD bread had different crumb b* values compared with B and H breads, being less yellow. Increasing H amount made the color to move to redness in both crust and crumb due to its skin content, as seen in both crust and crumb a* values. The effect of H on L* and a* values was also observed by other authors (Anil, 2007). In terms of regression models, for crust L* values, a significant model ($p < 0.1$, adjusted $R^2 = 0.98$, normally distributed residuals) was calculated. Model of crust a* values was not significant. Reduction in crumb luminosity due to the addition of fiber sources or nut flours were also reported elsewhere (Kurek & Wyrwisz, 2015; Pycia & Ivanišová, 2020). The ΔE values were calculated to quantify the color differences between STD and the other samples. ΔE values were higher than 3, suggesting that color differences with respect to STD were detectable to the naked eye (De Souza & Fernández, 2011). The crust color most similar to STD bread was observed in B-containing breads. Addition of H in the formulation caused darker colors in both the crust and crumb, with consequently higher ΔE values (Table 4).

Softness and volume of bread are two important textural and visual characteristics that affect consumer preference and satisfaction. The highest specific volume and the lowest hardness were observed in STD bread. The objective of this study was to keep specific volume and hardness parameters as close as possible to those of STD bread, but at the same time to improve GF bread in terms of fiber and proteins. However, increasing amounts of B and H adversely affected the appealing characteristics. Thus, to generate the contour plot for bread optimization (Fig. 1), the specific volume and hardness of STD bread were considered, as well as intermediate values of experimental observations. In details, the optimization plot was generated considering the following constraints: 0.5–7 N for hardness; 3–7 mL/g for specific volume. The white area shows possible formulations with low hardness and relatively high specific volumes (closest to STD bread). The formulations within this range contain 15% replacement flours (B, H, or both). Among all the formulations, the addition of 15% H gave the appealing characteristics closest to STD formulation, while contributing to the nutritional value of bread. Indeed, according with the European regulation on nutrition and health claims made on foods (Reg. (EC) No. 1924/2006), if a product contains at least 3 g of fiber per 100 g or at least 1.5 g of fiber per 100

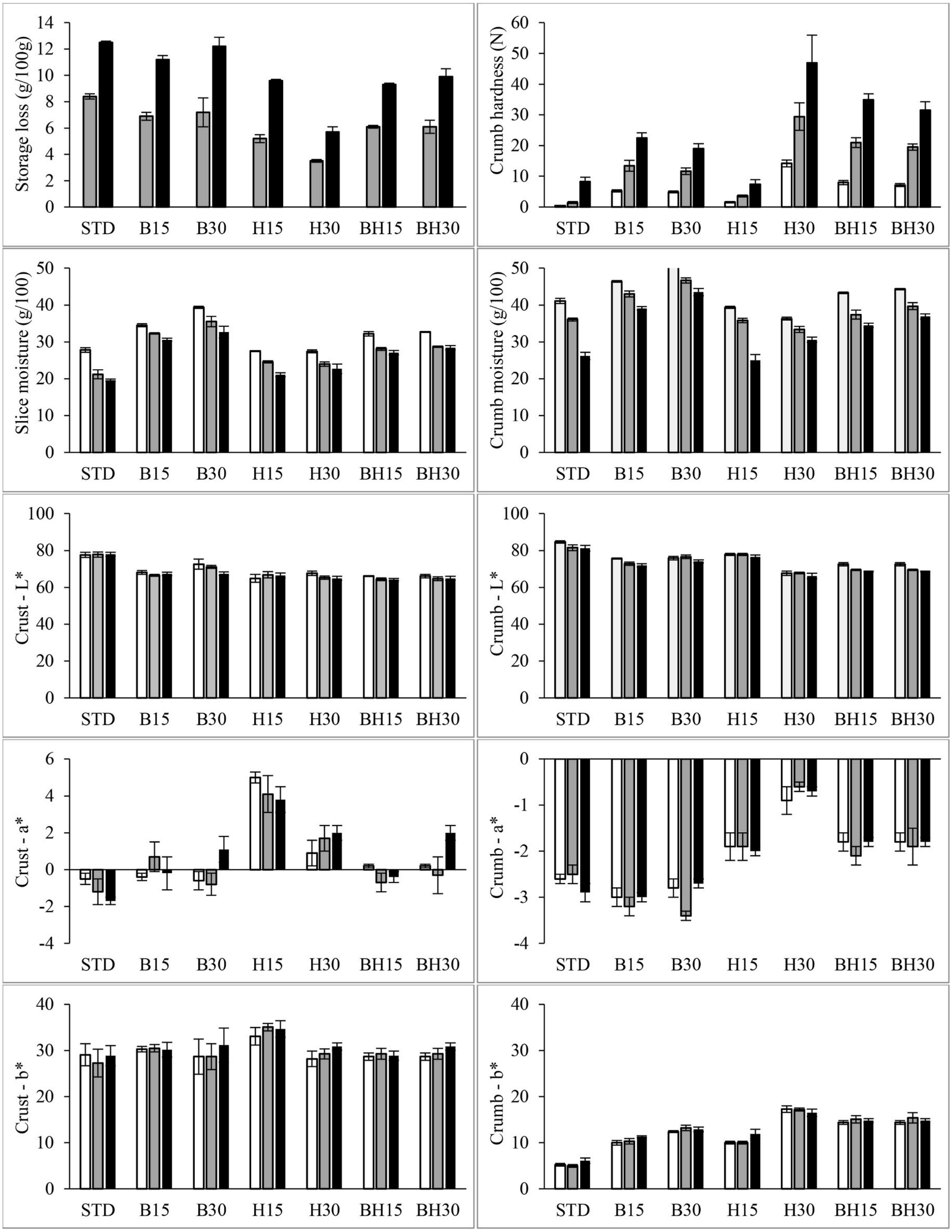

Fig. 6. Properties (n ≥ 4) of gluten-free bread samples during storage. STD, reference gluten-free bread formulation containing only rice flour and corn starch as flour mixture; B15 and B30, bread containing 15 and 30% respectively of white bean flour (B) in flour mixture; H15 and H30, bread containing 15 and 30% respectively of hazelnut flour (H) in flour mixture; BH15 and BH30 bread containing 15 and 30% respectively of B and H in equal amounts in flour mixture. White, grey, and dark grey colors indicate breads at t = 0, t = 24 h and t = 48 h, respectively.

kcal the claim "Source of Fiber" can be used. H15, H30, and BH30 bread formulations had 3.34, 4.48, and 3.27 g fiber/100g bread, respectively (calculated according with EU legislation, Reg. (EC) No. 1169/2011), thus they can be labeled with the "Source of Fiber" claim. Considering the optimal area in the contour plot in Fig. 1, and the calculated fiber content, it can be concluded that the H15 formulation meets the requirements of specific volume (>3.0 mL/g) and hardness (<7 N) and can bear the claim "Source of Fiber" (fiber content >3 g/100g).

3.5. Effect of storage on bread characteristics

Bread samples stored for 24 and 48 h were evaluated for some quality properties (Fig. 6). Water activities ranged among 0.985 to 0.883 (data not shown) according to the storage time and few differences were evidenced among the bread samples. Weight loss during storage was higher in STD and B breads. The lowest weight loss was observed in H30 which was able to retain water due to the high fiber content. As expected, moisture of samples decreased during storage, however B breads had the highest moisture at the end of 48h storage period, due to the higher amount of water added to the dough (i.e., farinographic water absorption). As an indication of staling, gradual increase in hardness was observed in all stored samples. The increase was higher in formulations including both B and H, whereas H15 sample at the end of storage was even softer than STD. During storage, STD and H15 breads showed similar changes in moisture, but with a lower weight loss in H15, thus confirming H15 as the best GF formulation. Color parameters showed patterns similar to those of fresh breads such as the highest L* values in STD breads, and the positive a* values in hazelnut breads (H15 and H30) as a result of the skin presence in H flour.

4. Conclusion

Inclusion of hazelnut and white bean flour in GF rice flour-corn starch mixture was studied in bread making with an extreme vertex mixture design. Results showed that the fiber content of bread samples can be increased to at least 3 g/100 g bread, which allowed the use of the claim "Source of Fiber" in label, according with the European legislation. The standard bread formulation with no legume nor nut flour had the highest specific volume and the lowest hardness, but bread replaced with 15% H was found to have the second highest specific volume, and the lowest hardness value among all legume and nut bread samples. Indeed, the optimal experimental region calculated using the significant models obtained for specific volume and crumb hardness indicated the H15 formulation as the best one. Moreover, during storage, H15 was found to have the characteristics closest to STD in terms of moisture and hardness, with a lower storage weight loss. Among stored breads, formulations with B had the highest moisture and water activity levels.

Findings of this study showed that the use of legume flours, such as bean flour, along with hazelnut flour could increase the nutritional value of GF bread formulations, by keeping acceptable quality properties. Of course, it has to be considered that the results obtained are valid for the tested flours and the bread making procedure applied: different commercial flours and/or baking conditions could affect GF bread properties and scaling-up of the production.

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CRediT authorship contribution statement

Ayça Tuna: Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Carola Cappa:** Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Figen Tokatli:** Conceptualization, Investigation, Resources, Data curation, Writing – review & editing. **Cristina Alamprese:** Conceptualization, Investigation, Resources, Data curation, Supervision, Project administration, 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

Data will be made available on request.

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